Aalborg Universitet

AALBORG UNIVERSITY
DENMARK

Ex-Ante LCA for Microalgae-Based Veterinary Molecules in Finfish Aquaculture: How to Assess the Environmental Performance of Unknown Molecules ?

Jouannais, Pierre; Pizzol, Massimo

Publication date:
2021

Document Version
Publisher's PDF, also known as Version of record

Link to publication from Aalborg University

Citation for published version (APA):
Jouannais, P., & Pizzol, M. (2021). *Ex-Ante LCA for Microalgae-Based Veterinary Molecules in Finfish Aquaculture: How to Assess the Environmental Performance of Unknown Molecules ?*. Abstract from Setac Europe 31st annual meeting, Sevilla, Spain.

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

Take down policy
If you believe that this document breaches copyright please contact us at vbn@aub.aau.dk providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from vbn.aau.dk on: April 19, 2024

SETAC

EUROPE2021

SETAC EUROPE 31ST ANNUAL MEETING

ABSTRACT BOOK

GLOBAL CHALLENGES. AN EMERGENCY FOR ENVIRONMENTAL SCIENCES.

3–6 MAY 2021 | VIRTUAL

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, electrostatic, magnetic tape, mechanical, photocopying, recording, or otherwise, without permission in writing from the copyright holder. SETAC Europe's consent does not extend to copying for general distribution, for promotion, for creating new works, or for resale. Specific permission must be obtained in writing from SETAC for such copying. Direct all inquiries to SETAC Europe.

PRINT ISSN 2309-8031 - ONLINE ISSN 2310-3043 © 2021
Society of Environmental Toxicology and Chemistry Europe (SETAC Europe)

ABSTRACT BOOK
SETAC EUROPE 31ST ANNUAL MEETING

TABLE OF CONTENTS

Keynote Abstracts:	1
Platform Abstracts:	3
Poster Abstracts:	128
Keyword Index:	291
Author Index:	295

This book compiles the abstracts from the platform and poster session presentations at the 31st Annual Meeting of the Society of Environmental Toxicology and Chemistry - Europe (SETAC Europe), conducted as a virtual conference from 3–6 May 2021.

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 underlined.

SETAC Europe Office
Avenue des Arts, 53
B-1000 Brussel
Belgium
T +32 2 772 72 81

setaceu@setac.org
setac.org

SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY

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®, to timely and effective communication of research, and to 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). Environmental Toxicology and Chemistry 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.

Keynote Lecture Abstracts

K1

On the Road To a Resilient, Zero-Carbon Future, We Must Do It Right!

Erwan Saouter, Managing Director at Saouter's Consulting

The quality of life we have reached in developed economies is dependent on easy and prolific access to (concentrated) energy. Energy (with an 80% fossil fuel mix) is behind every activity and benefits we experience daily. A large part of the world still needs access to basic needs, requiring increased energy demands. Projections establish that global energy consumption and associated raw material consumption will double by 2050. With a demographic expansion soon reaching 10 billion individuals, we also need to double current food production levels.

Is it possible to sustain our appetite for goods and their energy demand? Solutions exist. Alternatives like 'negative growth,' 'circular economy,' 'full renewable energy,' and others have existed for decades. Sadly enough, while these solutions exist, our global emissions keep rising. By now, the world knows. Companies know. Countries and politicians know (or at least most of them). We must decarbonize our economy globally. The challenge is to act fast and lead a transition that is inclusive of all economies. But first and foremost, we must do it right.

More than 100 countries and regions have pledged to net-zero carbon strategies. Over 2000 companies have joined the movement, representing a potential reduction of 2.8 gigatons of CO2 (about the size of India's emissions). Close to 1200 of these companies committed to Science-Based Targets and half have their targets already certified. The potential impact of these new commitments still needs to be translated into actual GHG reduction but is very encouraging.

However, it is crucial to base our transition to a carbon-free (and fossil-free) economy on strategies that assess all potential impacts holistically. We must take a systemic approach to understand long-term effects and avoid seeing short-term solutions for today's climate crisis become the next big environmental issues.

In this talk, we wish to provide a fresh perspective on this complex problem. We will discuss how proposed alternatives, like solar panels, windmills, biomass, biogas, or hydrogen, among others, do not come free of impacts. These energy sources are low carbon and are essential solutions to fight climate change. But we must understand the implications on land use, resource needs (steel, concrete, sand, metals, water, rare earth elements), or toxic waste emissions. We will present where we stand in such an assessment and emphasize the urgency to do a lot more and where SETAC could play a critical role in generating this knowledge.

K2

Fostering Responsible Research Practices Needs Attention From All Stakeholders

Lex M. Bouter, Vrije Universiteit Amsterdam, Netherlands

Traditionally research integrity focused on detecting and sanctioning research misconduct. But recently it has become clear that promoting responsible research practices and preventing questionable research practices, like p-hacking, HARKing (Hypothesizing After the Results are Known) and selective reporting, may avoid many harms to the validity and the trustworthiness of research. Especially the ongoing replication crisis has shown that more transparency and the adoption of open science practices are essential. The weak points of the research system become especially clear under pressure, like during the current Covid 19 pandemic. Only when the important stakeholders (researchers, research institutes, funding agencies, and scholarly journals) collaborate closely meaningful progress can be made.

K3

Small(er) Plastics, Big(ger) Problems? Fate, Transport and Implications of Nano- and Microplastics in the Environment

Prof. Denise M. Mitrano, Department of Environmental Systems Science, ETH Zürich, Switzerland

Numerous studies have made the ubiquitous presence of plastic in the environment undeniable, and thus it no longer comes as a surprise when scientists measure the accumulation of macroplastic litter and microplastic fragments in both urban and remote sites. Ultimately, the different physical and chemical characteristics of the different size classes of plastic pollution (macroplastic, microplastic and nano plastic) will result in divergent fate and hazards. Quantitative data are still limited due to analytical difficulties to detect nano plastics in complex matrices, and thus mechanistic studies to understand the fate, transport and biological interactions of these materials are limited. While progress is still ongoing to develop protocols to measure particulate plastic in field studies, researchers who study these processes in benchtop or pilot scale studies can take advantage of an entirely different approach. In the last years, we have synthesized a variety of particulate plastics with an embedded inorganic fingerprint which can be used as a proxy to detect plastic by common analytical techniques for trace metals analysis. In practice, this affords for quicker and more accurate sampling and subsequently allows us to investigate the basic processes and pathways which control particulate plastic fate and impacts. To highlight the utility of this approach, we have used these materials in a number of different test systems including, 1) mass balance and flux of plastic through pilot-scale wastewater and drinking water treatment plants, 2) application of sewage sludge in agriculture and plastic mobility through porous media and 3) the interaction and uptake of nano plastics with plants and organism. As environmental nanoscientists, we try to place nano plastic in the context of global plastic pollution by assessing its source and risk, but also by assessing commonalities nano plastics may share with other nano-sized objects in environmental systems, such as engineered nanomaterials and natural colloids. The presence of plastic in the environment has sparked considerable discussion amongst scientists, regulators and the general public as to how industrialization and consumerism are shaping our world. Restrictions on the intentional use of primary microplastics are under discussion globally, despite uncertain microplastic hazards and prioritization amongst options for action. Regulations should have a precise focus and must be enforceable by measurements. Policy must carefully evaluate under which contexts microplastic use may be warranted and where incentives to replace certain microplastics can stimulate innovation of new, more competitive and environmentally conscious materials. Collectively, our research aims to understand the implications of (nano- and micro)plastics in the environment and provide information to make more sound and sustainable choices in relation to plastic use and waste management.

K4

Risk Communication During the COVID-19 Pandemic – What Can We Learn for Risk Communication in General

Mirjam Jenny, Robert Koch Institute, Germany

The COVID-19 pandemic has increased the need for transparent and clear risk and science communication. Increasing citizens' risk literacy through effective communication can improve pandemic outcomes. During my talk, I will define risk literacy, discuss core elements in risk communication and give an overview of risk communication tools that can help public health institutes and other public or science organizations increase citizens' risk literacy. I will also highlight gaps in current risk communication strategies. Wherever possible, I will distil some lessons learned into more general principles for risk communication.

Platform Abstracts

Alternative Approaches to Animal Testing for Ecotoxicity and Environmental Risk Assessments

1.01.02

The Utility of a Primary FIsh Gill Cell Culture System (FIGCS) for Ecotoxicity Testing of Pharmaceuticals

S. Schnell, King's College London / Department of Nutritional Sciences; P. Kille, Cardiff University / School of Biosciences; N. Bury, University of Suffolk / School of Engineering, Arts, Science and Technology; C. Hogstrand, Kings College London / Department of Nutritional Sciences

Large numbers of fish are used worldwide to assess environmental effects of pharmaceuticals that may be released into aquatic ecosystems. In fish, the gill is one of the most critical sites for both absorption and toxicity to waterborne chemicals. We therefore developed a primary gill cell culture model and tested its potential to replace fish in ecotoxicity testing. The FIsh Gill cell Culture System (FIGCS) is constructed from rainbow trout gill cells grown into a tight epithelium on cell culture well inserts. This epithelium tolerates culture in water on the apical side, allowing the cells to be directly exposed to water samples. We investigated if FIGCS can predict toxicity of pharmaceuticals on fish. Transcriptomics and network analysis were used to identify targets of effects for seven diversely acting pharmaceuticals: clofibrate, diclofenac, ethyl oestradiol, fluoxetine, ivermectin, tetracyclin, and triclosan. Next, marker genes of effects on top hit networks were identified and their dose-responses to the pharmaceuticals analysed. Interestingly, the most sensitive networks affected by the pharmaceuticals were more often than not different from those predicted based on their therapeutic targets. Whilst cytotoxicity assays were not sufficiently sensitive to be used as a surrogate toxicity endpoints for pharmaceuticals, the molecular markers responded well and changes in their expression were observed at concentrations overlapping with published LC50 and in some cases NOEC in fish. Therefore, the utility of FIGCS shows promise as a low tier toxicity testing tool for environmental pharmaceuticals.

1.01.07

Regulating a SWiFT Transition: A Bayesian Network As Weight-Of-Evidence Approach to Replace the AFT With the FET

R. Wolf, NIVA - Norwegian Institute for Water Research / Section for Ecotoxicology and Risk Assessment; S.E. Belanger, The Procter & Gamble Company (retired) / Global Product Stewardship; T. Braunbeck, University of Heidelberg / AquaTox Centre for Organismal Studies; K.A. Connors, Procter & Gamble Company / Global Product Stewardship; M.R. Embry, Health and Environmental Sciences Institute (HESI); A.L. Madsen, Hugin Expert; J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; K. Schirmer, Eawag / Environmental Toxicology; S. Scholz, Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology; A. Lillicrap, NIVA / Section of Ecotoxicology and Risk Assessment

In 2006, the fish embryo toxicity (FET) test was submitted as a new proposed OECD test guideline. The draft proposal explicitly stated that the FET constitutes an alternative to acute fish toxicity (AFT) testing. The FET was adopted as OECD guideline 236 in 2013, after a long period of careful development and extensive laboratory ring trials; however, the test was not unanimously accepted before the term “alternative approach” had been removed from the introduction section of the guideline. Regulatory agencies, such as the European Chemicals Agency (ECHA), have declared to be open to the use of FET data within a weight-of-evidence (WoE) approach. However, industry and other stakeholders have been challenged to develop a methodology that would be needed for such a WoE implementation. As a response to the challenge posed by ECHA, a preliminary Bayesian network (BN) was developed to predict AFT toxicity intervals from a combination of FET data with other lines of evidence. These additional lines of evidence included physico-chemical properties, chemical category, and toxicity to other taxa, represented by algae and daphnids. This preliminary BN has been successfully implemented and evaluated for a small set of chemicals in a proof-of-concept WoE approach. All lines of evidence of the preliminary BN represent regulatory accepted methods, but the model lacks details on biotransformation, mode of action, and uncertainty of the toxicity nodes. The preliminary BN therefore needs to be expanded to increase its predictive power by including more information. Additional lines of evidence can include, amongst others, information on biotransformation, cytotoxicity, neurotoxicity, and chemical grouping approaches. The SWiFT (Strengthening Weight of evidence for FET data to replace acute Fish Toxicity) project aims to provide a supportive framework for regulatory acceptance of FET data to fulfil the requirements for acute fish testing. The project will deliver an advanced and restructured BN model to predict the toxicity intervals of AFT for any structurally defined chemical. Additionally, an interactive web interface will provide public access to the model. This advanced BN will represent a quantitative WoE approach with increased predictive power compared to traditional qualitative WoE approaches.

1.01.08

Are Two Negative Controls Needed When a Solvent Is Used in the Fish Early Life Stage Test (OECD TG 210)?

J.W. Green, JohnWGreen-ecostats.com / Data Science and Informatics; C. Fassbender, G. Stoddart, PETA International Science Consortium Ltd.; M. Halder, European Commission Joint Research Centre / DG Joint Research Centre IHCP EURL ECVAM; L. Weltje, BASF SE / Ecotoxicology; J. Handley, Envigo; K.A. Connors, Procter & Gamble Company / Global Product Stewardship

Data from the fish early life stage test (OECD Test Guideline [TG] 210) is often required to establish the environmental safety of products such as biocides, pesticides and industrial chemicals before they receive regulatory approval. With difficult-to-test substances, discussed in OECD Guidance Document 23 (OECD 2019), it is sometimes necessary to use a low concentration of a solvent to maintain an adequate and stable supply of the test chemical throughout the study. Currently, TG 210 requires both water and solvent controls when a solvent is used, which increases the number of animals used per study by 17%. Omitting the water control could significantly reduce animal use, therefore, an international team of scientists led by the United States and the International Council on Animal Protection in OECD Programs is exploring whether both controls are necessary in TG 210 studies when a solvent is used. This presentation explores the justification for omitting the water control from TG 210 studies and presents preliminary results. A TG 210 control database including new and previously published data (Oris *et al*., 2012) was compiled. The data predominantly includes fathead minnow studies using the solvent dimethylformamide. Additionally, full concentration-response data is available for some studies. The control database provides an adequate basis for extensive computer simulations to determine: · The ability to detect or estimate biologically meaningful effects of the test chemical on required TG 210 responses with both water and solvent controls and with only a solvent control; · Whether there are systematic differences between the two controls for one or more responses; · Whether there is an appreciable loss in power or sensitivity if the water control is not included in the study design when a solvent is used. Furthermore, concentration-response data is used to investigate the treatment effect (NOEC, EC_x regressions) when using the water, solvent or pooled controls. The biological, statistical, and regulatory concerns regarding the need for, and use of, both water and solvent controls in TG 210 studies are explored and a critical understanding is developed of the usefulness of having two controls to determine study acceptability and what, if anything, is lost when only one control is included in TG 210 studies.

1.01.09

Biotransformation Potential of Zebrafish (Danio rerio) Early Life Stages and the Zebrafish-Derived PAC2 Cell Line

A. Tierbach, Oekotoxzentrum; K.J. Groh, Eawag - Swiss federal Institute of Aquatic Science and Technology / UTOX Environmental Toxicology; R. Schoenenberger, Eawag - Swiss federal Institute of Aquatic Science and Technology; K. Schirmer, Eawag / Environmental Toxicology; M.J. Suter, Eawag - Swiss federal Institute of Aquatic Science and Technology

Zebrafish (*Danio rerio*) test systems, such as early life stages and isolated cell lines, provide an indispensable tool for chemical risk assessment, due to their genetic similarity to humans and the availability of well-established high-throughput techniques. However, despite the identical genetic constitution, the biotransformation capacity of embryos and the embryonic cell line PAC2 can vary due to differences in the repertoire and activity of biotransformation enzymes. For an accurate interpretation of toxicity results, it is therefore important to understand the similarities and differences of the zebrafish test systems. Glutathione and the associated phase II biotransformation pathway, i.e., the mercapturic acid route, are important for the clearance of electrophilic compounds and phase I biotransformation products. The most important reaction of this pathway is the initial conjugation of glutathione and the electrophile, catalyzed by glutathione S-transferases (GSTs). Thereafter, the glutathione conjugate is further biotransformed in multiple, spatially separated steps to the mercapturate, which is then excreted. With the aim to characterize the mercapturic acid pathway in zebrafish early life stages and the PAC2 cell line, we studied the GST protein repertoire (*via* targeted proteomics) and monitored the different biotransformation products of the model substrate 1-chloro-2,4-dinitrobenzene (CDNB) in the two test systems (*via* high-resolution mass spectrometry). In the two test systems, we found that, while the GST repertoire differs between zebrafish early life stages and the PAC2 cell line, it does not affect the functionality of the mercapturic acid pathway. For both test systems, the majority of biotransformation products and in both cases the final product were detected, demonstrating that the mercapturic acid pathway is fully functional. The ability of the zebrafish early life stages and PAC2 cells to biotransform chemical compounds within the mercapturic acid pathway confirms their potential for use in chemical screening applications.

1.01.10

Qsar-Based Prediction of the Biomagnification of Organic Chemicals in Fish
L. Bertato, University of Insubria / QSAR Research Unit/Department of Theoretical and Applied Sciences; O. Taboureau, Université de Paris; E. Papa, University of Insubria / Department of Theoretical and Applied Sciences
Studies and experiments carried out to address the bioccumulation of chemicals are of great importance to provide useful information to perform risk assessment. Several metrics have been used to describe chemical accumulation in biota such as the biomagnification factor (BMF). However, since the experimental quantification of this parameter is expensive and time consuming, *in silico* models such as those based on Quantitative Structure Activity Relationships (QSAR) can be used as a valid alternative. In this study a large set of fish laboratory-based BMF values composed of 736 data measured for 320 heterogeneous compounds was investigated and used to develop QSARs. After data curation multiple data available for a single compounds were averaged and log transformed and cover a wide range of log BMF from -5.70 to 1.26. Linear and non linear regression and classification techniques were applied to generate QSARs after calculation of theoretical molecular descriptors from the molecular structures.The models were developed taking into account regulatory needs such as OECD requirements for model's transparency, statistical validity, defined domains of applicability as well as interpretation of the structural information found to be relevant in the models. The good performances of the proposed regression and classification models are described in this study and compared to former literature results. Our results suggest that the here proposed QSARs, which are based on a larger chemical domain than former models, can be used to generate reliable predictions of fish BMFs. These models and their predictions are useful to support bioaccumulation assessment for existing and new chemicals, which is an important factor to evaluate risks that they may pose in the environment.

1.01.15
In Vitro Assays As Non-Animal Alternatives to Assess Metals and Nanoplastic Effects in Embryos of Xenopus laevis
D. Brotas, University of Aveiro / Department of Biology; M. Oliveira, University of Aveiro & CESAM / Centre for Environmental and Marine Studies (CESAM) & Department of Biology; I. Lopes, University of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies
Over the last 40 years amphibian populations are reported to be increasingly declining. This decline is mostly attributed to anthropogenic disturbances and to the negative effects they pose to this group of vertebrates. Until recently, the risk assessment of chemicals for amphibians was based on the data generated for fish and for birds and mammals. However, it has been reported that the use of data from these three groups of vertebrates may underestimate the risk of some chemicals to amphibians. It is, therefore needed to generate toxicity data specifically for amphibians in order to promote its accurate protection and conservation. The present study aimed at assessing the toxicity of two metals and a nanoplastic to aquatic early life stages of *Xenopus laevis*. It was also intended to assess the adequacy of using *in vitro* assays, with amphibian cell lines, as surrogates of the *in vivo* assays. Embryos and tadpoles of *X. laevis* were exposed to a set of concentrations of silver, lead or nanoplastics of polystyrene and the following endpoints were monitored: for embryos – mortality, malformations, hatching rate and body length; and for tadpoles – mortality, growth rate (as weight gain and body length increase), developmental stage, and heart beat rate. Results obtained with the embryos and tadpoles assays, showed significant adverse effects at lead concentrations equal or above 0.22 mg/L, while the *in vitro* assay with lead revealed a significant reduction of cells viability at concentrations equal or higher than 0.078 mg/L. Regarding silver, of 0.013mg/L induced the appearance of malformations, an early hatching and increased snout-to-vent length of larvae, increase in the tail and total body growth rates. The *in vitro* assay revealed to be very sensitive to silver, concentrations as low as 0.0004 mg/L of silver reduced the viability of cells. Nanoparticles of polystyrene induced no significant effects on both embryos and tadpoles. The *in vitro* assays performed with lead, revealed that cell lines are slightly more sensitive to lead than embryos or tadpoles of *X. laevis*. In the case of silver, toxic effects in the cell lines were observed at concentrations much lower than those inducing effects in embryos and tadpoles. These results suggest that for early stages of risk assessment frameworks, *in vitro* assays may be used for a first toxicity screening in order to avoid running animal experimentation.

1.01.17
In Vitro Studies Using Blood and Skin Biopsies to Evaluate Toxicity of PBDE-47, DEHP and Chrysene in Caretta Caretta (Linnaeus, 1758)
I. Caliani, University of Siena; A. Di Noi, University of Siena / Department of Life Sciences; T. Campani, University of Siena / Department of Physical, Earth and Environmental Sciences; L. Bini, L. Bianchi, University of Siena / Department of Life Sciences; S. Casini, University of Siena / Scienze Fisiche della Terra e dellAmbiente
The loggerhead sea turtle (*Caretta caretta*) is a long-lived carnivorous reptile included in the IUCN Red List. The IUCN assessment for this species underlines the lack of information regarding pollution and pathogen effects. A complete ecotoxicological assessment of this species includes not only the non-invasive sampling and monitoring of alive specimens but also the development of *in vitro* alternative approaches to evaluate ecotoxicity of persistent, bioaccumulative, and/or toxic compounds, including emerging contaminants. In this study, *in vitro* experiments were performed on blood and skin biopsy, taken from hospitalized specimens of *C. caretta*, treated with three different contaminants (Polybrominated diphenyl ethers-47 (PBDE-47), Di(2-ethylhexyl) phthalate (DEHP), chrysene). Immune system (lysozyme activity, respiratory burst, complement system) endpoints, genotoxicity biomarkers (Erythrocytic Nuclear Abnormalities (ENA) assay, comet assay), oxidative stress (TAS assay-Total Antioxidant Status assay) were evaluated in treated blood, while proteomic analysis were performed on the treated biopsies. DEHP and PBDE-47 were able to cause genotoxic effects. A statistically significant positive correlation was found between ENA and comet Assay ($r = 0.46$). All tested compounds produced an increase in the response of two of the immune system endpoints, the respiratory burst and complement activity. Complement system activity showed a significant positive correlation with micronuclei frequency ($r = 0,43$). Using the proteomics approach, 17 statistically significant quantitative differences were identified between the DMSO control and PBDE-47 treatment, of which 8 indicate an overexpression of proteins identified after exposure to PBDE-47 and 9 show a protein under-expression following the same treatment. Furthermore, 2 statistically significant qualitative differences were identified; in both cases the protein was present only in the control.

Alternative Methods for Safety Assessment of Nanomaterials in the Aquatic Environment

1.02.01
New Approach in Mussel Hemocytes Immunotoxicity Test to Assess Nanomaterials
J. Blasco, Instituto de Ciencias Marinas de Andalucia (ICMAN-CSIC) / ECOLOGY AND COASTAL MANAGEMENT; M. Sendra, CSIC - Spanish National Research Council / ECOLOGY AND COASTAL MANAGEMENT
In vitro assays have been promoted by EU as a sustainable alternative to animal testing and safety assessment of chemicals. These methodologies consist in a proper replacement, reduction and refinement of animal testing. The Directive on the protection of animals (2010/63), the Regulation on cosmetic products (1223/2009), REACH (2007/2006) and Classification, Labelling and Packaging (CLP) (1272/2008) are examples of EU strongly encourage the replacement of animal testing. Invertebrates represent more than 90% of the extant aquatic species, and in particular marine mussel *Mytilus galloprovincialis* due to sessile status, filtration capacity and cosmopolitan characteristics has shown to be a sensitive target to test the impact of numerous pollutants for a large number of environmental pollutants. In vitro immunotoxicity test has been proposed to be a tool to assess the immunomodulatory effect of nanomaterials (NPs); (Canesi et al., 2015). Despite the advantage, *M. galloprovincialis* mussels have a pan-genome and its genes are subject to presence-absence variation, which 25% of them may be entirely missing in several individuals (Gerdol et al., 2020). Due to this variation the variability among organisms and the response ways could be an artefact in the results of toxicity tests when small pools of different organisms are assessed. Among the emergent pollutants assessed in nowadays, nanoplastics is a pollutant of emerging concern due to high environmental concentration of plastic litter in marine ecosystems, due to small size and its interaction with cells and surrounding media and to be not only a common pollutant but soup of pollutants due to a large number of additives incorporated in plastic synthesis. In this study is proposed a new methodology to use pools in *in vitro* assays as well as a new methodology by flow cytometry to perform the simultaneous analysis of hemocytes cytotoxicity.

1.02.02
Alternative Methods for Safety Assessment of Nanoplastics in the Marine Environment
M. Auguste, Unversity of Genova / DISTAV; T. Balbi, university of genoa / DISTAV; C. Ciacci, University of Urbino / DIBS; L. Canesi, university of genoa / DISTAV
Nanoplastics-NPs (mainly from degradation of microplastics) and their potential impact on aquatic biota are receiving increasing attention. Therefore, there is a need to develop methods for risk assessment of this group of emerging pollutants. Filter feeding invertebrates, such as the marine bivalve *Mytilus galloprovincialis*, represent important targets for nanomaterials, including NPs. Data are here summarized on the effects of NPs on *Mytilus* evaluated in both alternative experimental settings (embryotoxicity tests and cellular *in vitro* toxicity tests) and standard exposure conditions (*in vivo* exposure of adult specimens), utilizing commercial surface modified nanopolystyrenes (PS-NH_2 and PS-COOH) as a proxy for NPs. PS-NH2 (50 nm) significantly affected early larval development in the 48 hpf embryotoxicity assay from 10 µg/L and with an $EC50 = 142$ µg/L. Alterations of shell formation and of transcription of genes involved in different

physiological processes were observed.Short term *in vitro* exposure of hemocytes to different types of NPs (30 min at low µg/mL), rapidly affected functional immune parameters and cell morphology, depending on NP size and surface characteristics, as well as on their behaviour exposure medium (ASW or hemolymph serum-HS) and formation of a protein corona in HS.The immunomodulatory effects of PS50 were confirmed *in vivo*, in single exposure experiments (24-96 h) together with changes in hemolymph microbiota composition. Repeated exposure experiments also revealed both tolerance and potentiation as compensatory mechanisms to maintain immune homeostasis, representing a form of immune memory. Overall, the results underline that alternative tests in *Mytilus* can be successfully utilized for safety assessment of NPs in the marine environment. 1) the standard embryotoxicity assay provides robust information for screening the impact of NPs on sensitive larval stages, also encompassing predicted environmental levels; 2) development of simple cellular assays on hemocytes (in vitro, non invasive) allows for the fast screening of NP immunosafety and nano-biointeractions in physiological media; 3) the *in vitro* effects can be then confirmed by classical *in vivo* exposure experiments, at much lower concentrations. The application of different approaches can give an overall estimation of the risk of exposure to NPs in mussels as model organisms wordlwide utilized in biomonitoring of marine coastal areas.

1.02.03

Intracellular Fate of Polystyrene Nano and Microplastics With or Without Benzo(A)Pyrene in Mussel Haemocytes

A. Katsumiti, PiE-University of the Basque Country / Zoology and Animal Cell Biology; M. Losada Carrillo, M. Barros, M.P. Cajaraville, University of the Basque Country / CBET Research Group, Dept. Zoology and Animal Cell Biology Faculty of Science and Technology and Research Centre for Experimental Marine Biology and Biotechnology PIE

Nanoplastics (NPs) and microplastics (MPs) can cause harmful effects and are able to adsorb and act as vectors of hydrophobic pollutants to aquatic organisms. In this study, the intracellular fate of NPs and MPs with or without benzo(a)pyrene (BaP) was investigated in mussel haemocytes using confocal microscopy, transmission electron microscopy (TEM) and a phagocytosis assay. For confocal microscopy, cells were exposed for 24h to 0.05µm NPs (10^{12} part/mL), 0.5µm MPs (10^{9} part/mL) and 4.5µm MPs (10^{8}part/mL) with or without BaP and to BaP alone (1µM). For TEM, haemocytes were exposed to the same doses of NPs and MPs without BaP. To assess haemocytes phagocytic activity, cells were exposed for 24h to 0.05µm NPs (10^{2}-10^{12} part/mL), 0.5µm MPs (10^{2}-10^{9}part/mL) and 4.5µm MPs (10^{2}-10^{8} part/mL) with or without BaP and to BaP alone (0.12, 0.56 and 1µM). In confocal microscopy, 0.05µm NPs were not discernible. In cells treated with 0.5µm MPs, structures resembling particle aggregates were found in cells cytoplasm and apparently in lysosomes. In haemocytes treated with 4.5µm MPs, MPs were easily distinguished in cells cytoplasm. In cells treated with NPs and MPs with BaP, BaP fluorescence was detected mainly in the cytoplasm, both outside and inside lysosomes. BaP alone was found mainly in lysosomes. Regarding TEM analysis, 0.05µm NPs were localised in the cytosol, possibly internalised by passive transport. 0.5µm MPs were surrounded by cell surface extensions and found inside lysosomal vesicles, possibly incorporated by macropinocytosis. 4.5µm MPs were engulfed through phagocytosis and found inside vesicles (possibly phagosomes or phagolysosomes). Phagocytic activity increased in haemocytes treated with 10^{5}-10^{12} part/mL 0.05µm NPs and 10^{6}-10^{9} part/mL 0.5µm MPs. When combined with BaP, phagocytic activity decreased at the highest doses of NPs and MPs. Phagocytic activity was also reduced in haemocytes treated with BaP alone. In conclusion, polystyrene NPs and MPs with or without associated BaP were taken up by mussel haemocytes and found both outside and inside lysosomes and other membrane-bound vesicles. These data indicate the ability of NPs and MPs to act as vehicles of BaP into mussel haemocytes. Further, NPs and smaller MPs triggered phagocytic activity but this was counteracted when associated with BaP, suggesting a differential immunomodulation by NPs and MPs with or without BaP.

1.02.05

Insight Into Nanoplastics-Cell Interactions: The Role of Surface Charges in Uptake and Toxicodynamics in Immune Cells of the Sea Urchin Paracentrotus lividus

C. Murano, University of Siena / Biology and evolution of marine organisms; E. Bergami, University of Siena / Physical, Earth and Environmental Sciences; G. Liberatori, University of Siena / Department of Physical, Earth and Environmental Sciences; A. Palumbo, Stazione Zoologica Anton Dohrn Naples / BEOM; I. Corsi, University of Siena / Physical, Earth and Environmental Sciences

Nano-sized polystyrene (PS) has been recently detected in seawaters of the Mediterranean Sea, becoming a source of concern due to its dual nature, chemical as well as related to the nanoscale, which can cause harm to marine biota. As a result of a weathering process, which includes changes in crystallinity and surface oxidation, NPs in the marine environment are expected to present oxygenated moieties, such as carboxylic groups (-COOH), and thus a negative surface charge. In this context, this study investigates the toxicokinetics and toxicodynamics of negatively charged carboxyl-modified PS NPs (PS-COOH, 62 nm), as a proxy for nanoplastics, on short-term primary cultures of *P. lividus* coelomocytes. The immune response of coelomocytes to PS NPs was evaluated through cell viability, lysosomal membrane stability and phagocytic activity after short-term (4 h) *in vitro* exposure to PS-COOH at 5 and 25 µg mL^{-1}. Positively charged amino-modified PS NPs (PS-NH_2, 50 nm) at 25 µg mL^{-1} were tested as a positive control. Dynamic light scattering analysis of PS NP suspensions in coelomic fluid (CF) revealed that both PS NPs were still found as nano-scale agglomerates after 4 h of exposure, though with a high polydispersity index (> 0.3 for both NPs). Once suspended in CF, PS-COOH and PS-NH_2 showed a low negative charge, as indicated by a ζ-potential of -11.1 ± 3 mV and -12.1 ± 4 mV, respectively. After 4 h of exposure to PS-COOH, no effect on cell viability was observed, but a significant decrease in lysosomal membrane stability (23.7 ± 4.8%) and phagocytic capacity (63.4 ± 3.4%) was found at the highest concentration (25 µg mL^{-1}). Positively charged PS-NH_2 led to the most prominent immune response and significantly reduced cell viability, lysosomal membrane stability and phagocytosis at 4 h, in agreement with previous studies. To gain insight into NP–coelomocytes interactions, time-resolved cellular uptake of fluorescently labelled PS-COOH NPs (5 and 25 µg mL^{-1}) was monitored at different time points (0, 0.5, 1, 2, 3, 4 h) by qualitative (optical and confocal fluorescence imaging) and quantitative (fluorimetric) analysis. PS NP uptake in phagocytes started within 1 h of exposure and occurred in a time-dependent manner. Overall, these findings improve our understanding of the toxicokinetics and toxicodynamics of negatively charged nanoplastics in *P. lividus* coelomocytes, which are confirmed as prominent biosensors for emerging stressors.

1.02.06

Transcriptomic Profile in Understanding the Mode of Action of Amino-Modified (PS-NH2) Polystyrene Nanoplastics in Ascidian Ciona Robusta Embryogenesis

M. Eliso, Universita degli Studi di Siena / Department of Physical Sciences, Earth and Environment; I. Corsi, University of Siena / Physical, Earth and Environmental Sciences; A. Spagnuolo, Zoological Station Anton Dohrn / Department of Biology and Evolution of Marine Organisms

The growing concern around nanoplastics (NPs) has led to a wide investigation of their effects on the embryogenesis, survival, immune system and behavior of different aquatic organisms. Nowadays, there is a growing need to understand the mode of action (MoA) of NPs, using the omics technologies such as transcriptomic approach (RNA-seq). In this regard, *Ciona robusta* embryos (2-cell stage) were exposed to 0, 10 and 15 µg mL^{-1} of Polystyrene Nanoparticles amino-modified (PS-NH_2, 50 nm) for 13 h. A total of 42 and 186 differentially expressed genes (DEGs) were detected in response to exposure to 10 and 15 µg mL^{-1} of PS-NH_2. The gene function and pathways were identified through GO and Pathways Analyses, with the resulting affection of the following pathways: (i) “glutathione synthesis and recycling”, (ii) “neurotransmitter clearance” (iii) “passive transport by aquaporins”, (iv) “fructose and mannose metabolism”, (v) “starch and sucrose metabolism” (vi) “glycolysis”. This study provides knowledge of the response upon PS-NH_2 exposure on *C. robusta* embryos, providing insights and useful information into the MoA nanoplastic proxies during the embryogenesis of a chordate model.

1.02.07

Uptake and Cellular Trafficking of PS NPs Through an In Vitro Assay With Zebrafish Embryonic Cell Line (ZF4)

M. Sendra, CSIC - Spanish National Research Council / ECOLOGY AND COASTAL MANAGEMENT; P. Pereiro, Marine Research Institute IIM-CSIC; P. Yeste, University of Cadiz

Plastic litter is the most common item found in the ocean. Through fragmentation and degradation processes plastics become into small fragments called nanoplastics (NPs < 1 µm). The NPs. due to their small size, are able to interact with cells and their surrounding environments. Microplastics (MPs; < 5 mm) and NPs have been observed in different organs from aquatic organisms and they can be translocated into cells, increasing the time of residence in the organisms and the unwanted effects triggered by these micropollutants. Despite the large number of studies about bioaccumulation and effects of MPs and NPs in different aquatic organisms, a lack of knowledge exists about the uptake mechanisms and the trafficking and internalization kinetics of MPs and NPs into cells. To throw light to this gap of knowledge, the goal of this study was to reveal the uptake mechanisms, trafficking and fate of polystyrene (PS) NPs into cells through an *in vitro* approach by using a zebrafish embryonic cell line (ZF4; ATCC CRL-2050).This study revealed that the cellular distribution and uptake mechanisms of the 50 nm and 1 µm PS NPs were different. Although small NPs are more susceptible of internalization their depuration is also higher in comparison to bigger NPs. The different endocytic pathways observed between 50 nm (dependent dynamin routes) and 1 µm PS NPs (dependent phagocytic routes) were marked. Interestingly, the inhibition of the uptake routes for the 50 nm NPs was not higher than 20%in any case, suggesting alternative uptake routes for small

NPs, such as passive transport. In this work it was also revealed that the fate organelle of NPs are the lysosomes, triggering in this organelles impairments in its functionality such as decrease in the pH, and gene regulation of cathepsins.

1.02.08

Toxicity of Graphene Oxide Alone and With Sorbed Benzo(A)Pyrene on Mussels Mytilus galloprovincialis

N. Gonzalez Soto, University of the Basque country (UPV/EHU); N. Blasco, University of the Basque country (UPV/EHU) / CBET Research Group Dept Zoology and Animal Cell Biology Faculty of Science and Technology and Research Centre for Experimental Marine Biology and Biotechnology PIE; M. Irazola-Duñabeitia, University of the Basque country UPVEHU / Dept Analytical Chemistry Faculty of Science and Technology and Research Centre for Experimental Marine Biology and Biotechnology PIE; E. Bilbao, University of the Basque country (UPV/EHU) / CBET Research Group, Dept. Zoology and Animal Cell Biology Faculty of Science and Technology and Research Centre for Experimental Marine Biology and Biotechnology PIE; L. Guilhermino, ICBAS & CIIMAR University of Porto / Department of Biology; M.P. Cajaraville, University of the Basque Country / CBET Research Group, Dept. Zoology and Animal Cell Biology Faculty of Science and Technology and Research Centre for Experimental Marine Biology and Biotechnology PIE

Among nanomaterials, graphene oxide (GO) has generated a great scientific and economic interest due to its unique properties. As the incorporation of GO in consumer products is rising year after year, GO will end up in the ocean through direct release, transport or/and waste management. Moreover, due to its high surface/volume ratio and hydrophobicity, GO can adsorb persistent organic pollutants (POPs), such as benzoapyrene (BaP), and act as carrier of POPs, increasing bioavailability of these compounds to marine organisms (the so-called "Trojan horse" effect). Thus, GO uptake and effects on marine biota represent a major concern. This work aims to assess the potential hazards of GO alone or with sorbed BaP on marine mussels *Mytilus galloprovincialis*. Mussels were exposed to GO (500 µg/L), GO with sorbed BaP (GO+BaP) (500 µg/L+100 µg/L GO/BaP) and BaP (96,7 µg/L) for 7 days. GO was detected in the lumen of the digestive tract and in feces of mussels exposed to GO and to GO+BaP. BaP was bioaccumulated in mussels exposed to GO+BaP, but especially in mussels exposed to BaP. Cell viability of hemocytes decreased significantly in mussels exposed to GO+BaP and to BaP. Activity of acetylcholinesterase (Ache) in muscle, isocitrate dehydrogenase in digestive gland and glutathione S-transferase (GST), glutathione peroxidase and superoxide dismutase (SOD) in digestive gland and gills were not altered after GO exposure. The Trojan horse effect was observed in the activity of SOD in digestive gland, with a higher activity in mussels exposed to GO+BaP than in mussels exposed to GO. GO+BaP also inhibited GST activity in digestive gland of mussels in comparison to control mussels. In mussels exposed to BaP, Ache and digestive gland GST and SOD were inhibited with respect to controls. At tissue level, an inflammatory response was observed in digestive gland and gonad of exposed mussels. In addition, oocyte atresia in stages 2 and 3 was observed in exposed mussels, with the highest intensity found in mussels exposed to GO+BaP. Overall, exposure to GO alone and to GO+BaP caused deleterious effects at different biological levels in mussels *M. galloprovincialis*. Moreover, GO acted as a carrier of BaP in mussels. However, overall toxicity of GO+BaP was lower than toxicity of BaP. Further work is still required in order to understand the hazards posed by GO, especially at long-term, and its relevance as carrier of other pollutants in the marine environment.

1.02.11

Comparison Between Twelve Nanomaterial's Accumulation and Elimination Behavior on Microcrustaceans (Daphnia magna)

A. Rivero Arze, INERIS / Direction de Risques Croniques; C. Mouneyrac, A. Chatel, Université Catholique de lOuest / Université Catholique de lOuest; N. Manier, INERIS

Nanomaterials (NMs) production and usage have been raising concerns for the last decades. Since such substances are particulate contaminants, their first contact with organisms is a physical encounter ruled by physicochemical processes that can determinate the potential NMs accumulation, toxicity, and trophic transfer. There is still a lack of understanding on the main NMs properties that drive the accumulation and epuration processes. This work aims at studying and comparing the accumulation and epuration profiles of a set of twelve well characterized NMs with the microcrustacean *Daphnia magna* in equal conditions and to investigate the NMs properties that influence this phenomenon. The NMs set is constituted by 6 TiO_2, 3 SiO_2, 2 CeO_2 and 1 ZnO NMs. The accumulation phase of the study was carried out during 24h of waterborne exposure to 1mg/L NMs suspension. Then, the organisms passed to the elimination phase of 120h in clean media. Samples were taken during the whole accumulation and elimination phases and were analyzed by ICP-OES. Using the biodynamic metal bioaccumulation model, the influx and loss rate constants have been found and compared. Differences have been determined on the accumulation and epuration kinetics of the NMs. These differences appeared to be linked to the NMs physicochemical properties as zeta potential measures can be correlated to the elimination rate constants. Similar observations were made concerning the same NMs and another type of organisms as microalgae, which highlights the importance of the physicochemical properties' role on the nano-bio interaction. These findings open the possibility of grouping the NMs based on their relationship with organisms for risk assessment purposes.

1.02.12

Innocent Until Proven Guilty: Chronic Toxicity of AgNPs for Water Remediation Reveals the Need to Reconsider Ecosafety Testing

A. Bellingeri, University of Siena / Department of Physical, Earth and Environmental Sciences; I. Venditti, C. Battocchio, Roma Tre University of Rome / Department of Sciences; G. Protano, University of Siena / Department of Physical, Earth and Environmental Sciences; I. Corsi, University of Siena / Physical, Earth and Environmental Sciences

Silver nanoparticles (AgNPs) are widely employed in everyday consumer products, mainly because of their antimicrobial potential. However, since the consequences of non-target species exposure are often severe, the potential impact on aquatic organisms should be considered already during the design and development of novel nanoscale materials. We tested the acute and chronic toxicity of novel AgNPs with a surface coating of citrate and L-cysteine (further called AgNPs) to be applied for the remediation of Hg contaminated waters. AgNPs behaviour and Ag release were assessed by DLS and ICP-MS, respectively, in all media used for toxicity testing. AgNPs potential toxicity was tested on organisms from both fresh and marine waters: two microalgal species, *Raphidocelis subcapitata* and *Phaeodactyum tricornutum* (1-1000 µg/L) and two microcrustacean with both acute and chronic toxicity tests, *Ceriodaphnia dubia* (1-100 µg/L), and *Artemia franciscana* (0.1-100 mg/L). DLS confirmed the nano-dimension of AgNPs while ICP-MS showed a very low release of Ag ions in freshwater media (0.43% of total Ag) and a higher release in seawater media (4.38% of total Ag). Ecotoxicological data showed very low toxicity for both microalgae with a maximum inhibition of growth rate compared to control of 21.23 ± 8.47 % for *R. subcapitata* at the highest concentration tested and no effects for *P. tricornutum*. In the acute test (48 h) *C. dubia* showed high sensitivity to AgNPs exposure already at the lowest concentration tested without reaching, however, a 100% immobilization rate at the highest exposure concentration, with a EC_{50} of 95.88 µg/L. Chronic exposure (7 d) confirmed the severity of 1 µg/L exposure but showed a significant impairment of the reproductive capacity only at 100 µg/L, together with the production of molts with undeveloped eggs on their inside. No acute toxicity was observed in *A. franciscana* (48 h) up to 100 mg/L but ingested particles in a concentration dependent manner were evident in gastrointestinal tract, apparently with no consequences on health status. Chronic exposure (14 d) led to a 100% immobilization rate already at 10 mg/L with a calculated EC_{50} of 5.087 mg/L. Surface coating is shown to play a significant role in reducing the toxicity of AgNPs in both freshwater and marine water scenarios. Our data also highlight the importance of chronic testing and of how acute data alone can bring to a strong underestimation of the potential toxicity of NPs.

Eco- and Human Neurotoxicology

1.03.01

Automated Behavior-Based Identification of Developmentally Neurotoxic ENvironmental Chemicals That Cause Deficits in Learning and Short-Term Memory in Larval Zebrafish

D. Leuthold, Helmholtz Centre for Environmental Research - UFZ GmbH / Bioanalytical Ecotoxicology; N. Kluver, N. Schweiger, Helmholtz Centre for Environmental Research UFZ / Bioanalytical Ecotoxicology; S. Scholz, Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology; T.L. Tal, Helmholtz-Centre for Environmental Research - UFZ / Bioanalytical Ecotoxicology

Habituation represents a non-associative form of learning that is conserved from unicellular organisms to invertebrates and humans. It is characterized by iterative response decrements to repeated inconsequential stimuli in order to reduce unnecessary physiological cost. Impairment of habituation is prevalent in human disorders including autism, schizophrenia, attention deficit hyperactivity disorder, and drug addiction. In addition to genetic predisposition, exposure to environmental factors, including xenobiotics, is linked with adverse neurological outcomes. However, there is a distinct lack of automated screening assays to detect the effects of developmental exposure to environmentally relevant chemicals on learning- and memory-related outcomes. Teleost fish, including zebrafish, exhibit a rapid escape motor behavior termed the acoustic startle response (ASR). Upon repeated acoustic stimulation, larvae undergo habituation such that the organism learns not to respond to the non-threatening stimulus. While acute pharmacological modulation of ASR by serotonergic, glutamatergic, and dopaminergic agents has been demonstrated in zebrafish, little is known regarding the effect of developmental exposure to environmental chemicals on learning and memory formation, nor has habituation been tested in parallel with other more commonly used automated behavior assays. Therefore, we established a sequential, automated zebrafish behavior-based pipeline to evaluate locomotor

responses to light stimuli in addition to acoustic sensitivity, habituation, and recovery. The assay was systematically optimized for age, stimuli frequency, acclimation requirements, and inter-test, inter-bout, and inter-stimulus intervals. We report that larval zebrafish at 5 but not 4 days post-fertilization demonstrate robust habituation behavior and that a 6 min inter-test interval is required to achieve ASR recovery. In line with previous pharmacological studies, acute exposure to the agonist NMDA (1.4 µM) and the NMDA antagonist ketamine (1 mM) reduced habituation learning, demonstrating assay functionality. Together, the assay will ultimately bridge a gap between environmental and human neurotoxicology via screening xenobiotics for developmental disruption of an evolutionarily conserved mechanism of habituation learning.

1.03.02

Similar Target, Similar Toxicity? Toxicogenomic Profiles of Neuronal Targeting Insecticides in the Zebrafish Embryo Model

H.A. Reinwald, Fraunhofer-Institut für Molekularbiologie und Angewandte Oekologie IME / EconOMICs ATTRACT; J. Alvincz, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Fraunhofer Attract EconOMICs; S.U. Ayobahan, Fraunhofer IME / Department of Aquatic Ecotoxicology; H. Hollert, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology; G. Salinas, NGS - Integrative Genomics Core Unit / Human Genetics; C. Schäfers, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; S. Eilebrecht, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Fraunhofer Attract EconOMICs

EU legislation requires environmental risk assessment for the registration of any pesticides, biocides or pharmaceuticals. This necessitates a large number of time consuming non-human animal studies. Consequently, alternative approaches to screen for adverse effects are required. With the possibility to assess the responses of thousands of genes and proteins from a single sample, omics provide sensitive and mechanistic information to support hazard assessments. To derive accurate ecotoxicological predictions from molecular signals, activity-specific markers need to be identified. Using transcriptomics, our study aims to identify specific ecotoxicogenomic fingerprints in the zebrafish (*Danio rerio*) embryo model as aquatic non-target organism to six neuronal targeting insecticides. In a modified version of the fish embryo toxicity test (OECD TG 236), eggs 3 hours post-fertilization were exposed to different sublethal concentrations of abamectin, carbaryl, chlorpyrifos, fipronil, imidacloprid and methoxychlor. Here we present the results from differential gene expression and gene set enrichment analysis (GSEA) obtained from the expression profiles. Although no physiological effects were detected for the lower exposure treatments across all tested substances, we found a considerable number of differentially expressed genes (DEG) in those conditions. The overlap of DEGs between high (HE) and low exposure (LE) levels was between 54 and 91.7 % (82.1 % on average with respect to LE). A set of 232 potential biomarker genes were identified, showing a concentration-dependent behavior. With increasing exposure concentration there were more DEGs as well as significantly enriched GO terms and KEGG pathways identified. Pairwise comparison among all treatments of normalized enrichment scores (NES) from common significantly enriched terms / pathways show a tendency of LE conditions to be less positively associated with terms / pathways from a another tested substance. Higher exposure levels however show a strong positive association. Our results hint towards differentiating underlying molecular initiating events observable at low exposure levels which blend into more systemic effects at higher levels of exposure. These findings will help in the development of an ecotoxicogenomic signature-based molecular screening assay for a time- and cost-efficient ecotoxicological hazard prediction.

1.03.03

The Bioavailability of Antidepressants in Larval Zebrafish and Their Effects on Brain Activity and Behaviour

S.L. Gould, University of Exeter / Biosciences; M.J. Winter, The University of Exeter / College of Life and Environmental Sciences; M. Trznadel, University of Exeter / Biosciences; C.R. Tyler, University of Exeter / Biosciences College of Life and Environmental Sciences

Antidepressant drugs are one of the most commonly prescribed classes of pharmaceuticals in the treatment of psychiatric conditions, such as anxiety and depression. As a result, they have been widely detected in the aquatic environment, albeit at relatively low concentrations. Antidepressants act predominantly upon the serotonergic/adrenergic systems, which are well-conserved across diverse animal phyla. As a consequence, non-target organisms in the aquatic environment may be at risk from the effects of exposure. Here, we report on the bioavailability, brain neuronal responses, and behavioural effects for exposure to representatives of the three major pharmacological classes of antidepressants in larval zebrafish (*Danio rerio*). Behavioural endpoints measured included thigmotaxis, an index of anxiety and general locomotor activity, quantified using an automated videotracking system. This assay incorporated a predefined light regime of three 5-minute light-dark cycles, to stimulate larval movement and induce anxiety-like behaviours. Brain neural activity in response to exposure to the antidepressants was measured using a transgenic zebrafish with a genetically-encoded pan-neuronal Ca^{2+} indicator, in combination with light sheet microscopy. Uptake, measured by liquid chromatography-tandem mass spectrometry, was found to be variable both within and across these classes of antidepressants, with bioconcentration factors ranging from of 0.6 to 95.5. There were no effects of the drugs on thigmotaxis (with the exception of sibutramine that was attributed to more general toxic effect), however, all drugs caused a reduction in fish locomotor activity levels, and these effects were generally more pronounced during darkness. Differences between antidepressant-evoked neural patterns were also found – for example, amitriptyline exposed animals exhibited brain-wide, smaller magnitude, less frequent bursts of neural activity compared with the controls, whereas those under duloxetine exposure presented with more intense and greater amplitude neural bursts. This preliminary work suggests that neural activity profiles in zebrafish may be able to differentiate between antidepressant classes. We are now seeking to investigate whether antidepressant exposure effects stress-evoked neural responses in fish.

1.03.06

Insecticides Trigger Olfactory-Mediated Aversive Responses in Larval Zebrafish

S. Könemann, Eawag - Swiss federal Institute of Aquatic Science and Technology / Environmental Toxicology; S. Meyer, Ecole Polytechnique Federal de Lausanne; A. Betz, Eawag / UTOX; A. Zupanic, National Institute of Biology / Biotechnology and Systems Biology; C.M. vom Berg, Eawag Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology

Insecticides inevitably find their way into surface water bodies, which leads to the exposure of aquatic non-target organisms, such as fish. Due to the similarities between the fish and insect nervous system, non-target neurotoxic effects are likely to occur. Such neurotoxic effects may be caused by an altered function of neurons. The presence of insecticides in the water might also alter fish behavior, affecting the survival of the individual as well as the whole population. For the detection of chemical cues, fish use different sensory modalities, such as olfaction. The sensory signals are further integrated in the brain to generate an appropriate behavioral response, such as attraction or avoidance. Insecticides potentially induce chemotactic behavior, which in turn affects exposure time and the severity of the toxic effect. This study investigates whether different classes of insecticides induce attractive or aversive behavior in zebrafish larvae. It further identifies brain regions responsible for generation of the measured behavioral response. By correlating neuronal activity and behavior, we aim to better understand how insecticides influence fish behavior and to better predict their ecological impact. The behavior of zebrafish larvae was tracked with an automated video recording system, while the test substance was added to one side of the test chamber. The difference between the mean position before and after the injection of the substance yields the mean displacement and is used as a measure for attraction and aversion. Olfactory-deficient larvae were used to assess whether the response is mediated by the olfactory system. To further analyze underlying brain regions, neuronal activity is visualized by antibody staining of the activity indicator pERK. The larvae responded with significant aversion to the positive control taurocholic acid (TCA, 0.2 mM), imidacloprid and diazinon at a concentration of 0.2 µM, while for the other tested insecticides no significant response was measured. In olfactory-deficient larvae the aversive responses were omitted, indicating that the olfactory system is involved in the perception of imidacloprid and diazinon. Further looking into neuronal activity it was observed that the three aversive chemicals evoked different activation patterns. Largest effect sizes were predominantly detected for regions and neuron clusters in the hypothalamus, an important control center of involved in stress response. Further, brain regions, such as the pituitary, the locus coeruleus or the RoL-R1, recently shown to be involved in avoidance behavior were identified. Unexpectedly, the neuronal activity in the chemical treatments, especially in the insecticide treatments, was lower than in the negative control. Decreased neuronal activity may indicate general neurotoxicity, as a reduction in neural firing upon exposure to imidacloprid was recently reported. In summary, our study contributes to a better understanding of how fish perceive and interpret anthropogenic chemicals in their environment and thus helps to assess the environmental impact of insecticides on fish populations.

1.03.07

Examining Ppar As a Host-Microbiome Nexus That Is Sensitive to Xenobiotic Disruption

T. Tal, Helmholtz Center for Environmental Research (UFZ) Leipzig / Department of Bioanalytical Ecotoxicology; L. Wehmas, X. Howey, S. Gaballah, L. Bertotto, U.S. EPA / CCTE; C. Wood, U.S. EPA / CPHEA; D. Leuthold, Helmholtz Centre for Environmental Research - UFZ GmbH / Bioanalytical Ecotoxicology; N. Schweiger, N. Kluver, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology

Individual susceptibility to xenobiotic exposure is variable. One factor that might account for this is the microbiome, which encompasses all microorganisms, and their encoded genes and associated functions, that colonize a host organism. We have previously shown that axenic (i.e. microbe-free) zebrafish are hyperactive

relative to colonized zebrafish. To understand mechanisms by which microbes influence neurobehavioral development, unbiased RNA sequencing was performed in head tissue isolated from axenic, conventionalized, or conventionally colonized zebrafish. We identified 504 differentially expressed genes (>2-fold, 0.05 FDR) when comparing both colonized groups to the axenic cohort. The peroxisome proliferator-activated receptor (ppar) was identified as one putative upstream regulatory pathway disrupted in axenic head tissue. As a case study to evaluate host-microbiome interactions in the context of chemical exposure, RNA sequencing was conducted on zebrafish head tissue obtained 24 and 48 h prior to the onset of hyperactivity in zebrafish exposed to non-teratogenic concentrations of the per- and polyfluoroalkyl substances (PFAS) PFOS (0.5-1.6 µM) or PFHxS (7.9-25.1 µM), or 0.4% DMSO. Transcriptomic benchmark concentration response modeling was concordant with the *in vivo* lowest observed effect concentrations for hyperactivity. ppar was a key predicted upstream regulator, setting the stage to test whether manipulation of ppar-dependent signaling blocks PFOS or PFHxS-induced hyperactivity. These data support the concept that microbial products and xenobiotics converge via classical toxicological signaling pathways to affect neurobehavioral development in zebrafish. *This abstract does not reflect EPA policy.*

1.03.11
A High-Throughput Assay for Neurite Outgrowth Inhibition in SH-SY5Y Cells to Identify Mixture Effects in Environmental Samples
J. Lee, Helmholtz centre for environmental research - UFZ / Cell Toxicology; B. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; S. Scholz, Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology; R. Schlichting, Helmholtz centre for environmental research - UFZ / Cell Toxicology
Despite the presence of neurotoxic chemicals in the environment, there is a lack of robust assays that are amenable to testing of mixtures of neurotoxicants in environmental samples. We aim to develop a high-throughput method for screening inhibiting effects of environmental chemicals and complex environmental samples on neurite outgrowth, which allows us to classify compounds and samples as neurotoxic based on their selectivity or specificity of the neurite length endpoint in relation to cytotoxicity. Human neuroblastoma SH-SY5Y cells were differentiated, seeded and exposed to controls for neurite outgrowth inhibition and baseline toxicants. Phase-contrast images and fluorescence images after staining live/dead cells were acquired and analyzed for neurite length and cell viability, respectively. The known neurotoxicants showed higher selectivity considering the ratio of effect concentrations from cytotoxicity and neurite outgrowth inhibition than the baseline toxicants, indicating their selective effects on neurite outgrowth. This bioassay enables us to capture neurotoxicity specifically and account for masking effects of cytotoxicity by measuring two endpoints simultaneously. Furthermore, we could classify individual compounds according to their selectivity or specificity and demonstrate the applicability of this screening tool to diverse environmental samples.

Effects of Contaminants in Wildlife: Endocrine Disruption, Immunomodulation, Oxidative Stress and Other Biomarker Responses

1.04.02
Sublethal Effects of Neurotoxic Pesticides on Tropical Bats: From Cells to Behaviour
N.I. Sandoval Herrera, University of Toronto Scarborough / Ecology and Evolutionary Biology; K.C. Welch, University of Toronto Scarborough / Biological Sciences
Agricultural intensification and the subsequent increase in pesticide use have been considered as a threat for bat populations. However, little research on this topic has been conducted in tropical agro-systems, where most of the cultivated land is intensively treated with pesticides and the highest diversity of bats is found. Insectivorous bats, for example, can eat more than 90% of their body mass every night, species that forage preferentially in crops could be highly exposed to pesticides through contaminated prey. Pesticides commonly used worldwide such as organophosphates (OPs) are highly neurotoxic for non-target vertebrate species but have low persistence in the environment. Even low doses can impair essential processes such as immune function, locomotion, and cognition, threatening animal survival and its ecological function in the longer-term. Our study seeks to determine how vulnerable bats foraging in or near crops are to pesticides and what are the sublethal effects of organophosphates on their physiology and behavior. We first assessed the risk of exposure by quantifying the foraging activity of bats in crop fields in Belize, Mexico and Costa Rica. Nocturnal insects from the same locations were screened for pesticide residues to estimate daily intake through diet. We used an integrative approach to study the toxic effects of OPs on captive and wild bats. We measured multiple biomarkers in a wild population roosting close to croplands in Mexico and Belize. To study the effects of such exposure, we used an integrative approach that involves measuring molecular (enzyme activity), physiological (metabolic rate, immune response), and behavioral (spatial memory) biomarkers, aiming to extrapolate these responses across levels of biological organization. We present evidence of the suitability of some of these biomarkers to monitor wild populations and we propose a simple, yet sensitive behavioral test to evaluate spatial memory as an ecologically relevant behavior in bats. Understanding the sublethal effects of pesticides at different scales will enable us to better predict the implications on bat populations and help to inform conservation strategies.

1.04.03
Multi-Biomarkers Approach in Three-Spined Stickleback (Gasterosteus Aculeatus, L.) Using an ENvironmental Biomonitoring Analysis : Genotoxic Biomarkers Integration
A. Cant, UMRI 02 INERIS-URCA-ULH SEBIO / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); M. Bonnard, Université de Reims Champagne Ardenne / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); J. Porcher, INERIS / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); J. Prygiel, Agence de l'eau Artois-Picardie; A. Catteau, INERIS / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); L. Delahaut, Université de Reims Champagne Ardenne / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); O. Palluel, INERIS / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); C. Turiès, INERIS / UMR I-02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); A. Geffard, Université de Reims Champagne Ardenne / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); A. Bado-Nilles, INERIS / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring)
In order to improve the diagnosis of environmental contamination of freshwater, multi-biomarkers approaches have been developed to better reflect the physiological state of an organism and potentially detect exposures to xenobiotics. Genotoxic biomarkers seem to be quite relevant due to the multitude of substances interacting with the DNA molecule which can affect its integrity in somatic and germ cells. Among the existing tools used to highlight the genotoxicity on fish erythrocytes, the alkaline comet assay (SCGE) is the most widely used method to detect primary DNA strand breaks and flow cytometry (FCM) is becoming a fast and convenient tool to notice chromosomal damages for aquatic biomonitoring. The present work proposes to evaluate the relevance to include two genotoxic biomarkers (primary DNA strand breaks and chromosomal damages) in the existing battery of multi-biomarkers developed on three-spined stickleback (Gasterosteus aculeatus). After 21 days of exposure, biological samples were recovered from sticklebacks caged in six sites in Northern France to constitute the battery. The inclusion of genotoxicity biomarkers seems to bring a little improvement to the discriminating power of sites as obtained by a Principal Component Analysis (PCA) explaining 30.8% of the variance, although they provide a complementary information on the toxicity of sites. The “Rhonelle” river was the most affected by primary DNA strand breaks measured in fish but this parameter was not well represented on axis, in opposition to fish caged in “Deule” and “Sensée Bouchain” sites showing lower levels of chromosomal damages, in accordance with the first component axis of the PCA.

1.04.04
Clinical Effects of Microplastics Ingestion in Birds: A Long-Term Experimental Study With Japanese Quails
L. Monclus, E. McCann Smith, Norwegian University of Science & Technology (NTNU) / Department of Biology; M. Wagner, Norwegian University of Science and Technology / Department of Biology; V. Jaspers, Norwegian University of Science & Technology / Biology
Microplastics are ubiquitous pollutants that are ingested by several wild bird species, especially seabirds. Most studies have focused on the occurrence and amount of plastic ingested by wild birds while very few have investigated their toxicity in controlled laboratory experiments. In addition, most of the studies performed in birds refer to plastics ingestion of > 1 mm, and as such, there is an important gap of knowledge on the potential effects of smaller plastics in birds. In a controlled experiment with a well-established bird model, Japanese quail (*Coturnix japonica*), we investigated the clinical effects of oral exposure to different size classes of microplastics made of a mixture of three polymers. To this purpose, we collected different plastic items in the marine environment (Mausund, Norway) and analyzed the polymer type using µFTIR and Raman spectroscopy. Three items made of polyethylene, polystyrene and polypropylene were selected. They were prepared as powder of less than 125 µm and 3 mm circular disks. Mixtures of the three polymers were used for the experiment. Fifty-six quails were randomly distributed in four groups, one control and three treatments groups that were orally exposed to < 125 µm powder, 3 mm disks and a mixture of both. The amounts and types of polymers used were the same in all groups. Quails were exposed to microplastics every third day from the age of one week onwards until they were six weeks old. Doses of microplastic increased three times with the age

and growing of the quails, ranging from 25 to 75 mg per exposure. We evaluated the growth rate of chicks and blood biochemical parameters as well as the plastic amount found in the stomach. Body weight and biometric measures (tarsus, head and bill, and wing length) were determined every week. At day 42 of age, quails were euthanized and blood was collected. Plasma was obtained and stored at -80°C until biochemical analyses were performed. The proventriculus and gizzard were also stored at -80°C. The results for the bird morphometrics, blood biochemical parameters (glucose, cholesterol, blood proteins, liver and kidney function parameters) and the amount of plastic found in the stomach will be presented at the conference. This study is part of a more extensive project which will enable us to better understand the potential negative effects of the ingestion of small microplastics in birds.

1.04.05

Inter-Relationships of Perfluoroalkyl Acids, Thyroid Activity and Diet in Free-Ranging Passerine Birds

K.J. Fernie, Environment and Climate Change Canada / Ecotoxicology & Wildlife Health Division; K. Hopkins, McGill University - Macdonald Campus / Dept of Natural Resource Sciences; M.A. McKinney, McGill University / Department of Natural Resource Sciences; R.J. Letcher, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health Division

Perfluoroalkyl substances are a large, widely-used diverse group of synthetic chemicals that include several perfluoroalkyl acids (PFAAs). Production of perfluorooctane sulfonyl fluoride- (PFOSF-) based compounds was voluntarily phased-out (2000-2002), and perfluorooctane sulfonate (PFOS) (2009) and perfluorooctanoic acid (PFOA) (2019), their salts and related substances, listed under the Stockholm Convention. Internationally, replacement short- and long-chain perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs) are now high-priority chemicals for risk assessment and management. Little is known about the exposure, accumulation and potential associated effects on wildlife of these priority PFCA and PFSAs. Our study with the passerine species, tree swallow (*Tachicyneta bicolor*), characterized the birds' exposure and accumulation of these priority chemicals, in relation to the birds' diet and associated changes in thyroid structure, activity and circulating hormones. The study was conducted in Canada with wild tree swallow chicks raised by their parents in nest boxes located 300 m from the effluent discharge of a large, urban waste water treatment plant (WWTP) or at a reference site adjacent to a large, constructed reservoir. Compared to reference chicks, chicks near the WWTP accumulated greater hepatic concentrations of sum-PFCAs, PFOS, PFDA, PFDoA, and PFTeDA, but had lower hepatic concentrations of sum-PFSAs, PFOA, PFNA, PFUdA, and PFTrDA. Hepatic sum-PFCAs, sum-PFSAs, and individual PFAAs were associated with the chicks diet (trophic position: $\delta^{15}N$; foraging: $\delta^{13}C$), with evidence of dietary differences between the sites. There was also strong evidence of differences in thyroid activity between the sites, notably with structural changes in the thyroid glands of WWTP chicks (i.e., depleted colloid (area, perimeter), increased epithelial height) that were consistent with increased glandular activity. Changes were evident in circulating triiodothyronine but not circulating thyroxine. Among multiple factors that can influence avian thyroid function, our early investigations have identified significant correlations with hepatic sum-PFCAs, sum-PFSAs, some of the measured individual PFAAs, or dietary tracers ($\delta^{15}N$, $\delta^{13}C$), and the thyroid gland (structure, activity) but not circulating thyroid hormones of the birds, that warrant further investigation.

1.04.06

Mechanisms of Immunomodulation of Cd in Small Mammalian Wildlife, a Mechanistic In Vitro, In Vivo and Field Level Weight of Evidence Approach

D. Garcia Mendoza, H.H. van den Berg, Wageningen University / Division of Toxicology; N. van den Brink, Wageningen University / Dept of Toxicology

Wild small mammal species can act as potential hosts and reservoirs for zoonotic diseases like for instance Lyme disease, and are as such important in their environmental spread. Exposure to immunomodulatory chemicals, may impair immune responses to infections and therefore facilitate transmission of pathogens between organisms and increase their prevalence. Studies have shown that heavy metals like Cd can impair host immunity, although limited information is known about the mechanisms of toxicity in wildlife. In order to gain mechanistic insights in potential effects of cadmium on the humoral immunity of small mammals, several studies were performed using Cd, ranging from detailed in vitro studies to field observations. In an in vitro study with challenged B cells, $CdCl_2$ reduced DNA synthesis at concentrations two orders below cytotoxic levels. Secretion of IgM by B cells was not affected, however, the reduced numbers of B cells may reduce the levels of circulating antibodies during humoral responses *in vivo*. In challenged T cells similar $CdCl_2$ concentrations increased metabolic activity and IL-4 secretion, while not affecting DNA synthesis, potentially skewing immune responses towards Th2 responses and switching immunoglobulin classes from IgM/IgG to IgE. In a subsequent *in vivo* study, NMRI mice were exposed to different levels of cadmium and immunized with sheep red blood cells (SRBC). Control female mice appeared to be more immunocompetent than males, and Cd decreased haemolytic titers and IgM/IgG levels only in immunized female mice. IgM/IgG plasma levels in females significantly decreased at elevated Cd exposures and were highly correlated with the number of splenic plaque forming cells (PFCs). Cadmium exposure reduced circulating antigen-specific antibody levels in female mice by reducing B cell numbers, in line with the previous *in vitro* experiments. In a field study using wood mice (*Apodemus sylvaticus*) site-specific decreases in PFCs were observed with increasing cadmium exposure, similar to the lab study. These studies at different levels of biological integration show that humoral responses of wildlife may be impaired by Cd exposure, even at low environmental levels, potentially increasing their susceptibility to pathogens and their reservoir function.

1.04.14

Transcriptional Response of Diamesa Zernyi (Chironomidae) Reveals Metabolic Alterations Due to Chlorpyrifos Exposure in Glacier-Fed Streams

A. Muñiz-González, UNED / Mathematical Physics and Fluids; V. Lencioni, MUSE-Museo delle Scienze Trento / Dept. of Invertebrate Zoology and Hydrobiology; J. Martinez-Guitarte, UNED / Fisica Matematica y de Fluidos

Increased demand for agricultural production has resulted in greater and inadequate use of insecticides around the world, with consequent freshwater pollution. Chlorpyrifos (CPF) is an organophosphorus insecticide extensively used also in alpine orchards. Due to the drift effects, CPF has been transported at medium-high distance from the fields, trapped in glaciers and released in melting waters. Concentrations of 6-10 ng/L were detected in several glacier-fed streams in the Trentino Province (NE-Italy, Southern Alps), among which that one where the larvae of the target species we selected (*Diamesa zernyi*; Diptera Chironomidae) for this study were collected (Amola glacier-fed stream; September 2019). This species is cold-stenothermal, typical of kryal habitats (habitats fed by icemelt). Previous studies highlighted negative effects of CPF on *D. zernyi* larvae exposed at sub-lethal concentrations corresponding to 1/100 LC10 (0.011 µg/L) and 1/10 LC10 (0.11 µg/L) and LC10 (1.1 µg/L), from mobility inhibition and biochemical alterations (i.e., lipid peroxidation and protein carbonylation due to oxidative stress). We exposed the larvae at these conditions listed above, for 24 h, in controlled laboratory conditions (8 light:16 dark hours; 2°C= environmental water temperature). Sub-organismal response was evaluated using an array of genes designed from the sequences previously obtained by "de novo transcriptome". After 24h, eight genes resulted altered: *EcR*, *E75*, *E93*, and *Met* (endocrine system); *GST* omega and sigma (detoxification response); *hsp75*, *hsp83*, *HYOU1* (stress responses). According to these results, CPF seems to act as endocrine disruptor, altering genes essential for the insect correct development. The increased expression of both GST reinforces the idea of the CPF acting as oxidative compound. Besides the stress response activation can indicate a strong disruption on its homeostasis deriving in defective protein folding. Our findings confirm the high environmental risk of CPF, affecting the metabolism of aquatic insects and results raise the level of concern about this pesticide for high altitude water bodies, considered generally pristine. We confirm also the importance to include molecular approaches in the toxicology evaluation to detect early adverse effects on wildlife. In conclusion, biomarkers should be included in biomonitoring programs besides the organismal response, because detect early adverse effects on the health of the organisms.

Endocrine Disrupting Chemicals in Vertebrates: New Developments and Challenges

1.05.08

Eye Development As a New Endpoint for Thyroid Disruptor Testing With Fish

L.A. Baumann, University of Heidelberg / Aquatic Ecology and Toxicology; T. Braunbeck, University of Heidelberg / AquaTox Centre for Organismal Studies; M.P. Fagundes, University of Heidelberg; L. Golz, COS University of Heidelberg / Aquatic Ecology & Toxicology, COS; H. Holbech, University of Southern Denmark / Biology; D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; P. Pannetier, University of Heidelberg / Aquatic Ecology & Toxicology, COS; L. Vergauwen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences

Early vertebrate development is at least partly regulated by thyroid hormones (THs) and can therefore easily be disrupted by environmental pollutants, which interact with the TH system (thyroid disrupting chemicals, TDCs). THs serve multiple functions; Among others, THs regulate neuromorphological, specifically eye development in vertebrates. Also in fish, eye development is known to be directly regulated by THs and can, therefore, be used as thyroid-related endpoint in endocrine disruptor testing with fish. Chemical-induced impairment of the visual system can have severe consequences for survival rates in the field, especially for developing fish, which are thought to be very sensitive to TDCs. However, the interaction between TDCs and eye development in fish has not yet been fully explored. Especially with regard to the environmental consequences of impaired eye development, it is important to understand the full sequence of events from molecular interaction with the TH system over morphological and

physiological changes up to the adverse outcome at the individual and population levels. For development of an Adverse Outcome Pathway (AOP) for disrupted eye development by TDCs in fish, we performed a detailed literature review covering approximately 80 studies into the effects of modification of TH levels on eye development in different life stages of 12 fish species. Additonally, we performed exposure experiments with zebrafish and multiple TDCs with different modes of action. Endpoints assessed range from gene expression and hormone level analyses to cellular changes in the eyes (e.g. cell size, cell layer structure and organization, amount of photoreceptors, pigmentation etc.), morphological changes (e.g. eye size or shape), but also physiological and behavioral changes (e.g. optokinetic response, light response, swimming activity, etc.). Our literature analysis, combined with results from own experiments provide clear evidence that different modes of TDCs result in impaired eye development of zebrafish. Results confirm the growing evidence that fish eye development is sensitive to TDC treatment and might represent a promising endpoint for the assessment of thyroid-related effects in fish. The AOP will build the basis for further research on the consequences of thyroid disruption in fish, a research field with many knowledge gaps.Funding by: European Union's Horizon 2020 research and innovation program, under grant agreement No. 825753 (ERGO).

1.05.12

Gaps and Obscurities Concerning the Performance of the Fish Short Term Reproduction Assay (FSTRA) and the Amphibian Metamorphosis Assay (AMA)

S. Hoger, Innovative Environmental Services (IES) Ltd; J. Schreitmüller, A. Peither, Innovative Environmental Services (IES) Ltd / Ecotoxicology; P. Hvorslev, Innovative Environmental Services (IES) Ltd

Testing for endocrine disruption potential is an important part of the regulation of chemical substances in the EU and in the USA. Key screening tests are the Fish Short Term Reproduction Assay (FSTRA), OECD 229 (OCSPP 890.1350), and the Amphibian Metamorphosis Assay (AMA), OECD 231 (OCSPP 890.1100), for the hypothalamus-pituitary-gonadal axis and the hypothalamus-pituitary-thyroid axis, respectively. Especially for registration of plant protection products in Europe these tests are critical, since indication of an effect in these tests trigger higher tier tests with consequent additional costs and additional use of animals and, in case of a confirmed endocrine disrupting effect of the substance, a ban on the substance in the EU. IES has conducted FSTRA in fathead minnow (*Pimephales promelas*) and AMA in African clawed frog (*Xenopus laevis*) since 2019. During implementation and performance of the tests in 2019 and 2020 it became clear that both the OECD and the OPPTS (OCSPP) guidelines have gaps in their description of the test performance which can result in test repetitions or serious questions from the authorities. In addition, without a clear description how a test has to be conducted, the tests are not comparable to each other. For this reason we will provide a summary of our findings during the planning and performance of these tests. The following endpoints are discussed: fecundity, nuptial tubercle score, and vitellogenin determination for the OECD 229 and Nieuwkoop-Faber staging and measurement of the hind-limb and snout-vent length for the OECD 231. In addition, the determination of the best concentrations for the tests are discussed. Data from IES studies are compared to data from the EPA endocrine disruptor screening programme and suitable procedures that have been developed at IES are described. In particular, for parameters with a degree of subjectivity, it is important that one person conduct the procedure on all animals in a study. In addition we recommend that a Q&A document be published with best ways to perform the tests and interpret the data for each guideline to support the authorities, the industry and the laboratories to provide high quality animal studies.

1.05.13

Population Relevant Effects on Fish - an Update on ZEOGRT Validation

M. Teigeler, Fraunhofer IME / Ecotoxicology; E. Eilebrecht, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecotoxicology; C. Schaefers, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; G. Maack, German Environment Agency / Ecotoxicological Assessment of Pharmaceuticals; S. Walter-Rohde, German Federal Environment Agency (UBA)

In 2015, OECD adopted a protocol for an Extended One Generation Reproduction Test (EOGRT) with Medaka (*Oryzias latipes*) as Test Guideline 240. This test protocol includes the exposure of a parental generation (F_0), a full Filial 1 (F_1) generation and a Filial 2 (F_2) generation. As this protocol was designed and validated for a single test species only, there was an initiative from Germany at OECD to develop a similar test approach with zebrafish (*Danio rerio*). Data from the initial phase of validation are presented. The aim of the phase 1 of validation - including the performance of four ZEOGRT studies - was to proof the applicability of the test protocol for the test species zebrafish. The obtained effect concentrations were compared with existing data from the MEOGRT validation studies and from the literature. The studies were conducted with tamoxifen-citrate, prochloraz, dienogest and dexamethasone. The list of relevant life stages and endpoints included reproduction of the F_0 generation, early life stages, juvenile growth, sexual maturation, and reproduction of the F_1 generation, and the early embryonal stage of the F_2. Blood plasma samples of the adult fish of both generations were measured for vitellogenin (VTG) concentrations; a histopathological examination of the fish gonads of the adult fish of F_0- and F_1- generation was performed. To ensure consistency with fish test guidelines on endocrine acting properties, the list of test acceptance criteria contains values and ranges for the different parameters regarding test conditions and biological performance, as defined in OECD TG 210, 229, 234 and 240. For nearly all of the biological data obtained in the four studies, compliance with the defined validity criteria was confirmed. Considering the control variability, the reproduction parameters showed low variability, while sex ratio variability was higher. The growth parameters expressed sex specific differences. The VTG concentrations in females were similar for both F_0 and F_1, while VTG concentrations in males were at a low level as expected. The test protocol applied is feasible to detect population relevant endpoints for zebrafish at sufficient sensitivity. The results obtained were in good compliance with available data from the literature. Finally, it can be stated that the ZEOGRT setup is suitable to assess endocrine effects beside receptor modulated mechanisms and steroidogenesis inhibition (EAS).

1.05.15

OECD Validation Exercise for the Rapid Estrogen ACTivity In Vivo (REACTIV) Assay

A. Tindall, D. Du Pasquier, C. Zany, Watchfrog S.A.; I. Katsiadaki, Cefas / Environment and Animal Health; M. Sebire, Cefas; H. Yamamoto, National Institute for Environmental Studies / Center for Health and Environmental Risk Research; M.K. Sellin Jeffries, Texas Christian University / Department of Biology; G. Lemkine, Watchfrog S.A.

Under European REACH legislation, estrogen axis activity is being evaluated for a large number of chemicals, including obligatory testing for plant protection products, leading to the use of huge numbers of laboratory animals as well as saturation of testing capacity. The availability of whole animal, physiologically relevant, embryonic fish models for medium throughput screening and prioritisation of chemicals would alleviate these problems. We developed an assay based around a transgenic medaka fish eleuthero-embryonic model, harbouring the *choriogenin h* promoter driving expression of *gfp* (*chgh-gfp*), the Rapid Estrogen ACTivity In Vivo (REACTIV) assay. The structural role of choriogenin proteins in egg formation suggests that this biomarker may not only reveal estrogen axis activity, but may also accurately predict impaired reproduction. An international interlaboratory validation exercise is currently in progress to evaluate the REACTIV assay as a potential level 3 medium throughput OECD test guideline. The REACTIV assay is performed by exposing eight newly hatched fry of the *chgh-gfp* line to the test chemical in the presence and absence of testosterone (30 µg/L), allowing quantification of the effects of chemicals acting via MoAs requiring the presence of an aromatisable androgen (eg. aromatase inhibition, estrogen receptor antagonism, 5α-reductase inhibition) as well as those more easily detected in the absence of an aromatisable androgen (eg. estrogen receptor agonists). Exposure studies are carried out in six-well plates for 24 h. Readout is by fluorescence imagery. Results so far indicate that the assay can be transferred between laboratories with similar results in terms of activity and sensitivity whether the test chemical acts on estrogen axis signalling at the receptor level or on downstream steroidogenesis. The selectivity of the assay has also been shown by the evaluation of a number of biologically active chemicals which are not expected to have estrogen axis activity. The REACTIV assay allows simple and rapid (24h) determination of the estrogenic activity of single chemicals. In addition, this model, based on the use of early life stages outside of the EU definition of a laboratory animal, provides ethical advantages in line with the three R's principles. The ongoing OECD interlaboratory validation exercise will determine the utility of this assay for level 3 medium throughput screening of single chemicals in a regulatory context.

1.05.16

Finalised Results for the Interlaboratory OECD Validation of the Rapid Androgen Disruption Adverse-Outcome Reporter (RADAR) Assay

A. Tindall, S. Bancel, M. Chabanon, Watchfrog S.A.; I. Katsiadaki, Cefas / Environment and Animal Health; M. Sebire, Cefas; A. König, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; E. Eilebrecht, Fraunhofer IME / Ecotoxicology; L. Kosak, Fraunhofer Institute for Molecular Biology and Applied Ecology; J. Rieder, H. Segner, University of Bern / Centre for Fish and Wildlife Health; T. Okamura, M. Sakurai, IDEA Consultants, Inc.; G. Lemkine, Watchfrog S.A.

Increasing interest is being paid to the identification of chemicals interfering with the normal functioning of the androgen axis. Two key studies identified 66/200 and 37/134 pesticides tested as anti-androgenic *in vitro*. However, the effects of many of these pesticides have yet to be confirmed *in vivo* due to the absence of medium throughput *in vivo* assays. We developed a transgenic medaka line capable of revealing the level of activity of the androgen axis by emission of green fluorescence. This stable transgenic line harbours a portion of the spiggin1 gene

promoter upstream of GFP coding sequence, responds specifically to androgens and is capable of identifying pro-androgenic and anti-androgenic chemicals acting via a variety of modes of action (MoAs). Sensitivity has been shown to be similar to that of the 21-day androgenised female stickleback screen (AFSS). Using eleuthero-embryonic life stages of this transgenic line, which are non-compliant with the EU definition of a laboratory animal, we developed the 72 h Rapid Androgen Disruption Adverse-outcome Reporter (RADAR) assay. The RADAR assay was performed in five different laboratories in France, the UK, Switzerland, Germany and Japan. Test chemicals covered a wide range of MoAs. Twenty newly hatched fry of the spiggin1-gfp line were exposed to test chemicals in the presence and absence of 17MT 3 µg/L, allowing quantification of the effects of chemicals acting via MoAs requiring the presence of an aromatisable androgen (eg. aromatase inhibition, androgen receptor antagonism) as well as those more easily detected in the absence of an aromatisable androgen (eg. androgen receptor agonists). The exposure studies were carried out in six-well plates for 72 h with media renewal every 24 h. At the end of the exposure, GFP expression was quantified by fluorescence imagery. The results were coherent between each of the partner laboratories and showed a similar sensitivity. The transferability of the RADAR assay has been confirmed as well as its ability to be read using a variety of different fluorescence imaging systems. This transferability includes not only the assay itself but the ability to perform it with embryos shipped from a supplier or with embryos bred on site. The experimental results matched the expected results and a draft test guideline has been written which is currently under evaluation by the OECD.

1.05.17

Acute Toxicity, Teratogenic and Estrogenic Activity of Test Chemicals Using a Combination of Two Zebrafish Embryos-Based Assays Using the Cyp19A1B-Gfp Line

A.M. CHRISTOPHE, INERIS; S. Ait-Aissa, INERIS / UMRI SEBIO ECOT; B. Piccini, INERIS; P. Pandard, INERIS / Direction of Chronic Risk Assessment; F. BRION, INERIS

The zebrafish embryo has become an essential alternative vertebrate model to animal testing for the environmental hazard and risk assessments of chemicals. Further interest of the zebrafish embryo model has gained through the development of transgenic models and their use to assess endocrine disrupting chemicals (EDCs). Among them, the EASZY assay was designed to detect and quantify the estrogenic activity of chemicals acting through estrogen receptors (ERs) to induce GFP driven by the ER-regulated brain aromatase cyp19a1b gene. This assay, for which a draft OECD TG is currently under adoption, is considered as a level 3 assay of the OECD conceptual framework for EDCs. However, its integration within fish testing strategy for the hazard and risk assessments of chemicals needs to be experienced. The objective of this study was therefore to set-up a functional testing strategy based on cyp19a1b-GFP embryos combining a refined FET assay and the EASZY assay to assess the toxicity, teratogenicity and estrogenic activity. This demonstration study was undertaken and applied to a panel of bisphenol A substitutes for which such toxicological information was lacking. Eleven bisphenols (BPs) were sequencely tested in the refined FET and EASZY assays. Mortality (LC50), teratogenic effetcs (EC50) were assessed for each compound demonstrating that BPs have variable toxicities and elicited various teratogen phenotypes, some BPs being classified as teratogens. The use of cyp19a1b-GFP embryos in the refined FET provided preliminary information on their potential estrogenic activity which was further investigated in EASZY. Chemical exposures to BPA and BPA substitutes led to concentration-dependant inductions of GFP except for TCBPA. BPs could be ranked, based on their EC50, from the most to the least active, highlighting that most of them elicit higher estrogenic activities than BPA. Altogether, we successfully combined two zebrafish-based embryo assays, FET and EASZY, through the use of transgenic cyp19a1b-GFP embryos within a functional testing strategy to efficiently acquire novel data on acute toxicity, teratogenic and estrogenic activity of BPA substitutes thereby contributing to their hazard assessment. This study also provides further evidence that chemicals modulate cyp19a1b expression during early brain development which potential adverse effects still need to be investigated to accuratly assess the risk posed by EDCs acting in brain.

1.05.20

Update on the ERGO Project (Breaking Down the Wall Between Human Health and Environmental Testing of Endocrine Disrupters)

H. Holbech, University of Southern Denmark / Biology; S. Volz, Syddansk Universitet / Biology; M.L. Christiansen, University of Southern Denmark / Biology; D. Knapen, L. Vergauwen, I. van Dingenen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; M. Hansen, Aarhus University / Department of Environmental Science; R. Poulsen, Aarhus University / Department of Plant and Environmental Sciences; F. flamant, Ecole Normale Normale Superieure de Lyon; T. Braunbeck, University of Heidelberg / AquaTox Centre for Organismal Studies; L. Gölz, University of Heidelberg / Aquatic Ecology and Toxicology, Centre for Organismal Studies; L.A. Baumann, University of Heidelberg / Aquatic Ecology and Toxicology

The ERGO project aims at breaking down the wall between mammalian and non-mammalian vertebrate regulatory testing by identifying, developing and aligning thyroid-related biomarkers and endpoints for linkage of effects between different vertebrate classes. For this end, an Adverse Outcome Pathway (AOP) network covering various modes of thyroid hormone system disruption in multiple vertebrate classes is under development. The AOP network will provide the scientifically plausible and evidence-based foundation for the selection of assays and biomarkers in lower vertebrates predictive of human health outcomes. These assays will be prioritized for validation in ERGO. We present some of the early findings from ERGO experimental work with fish, amphibian and mammalian models exposed to selected thyroid hormone system disrupting chemicals. We also present progresses with AOP developments for thyroid disruption in fish.

1.05.21

Comprehensive Steroid Profiling in the Context of Reproductive and Neurodevelopmental Endocrine-Mediated Toxicity

S. Evangelista, P. Vazakidou, P. Leonards, Vrije Universiteit Amsterdam / Environment & Health; M. Lamoree, VU University, Department Environment & Health / Department of Environment and Health; M. van Duursen, Vrije Universiteit Amsterdam / Environment & Health

It is well known that steroid hormones play important roles in regulation of growth, development, sexual differentiation and reproduction. Steroidogenic pathway analysis has been used so far to unveil pathologies causations and the discovery of new diagnostic biomarkers in the context of Endocrine disruptors (EDCs) exposure. A growing number of data reveal how the exposure to EDCs during pregnancy and early stages of life can disrupt normal patterns of brain development and reproductive function leading to severe pathologies. As part of the ENDpoiNTs and Freia Horizon 2020 projects, we developed a LC-ESI(+/-)MS/MS method for the quantification of 21 unconjugated steroids to evaluate the effects of a panel of EDCs on steroidogenesis. The method was tested and will be used on biological matrices of interest of the projects as rat plasma samples. Accurate measurement of steroids is challenging due to the non-polar structure and low proton affinity of these compounds, so special attention should be given to maximize their ionization power when analyzed without derivatization. Moreover steroids basal levels in biological matrices are strongly gender and age dependent, which implies that the methods should cover a wide dynamic range. In the current work, we followed a number of optimization strategies to increase the mass spectrometry response and the sample preparation. Preliminary results on adult rat plasma samples (data not shown) demonstrate that the method allows simultaneous quantification of androstenedione, 11-deoxycorticosterone, 11-deoxycortisol, 17-α OH progesterone, 17-α OH pregnenolone, 17-α OH dihydroprogesterone, 5-α THDOC, 5-α DHT, 5-α androsterone, corticosterone, cortisol, cortisone, etioch-3α 17-one, pregnenolone, progesterone, testosterone, DHEA, E1, E3, 17-β E2, and 17-α E2 in 100 µL of matrix with a total run time of 12 min. Sample treatment consisted of a deconjugation reaction and centrifugation prior to SPE extraction. A sensitive LC–MS/MS method was developed allowing limits of detection ranging from 10 pg/ml to 1 ng/ml without derivatization.

Environmental Epigenetics: Impacts of Stressors on the Epigenome, Short-Term and Long-Term Effects and Challenges for the Integration into Risk Assessment

1.06.01

Alterations of DNA Methylation Levels of Key Genes Related to BPA Exposures in Zebrafish Embryos

R. Martinez, IDAEA-CSIC / Environmental Chemistrty; R. Tauler, B. Piña, Institute of Environmental Assessment and Water Research (IDAEA) Spanish Research Council (CSIC) / Environmental Chemistry; L. Navarro-Martin, Institute of Environmental Assessment and Water Research / Environmental Chemistry

An emerging concern related to Endocrine Disrupting Chemicals (EDCs) is that are able to modify cellular regulatory mechanisms long after the actual exposure occurred, a phenomenon mediated by epigenetics. A recently published study has shown the alteration of methylation patterns of genes associated with the nervous system development in embryos exposed to BPA, which could be linked to abnormal swimming activity. On the other hand, in a previous study we have identified the alteration of two-key genes in zebrafish eleutheroembryos in eleutheroembryos exposed to BPA. Our results showed that the cytochrome P450a1b (*cyp19a1b*), responsible for the conversion of testosterone to estrogen and which is involved in the response to estrogenic compounds, was the most affected gene. On the other hand, we observed alterations in the retinol pathway and identified the aldehyde dehydrogenase 1a2 (a*ldh1a2*) gene as another key-player of the mode of action of BPA. Unfortunately, alteration of DNA methylation levels of these genes was not detected by WGBS using a threshold of 10%. For that reason, we exposed zebrafish embryos from 0 to 5 days post fertilization to 17.5 µM of BPA and performed DNA methylation analysis of these target genes. A total of 10 biological replicates per condition were collected and DNA extracted. Then, bisulfite conversion was carried out and the BisPCR^2

method used. Our results revealed significant differences ranging from 0.1 to 2 in the % of DNA methylation in several CpG positions of the two analysed genes. We observed higher DNA methylation levels in the *cyp19a1b* promoter region in eleutheroembryos treated with BPA when compared with controls. In contrast, 3 CpG positions at the beginning of the first intron were found more methylated in control than in exposed ones. In the case of *aldh1a2*, our results show that control animals presented significant higher methylation levels than BPA treated and that the majority of these CpG were located in the promoter region. Interestingly, although both mRNA levels of *cyp19a1b* and *aldh1a2* were found higher in exposed than in control animals, differences in methylation levels were found in opposite directions, suggesting that regulation of expression of both genes might be mediated by different mechanisms. Integrating transcriptomic and epigenetic platforms is the next step to identify and develop cost-effective high-throughput methodologies to screen for epigenetic signatures after EDCs exposures.
Supported by ERC-32073-Chemageb, MSCA-IF-RI-2017-797725-EpiSTOX and FPU15/03332.

1.06.04

Multigenerational Effects of Endocrine Disrupting Compounds: Understanding Population Level Implications Using Modelling Approaches

S.M. Brander, Oregon State University / Environmental and Molecular Toxicology; J.W. White, Oregon State University / Department of Fisheries and Wildlife; B. DeCourten, University of North Carolina, Wilmington / Department of Environmental and Molecular Toxicology; K.M. Major, Oregon Department of Environmental Quality / Water Quality Standards; R.E. Connon, University of California, Davis / School of Veterinary Medicine Anatomy, Physiology and Cell Biology; A.C. Mehinto, Southern California Coastal Water Research Project / Toxicology

Emerging research demonstrates that endocrine disrupting compounds (EDCs), which agonize, antagonize, and / or synergize the effects of endogenous hormones, can cause deleterious effects as a result of early-life exposure in fishes, as well as effects across generations. To quantify possible multi-generational effects, we explored the effects of a suite of EDCs (ethinylestradiol, bifenthrin, levonorgestrel, and trenbolone) at low ng/L concentrations on the euryhaline model *Menidia beryllina*. Fish were exposed from fertilization until 21 dph, then reared through three generations in clean water. Early life exposure led to changes in sex ratio, phenotypic deformities, as well as differential gene expression and methylation across generations. Taken together, early life EDC exposure caused functionally relevant changes in the epigenome, transcriptome, and reproductive and immune phenotypes in directly (P0), indirectly (F1), and unexposed individuals (F2). To examine the consequences of these individual-level effects on overall population dynamics, we used these data to parameterize a size-and-age-structured integral projection model (IPM). This approach represented empirically-determined effects on growth, survival, and fecundity. Additionally, we tracked the distribution of multi- and transgenerational effects in the model using a delay coordinate approach. We then quantified the effects of both single and chronic exposures to a range of EDCs at environmentally relevant concentrations, both for population persistence and population variability. Model analysis revealed that a) single EDC exposures have longer-term effects on population variability (i.e., oscillations in abundance), and b) chronic EDC exposures were more likely to lead to population collapse when multigenerational effects were accounted for than when not. These projected effects demonstrate that EDC exposures can have far-reaching effects on unexposed generations, highlighting the need for these types of cross-generational effects to be considered in risk assessment.

1.06.06

Potential Role of DNA Methylation and miRNA Expression in the Regulation of the Plant Response to Ionising Radiation in Field and Lab Conditions

N. Horemans, Belgean Nuclear Research Centre (SCK CEN) / Biosphere Impact Studies; M. Mysara, Belgian Nuclear Research Centre SCKCEN; J. Kariuki, P. Laanen, R. Nauts, E. Saenen, Belgian Nuclear Research Centre (SCK CEN); A. Cuypers, Universiteit Hasselt

The impact on plants of long-term (multigenerational) exposure to radiation coming from nuclear accidents like Fukushima and Chernobyl is investigated in field collected samples and compared with lab experiments in *Arabidopsis thaliana* and *Oryza sativa* plants chronically exposed to radiation for one or more generations and/or allowed to recover. The hypothesis is that changes in DNA methylation or miRNAs form the basis of altered gene expression leading to changes in growth and development in plants chronically exposed to radiation. For the field collected samples only for Chernobyl a significant difference in DNA methylation was found. In the multigenerational exposed plants after bisulphite sequencing a clear increase in IR-induced DMRs was seen over the three generations where Gen 2 had a markedly higher number of DMRs than the previous two generations (Parent and Gen 1). A large number of DMRs associated with transposable elements, the majority of them being hyper methylated, likely leading to more genetic stability. In all generations and gamma treatments a limited number of DMRs were found in upstream, promoter-associated regions of genes related to development as well as various stress responses, including DNA repair, chromosome organisation, cell wall organisation, and (a)biotic stress responses. Small RNA sequencing libraries were generated from rice leaves directly after exposure and after 14 days in exposed and newly grown leaves. A total of 40 known rice miRNAs were differentially expressed 60% of which were differentially expressed in irradiated plants and the remaining 40% were differentially expressed in recovering plants. Functional analysis of the target genes revealed that most of the identified known miRNAs were involved in regulating various cellular processes that contribute towards growth and development, antioxidative defence, hormone and MAPK-mediated signal transduction and regulation of gene expression. Although not so evident from field samples, lab experiements clearly showed a strong involvment of DNA methylation and miRNA in the regulation of the plant response to ionising radiation in both multiples generations as well as in plants recovering from exposure. Especially the possible role of transposable elements will be further investigated together with changes in hormone homeostasis in both field and lab exposed plants. *Acknowledgement* - COMET (Fission-2012-3.4.1-604794) and FWO-Vlaanderen (VS01719N).

1.06.07

Transmitted Effects in the Fish Model, Danio rerio, After Multigenerational Gamma Irradiation Lead to F1 Altered Sex Ratio in Link With Epigenetic Mark Modifications

N. Guirandy, IRSN/PRP-ENV/LECO; O. Simon, IRSN; F. Pierron, Université de Bordeaux / UMR EPOC CNRS 5805; P. Gonzalez, University of Bordeaux / UMR EPOC CNRS 5805

Ionising radiation (IR) are known to induce deleterious effects in aquatic organisms. Several studies have shown a great impact at life sensitive stages aimed, such as embryo-larval stage. However, few studies have been aimed to evidence effects in all life cycle and along multigenerational exposure, including the potential transmission of effects across generations. Such exposure conditions may highlight a worsening effects over generations and represent a major challenge for the understanding of effects at ecosystem scale and their consequences for the ecological risk assessment. In this study, fish model *Danio rerio* were irradiated at 3 dose rates (0.05, 5 and 50 $mGy.h^{-1}$) before producing F1 offspring. F1 offspring from irradiated adults was then irradiated (F1-I) or non-irradiated (F1-Recovery). Both F1 generation population were reared to adult stage (131 days) for dose rates of 0.05 and 5 $mGy.h^{-1}$. Following of F1 mortality rate allowed to highlight in *Danio rerio* a transmission of effects from parents to offspring at 5 and 50 $mGy.h^{-1}$. Thus, after adults' exposure at 50 $mGy.h^{-1}$, offspring was not viable with 100% of mortality at 120 hours post fertilization (hpf). At lower dose rate (5 $mGy.h^{-1}$), adults' exposure lead to altered sex ratio in F1 irradiated and recovery. Indeed, sex ratio was of 1 female to 4 males. No sex ratio disruption was highlighted at 0.05 $mGy.h^{-1}$. Larval mortality and altered sex ratio could have a significant effects on population dynamic. This surprising phenotype could be explain by gene expression modulation. Thus, *cyp19a1a* methylation have shown a different methylation rate in its promoter in F1 female gonads at 5 $mGy.h^{-1}$ compared to controls. Expression levels of this gene have to be confirmed by qPCR. Other hypothesis can explain the great impact of IR on sex ratio disruption. Cortisol is a stress hormone, known to alter sex ratio that could be link to this phenotype. Major transmitted effects to the next generation were observed. This study highlights the interest to include multigenerational exposure to ecological risk assessment.

1.06.09

Exposure to Methylmercury During Early Development Results in Increased Tolerance Later in Development in the Zebrafish Embryo Model

P. Robinson, University of Exeter / Biosciences; K. Moore, A. Jeffries, A. Farbos, University of Exeter / Exeter Sequencing Services; H. Littler, University of Exeter / Biosciences College of Life and Environmental Sciences; E. Dempster, J. Mill, University of Exeter / Exeter Medical School; R. van Aerle, Centre for Environment Fisheries and Aquaculture Science / Biosciences College of Life and Environmental Sciences; E.M. Santos, University of Exeter / Biosciences College of Life and Environmental Sciences

Mercury is a toxic metal pollutant that has been shown to have neurotoxic and genotoxic effects at low doses, and bioaccumulates in aquatic environments following conversion of inorganic to organic methylmercury compounds by anaerobic bacteria. This toxicant has been shown to cause epigenetic alterations, leading to persistent effects that can manifest long after the exposure period. However, little work has been done to determine whether the susceptibility to epigenetic alteration varies during development, and results in altered tolerance to subsequent exposures. In order to address this knowledge gap, zebrafish embryos were used as a model as their epigenetic reprogramming periods have been studied in detail, so periods of putative sensitivity could be postulated. We hypothesised that embryos are particularly sensitive to environmental stressors during epigenetic reprogramming, leading to alterations of sensitivity to re-exposure later in development. To test these hypotheses, we exposed embryos to methylmercury during and after the reprogramming period to determine the

impacts on embryo physiology. We then re-exposed zebrafish larvae to determine if early-life non-lethal exposure impacted later response, and to understand the mechanisms underpinning any differences observed. The timing of exposure initiation caused no apparent difference in embryo mortality after 24 or 48 hours of exposure, however embryos exposed to methylmercury at LC5 concentrations for 24 hours during early development showed an increase in tolerance to larval methylmercury re-exposure from 96-120 hpf, when compared to naïve larvae. This effect appeared to be more pronounced for embryos pre-exposed during the epigenetic reprogramming period, leading to the hypothesis that methylmercury may cause more profound effects in the epigenome during this window of development. We are now investigating the mechanisms underpinning the effects observed at the phenotypic level using a combination of global transcriptomics and methylation measurements. Understanding how pre-exposures alter an individual's response is of fundamental importance to assess the risk that chemicals pose to fish populations and implement management strategies to protect them in the natural environment.

Fish Model Species in Human and Environmental Toxicology

1.07.03

Sensitivity of O. Melastigma Embryos to Cardiotoxic and Neurotoxic Compounds - Is the Marine Medaka a Promising Teleost Model for Marine Ecotoxicology?

S. Johann, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology; J. Pfefferle, Goethe University Frankfurt; R. Massei, University of Antwerp; H. Hollert, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology

Oryzias melastigma ("marine medaka") is emerging as a potential laboratory fish model species for the brackish and marine environment. While freshwater species are widely used and studied, the marine research basically lacks standardized fish models. Understanding the sensitivity of a species is of high importance for establishing a marine standardized model species. Our research on *O. melastigma* focuses on its applicability as a marine alternative to the well-established freshwater model zebrafish for early life stage testing. Several external (medium-related) and internal (species-related) factors need to be considered for the sensitivity interpretation. Marine exposure conditions strongly affect the chemical characteristics of a compound and hence the bioavailability. Additionally, the chorion of the marine medaka is thicker compared to its freshwater counterpart, which might act as a stronger barrier for chemical uptake. Since cardiotoxicity and neurotoxicity are dominant embryotoxic mechanisms for a variety of pollutants, cardio- and neurotoxic model compounds in addition to different carrier solvents were selected to investigate acute and mechanism-specific toxicity. We successfully adapted the acute fish embryo toxicity test (FET) with the marine medaka according to verified international guidelines. The pre-hatching development of *O. melastigma* is elongated nearly two times the stages in zebrafish. Experiments with single compound exposure starting from different developmental stages showed the earliest developmental time window to be the most sensitive, leading to recommendation for latest exposure start within standardized FET. In order to investigate the impact of solvents on chorion permeability and, hence, the xenobiotic uptake, a lipophilic fluorescent dye was used to visualize the uptake through the chorion. First results indicate a reduced chorion permeability, which is in line with a reduced sensitivity of *O. melastigma* exposed to single cardio- and neurotoxic substances. However, potentially earlier onset of xenobiotic biotransformation within pre-hatching stages have to be considered. Chemical exposure resulted in a strong xenobiotic metabolism, oxidative stress response as well as AChE inhibition by means enzymatic activity particularly in late pre-hatching developmental stages. The potential application of behavioural endpoints including a light-dark transition test is currently under investigation.

1.07.06

Effects of Isothiazolinones on Development and Thyroid Endocrine System of Zebrafish Larvae

s. lee, researcher / occupational environmental health; K. Ji, Yongin University / Department of Occupational and Environmental Health

Benzisothiazolinone (BIT), methylisothiazolinone (MIT) and octylisothiazolinone (OIT) are used as preservatives and biocides in consumer products to prevent product decay or deterioration. Several studies based on skin corrosion and rodent inhalation toxicity of isothiazolinones have been reported; however, studies on the effects of these ingredients on aquatic organisms are limited. In the present study, the effect of exposure of BIT, MIT, and OIT on the development and thyroid endocrine system of zebrafish embryos was investigated. In accordance with OECD TG 236, zebrafish embryos were exposed to various concentrations (0, 0.03, 0.3, 3, 30, and 300 μg/L) of BIT, MIT, and OIT for 96 h. Coagulation, hatchability, hatch time, survival rate, and wet weight of larvae were observed. At the end of exposure, changes in the expression of genes related to the thyroid endocrine system and thyroid hormones (i.e., T3 and T4) were measured by real-time polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. The zebrafish embryos exposed to 300 μg/L of OIT died before hatching. In fish exposed to 300 μg/L of BIT and MIT, wet weight, thyroid hormones, and the expression of genes related to the thyroid endocrine system (e.g., thra and deio2) decreased significantly compared to the control group. The results suggest that isothiazolinones could affect the thyroid endocrine system of aquatic organisms and ultimately cause growth retardation. The highest concentration of MIT used in the test was 50 times lower than that in a product that can be used in wash-out cosmetics, indicating that exposure to this concentration may disrupt the thyroid hormone endocrine system. The potential effects of long-term exposure to isothiazolinones and mixture effects of several components should be further investigated. Acknowledgement: This study was supported by National Research Foundation of Korea (NRF; Project no. 2019R1A2C1002712). Keywords: Thyroid endocrine system, Isothiazolinone, Zebrafish larvae, Developmental toxicity

1.07.08

Unraveling Molecular Effects of Chemical Mixtures With the Proteome Integral Solubility Alteration Assay in Zebrafish Embryo

V. Lizano-Fallas, Linköping University; A. Carrasco del Amor, S. Cristobal, Linköping University / Biomedical and Clinical Sciences

The development of novel methodology to unbiased identification of mechanisms of action of chemicals mixtures, including novel or poor-studied compounds without any previous solid knowledge, or even compounds in a phase of chemical characterization, would address one of today´s challenges of environmental and human toxicology. Traditional methodologies evaluate the impact in well-known pathways that have been previously described as affected by one specific compound. Therefore, there are several assays that are routinely evaluated but will not offer a specific response for one single compound. Here, we envisioned that the application of thermal proteome profiling approaches could be a promising alternative. Zebrafish embryos offer a unique opportunity to explore the proteome integral solubility alteration (PISA) assay to study the proteins that increase or decrease their solubility as protein targets of the chemicals and chemical mixtures in an organism at a developmental stage. We performed the PISA assay in the soluble proteome of zebrafish embryo at 5 days post fertilization with different types of compounds of environmental and health concern: i) endocrine disrupting chemical, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and ii) complex chemical mixtures composed by TCDD + alpha-endosulfan + bisphenol A (BPA) to unbiased outline the pathways compromised under exposure. Our results demonstrated the unique opportunities of PISA assay in zebrafish for toxicology. We remark some specific findings of the application of PISA in toxicology. First, we can analyze both targets arising from an increase in solubility and in those decreasing, as both would be involved in modulation the cellular functions, differing from the original application of PISA for drug target deconvolution. Second, the method is applicable to compound before chemical characterization or a chemical mixture of unknown stoichiometry. Third, the results from zebrafish embryo could provide a high-resolution, and throughput prediction of the mode of actions of chemicals in support of the 3R, and 3M principles.

1.07.13

A Zebrafish Behavioral and Transcriptomic Model to Predict General Toxicity and Endocrine Disruption That Transitions Animal to Non-Animal Testing

C. Woodland, Government of Canada- Health Canada / Health Canada; L.D. Ellis, J.C. Achenbach, M.G. Morash, National Research Council of Canada / Aquatic and Crop Resource Development

To transition from animal to non-animal testing in chemical risk assessment, the global regulatory community has identified the need for a robust whole organism predictive tool that is alternative to the rodent model. In 2018 the Government of Canada initiated research to investigate the potential of the zebrafish embryo (ZFE) as a predictive tool for ecotoxicity and as an alternative to the rodent model for predicting human health hazard in regulatory decision-making. The ZFE has been established as a high-throughput screening tool for assessing developmental toxicity given its high gene homology to humans (70% sequence identity), high fecundity, rapid maturation, small size, transparency and lower husbandry costs. This research will serve as a means to validate non-animal assays for their ability to predict human health and environmental health hazard in response to chemical exposure, thus supporting the three R's of reduction, replacement and refinement of animal testing. Based on existing international ZFE models NRC Canada has developed protocols to evaluate developmental, general and behavioral toxicity. These are being further refined to include toxicokinetic and transcriptomics platforms for both human health and environmental health risk assessments. The toxicokinetic platform will investigate the role of intact chorion and static exposure on chemical bioavailability and metabolite production. Transcriptomic profiling, in correlation with phenotypic anchoring is being developed to be a more sensitive predictive tool for chronic toxicity. A platform for investigating ecotoxicity is being developed separately from the human health platform which is investigating the potential toxicity of twenty substances that were selected in collaboration with the US National Toxicology Program (NTP) Systematic

Evaluation of the Zebrafish in Toxicology (SEAZIT) project. For the human health platform, results for the phenotypic and transcriptomic evaluations for endocrine disruption, and general and behavioral toxicity will be presented, as well as results for toxicokinetic evaluations. Analysis of this promising method beyond historical application as a screening tool and more as an alternative to the rodent model for use in quantitative risk assessment of repeated dose exposures, will help bridge the transition away from rodent studies to modern toxicity testing that meets the 3R's criteria but maintains biological relevance by using a whole organism model.

1.07.14

Applicability of a Multi-Omics Approach for Neurotoxicity Testing

A. Haigis, Institute for Environmental Research, RWTH Aachen / Department of Ecosystem Analysis ESA; P. Leonards, Vrije Universiteit Amsterdam / Environment & Health; J. Legradi, Vrije Universiteit Amsterdam; H. Hollert, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology; C. Preisinger, L. Gan, Interdisciplinary Center for Clinical Research Aachen (IZKF Aachen) / Faculty of Medicine, RWTH Aachen University

Neurological disorders are of major concern for society since they are assumed to cost billions of dollars and are incurable. Exposure to neurotoxic substances, especially during neurogenesis may increase the risk to develop a neurological disorder. For example, organophosphate (OP) pesticides are suspected to promote Attention-Deficit Hyperactivity Disorders (ADHD). To make things worse, endocrine disrupting compounds may also interfere with the nervous system development, as was suggested for thyroid hormone disrupting compounds. Thyroid hormones are involved in neuronal differentiation, migration and proliferation in cerebral areas and regulate dopaminergic and cholinergic systems. Thyroid system disruption during early development may thus have severe consequences. But so far it was not possible to establish a causal relationship between substance exposure and neurological perturbations. In this work aim at investigating the molecular mechanisms induced in zebrafish (*Danio rerio*) embryos following exposure to several pesticides and endocrine disruptors. Non-target transcriptome, proteome and lipidome as well as neurotransmitter analyses were used to cover different levels of cellular organisation. First results from transcriptome, proteome and neurotransmitter analyses indicate effects different from the suggested mode of action. For example, dichlorvos a known acetylcholinesterase inhibitor, was found to affect phototransduction (proteome) and the dopamine system (neurotransmitter).

1.07.19

Effect-Based Monitoring of a Wastewater Effluent Using the Spontaneous Tail Coiling (STC) of Danio rerio Embryos

A.O. Ogungbemi, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology; E. Küster, Helmholtz Centre for Environmental Research, Dept.Bioanalytical Ecotoxicology / Bioanalytical Ecotoxicology; S. Scholz, Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology; R. Massei, Helmholtz Centre for Environmental Research UFZ

In the last decades, the rise in the menace of neurological diseases, as well as the increased environmental detection of neuroactive substances, has motivated to develop new, fast and sensitive toxicological tools to screen neuroactive substances in the environment. Although chemical monitoring techniques are commonly used, a holistic approach for risk assessment requires appropriate effect-based tools to detect neurotoxic effects. In this context, zebrafish behavior tests are considered to be sensitive and specific to detect neurotoxic effects since they measure endpoints such as locomotor activity which may be directly or indirectly related to the nervous system. Organism behaviors are also ecologically relevant because they can reduce fitness and lead to population decline. Such behavior tests have been utilized for both drug development and toxicity testing in animals such as rodents, fishes and amphibians. In this study, we used the STC test as an effect based tool to screen a wastewater effluent sample for potential neuroactivity. Goal of the study was: 1) To evaluate the use of STC as an effect based tool in monitoring 2) To assess the use of STC as a diagnostic tool for chemical identification and prioritization. Water extracts were re-dissolved in methanol and diluted to relative enrichment factors (REF) of 10, 7.5, 5, 2.5 and 1 representing concentrations below the LC_{10}. The diluted samples at varying REFs were exposed to zebrafish embryo from 2hpf) and STC assessment was conducted at 24hpf by video recording and analyzing the videos in a KNIME workflow. The STC results show that Mulde sample at REF 7.5 and REF 10 caused hyperactivity effect in 24hpf zebrafish embryo. An AChE inhibition test at 24hpf was performed to investigate the observed hyperactivity was associated with AChE inhibition. The result shows that REF 7.5 and REF 10 also inhibit AChE activity. Nevertheless, more tests are required to further confirm the effects observed since the complex mixture of Mulde sample can also cause side effects. Therefore, we plan to conduct further tests such as determining the influence of toxicokinetics and measuring neurotransmitters in the zebrafish embryo.

1.07.20

Can a Hepatotoxin Cause Sublethal Effects on Kidney of a Neotropical Catfish?

A. Lima da Silveira, Federal University of Paraná / Ecology and Conservation Program Post-Graduation; S.L. Calado, Federal University of Paraná; H. C. Silva de Assis, Federal University of Paraná / Department of Pharmacology

Microcystins are hepatotoxic with the most frequent record in freshwater. Consequently, the liver is target-organ on the toxins exposure, but there are reports in toxicity on other tissues, such as the kidney. Therefore, to assess contamination and toxic effects is important, and using biomarkers in fish can be a good tool. The aim of this study was to investigate if microcystins in environmental concentrations can cause sublethal effects on kidney (non-target organ) of a Neotropical catfish *Rhamdia quelen*. Fish specimens were exposed for 96 hours (waterway) to 1.0 and 10.0 µg/L of microcystins obtained from toxic *Microcystis aeruginosa* extract. Three conditions were tested, considering the control (n=12). Before kidney collection, fish were anesthetized and euthanized. Biochemical biomarkers such as activities of catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and superoxide dismutase (SOD) besides non-protein tiol/glutathione (GSH) and lipid peroxidation (LPO) levels were analyzed in kidney. GSH levels showed significant decrease in 1.0 µg/L of microcystins and an increase in 10.0 µg/L when compared to control with mean respectively 20.5, 23.1 and 11.8 nmol/min/mg protein. The GPx activity decreased in higher microcystins concentration and means in control and 10.0 µg/L were respectively 20.5 and 11.8 nmol/min/mg protein. In contrast, SOD activity expressed significant increase in higher microcystins concentration and means in control and 10.0 µg/L were respectively 91.3 and 133.8 U/mg protein. The absorption of toxin by the kidney cells caused depletion on GSH levels in lower microcystins concentration. When the microcystins enter cells stimulates GSH expulsion, because the same cell transportation mechanism is used. Increase in GSH levels and SOD activity on higher microcystins concentration may reflect the efforts of antioxidant system to neutralize reactive oxygen species (ROS) that possibly also caused a decrease in the GPx activity, due to enzymatic oxidation. In addition, other biomarkers are being carried out that will contribute to the final discussion of this study. Our data showed that environmental concentrations of microcystins can cause sublethal effects on kidney of *Rhamdia quelen*. Therefore, the evaluation of different tissues and multibiomarkers are required to understand the potential effects of microcystins that is not yet fully understood.

Integrating New Approach Methodologies (NAMs) and Quantitative Adverse Outcome Pathways (qAOPs) from Development to Deployment into Risk Assessment and Decision-Making

1.08.01

Evaluation of Transcriptional Responses in Duck Mussel (Anodonta anatina) As Biomarkers of Chemical Stress

G.M. Ekelund Ugge, Lund University / Department of Biology; A. Jonsson, University of Skövde / Department of Bioscience; O. Berglund, Lund University / Dept of Biology

In a series of laboratory experiments we exposed duck mussels (*Anodonta anatina*) to copper or industrial wastewater for 96 h, to assess the sensitivity and robustness of commonly used transcriptional stress biomarkers. Specifically, we measured gill and digestive gland expression of catalase (*cat*), glutathione-S-transferase (*gst*), heat shock proteins 70 (*hsp70*) and 90 (*hsp90*), metallo-thio-nein (*mt*) and superoxide dismutase (*sod*) by reverse transcription quantitative polymerase chain reaction. Across experiments, digestive glands demonstrated low sensitivity in all selected markers. Most markers did however demonstrate concentration-dependent responses in gills at exposure to a wide range of Cu concentrations. Yet, consistently throughout experiments, response magnitudes have generally been small even in gills. For instance, the most sensitive gill marker (*hsp70*) demonstrated responses of less than 3-fold increases at exposures of nearly lethal Cu concentrations (up to 1 600 µg/l). Furthermore, biomarker robustness is potentially affected by mussel gravidness, which occurs from late summer until early spring. Sex-specific differences, independent of treatment, have been observed in *cat*, *mt*, *hsp70*, while for instance *hsp90* demonstrated different responses to industrial wastewater in gravid and non-gravid mussels. Such differences in baseline expression and response patterns may add to background noise, potentially obscuring low-magnitude stress responses. Results from our experiments will be presented and discussed, with main focus on the sensitivity and robustness of selected gene responses as biomarkers.

1.08.02

Transcriptome Sequencing of Brassica Napus Highlights the Complex Issues of Soil Supplementation With Sewage Sludge

M. Jaskulak, Czestochowa University of Technology; F. Vandenbulcke, LGCgE, Universite de Lille 1 / LGCgE

The recent increase in soil supplementation with waste products, including sewage sludge, is gaining a lot of interest, and the proper evaluation of its safety is

therefore critically needed. Contaminants that are often seen in sewage sludge such as heavy metals, pharmaceuticals, and persistent organic pollutants, pose a direct threat to all members of the food chain. Still, our knowledge and understanding of the specific effects of sewage sludge on plant physiology are strictly limited. **The main aim of the experiment was to assess the transcriptomic changes in leaves of *Brassica napus* after six months of growing on agricultural soil supplemented with municipal sewage sludge. Thus, the purpose was to identify the ways in which sewage sludge application is influencing plants on the transcriptome level to broaden our understanding of the environmental impact and the safety of the use of sewage sludge as a fertilizer in agricultural soils. The experiment was performed in real field conditions in order to assess the actual changes to plant transcriptome after soil is fertilized with sewage sludge.** All the cDNA libraries of *B. napus* were sequenced using an Illumina HiSeq™ 4000 platform. The sequencing generated 21 688 894 to 29 810 359 raw paired reads for each sample. **The soil supplementation with sewage sludge caused 555 genes to be differentially expressed compared to agricultural soil without sludge. Out of those, 313 were down-regulated by supplementation with sewage sludge, whereas 242 were up-regulated. Moreover, the expression of 79 genes was completely suppressed after supplementation with sludge, and 60 were induced by such action.** The gene ontology analysis showed that the majority of differently expressed genes were related to plants' response to stress. **The project consisted of a first study dealing with the influence of long-term sewage sludge application on plant whole transcriptome.** RNAseq analysis illustrated the impact of sewage sludge on plants' metabolic processes at the transcriptomic level. Those genes were mainly involved in response to stress, transport, biosynthetic processes, the development of anatomical structure, lipid metabolism, and others. **The identification of mechanisms in which sewage sludge influences specific gene expression in plants can be used to assess the potential risks of soil supplementation with sewage sludge.** *Acknowledgement* - Research was funded by National Science Centre, Poland, 2018/31/N/NZ9/01742

1.08.04

Subcellular Distribution of Dietary Methyl-Mercury in Gammarus fossarum and Its Impact on the Amphipod Proteome

C. Cosio, University of Reims Champagne-Ardenne / UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); D. Degli Esposti, INRAE / UR RIVERLY Laboratoire Ecotoxicologie; C. Almunia, CEA / Laboratoire Innovations technologiques pour la Détection et le Diagnostic; V. Gaillet, Université Reims Champagne Ardenne / UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); H. Sartelet, University of Reims Champagne-Ardenne / Medyc; J. Armengaud, CEA / Laboratory Innovative technologies for Detection and Diagnostics; A. Chaumot, INRAE / UR RIVERLY Laboratoire Ecotoxicologie; O. Geffard, Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) / Unité de Recherche RiverLy; A. Geffard, Université de Reims Champagne Ardenne / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring)

Hg threats ecosystems and human health, because of the biomagnification of methyl-Hg (MeHg) through the food chain. Nonetheless, we still lack knowledge on its toxicity through diet. Gammarids play a major role in European lotic ecosystems. Here, we aimed to determine the trophic transfer of two sources of dietary MeHg in *Gammarus fossarum* and its impact at the proteomic level. *Elodea nuttallii* were exposed to MeHg (low and high concentrations) and washed to retain Hg from intracellular (I) or cell walls (C) compartment. *G. fossarum* were fed 7 days with washed shoots. Differential centrifugation was used to determine subcellular fate of Hg. Proteins were identified and quantified by next-generation shotgun. Hg bioaccumulation in gammarids spanned concentration found in clean to contaminated sites, with higher THg concentrations in animals fed with cell wall. High-MeHg diet resulted in increased concentrations in the metal sensitive fraction (MSF) and biologically detoxified metal (BDM) fraction for animals fed with intracellular and cell wall respectively. The number of proteins modulated after MeHg exposure was congruent with MeHg proportion in MSF. Proteins involved in autophagy and mitochondrion organization increased with MeHg doses, suggesting a higher impact on cell integrity and energy metabolism. Data showed a contrasted modulation of proteins involved in development, growth, energy, regulation and reproduction, supporting a higher impact of MeHg from the cell wall compartment. Data suggest that in response to increasing doses of MeHg, *G. fossarum* compensates for the loss/impairment of certain pathways caused by the toxicant via activation and deactivation of biochemical processes (i.e. adaptation processes). As such, several thresholds of proteomic response are triggered by increased bioaccumulation in each subcellular fraction and correlated with Hg exclusively bound to the MSF, while the increase in BDM likely had a cost for fitness. The cell wall constituents are expected to remain for a long period in the environment because of their recalcitrant nature. Our data confirmed that Hg bound in cell walls of plants can be assimilated by *G. fossarum*, which is consistent with its feeding strategy, hence pointing cell walls as an important source of Hg transfer and toxicity to primary consumers. Moreover, proteomic analysis revealed contrasted responses for intracellular and cell wall treatments, supporting that the different binding sites and speciation in shoots subsequently results in different cellular toxicity pathways to consumers. In this context feeding behavior of consumers appears central for the fate and impact of Hg in food webs.

1.08.05

Substance-Specific Fingerprints of Thyroid Disruption in Zebrafish Embryos

S.U. Ayobahan, Fraunhofer IME / Department of Aquatic Ecotoxicology; H. Reinwald, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Fraunhofer Attract EconOMICs; A. König, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; J. Alvincz, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Fraunhofer Attract EconOMICs; L. Sipos, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; G. Böhle, Fraunhofer IME; B. Göckener, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; O. Shomroni, NGS - Integrative Genomics Core Unit / Human Genetics; H. Hollert, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology; G. Salinas, NGS - Integrative Genomics Core Unit / Human Genetics; C. Schäfers, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; E. Eilebrecht, Fraunhofer IME / Ecotoxicology; S. Eilebrecht, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Fraunhofer Attract EconOMICs

Endocrine active chemical substances targeting thyroid hormone-related physiological processes could trigger far-reaching effects on environmental populations, thus justifying a refusal of market approval for chemicals with thyroid disrupting (TD) properties. Currently, hazard assessment of TD effects mostly relies on large numbers of amphibian studies, which are expensive, both in terms of resources and animal use. To this end, we used transcriptomics and proteomics for identifying molecular signatures of interference with thyroid hormone signalling preceding physiological effects in zebrafish embryos (*Danio rerio*). In the present study, zebrafish embryos were exposed to the thyroid hormone 3,3',5-triiodothyronine (T3) and the thyroid peroxidase inhibitor 6-propyl-2-thiouracil (6-PTU) as model substances for detecting increased and repressed thyroid hormone signalling in a modified zebrafish embryo toxicity test (zFET). We observed a coherent reduction in swim bladder inflation and a statistically significant hatching delay upon T3 and 6-PTU exposure, respectively. In conformity with these physiological changes, we identified consistent gene expression fingerprints for both modes-of-action at sublethal test concentrations. T3 and 6-PTU both significantly target the expression of genes involved in muscle contraction and functioning in an opposing fashion, allowing for an in-depth understanding of the observed physiological changes and a mechanistic refinement of key event relationships in thyroid-related adverse outcome pathways in fish. Furthermore, our fingerprints identify biomarker candidates for thyroid disruption hazard screening approaches. Eventually, our findings will promote the AOP-based development of *in vitro* assays for the assessment thyroidal endocrine disruptors, which in the long term will contribute to a reduction of regulatory animal tests.

1.08.08

Co-Expression Network Analysis Identifies Distinct Protein Modules and Pathways Associated With Cadmium and Pyriproxyfen Testicular Toxicity in Gammarus fossarum

N.K. Koenig, INRAE Centre de Lyon-Grenoble Auvergne-Rhône-Alpes / UR RIVERLY Laboratoire Ecotoxicologie; C. Almunia, CEA / Laboratoire Innovations technologiques pour la Détection et le Diagnostic; J. Armengaud, CEA / Laboratory Innovative technologies for Detection and Diagnostics; A. Chaumot, INRAE / UR RIVERLY Laboratoire Ecotoxicologie; O. Geffard, INRAE Lyon / UR RIVERLY Laboratoire Ecotoxicologie; D. Degli Esposti, INRAE / UR RIVERLY Laboratoire Ecotoxicologie

In ecotoxicology, omics approaches are promising tools to improve our comprehension on the mechanisms of action of contaminants in environmental sentinel species. Co-expression network approaches provide an excellent framework for studying, without any *a priori* knowledge, the rich content of availlable omics datasets to describe species-specific molecular pathways involved in response to a contaminant exposure . This study aimed to find key pathways and proteins related to the testicular toxicity in the freshwater crustacean *Gammarus fossarum* after exposure to two pesticides and a heavy metal, using a weighted protein co-expression network analysis (WGCNA). The analysis was performed on a shotgun proteomics dataset of 40 samples consisting of male gonads obtained from amphipods exposed to two concentrations of cadmium (Cd), pyriproxyfen (Pyr) and methoxyfenozide (Met) in laboratory conditions. Four distinct modules were identified as significantly correlated to contaminants' exposure. Protein set enrichment analysis identified modules involved in cytoskeleton organization and oxidative stress response associated with the Cd exposure. The module associated with Pyr exposure was associated with endoplasmic reticulum stress (ER) response. Our study provides the first evidence of a possible implication of ER stress as an endocrine disruptive mode of action in testicular toxicity in invertebrates. The module correlated with Met exposure was characterized by a significant proportion of proteins whose functions are still not

characterized, highligthing taxon specific proteins responses in contaminant impacts, and the importance of expanding the biological diversity in molecular ecotoxicology. Our results show that co-expression networks are efficient and adapted tools to exploit data from shotgun proteomics in species for which annotated genome is not availble. These approaches will help shed light on the mechanisms of action of contaminants and identify molecular actors for future in-depth functional analyses.

1.08.10

Multiomics Dose-Response Modeling Identifies Low-Dose Hazards of Ionizing Radiation to Daphnia and Supports an Adverse Outcome Pathway Network

Y. Song, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; K. Zheng, D.A. Brede, B. Salbu, Norwegian University of Life Sciences (NMBU) / Centre for Environmental Radioactivity (CERAD); K. Tollefsen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment

Elevated levels of ionizing radiation from radionuclides released due to nuclear accidents, authorized releases and from naturally occurring radioactive materials may pose hazards to aquatic organisms. While adverse biological effects of acute high-dose ionizing radiation have been extensively investigated, knowledge on low-dose chronic effects is scarce. The aims of the present study were to: 1) Identify low-dose hazards of ionizing radiation to *Daphnia magna* using multiomics dose-response modeling; 2) demonstrate the use of omics data to support an adverse outcome pathway (AOP) network developed for ionizing radiation. Neonatal *D. magna* were exposed to gamma radiation for 8 days. Transcriptomic analysis (RNA-seq) was performed after 4 days and 8 days of exposure, whereas metabolomics (UHPLC-HRMS/MS) and assays for functional endpoints, such as reactive oxygen species (ROS) formation, mitochondrial membrane potential (MMP) and whole-organism ATP content, were conducted after 8 days of exposure. Dose-response modeling and functional integration of the multiomics data were performed using the R package DRomics and MetaboAnalyst, respectively.Benchmark doses (BMDs, 5%) as points of departure (PODs) were estimated for both dose-responsive genes/metabolites and the enriched KEGG pathways. The PODs of relevant pathways and functional endpoints were then overlayed with a previously published AOP network. The results showed that several KEGG pathways were highly relevant to the known modes of action of gamma radiation, including oxidative stress, DNA damage, mitochondrial dysfunction, protein degradation and apoptosis. The functional assays showed increased ROS production, and decreased MMP and ATP. Ranking of PODs at the pathway and functional levels showed that oxidative damage related functions had relatively low PODs, followed by DNA damage, energy metabolism and apoptosis. These were in agreement with the proposed AOP network for ionizing radiation. The present study employed multiomics dose-response modeling to identify low-dose hazards of ionizing radiation to *Daphnia*. This approach yielded promising results and can potentially provide additional empirical evidence to support AOPs. ***Acknowledgement*** - This project was funded by the Research Council of Norway through the Centre of Excellence project 223268 (www.niva.no/en/projectweb/cerad), and supported by the NIVA Computational Toxicology Program (www.niva.no/nctp).

1.08.12

Piecewise Structural Equation Modeling Aided Construction of a Quantitative Adverse Outcome Pathway Network

Y. Cao, Orebro University; J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; R. de Bin, University of Oslo / Department of Mathematics; K. Tollefsen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; Y. Song, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment

Quantitative adverse outcome pathway (qAOP) is gaining momentum due to the predictive nature and alignment with quantitative risk assessment. A wide range of modeling approaches can potentially assist the construction of qAOPs. Among these, piecewise structural equation modeling (PSEM) is considered highly suitable for qAOP network construction. The present work aimed to demonstrate the usefulness of PSEM for qAOP development. A previously published AOP network linking excessive reactive oxygen species production to mortality (AOPWiki, AOP 327-330) was used as a case study. The AOP network was comprised of four linear AOPs. The case study intended to answer: 1) which linear AOP in the network contributed the most to the AO? 2) can any of the upstream KEs accurately predict the AO; 3) is PSEM a suitable approach for qAOP development? Briefly, each set of response-response relationships was estimated independently (or locally) in PSEM. This process decomposed the network into the corresponding simple or multiple generalized linear regression models for each response. Each response was evaluated separately, and then combined to generate inferences of the entire SEM. Model fit for PSEM was tested using d-separation. The test examined if any linear AOP was missing from the model, and whether the model would be improved with the inclusion of the missing AOP. The predictive ability of a linear AOP in the network was evaluated using the area under the receiver operating characteristic curve (AUC). By estimating the regression coefficients for all adjacent KEs, one linear qAOP was found to be highly significant. Both multiplicative model and additive multivariate logistic regression models were fitted, and the AUCs of both models were larger than 0.9, indicating strong predictive ability of the qAOP. The present study shows that PSEM is a suitable approach for constructing complex qAOP networks. Besides quantification of response-response relationships, the approach can also identify the most influencing AOP in a network and evaluate the predictability of this AOP. Follow-up studies will also identify the most influencing KE(s) in the network and how accurately this KE can be used to predict the final AO. ***Acknowledgement*** - This project was funded by the Research Council of Norway (RCN), grant no. 301397 “RiskAOP” (www.niva.no/en/projectweb/riskaop), and supported by the NIVA Computational Toxicology Program (www.niva.no/nctp).

1.08.13

An Integrated Metabolomics and Proteogenomics Approach Reveals Metabolic Alterations Following Carbamazepine Exposure in the Mussel Mytilus galloprovincialis

T. Dumas, Université de Montpellier - UMR 5151 Hydrosciences / UMR 5569 Hydrosciences Montpellier; E. Gomez, HydroSciences Montpellier, IRD, CNRS, University of Montpellier / UMR 5569 Hydrosciences Montpellier; C. Almunia, CEA / Laboratoire Innovations technologiques pour la Détection et le Diagnostic; J. Boccard, University of Geneva / School of Pharmaceutical Sciences; G. DUPORTE, HydroSciences Montpellier, IRD, CNRS, University of Montpellier / UMR 5569 Hydrosciences Montpellier; H. Fenet, Université de Montpellier, Hydrosciences Montpellier UMR 5569 / UMR 5569 Hydrosciences Montpellier; F. Courant, HydroSciences Montpellier, IRD, CNRS, University of Montpellier / UMR 5569 Hydrosciences Montpellier

The antiepileptic carbamazepine (CBZ) is among the most frequent pharmaceutical active compounds detected in the environment, such as marine ecosystems, with proven adverse effects on organisms, including bivalves. Investigations of CBZ effects on those organisms are often hypothesis-driven and could not be exhaustive. In this context, it is necessary to apply holistic approaches to further investigate and understand (sub)individual adverse effects of CBZ on marine bivalves. Omics approaches are now recognised as suitable to overcome this issue. Although multi-omics strategy is less common in ecotoxicology, it can provide a more precise overview of contaminant effects. The present study aims to combine the metabolomic and proteogenomic approaches to study underlying mechanisms of CBZ and its potential toxicological effects on the marine mussel *Mytilus galloprovincialis*. Mussels were exposed for 3 days in controlled laboratory conditions at an environmental dose (80 ng/L) and a higher dose (8 µg/L) of CBZ. Non-targeted metabolomic and shotgun proteomic analyses were done on digestive gland of male mussels using liquid chromatography–high resolution mass spectrometry. A mid-level data fusion strategy was implemented in this work, as well as different bioinformatics tools to highlight biological processes altered by CBZ exposure. The results reflect a state of cellular stress induced by CBZ, even at an environmental dose. This stress is mainly expressed by a disturbance of protein and lipid homeostasis and can reveal an alteration of lysosomal membrane stability and a peroxisome proliferation. As our results show, the induction of oxidative stress leading to cell death (apoptosis) can probably be the consequences of such exposure. This study supports the interest of combining the metabolomic and proteomic approaches in order to benefit from their complementarity for a better understanding of the effects caused by pharmaceutical active compounds.

New Methods and Tools for the Challenge to Emerging Contaminant Issue

1.09.01

Artificial Intelligence and Systems Toxicology for the Construction of a AOP Network Related to Metabolic Disorders Induced by an Endocrine-Disrupting Chemical Mixture

E. Zgheib, M. Kim, F. Jornod, Université de Paris/U1124; E. Blanc, K. Bernal, X. Coumoul, Université de Paris/Inserm U1124; N. Benhajkassen, ANSES; C. Rousselle, ANSES / Unit of Chemical Substances Risk assessment Department; R. Barouki, K. Audouze, Université de Paris/ Inserm U1124

Metabolic disorders are among the main adverse health outcomes that have been associated with endocrine disrupting chemicals (EDCs). It is essential to have a better understanding of the mode of action of suspected EDCs, and the biological pathways that they may be perturbed to identify their real impact on the human population. The concept of Adverse outcome pathways (AOP) provides a practical organizing framework of perturbations at different levels of the biological organization by linking molecular initiating events (MIE) to an adverse outcome (AO) across several intermediate key events (KE). We investigated the applicability of an integrated systems toxicology approach to develop a AOP

network related to metabolism disorders. First, a new tool called AOP-helpFinder was used to identify metabolic effects associated with selected EDCs such as biphenols, PFAS, PCBs, brominated compounds. AOP-helpFinder combines text mining and graph theory to automatically screen abstracts from the PubMed database. The tool searched for co-occurring terms among two lists: one that contains the studied EDCs, and one with the biological events (from the AOP-wiki database and from *in house* experts). This step allowed to decipher links between each EDC and biological events, and was followed by a manual curation to select the most relevant publications, whose reliability was assessed by a dedicated tool. Then, an AOP network was proposed, which was enriched by integration of databases information (e.g. CompTox). The AOP-helpFinder tool allowed to establish linkage between the EDCs and 80 events from 15414 articles. The most relevant events were related to lipids accumulation/obesity, oxidative stress, diabetes, and liver steatosis. Among them, 20 events were related to nuclear receptors and transcriptional factors. After an individual analysis of each EDC, the findings were merged to mimic an EDC mixture. An AOP network that reflects biological key events that could be triggered by several EDCs was then proposed. These findings highlight the increasingly relevant use of computational tools in predictive toxicology.

1.09.03

High Throughput Incubation With Human Liver Extracts As a Tool for Screening Metabolites of Chemicals of Emerging Concern in Human Samples

C. Huber, T. Schulze, W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis; M. Krauss, Helmholtz centre for environmental research - UFZ / Department of Effect-Directed Analysis

Chemicals of emerging concern (CECs) are compounds of current use which are suspected to cause ecological or human health impacts. Including more CECs and their metabolites in human biomonitoring is essential to characterize the human exposome. Screening strategies based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) are a promising tool for this task. However, the annotation of metabolites in complex matrices is challenging, as often reference standards are not available. This study presents a high-throughput strategy to overcome these limitations by generating phase I metabolites of CECs *in-vitro* by incubation of parent compounds with highly pooled human liver S9 extract. The incubate mixtures are measured by LC-HRMS and the data is automatically processed to detect metabolites and to generate a spectral library database, which can then be used for identification and confirmation of those compounds in human samples (*in vivo*). We applied this strategy to over 60 compounds, including pesticides and ingredients of personal care products.

1.09.04

Lung Organoids and Microplastic Fibres: A New Exposure Model for Emerging Contaminants

A.S. Winkler, State University of Milan / Department for Environmental Science and Policy; N. Santo, L. Madaschi, State University of Milan / Unitech NOLIMITS, Imaging facility; A. Cherubini, F. Rusconi, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico / Laboratory of Regenerative Medicine - Cell Factory, Department of Transfusion Medicine and Hematology; L. Rosso, State University of Milan / Health Sciences Department; P. Tremolada, State University of Milan / Department of Environmental Science and Policy; L. Lazzari, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico / Laboratory of Regenerative Medicine - Cell Factory, Department of Transfusion Medicine and Hematology; R. Bacchetta, State University of Milan / Department of Environmental Science and Policy

Increasing report of airborne microplastics, including synthetic fibres from clothes and textiles, poses a potential risk to humans upon inhalation. In this study, we address microfibre release from dryer machines into the environment since this emission source is still poorly investigated. We analysed the effect of the environmentally relevant polyester fibres applying an innovative 3D model consisting of human lung organoids as test model representing a potential target of airborne contamination. Organoids are the most advanced *in vitro* models but are not yet applied to evaluate the biological effect associated with microplastic exposure. For this study, a variety of polyester clothes and tissues was washed and dried. Characterisation of released fibres into the air filter was performed by SEM-EDS. The organoids, established of human lung epithelial cells, were exposed to microplastic fibres in various concentrations (1, 10 and 50 mg/L) and effects were analysed by SEM and confocal microscopy. Gene expression analysis by qRT-PCR was performed to validate the airway cell composition of organoids further. Preliminary results show that lung organoids were affected by microplastic fibres at all concentration steps exhibiting deformations of the 3D cell structure and internalisation of the fibres. Our results suggest that organoids have the potential to replace animal models and primary human tissues (cell lines, 2D models) in the research of emerging contaminants to demonstrate human physiological responses comprehensively.

1.09.16

Assay Optimization to Detect Cyclooxygenase-2 Inhibitors in the Aquatic Environment

T. Jonkers, VU University / Department of Environment and Health; F. Verburg, Vrije Universiteit Amsterdam / Department of Environment and Health; C.J. Houtman, The Water Laboratory; J. Kool, Vrije Universiteit Amsterdam / BioMolecular Analysis group; M. Lamoree, VU University, Department Environment & Health / Department of Environment and Health; T. Hamers, VU University Amsterdam, Institute for Environmental Studies (IVM) / Department of Environment and Health

The high consumption of non-steroidal anti-inflammatory drugs (NSAIDs) wordwide leads to the detection of these drugs in the aquatic environment. Organisms may be adversely affected by the parent compound, but also metabolites and tranformation products may still be bioactive and toxic. Effect-directed analysis (EDA) –a platform that combines biological and chemical measurements– allows the identification of bioactive compounds that are not routinely monitored with targeted chemical screening techniques. Our aim was to develop a bioassay that can be used in such an EDA-study to identify COX-2 inhibitors in concentrated water extracts. NSAIDs inhibit the enzyme prostaglandin H synthase, often referred to as cyclooxygenase (COX). Inhibition of COX-2, an inducible isozyme of COX in mammals, prevents the conversion of arachidonic acid into products that would ultimately lead to inflammation and sensitivity to pain. In this study, we used human recombinant COX-2 (hCOX-2) to develop an assay to screen for COX-2 inhibitors. A sensitive read-out for the assay was determined by studying three indicator molecules, that monitored the hydroperoxidase reaction of the hCOX-2 enzyme. The oxidized indicators were measured as a photometric, fluorescent or luminescent read-out. The fluorescent indicator was selected as most suitable because of the stable product formation and high sensitivity in monitoring the enzyme activity. Further assay optimization steps included a decrease in the assay volume, a reduction of the hCOX-2 required in the assay reaction, and the determination of the Km-value. The sensitivity of the assay was assessed by exposures to 11 NSAIDs with a high prescribed use in the Netherlands. IC50-values (concentration where 50% enzyme inhibition occurs) were determined from the resulting dose-response curves. The most potent inhibitors were the propionic acid NSAIDs (IC50 in nM-range), whereas the enolic acids responded the least sensitive (IC50 in µM-range). Pharmaceuticals that were not expected to interact with COX-2 showed no cross-reactivity with the assay indeed (>100 µM), although the tested antioxidants did interfere with the assay read-out in the µM range (data not shown). In the presentation, we will demonstrate the use of the assay on fractionated environmental samples.

Extended submission 1 - Ecotoxicology and human toxicology: from molecules to organisms, from omics to in vivo

1.10.01

A Quick, Comprehensive and Reproducible Protocol for the Integrative Multi Stressor - Multi OMICS Analyses of ENvironmental Samples

I. Grgić, Ruđer Bošković Institute / Department of Physical Chemistry; M. Petrovic, ICRA; M. Rozman, Ruder Boskovic Institute / Department of Physical Chemistry

Man-made contaminants have become part of our everyday lives and their presence in the environment is readily acknowledged. Due to their omnipresence in the environment, microplastics and certain chemicals such as endocrine disrupting compounds (EDCs) and pharmaceuticals (PhACs) have become contaminants of emerging concern. The general approach in (eco)toxicological studies has mainly focused on the impact of individual stressors (e.g. EDCs, PhACs or microplastics) on aquatic biota. As individual stressors in natural habitats rarely acts in isolation and many ecosystems are characterized by the co-occurrence of stressors, it has been suggested that future studies should consider multiple stressor environments. The response of biological systems to stress can range from the individual to the community level. On the individual level, stress may alter the physiology of the cellular and molecular network. Incorporating stress information encoded at the molecular level will certainly provide a more layered, complete and informative view of the processes occurring in multiple stress environments. The integration of multiple measurements (i.e. quantification of stressors, proteomics and metabolomics) may provide a more informative view on the effects of m**ultiple stressors on aquatic organisms.** The possibility of extracting a variety of molecules and performing multiple measurements on single samples is very attractive, but also challenging and a first step is to develop and validate analytical methods for their extraction and determination in biotic matrices. Our study focused on the development of a rapid and comprehensive protocol for the simultaneous extraction of emerging contaminants (EDCs, PhACs and microplastics), metabolites and proteins for an integrative multi-stressor and multi-omics approach. We based the protocol on extraction with organic solvents and solid phase extraction clean up procedures. Special focus was placed on testing the purification efficiency of complex matrices, taking into account the values of the matrix effect and recovery, and on accelerating and simplifying the process of isolation of microplastic particles from biota samples. To evaluate the

broad applicability of this method, we performed extraction procedures on a variety of biotic samples. Here we present the results of the application of this protocol in a mesocosm study examining the impact of stressors on freshwater biota.

1.10.03

In Vivo Pulmonary Toxicity and Underlying Mechanism of Two Alkyl Organophosphate Flame Retardants

Y. Meng, Tongji University / College of Environmental Science and Engineering; X. Xu, Tongji University / School of Medicine; Y. Qiu, Tongi University / Key Laboratory of Yangtze River Water Environment (Ministry of Education), College of Environmental Science and Engineering

Tri-n-butyl phosphate (TnBP) and tris (2-butoxyethyl) phosphate (TBOEP), two typical alkyl organophosphate flame retardants (OPFRs), are ubiquitously detected in indoor dust. This study is to investigate the lung toxicity of TnBP and TBOEP by *in vivo* research and access the underlying toxic mechanism to provide evidence to the health risk of alkyl OPFRs. **Methods** Female Bal/C mice were randomly divided into control, TnBP (Low) (10 mg/kg/day), TnBP (High) (100 mg/kg/day), TBOEP (Low) (10 mg/kg/day), TBOEP (High) (100 mg/kg/day) group. Mice were continuously instilled intranasally daily for 3 weeks and sacrificed. Plasma or lung tissue were collected and stored at -80℃. Fixed lung tissues were embedded in paraffin and stained with hematoxylin and eosin for histological observation. SOD, GSH-Px, MDA and IL-6 were measured by SOD assay kit, GSH-Px Elisa kit, MDA Elisa kit and IL-6 Elisa kit according to the manufacturers' instructions. Total RNA was isolated by Trizol reagent and sequencing libraries were conducted by NEBNext® UltraTM RNA Library Prep Kit for Illumina with 150 bp paired-end reads generated. **Results** Reddish edema fluid was secreted in alveolar cavities with thickened alveolar septum after inhaled TnBP or TBOEP compared with the control. Erythrocyte diapedesis and mononuclear leukocytes infiltration could be seen in the thickened alveolar walls. Bronchial cartilage calcification and exfoliated bronchial epithelial cells were observed in higher dose exposure. No obvious difference was detected in SOD, GSH-px and MDA level when exposing to low-dose TnBP or TBOEP compared with the control, while significant increase of those biomarkers observed in high-dose groups, indicating the occurrence of oxidative stress. IL-6 participates in the early immune activation and inflammatory response. The expression of IL-6 was elevated obviously in TnBP or TBOEP group suggesting they can enhance inflammation via oxidative stress by secreting proinflammatory cytokines IL-6. 82 upregulated genes and 131 downregulated genes were identified between TnBP (High) group and control group ($P< 0.05$ and $|log2FC|>1$), and the expression of Map3k6 was upregulated significantly suggesting MAPK signal pathway was involved in TnBP group. The expression of Alas2 was downregulated, resulting in the occurrence of glycine, serine and threonine metabolism induced by TnBP. Compared with the control, 348 differential expressed genes (DEGs) were upregulated and 194 DEGs were downregulated in TBOEP (High) group. Calcium signal pathway was the key mediated pathway in TBOEP group with the expression of Atp2a2 upregulated notably, which is related to ATP binding, calcium ion transport and protein binding. Neuroactive ligand-receptor interaction was also involved in TBOEP group with Gzma downregulated obviously. **Conclusions** TnBP and TBOEP could cause lung damage through inflammation mediated by oxidative stress. MAPK signal pathway and glycine, serine and threonine metabolism were main regulators in TnBP group, while calcium signal pathway and neuroactive ligand-receptor interaction took crucial part in TBOEP group.

1.10.05

Proteomic Changes in the Solitary Ascidian Herdmania Momus Following Exposure to the Anticonvulsant Medication Carbamazepine

G. Navon, Tel Aviv University / Zoology; L. Novak, Tel-Aviv University; N. Shenkar, Tel-Aviv University / Zoology

The increasing use of pharmaceuticals in human and veterinary medicine, along with their poor removal rates at wastewater treatment facilities is resulting in the chronic release of pharmaceutically-active compounds (PhACs) into the marine environment, where they pose a threat to non-target organisms. A useful approach, as applied in the current study for assessing the effects of PhACs on non-target organisms, is the proteomic approach: the large-scale study of an organism's proteins. Using 'shotgun' proteomics, we identified differentially-expressed proteins based on peptide fragments in the solitary ascidian, *Herdmania momus*, following a 14-day laboratory experimental exposure to the PhAC carbamazepine (CBZ), an anticonvulsant and antidepressant medication, frequently detected in the aquatic environment. Individuals were exposed to environmentally relevant concentrations: 5 or 10 µg/L of CBZ, in addition to a control treatment. Out of 199 identified proteins, 24 were differentially expressed (12%) between the treatment groups. Seven of these proteins had been previously demonstrated to respond according to environmental stressors, and thus can potentially be developed as biomarkers for PhAC contamination. Ascidians' phylogenetic position within the closest sister group to vertebrates presents an advantage in examining the pathological effects of PhACs on vertebrate-related organs and systems. Together with the world-wide distribution of some model ascidian species, and their ability to flourish in pristine and polluted sites, they provide a promising tool through which to study the extent and effects of PhAC contamination on marine organisms.

1.10.08

Evaluation of the Affinity of Uranium for Synthetic Multiphosphorylated Peptides by Hydrophilic Interaction Liquid Chromatography (HILIC) Coupled to Elemental and Molecular Mass Spectrometry

L. ABOU ZEID, A. Pell, H. Isnard, CEA Paris-Saclay; P. Delangle, CEA Grenoble; C. Bresson, CEA Paris-Saclay

Understanding and limiting the impact of uranium (U) generated by research and industrial applications on humans and on the environment is a growing concern. In this context, our partner synthetized dedicated multiphosphorylated peptides that mimic U binding sites in proteins in order to deepen the knowledge on (i) U toxicity mechanisms at the molecular level and (ii) the key parameters responsible for U affinity towards its target proteins, unknown until now. The aim of our work is to develop an analytical method that will allow us to determine in a single step the affinity scale of U for these peptides. The analytical strategy consists in the implementation of the simultaneous coupling of hydrophilic interaction chromatography (HILIC), to an electrospray ionization source mass spectrometer (ESI-MS) and an inductively coupled plasma source mass spectrometer (ICP-MS) in order to acquire structural and elemental data in a single analysis. Firstly, the selectivity of stationary phases of different polarity was evaluated in order to define the best conditions for separating U-peptide complexes. Secondarily, the effect of the structure of the peptides on their affinity for U was assessed. Therefore, a competition of cyclic and linear tetraphosphorylated peptides for the complexation of uranium was conducted by modifying the U/peptide ratio. The U complexes formed with these two peptides were separated and a quantification method based on external calibration was developed by ICP-MS using the simultaneous coupling. The results obtained through this instrumental setup showed preferential complexation of U with the cyclic peptide. Overall, this method allowed to evaluate the effect of the structure of the peptide on U affinity.

Advances in Behavioural (Eco)Toxicology-the Missing Link Connecting Proximate and Ultimate Effects of Chemical Contaminants and Environmental Changes

2.01.01

The Role of Behavioural Ecotoxicology in Environmental Protection

G. Maack, German Environment Agency / Ecotoxicological Assessment of Pharmaceuticals; M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry; B.W. Brooks, Baylor University / Department of Environmental Science; A. Ford, University of Portsmouth / Biological Sciences

We are increasingly aware that a wide variety of contaminants and other environmental stressors adversely affect organismal behaviour and adverse outcomes in terrestrial and aquatic ecosystems. There is also a groundswell of concern that regulatory ecotoxicology does not adequately consider behaviour, primarily due to a lack of standardized toxicity methods. This has led to the exclusion of behavioural ecotoxicology studies from chemical risk assessments. To improve the understanding of the challenges and opportunities for behavioural toxicology within regulatory toxicology/risk assessment, a workshop at the German Environment Agency (UBA) was organised. International representatives from the fields of behavioural ecology, ecotoxicology, regulatory (eco)toxicology, neurotoxicology, test standardization, and risk assessment concluded on consensus perspectives and recommendations. These promise to serve as a roadmap to advance interfaces among the basic and translational sciences and regulatory practices. Evident from the workshop was a consistent shared perspective that considerable data exists highlighting that chemical pollutants can impact the behaviour of humans and wildlife. Similarly, experts agreed that behaviour is a sensitive indicator of disturbance and is linked to fundamental processes that influence individual fitness and can lead to population- and ecosystem-level adverse outcomes. There was also agreement that the field is still evolving and that the current body of research has limitations that will need to be overcome in terms of design, intra-species variability, cross-species extrapolation, repeatability, and being representative for the field situation. The commercial sector is making use of behavioural tests during e.g. drug development and is looking towards model aquatic species to replace mammalian species, for reasons of costs and ethics. Six recommendations for the improved use of behavioural endpoints in environmental risk assessment of chemicals were developed: - Improve the mechanistic understanding of contaminant-induced behavioural alterations - Develop new and adapt exiting standard toxicity tests to include behaviour - Develop an integrative approach to environmental risk assessment, which includes behaviour - Improve the reliability and reproducibility of behavioural endpoints. - Develop guidance and training on the evaluation and reporting of behavioural studies. - Better integrate human and wildlife behavioural toxicology.

2.01.04

Antidepressant Effects on a Freshwater Invertebrate Community and the Behaviour of Individual Species

L.M. Schuijt, Wageningen University & Research; A. Vonk, Wageningen University; P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences

Pharmaceutical contamination is one of the emerging threats to aquatic ecosystems. A group particular concerning are antidepressants, with fluoxetine being one of the most commonly prescribed and frequently detected in surface waters and sediments. However, little is know about the effects of fluoxetine on the invertebrate community and behaviour of freshwater invertebrates. Therefore, the aim of this study was to assess the effects of the chronic application of fluoxetine on the invertebrate community and the behaviour of some individual freshwater invertebrate species. We first performed a mesocosm experiment to investigate the effect on invertebrate community and which taxa are responding to fluoxetine. We exposed 18 outdoor mesocosm (water volume of 1,500L and 10 cm of sediment) to four different concentrations of fluoxetine (0.2, 2, 20 and 200 µg/L) for eight weeks with adjacent an eight week recovery period. Next, we evaluated the sensitivity of the two most responding taxa by performing single species laboratory experiments using similar fluoxetine concentrations and assessed whether the observed reduction in abundances can be related to behavioural changes. Here our main endpoints are swimming velocity, boldness, growth and feeding rates. In the mesocosm experiment, our results showed responses of macroinvertebrate community to especially the highest concentration. The most negatively responding taxa to fluoxetine were zygoptera larvae (NOEC of 2 µg/L) and the snail *Radix* sp. (NOEC of 20 µg/L). Since zygoptera larvae and the snail *Radix* sp. appeared to be the most negatively responding taxa to fluoxetine according the mesocosm experiment, we perfomed the lab experiments with those taxa. We found that fluoxetine does not affect acute mortality of zygoptera larvae *Coenagion pulchellum/puella*. In addition, we are analysing the behavioural data of the two invertebrate species, to gain more insights into the behavioral impacts of fluoxetine.

2.01.05

Simultaneous Online Chemical and Behaviour Measurements in the Field Indicate That Pesticide Peaks Alter Gammarid Behavior Patterns

A. Lopez Mangas, University of Applied Sciences and Arts Northwestern Switzerland (FHNW); H. Singer, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Chemistry; M. Langer, Oekotoxzentrum Eawag-EPFL / Aquatic Ecotoxicology

Existing pesticide risk assessment might not reflect the full risk that pesticide poses to aquatic organisms since key aspects – i.e mixture toxicity and pesticide dynamics - are often not covered adequately. We investigated the dynamics of pesticide peaks and their effects on behavior of the model organism *Gammarus spec.* simultaneously. For the first time, the MS2field, an completely autonomous online analytic MS lab with a with 20 min resolution, was run in parallel with a biomonitoring system that measures locomotor activity of introduced gammarids in a bypass of a small swiss stream located in an intensively agricultural area. To extract the influence of natural stressors, we studied the effects of pesticide pulses on the locomotor activity under controlled laboratory conditions in a second experiment. Here, the circadian rhythm of *Gammarus fossarum* was disrupted after pesticide pulses of low risk – i.e 0.17 and 0.08 TUs. In the field, rainfall and pesticide peaks followed a similar pattern. The detected pesticide pressure was low with a maximum pesticide peak of ≈ 0.03 TUs. During and after pesticide peaks, we detected changes of different time length on gammarids behaviour pattern. We propose that changes in locomotor activity is an endpoint worth exploring that could provide sensitive information.

2.01.09

Social Status Modulates the Behavioural and Physiological Consequences of a Pharmaceutical Pollutant in Fish Groups

E. McCallum, Swedish University of Agricultural Sciences; C. Dey, Fisheries and Oceans Canada; A. Bose, Max Planck Institute of Animal Behavior; D. Cerveny, Swedish University of Agricultural Sciences / Department of Wildlife, Fish, and Environmental Studies; T. Brodin, Swedish University of Agricultural Sciences (SLU) / Department of Wildlife, Fish, and Environmental Studies

The social environment is a commonly overlooked aspect of biology when scientists evaluate the effects of chemical contaminants. However, the social environment represents the arena in which individual-level performance shapes group- or population-level outcomes, and therefore may mediate many of the ultimate consequences of chemicals for wildlife. To evaluate the importance of social status and social structure on the consequences of a pollutant exposure, we exposed groups of juvenile brown trout (*Salmo trutta*) to an emerging pharmaceutical pollutant that is commonly detected in freshwaters (the benzodiazepine, oxazepam), and allowed them to form dominance hierarchies. Exposure affected dominant and subordinate fish differently, causing subordinate fish to become less aggressive at high doses but more competitively successful at low doses. These perturbations had further consequences for exposed dominant fish, who grew less and incurred more fin damage. Exposure modulated physiological stress (plasma cortisol) in the hierarchy, and social status covaried with how much oxazepam bioconcentrated in tissues, where subordinate fish bioconcetrated more than dominant fish in the same exposure. This potentially created a dynamic feedback loop that re-enforced the asymmetric effects of exposure on differing social statuses. Many effects followed a non-monotonic ("U-shaped") dose-response, highlighting the importance of non-linear, low dose effects. These findings underscore the need to consider an organism's natural social complexity when understanding the consequences of pollutant exposures for wildlife.

2.01.11

Glyphosate Targets Fish Monoaminergic Systems Leading to Oxidative Stress and Anxiety

J. Bedrossiantz, IDAEA CSIC / Environmental Toxicology; M.D. Faria, IDAEA-CSIC / Environmental Chemistry; M. Mayol, University Ramon Llull IQS / Analytical Chemistry Department; E. Prats, CID-CSIC; N. Reyero, US Army Engineer Research and Development Center; C. Gómez-Canela, University Ramon Llull IQS / Analytical Chemistry Department; L. Gómez-Oliván, Autonomous University of the State of Mexico / Chemistry; D. Raldúa, IDAEA-CSIC / Environmental Chemistry

Glyphosate is the most widely used herbicide in the world. There is controversy regarding the potential neurotoxicity of glyphosate, since although some international agencies have classified this compound from non-neurotoxic (US EPA) to unlikely to be neurotoxic (EFSA), different studies have provided evidences of its potential neurotoxicity. In this study, adult zebrafish have been exposed during 2 weeks to 0.3 and 3 ppb glyphosate in water in a semi static system. These concentrations were selected as they are in the range of the levels reported in different aquatic ecosystems. After the exposure, changes in complex behaviour of the fish, including exploratory behaviour, boldness and social behaviour, were analysed by using a battery of behavioural paradigms: the novel tank test, the light-dark test, the novel approach test, and the shoaling test. After the behavioural analysis, brains were dissected and changes in the neurotransmitter profiles were determined. Transcriptional changes in a battery of genes related with neurotoxicity were also analysed. Finally, biochemical determinations were conducted in the subcellular fraction of zebrafish brain tissue. Our results demonstrate that environmental relevant concentrations of glyphosate impair in fish some behaviours as exploratory behaviour, essential for survival in aquatic ecosystems.

2.01.12

Pharmacological Modulation of Phototactic Response of Daphnia magna to Fish. the Role of Cholinergic and GABAergic Signalling

C. Barata, CSIC / Environmental Chemistry; J. Bedrossiantz, IDAEA CSIC / Environmental Toxicology; I. Fuertes, IDAEA-CSIC; L. De Meester, Luven University; D. Raldúa, IDAEA-CSIC / Environmental Chemistry

Animal behaviour is closely related with individual fitness allowing animals to choose suitable mates or avoid predation. The central nervous system (CNS) regulates many aspects if not all of animal behaviour responses, therefore behavioural responses can be special sensitive to compounds with a neurodevelopmental or neuro-functional mode of action. Phototactic behavioural changes against fish in the freshwater crustacean *Daphnia magna* have been the subject of many ecological and more recently genomic investigations. The aim of this study was to identify which neurotramitter systems modulate the phototactic behaviour to fish kairomoines. We use a positive phototactic clone (P_I32,85) that shows a marked negative phototactism after exposure to fish kairomones. Fish conditioned water was obtained with juveiles of *Leuciscus idus*. Treatments include up to 14 known agonists and antagonist of serotonergic, cholinergic, dopaminergic, histaminergic and GABAergic receptors. A new custom designed vertical oriented two 50 mL chamber device with an apical white led light controlled by the Noldus software was used. Changes in preferred area (bottom, middle vs upper areas) were analysed using groups of animals upon exposure to 24 h to selected substances. Results indicated that agonists of the acetylcholine and GABA receptors and their equi- effective mixture decreased the negative phototactic response to fish kairomones whereas antagonists of the above mentioned receptors increased negative phototaxis. The analysis of the profile of neurotransmitters and related metabolites showed that changes in acetylcholine, dopamine and GABA could be anchored with the observed phototactic changes of fish kairomines, cholinergic and GABAergic compounds.

2.01.14

Can We Use Zebrafish (Danio rerio) in Behavioural Ecotoxicology?

L. Vossen, Uppsala University / Department of Anatomy, Physiology & Biochemistry; T. Brodin, Swedish University of Agricultural Sciences (SLU) / Department of Wildlife, Fish, and Environmental Studies; S. Winberg, Uppsala University / Department of Neuroscience

Pollution by psychoactive pharmaceuticals has been shown to disrupt the anti-predator behaviour of wild fish. The zebrafish is a model species that potentially

could be used in high-throughput screening for behavioural effect concentrations of many pharmaceuticals at many concentrations. To investigate if and how long zebrafish are affected by psychoactive pharmaceuticals, we conducted two experiments. In experiment 1, we compared the anti-predator behaviour of laboratory and wild-caught zebrafish exposed to the anxiolytic pharmaceutical oxazepam. Laboratory zebrafish lacked the species-specific response to alarm cue, and therefore the effect of oxazepam could not be investigated. Wild-caught females were affected at low concentrations (0.57 ug L^{-1}) but males were less sensitive (60.83 ug L^{-1}). In experiment 2, we used wild-caught zebrafish to investigate the effect of oxazepam (~7 ug L^{-1}) on behaviour and physiology after 7 and 28 days of exposure. At 21 days anti-predator behaviour was no longer affected, indicating that the zebrafish had developed behavioural tolerance to the drug. However at 28 days serotonin turnover was reduced. Effects on serotonin concentration and turnover have also been reported for anti-depressants fluoxetine and venlafaxine, and could be additive, therefore this finding requires further investigation. In summary, wild-caught zebrafish can be used but AB zebrafish may not be preferred in studies of anti-predator behaviours. Rather than arguing for a high-throughput screening approach, our findings highlight the importance of small-scale experiments on wild-caught fish, that can be tailored to the ecology of the species and the pharmaceutical under investigation.

2.01.17

Behavior As a Sensitive Marker for Caffeine Exposure

N. Santos, Universidad of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies; V. Picolo, University of Brasilia (UnB) / Dept.. of Biology; V.M. Perillo, University of Brasilia; I. Domingues, University of Aveiro / Biology Department & CESAM; C. Grisolia, University of Brasilia / Department of Genetics and Morphology; M. Oliveira, University of Aveiro & CESAM / Centre for Environmental and Marine Studies (CESAM) & Department of Biology

Coffee is among the most consumed stimulant beverages in the world. The active compound, caffeine (CAF) has been considered an emerging contaminant and indicator of human contamination due to its wide detection in aquatic systems. The existence of CAF in the environment may pose a risk to human and environmental health. Within this context, the goal of this work was to evaluate the effects of CAF on behaviours of zebrafish juveniles Locomotor behavioural analysis was performed after exposure to CAF into four concentrations of CAF (0; 0.5; 1.5; 300 µg. L^{-1}) for 8 days. Behavioural activities were analysed using the novel tank test (exploration behaviour) and mirror test (aggression behaviour). Behaviour was determined using the video tracking software ANY-Maze™ (Stoelting Co., USA) at 30 frames/s rates. In the novel tank test, the following parameters were analysed: total distance travelled and the number of transitions between each aquarium zone. In the mirror test, latency to the first approach to the mirror and mirror biting duration was evaluated in this test. In the novel tank test, control fish travelled less in the bottom of the aquaria than the fish exposed to CAF, a non-significant tendency to decrease the number of entries in the bottom zone was also observed from 0.5 to 300 µg. L^{-1}, while in the middle and top zones there is a reduction in the number of entrances in fish exposed to CAF. Zebrafish exposed to 0.5 and 1.5 µg. L^{-1} of CAF took less time to the first approach to the mirror and this fish the duration of the mirror biting tended to increase; this trend follows a dose-dependent concentration. In this work, concentrations of CAF, with environmental relevance produced important behavioural changes in zebrafish juveniles. The main effects of CAF include the increase in shyness, detected by the reduction of swimming the behaviour of fish in a new environment (novel tank test). This change in behaviour can induce direct negative effects on reproduction, which may reflect a reduction in the population of fish in nature. Additionally, fish exposed to CAF reacted to the mirror in less time than the control group, however, they have a lower number of attacks but with higher durability. Therefore, CAF concentrations did not increase aggressive behavioural responses caused by an opponent's zebrafish vision but did induce an anxiety response. Locomotor behaviour proved to be highly sensitive, highlighting the potential for behavioural endpoints to translate relevant ecological effects, such as feeding behaviour of antipredator behaviour disruption, elucidating the importance of considering the sublethal effects of CAF to better estimate their risk to human health and aquatic ecosystems.

2.01.23

Plasticizers: Negative Impacts on the Immune System and Metabolism

N. Saad, Uppsala University / Department of Organismal Biology; C. Bereketoglu, Iskenderun Technical University, Faculty of Engineering and Natural Sciences / Department of Biomedical Engineering; A. Pradhan, Orebro University / SCHOOL OF SCIENCE AND TECHNOLOGY

The immune system and metabolism are tightly regulated by each other; however, several pollutants can disrupt this homeostasis and make individuals susceptible to many diseases. Plasticizers have been shown to affect both fatty acid metabolism and the immune system. Plasticizers are multifunctional chemicals that are used in a wide variety of products including plastic materials, cosmetics, children's toys, fabrics and medical devices. They are not covalently bound to the plastic materials; hence they leach out and contaminate the environment. The increased production of plasticizers, their persistent nature and the risk to human health have raised concerns worldwide. di-(2-ethylhexyl) phthalate (DEHP) is one of the most widely used plasticizer, which is now regulated and banned in European markets due to its reported cases of toxicity. The phasing out of DEHP has resulted in introduction of alternative plasticizers, which are expected to show lower toxicity. However, our data using *C. elegans, Daphnia magna* and zebrafish (*Danio rerio*) suggest that the alternative plasticizer diethyl phthalate (DEP) also has adverse effects. Among the alternative plasticizers, the use of diisononyl cyclohexane-1, 2-dicarboxylate (DINCH) is rapidly increasing in the European market. Our preliminary data suggest that DINCH can negatively regulate lipid metabolism, immune system and behavior. Exposure to DINCH (0.01, 0.1, 1 and 10 µM) in zebrafish larvae from 0 to 6 days post fertilization (dpf) showed that it can inhibit hatching process at higher doses (1 and 10 µM); however, mortality and developmental defects were not observed up to 10 µM dose. Lipid staining using Oil Red O dye showed an increased lipid level in larvae after 6 days of DINCH exposure. Genes involved in lipid metabolism and the immune system were also altered. Behavior analysis showed that DINCH can alter the larval movement pattern in response to the light-dark cycle. This indicates that DINCH apart from having negative effects on lipid metabolism and the immune system, can also show detrimental effects to the nervous system. Our data suggest that alternative plasticizers are not completely safe and further risk assessments should be performed for the new emerging plasticizers. Evaluating the possible negative impacts of these compounds on different model systems is important to better understand their effects on the ecosystem.

Advantages of Using Invertebrates in Ecotoxicology: Challenges and Opportunities for Environmental Risk Assessment and Endocrine and Neuro-Endocrine Research

2.02.02

Influence of Laboratory Conditions on Development and Survival of Field Collected Mayflies (Cloeon dipterum), a Key Step Towards a Chronic Test Method

S. Claßen, gaiac - Research Institute for Ecosystem Analysis and Assessment; I. Roessink, Wageningen Environmental Research / Environmental Risk Assessment; E. Bruns, Bayer AG, Division Bayer CropScience / Ecotoxicology; K. Gergs, gaiac - Research Institute for Ecosystem Analysis and Assessment; J. Hager, Bayer Ag; L.L. Lagadic, T. Preuss, Bayer Ag / Environmental Safety

In recent years, the interest of testing non-standard species for the environmental risk assessment of pesticides has increased. Especially mayflies seems to be of interest due to their relevance in aquatic ecosystems and sensitive response to chronic exposure to certain pesticides. However, an optimized and standardized test method for an adequate use of mayflies in risk assessment is still lacking. In order to take the first steps in developing such a method an interlaboratory comparison was conducted using a cross design experiment and the mayfly *Cloeon dipterum* as the test species. The test design comprised different food items, i.e. pre-grown tiles with periphyton and pre-grown jars with biofilm, species origin and general housing conditions. Thus resulting in eight different combinations of larvae-food-lab. Test were conducted using Elendt M4 medium and had a duration of 28 days. In both labs, *Cloeon dipterum* instars originating from the other location did not perform well showing high mortality up to 43 percent at the end of the 28 day test period. Although lab settings had a big influence as well, the type of food greatly impacted development time and larval sizes. Periphyton on pre-grown tiles provided the fastest instar development and resulted in control survival between 80 and 87 percent, in both labs respectively. Based on these results recommendations for a ring test protocol can be made enabling further testing of an optimal experimental design plus validation criteria for control survival and development rates thus bringing a robust test method for mayflies a step closer.

2.02.07

Assessing the Toxicity of Sediment From Four Estuaries of the Bay of Biscay Using Standard and Novel Bioassays With Benthic Invertebrates

N. Garcia-Velasco, University of the Basque Country UPV/EHU / Dept. Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU); J. Carrero, University of the Basque Country UPV/EHU / Dept Analytical Chemistry; E. Urionabarrenetxea, University of the Basque Country UPV/EHU / Dept. Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU); L.d. Jiménez, University of the Basque country (UPV/EHU) / Dept. Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU); B. Zaldibar, University of the Basque Country UPV/EHU / Dept. Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU); A. Gredilla, University of the Basque Country UPV/EHU / Dept. Applied Chemistry; O. Liñero, University of the Basque Country UPV/EHU / Dept. Analytical

Chemistry; A. de Diego, University of the Basque Country UPV/EHU / Dept. Analytical Chemistry, Research Centre for Experimental Marine Biology and Biotechnology; U. IZAGIRRE, UNIV BASQUE COUNTRY / Dept. Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU); M. Soto, University of the Basque Country / Zoology and Animal Cell Biology

Estuaries own a historical trend of industrial and urbanistic development, leading to overexploitation and pollution. Estuarine sediments can accumulate pollutants over time and pose a risk for the environment and human health. In order to proceed with an accurate risk assessment and environmental management, chemical and ecotoxicological characterization of sediments should be integrated. Bioassays with benthic organisms can provide very useful information on sediment toxicity. The sea urchin embryo test (SET) is a standard toxicity bioassay broadly used for the screening of sediments. Among endobenthic organisms, the polychaete *Hediste diversicolo*r is widespread in sediments and has the capacity to accumulate pollutants. Presently, the potential adverse effects of sediments collected at different seasons in the Bay of Biscay were assessed with the aid of SET and innovative bioassays with *H. diversicolor.* In parallel, biomarkers and *in vitro* approaches were developed and validated for *H. diversicolor*. Tested sediments were sampled in the Nerbioi-Ibaizabal (4 sites: Udondo, Kadagua, Benedicta, Zorroza), Butroe (Plentzia), La Gironde (Plassac) and Charente (Rochefort) estuaries in October 2018 and April, July and October 2019. SET (ICES, 2012) was carried out following different guidelines of sediment elutriation and after 48h size increase and embryo developmental abnormalities were measured. Ragworms were maintained for 7 days in sampled sediments and survival, weight loss, metal accumulation and biomarkers (histopathology, coelomocytes viability, enzyme activities) were measured after exposure. The metal characterization of all sediment and elutriates used was done by ICP-MS. Both bioassays indicated severe effects (growth decrease, abnormalities, enzyme alterations) exerted by sediments with high contamination levels (Udondo and Benedicta). However, the metal accumulation in ragworm tissues, weight loss and measured biomarkers were highly dependent on the physico-chemical characteristics of sediments. Elutriation method compromised the toxic response of embryos too. Seasonal variations were not recorded. The bioassay and biomarkers developed for *H. diversicolor* appeared to be accurate for sediment toxicity testing. This work could contribute to render more robust sediment toxicity tests and might lead to fill the gaps in Sediment Quality Guidelines. Acknowledgements: Basque Gov. (IT1302-19, IT1213-19), U. Basque Country and MINECO (CTM2017-87766-R).

2.02.09

Filter Feeders in Port Areas: Key Species in a Poorly Known Socio-Ecosystem. How Does the Port's Hyper-Anthropic Environment Affect Their Metabolism?

V. HAMANI, LIENSs / LIttoral ENvironnement et Sociétés LIENSs; I. Brenon, P. Ory, P. Bodet, UMR 7266 LIENSs - CNRS University of La Rochelle, France / LIttoral ENvironnement et Sociétés LIENSs; M. Graber, LIENS-UMRi 7266 CNRS / LIttoral ENvironnement et Sociétés LIENSs; L. Murillo, UMR 7266 LIENSs - CNRS University of La Rochelle, France / LIttoral ENvironnement et Sociétés LIENSs

Ports are hyper-anthropic environments where life seems to have no place. However, significant communities grow, out of sight, protected by the pontoons' maze that the harbour ecosystem offers. Among the multitude of species inhabiting the port, one group seems particularly interesting to study: The Filter feeders. These organisms are characterized by the fact that they filter the water to collect their food and they live anchored to their substrate for part of their lives. Thus, due to their way of life, the Filters feeders have a strong interaction with the port environment. These species have a very high capacity to incorporate and retain pollutants and some have been proposed as a potential ecotoxicological biomonitoring species.Ports are complex environments governed by many factors. Among them, some factors such as metal pollutants, seem interesting to study because their presence in the water column make them easily available for Filter feeders. Several studies have shown the effect of these pollutants on the metabolism of similar organisms, but none have been carried out in similar context. As copper and zinc are particularly present in the water column of the Port of Les Minimes, we chose to study their effects on the metabolism of Filter feeders. This work is carried out in the laboratory, on organisms exposed to environmental concentrations of these metals. The analyses are then carried out on gill tissues, the first places of exchange between the organism and the environment.The studies carried out at the cellular level enable fine and rapid responses. This is why we have chosen to study the effect of this complex environment on the metabolism of these organisms using two complementary approaches:-High-resolution respirometry (oxygraphy) enables us to study the effect of conditions on mitochondrial respiration; the mitochondria being largely involved in the energy production of the cell. This approach allows an accurate study of mitochondrial respiration and allows to identify in detail how it is impacted. -Metabolomic allows to study the effect of the same conditions on all components of the cell. This approach allows to precisely identify the metabolic pathways impacted by these metals. The results obtained on zinc have already highlighted a sensitive metabolic response of the bivalves. This could reflect the potential effects of zinc on several biological processes, such as osmoregulation, energy metabolism and oxidative stress.

2.02.10

Estrogens Alter the Metabolome of Oysters in a Tissue-Specific Fashion

R. ISLAM, The University of Newcastle / Environmental and Life Sciences; S. Melvin, Australian Rivers Institute / School of Environment and Science, Griffith University; R. Man Kit Yu, University of Newcastle (Australia) / School of Environmental and Life Sciences; W. O'Conner, NSW Department of Primary Industries / Port Stephens Fisheries Institute; F.D. Leusch, Griffith University - Smart Water Research Centre / Australian Rivers Institute; G. MacFarlane, University of Newcastle (Australia) / School of Environmental and Life Sciences

The current study explored the effect of environmentally relevant mixtures of estrogens on the metabolome of the Sydney rock oyster, *Saccostrea glomerata*. Oysters were exposed for 7 days to a "low" and a "high" mixture of (xeno)estrogens representing relevant concentrations for Australian (low) and global receiving waters (high). Polar metabolites were measured from the digestive gland, gill, and gonad tissues using proton (^{1}H) Nuclear Magnetic Resonance (NMR) spectroscopy. Estrogens lowered the body mass and remarkably altered metabolite profile in the digestive glands. However, gills and gonads demonstrated relatively lesser sensitivity to the mixtures, with significant alterations observed for the high mixture only. The female gonad showed moderate sensitivity, whereas the male gonad did not respond to any estrogenic exposure. The major metabolites including amino acids, carbohydrates, intermediates of the tricarboxylic acid cycle and ATP were all down-regulated and exhibited tissue-specific patterns of alteration, with the greatest proportion of metabolites down-regulated in the digestive gland. Overall, exposure to (xeno)estrogen mixtures reported in receiving waters in Australia and globally can impact the metabolome and associated energy metabolism, especially in the digestive gland, translating to lower pools of available ATP energy for cellular homeostasis, somatic maintenance and growth, and the reproduction and fitness of oysters.

2.02.11

Development of Neuroendocrine Signalling in Early Bivalve Larvae As a Target for Endocrine Disrupting Chemicals

A. Miglioli, University of Genova / Dipartimento di Scienze della Terra; T. Balbi, university of genoa / DISTAV; L. Besnardeau, Institut de la mer de Villefranche-sur-mer / LBDV; L. Canesi, university of genoa / DISTAV; R. Dumollard, Institut de la mer de Villefranche-sur-mer / LBDV

Neuroendocrine mechanisms and neuropeptide hormones play a vital role in endocrine systems of invertebrates, including ecologically important groups, such as bivalve molluscs. In the marine bivalve, the mussel *Mytilus* different contaminants, including suspected endocrine disrupting chemicals-EDCs, such as Bisphenol-A (BPA) and Tetrabromobisphenol-A (TBBPA), have been shown to significantly affect early larval development, inducing shell malformations and developmental delay within 48 h post fertilization. However, the mechanisms of action of these compounds remain largely unknown, due to the lack of knowledge on the physiological mechanisms and pathways controlling larval development in bivalves. Accordingly, the study and characterization of the neuroendocrine system during bivalve development could shed a light on the processes guiding body patterning and morphogenesis, as well as the on the mechanisms of action of EDCs in these organisms. In this light, we investigated the onset of serotonergic (5-HT), GABA-ergic (aminobutiric) and dopaminergic (DOPA) systems during early developmental stages (from 24 to 48 hpf) of *M. galloprovincialis* larvae utilizing different approaches (molecular, immunological pharmacological). The results indicate that, in physiological conditions, the onset of different components of neuroendocrine signalling occurs from 24 hpf, followed by progressive differentiation of neuroendocrine cells up to 48 hpf. These processes are paralleled by progressive deposition of shell components (organic matrix and CaCO3) leading to the formation of the first shelled larva. Moreover, the effects of BPA and TBBPA on larval neurogenesis and morphogenesis were investigated. The effects of EDCs on development of 5HT- GABA- and DOPA-ergic signalling nicely correlated with alterations in deposition of shell components resulting in altered larval phenotypes (shell malformations, developmental delay and arrest). The results strongly support evidence for the morphogenetic function of the neuroendocrine system in bivalve larvae. Moreover, these data indicate that neuroendocrine signalling represents a key target for the action of tested EDCs in mussel larvae at nmolar concentrations. This study also provides the very first *in vivo* evidence of EDC neurotoxicity and consequent teratogenicity in bivalve molluscs.

2.02.12

Vulnerability of the Estuarine Copepod Eurytemora Affinis to the Endocrine Disruptor Tebufenozide: A Transcriptomic Approach

C. Arcanjo, UMRI 02 INERIS-URCA-ULH SEBIO / IRSN/PRP-ENV/LECO; G.

Tremolet, N. Giusti-Petrucciani, University of Le Havre / UMRI SEBIO; A. Duflot, University of Le Havre / UMR SEBIO; J. Forget-Leray, C. Boulange-Lecomte, SEBIO UNIVERSITY OF LE HAVRE / UMRI SEBIO

Since the 20th century, man-made pesticides have been widely used to protect crops from biological threat. Some of them – especially insecticides – are known to act as endocrine disruptors (ED). Their impact on ecosystems is of growing concern. The present work focuses on the effect of tebufenozide (TEB) on the copepod *Eurytemora affinis* by using a transcriptomic approach to identify potential biomarkers of exposure. TEB was used as a model compound. This insecticide acts as an agonist of the ecdysone receptor causing premature molting in Lepidoptera larvae. Despite its narrow spectrum, this molecule could affect non-targeted organisms with close endocrine systems. Among them, copepods are organisms of wide interest in pollutant risk assessment because of their importance in trophic chain. The transcriptomic analysis was achieved with adult males or females *E. affinis* sampled in the Seine estuary and exposed to TEB (0.5 and 50 µg/L) during 72h. Results indicated that few genes were mis-regulated after exposures at 0.5 µg/L for both sex with a higher proportion in females. A wider number of genes were mis-regulated after exposure at 50 µg/L with a higher proportion in males, suggesting a sex- and concentration-dependent response to exposures. A few commonly mis-regulated genes were found between sex at 50 µg/L (n=26) and between females for both concentrations (n=8). Interestingly, genes involved in molting and metamorphosis pathways were found at 50 µg/L in both sex and in females at 0.5 µg/L. Their mis-regulation could affect copepod cuticle integrity and lead to a greater vulnerability to pollutants. These results also highlight the effect of TEB as ecdysone agonist on a non-targeted species. Furthermore, genes involved in muscle contraction and neurotransmission were mis-regulated in both sex at 50 µg/L suggesting further alteration of these pathways in organisms and potential detrimental effects on locomotion for example. Reproductive and defense process could also be affected supported by the mis-regulation of genes involved in these pathways in males at 50 µg/L. Genes taking part in DNA methylation were also highlighted and are of interest regarding the potential transmission of these epigenetic marker to offspring. To complete the transcriptomic results, it would be interesting to perform analysis on higher biological scales to have an overview of responses triggered by TEB on non-targeted species.

2.02.13

Reproductive Impacts in a Costal Marine Amphipod From Protected Area

M. Tenório Botelho, University of São Paulo / Biological Oceanography Department; N. Fuller, University of Portsmouth / Biology; M. Vannuci-Silva, University of the state of rio de janeiro / Institute of Biology; G. Yang, K. Richardson, University of Portsmouth; G. Umbuzeiro, School of Technology-UNICAMP / LAEG Laboratory of Ecotoxicology and Genotoxicity; V. Gomes, University of São Paulo / Department of Biological Oceanography; A. Ford, University of Portsmouth / Biological Sciences

Sperm quantity and eggs number are important reproductive endpoints easily linked to population levels. Sperm count is broadly used in amphipods, however the relationships between sperm count and male size are not well understood. Amphipods are widely used as model organisms in environmental toxicology. *Echinogammarus marinus* is one of the most abundant amphipod species in coastal communities in the northeast Atlantic, being an important prey for wading birds. The aim of this study was to compare sperm quantity and its relationship with animal weight in amphipods collected at five UK sites with different water quality. Due to low sperm counts and an observed lack of relationship between sperm count and male weight, amphipods collected from a nationally protected area (Langstone Harbour, England), we compared datasets from this site over a decade. Eggs number and stage were also evaluated in females from this area to evaluate if females were also being affected and these results were compared to previous published works in the UK. Amphipods collected in all sites, except Langstone Harbour, presented significantly positively correlations between sperm count and male weight. Amphipods from Langstone Harbour also presented lower sperm counts and eggs number compared to reference sites and the values were similar to industrially polluted areas. Langstone Harbour is a national and international protected area for marine live and wading birds, however regular discharges from storm water overflows still occur in periods of heavy rains. Our results indicate that the population of an important food source for wading birds is possibly being impacted by unknown reproductive stressors, which could be related to storm water overflows. This study highlights the importance of reproductive endpoints.

2.02.14

Happy Crabs? -- Disruption of Neurohormonal Signalling in Decapod Crustaceans by Antidepressant Drugs

T. Knigge, Universite du Havre / UMR-I 02 Environmental Stress and Aquatic Biomonitoring (SEBIO); A. Chabenat, University Le Havre Normandie / UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); A. Robert, Université Le Havre Normandie / UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); C. Bellanger, Université de Caen Normandie / UMR 6552, Rennes 1-CNRS Animal and Human Ethology (EthoS); T. Monsinjon, Université Le Havre Normandie / UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO)

Antidepressant drugs target monoamine neurotransmitter systems. Since the introduction of fluoxetine in the late 1980s, so-called 'atypical' antidepressants belonging to the selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) have multiplied and their prescriptions have constantly increased. Consequently, residues of SSRIs/SNRIs are released into the water bodies, where they may interfere with the regulation of monoamines in non-target organisms. Here we show that physiological parameters as different as cardiovascular and respiratory functions, blood glucose levels and locomotor activity, camouflage or moulting can be affected by antidepressants, putatively by disrupting neurohormonal signalling in crustaceans. Injections of 0.5 M fluoxetine increased heart rate and ventilation of gill chambers in Shore crabs, Carcinus maenas, both in frequency and amplitude. Juvenile crabs exposed to 10 ng·L-1 fluoxetine+venlafaxine exhibited an enhanced locomotor activity, characterised as higher velocity and distance moved as well as more time spend moving. These observations are coherent with an increased blood glucose level following injection of 0.5 M fluoxetine. Indirect evidence suggests that the antidepressants stimulated the release of crustacean hyperglycaemic hormone leading to the mobilisation of glucose and increased levels of activity. A similar mechanism is likely to have stimulated the release of moult inhibiting hormone resulting in lower ecdysteroid levels. Furthermore, in juvenile C. maenas exposed to 10 ng·L-1 fluoxetine+venlafaxine colour change was reduced and less efficient, thus impairing the capacity of crabs to adapt to different backgrounds. At the same time, crabs showed enhanced cryptic behaviour by burying themselves deeper into the sediment. All things considered, the results of fluoxetine injection point to the disruption of neuroendocrine control mechanisms, which appear to be effective even at low environmental concentrations, at least in juveniles. SSRIs/SNRIs may, therefore, constitute endocrine disrupting chemicals in crustaceans. Keywords: neuropeptides, ecdysteroids, colour change, camouflage

2.02.15

Integrated Testing Tool to Identify and Quantify New Endocrine Disrupting Chemicals in Crustaceans

D. Degli Esposti, INRAE / UR RIVERLY Laboratoire Ecotoxicologie; R. Ruivo, CIIMAR University of Porto; T. Neuparth, University of Porto: CIMAR-LA/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental / Endocrine Disruptors and emergent contaminant group; F. Castro, CIIMAR - University of Porto; A. Chaumot, O. Geffard, Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) / Unité de Recherche RiverLy; M.M. Santos, CIIMAR/FCUP / Biology/Endocrine Disruptors and Emerging Contaminants

Endocrine disrupting chemicals (EDCs) are an important part of the anthropogenic chemical pollution that affects the entire ecosystem. Research on EDCs mainly focuses on vertebrates, leading to proposal specific ED targets (e.g. ERa, THR, AR) and *in vitro* assays that are widely recognized and currently proposed as tools for screening programs. While arthropods represent the vast majority of animal species, there is still a lack of knowledge on their endocrine systems, due to a great divergence from that of vertebrates. Consequently, no ecotoxicological screening tools for ED are currently available in these species. Among arthropods, crustaceans are among the most diverse species and are essential for the good function of aquatic ecosystems. Compared to insects, few annotated crustacean genomes are today available and detailed molecular knowledge on the genetic pathways involved in the major hormonal pathways is lacking. Here, we will present the first results of an integrated strategy combining bioinformatics and phylogenetic approaches with an *in vitro* functional screening for the identification of the Ecdysone Receptors (EcR) across different species of amphipods, crustacean species extensively used to assess the ecotoxicological status of water bodies. In particular, we will show how this strategy is leading us to: i) a contaminant classification as EDCs in crustaceans based on their mechanism of action, i.e. NR agonist/antagonist; ii) a prioritization scale in terms of hazard assessment based on the strength of the observed activation; iii) an ecotoxicological toolbox to validate the *in vitro* tests in both marine and freshwater species.

2.02.17

Stygophilous Amphipod Synurella Ambulans As a Potential Bioindicator of Metal Pollution in the Hyporheic Zone of the Sava River, Croatia

Z. Redžović, Ruđer Bošković Institute / Division of Molecular Medicine; S. Gottstein, Faculty of Science / Department of Biology; M. Erk, M. Cindrić, Ruđer Bošković Institute / Division of Molecular Medicine

Due to the growing impact of human activities on aquatic ecosystems, it is important to carry out biomonitoring programmes and assess the ecological status of waters in order to adequately protect aquatic ecosystems. The hyporheic zone (HZ) is a linking ecotone between surface and groundwater bodies. It is exposed to constant anthropogenic influences, which can affect the quality of surface

running waters as well as aquifers. The aim of this study was to investigate the accumulation of metals in the stygophilous crustacean species *Synurella ambulans* (F. Müller, 1846) in order to assess its potential as a bioindicator of water quality in HZ. Amphipods were sampled by Bou-Rouch pump in the HZ of the Sava River gravel bar on the average depth of cca 50 cm once per season in winter 2018, spring, summer and autumn 2019 at two localities. First was located about 3 km upstream from the wastewater outlet of the town of Zaprešić, and second about 13 km downstream from the outlet situated in the city of Zagreb. Through the sewerage system of the town of Zaprešić treated wastewaters from the pharmaceutical industry are discharged together with treated municipal wastewaters. Measured concentrations of Cd, Cr, Cu, Co, Fe and Mn in the interstitial water of HZ, were not significantly different between two locations in most seasons, while Pb and Zn were below the detection limit of the method. Ni was significantly elevated at downstream location, and Al at upstream location in most seasons. According to the environmental quality standards, the metal concentrations in the interstitial water of the Sava River do not exceed the maximum permitted concentrations for groundwater. Concerning the bioaccumulated concentrations, it was found that Pb, Ni, Cr and Al were significantly elevated in *S. ambulans* at downstream location, while for Cd, Cu, Co, Zn, Mn and Fe no difference was observed between locations in most seasons. Elevated concentrations of metals in *S. ambulans* compared to the interstitial water of HZ indicate their high bioaccumulation in organisms. Because of their capacity to bioaccumulate metals, stygophilous amphipods may be useful in research on the long-term risk of metal discharges into subterranean habitats. In conclusion, biomonitoring of HZ is crucial for protection of this transitional ecosystem and *S. ambulans* can be used as potential bioindicator of metal pollution.

2.02.19

Characterization of Cuticle Structural Properties on Palaemon Serratus. Are These Markers Appliable in an Ecotoxicological Approach?

M. Rollin, SEBIO UNIVERSITE LE HAVRE; N. Hucher, Normandie University, University of Le Havre / FR 3038 CNRS, URCOM, 76600 Le Havre, France; R. Coulaud, UMRI 02 INERIS-URCA-ULH SEBIO / UMR I-02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); A. Poret, SEBIO UNIVERSITY OF LE HAVRE / UMRI SEBIO; A. Duflot, University of Le Havre / UMR SEBIO; F. Le Foll, Université Le Havre / UMR I-02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); C. Picard, Universite du Havre; B. Xuereb, Le Havre Normandie University (ULHN) / UMR-I 02 Environmental Stresses and Biomonitoring of Aquatic Ecosystems (SEBIO)

Moult is a key physiological process for organisms with exoskeleton as crustaceans. Indeed, life-traits like growth, development and reproduction depend to the achievement of the moult cycle. The different physiological events occurring during moult cycle involve complex molecular mechanisms of regulation, which are potentially target for alteration by xenobiotics (review in Song et *al.*, 2017). Many xenobiotics have been shown to cause delay in moult cycle or ecdysis failure under laboratory bioassay conditions (Leblanc, 2007). Despite this fact, moult alterations remain difficult to diagnose *in natura*, due to a lack of adapted tools, and could represent an invisible form of chemical impact (Zou, 2005). In this context, the present study focused on the development of tools which may detect moult impairments with wild populations (*i.e.* out of a bioassay application) in a European marine prawn species, *Palaemon serratus*. This work was organized around an innovative and integrative biological response: the analysis of cuticle structural properties. These properties were assessed by determining cuticle composition and material structural organization with thermal approaches such as calorimetry (DSC) and thermogravimetry (TGA). To our knowledge, the use of cuticle structural properties to monitor moult quality 1) over a moult cycle and 2) in pollutant exposure assays has been rarely used. To develop this tool, a first step was made, consisting of the methodological optimization and the characterization of the response. Hence, thermal approaches permitted to determine that *P. serratus* cuticle is mainly composed of 3 materials: chitin, proteins, and inorganic compounds. Furthermore, no differences of structural properties were detected when several pieces of the same abdominal cuticle were compared. Then, the second step was to use finalized protocols to measure the response modulations due to a physiological factor: moult cycle. For both DSC and TGA analyses, moult cycle influences were detected on several parameters, specially those related to protein and inorganic compound levels. Finally, the new protocols were applied in an ecotoxicological approach, including *in vivo* and *in natura* assays. Results of these assays will be analysed during the next months, helping us to determine the power of selected biomarkers to detect moult alterations.

Adverse Effects of Chemicals on Host-Associated and Free-Living Microbial Communities

2.03.05

Metabolomic Unraveling of the Influence of ENvironmental Factors in the Responses of Aquatic Biofilms to the Herbicide Diuron

N. Creusot, INRAE BORDEAUX / AQUA/EABX; N. Mazzella, B. Chaumet, M. Eon, S. Morin, INRAE BORDEAUX / UR EABX

Facing increasing pollution of aquatic ecosystems, stream biofilms are increasinlgy used because of their diversity and their key role in ecosystem functions, in order to gain knowledge on the sustainibility of these ecosystems and associated services. Despite growing knowledge, a strong research effort is still required to better understand the response of these microbial communities to chemical stress and, in the global change context, the influence of environmental stressors in this response. For instance, although we recently showed that seasons and flow might influence the response of biofilms to chemical stress, the underlying (molecular) mechanisms remained poorly understood. To tackle this challenge, the high resolution mass spectrometry (HRMS) based-untargeted metabolomics is an approach of choice since it provides a comprehensive picture of both exposure and effect, as well as identify signalling pathways associated to these responses by providing a full picture of the molecular response. In this context, through the retrospective implementation of a metabolomic approach, this study aims to better understand the influence of various environmental confounding factors (duration of exposure, seasonality, flow) on the impact of the chemical stress on stream biofilms. To this end, biofilms have been previously colonized on a reference site and exposed to the model herbicide diuron at the laboratory in controlled conditions. Following these exposures, usual functional and structural descriptors (e.g. protein/polysacharides content, photosynthetic activity) and diuron bioaccumulation have been measured. In this study, we further characterize the associated metabolomic responses. Our results showed the influence of the exposure duration, the concentration of the contaminant, the season (i.e. combination of temperature/photoperiod) and the flow on the metabolomic responses whereas some usual descriptors were not sensitive to such environmental factors. In addition, we tentatively identified two plant-specific omega-3 fatty acids showing a strong inhibition in their production that could play a major in the sustainability of the trophic chain. Overall, this study demonstrates the relevance of the metabolomic approach to assess early and sensitive effect of chemical stress as the influence of confounding factors on these responses. Further investigations would help to identify metabolic pathways involved such responses and propose relevant biomarkers.

2.03.06

Are Laboratory Transferred Fungal Communities Representative for the Evaluation of Xenobiotic Stressors?

S. Carl, Goethe University Frankurt / IV; S. Mohr, German Environment Agency / IV; R. Sahm, Umweltbundesamt / IV; C. Baschien, Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures / BUG

Considering xenobiotic effects on aquatic fungal communities for the environmental risk assessment still needs fundamental methodological investigations. Although many studies have dealt with changes in fungal community structure under fungicide stress in laboratory studies, it still remains unclear to what extent natural fungal communities can be sustained under *experimental lab conditions*. Therefore, we investigated the development of natural fungal communities grown on black alder leaves in a reference stream in comparison to their development under laboratory conditions by using *fungal DNA metabarcoding*. After 2 weeks of leaf exposure in the reference stream a 3-week-laboratory experiment was conducted in aquaria- and flask-microcosms with a weekly sampling both in reference stream and in microcosms. *Five* different commonly used leaf conditioning treatments were compared by A) colonizing leaves in a reference stream, B+C) exposing colonized leaves to a standardized medium or sterile filtered stream water and D+E) using colonized leaves as inoculum for conditioning of sterile leaves in a standardized medium or unfiltered stream water. Total DNA was extracted from all leaf samples and the Internal Transcribed Spacer (*ITS2*) region of the fungal rDNA operon was sequenced *via* a metabarcoding approach on the *Illumina MiSeq* platform. Analysis of the fungal ITS dataset showed, that the fungal communities in the inoculation treatments had a lower species richness compared to the reference stream and the stream conditioned laboratory treatments, which is explained by absence or lower abundance of genera in the inoculation treatments. In contrast, differences in richness and community structure between the standardized medium and filtered or unfiltered stream water were comparably low. The findings suggest, that i) the community structure of aquatic fungi obtained from the field did not change significantly when held under laboratory conditions, ii) the fungal community grown under lab conditions in a standardized medium was comparable to the community held in stream water, and iii) that fungal communities on stream colonized leaves were more complex in terms of species richness and structure compared to leaves inoculated under laboratory conditions. These results might contribute to a better understanding of laboratory experiments and their results when effects of xenobiotics on natural aquatic fungi communities are examined.

2.03.08

Consistent Declines in Aquatic Biodiversity (Microbiota and Macrobiota)

Across Diverse Domains of Life in Rivers Impacted by Surface Coal Mining
M. Simonin, INRAE - IRHS / Biology; J.D. Rocca, Duke University / Biology department; J.R. Gerson, E. Moore, E. Bernhardt, Duke University / Biology
Appalachia – a biodiversity hot spot – is currently threatened by mountaintop mining of coal. Mountaintop coal mining alters river water chemistry, leading to increased specific conductivity associated with the alkaline mine drainage (elevated salts dominated by Ca^{+}, Mg^{+}, SO_4^{2-}, and HCO_3^{-}), in turn impairing aquatic life. We performed an eDNA regional assessment of the impact of this major land cover change on river microbiota and macrobiota (amplicon sequencing of 16S, 18S, 23S, fish 12S rRNA, arthropod COI genes) sampled from 93 rivers across Central Appalachia (West Virginia, USA) spanning a gradient of exposures to mountaintop coal mining. For each group of organisms, we identified the sensitive and tolerant taxa to this gradient of mountaintop mining exposure, and we calculated the thresholds to stream specific conductivity where large synchronous changes in diversity were observed using the Threshold Indicator Taxa Analysis (TITAN). We found that rivers exposed to mountaintop coal mining showed decreased microbial, algal and eukaryotic diversity and large shifts in community composition. Streams below mining operations had steep declines in diversity (-18 to -41%) and substantial shifts in community composition that were consistent across multiple taxonomic groups. Overall, large synchronous declines in bacterial, algal, and macroinvertebrate communities occurred even at low levels of mining impact at stream specific conductivity thresholds of 150 to 200 µS/cm that are substantially below the current EPA Aquatic Life Benchmark of 300 µS/cm for Central Appalachian streams. We show that extensive coal surface mining activities led to the extirpation of 40% of biodiversity from impacted rivers throughout the region and that current water quality criteria are likely not protective for many groups of aquatic organisms, especially microbiota and algae.

2.03.12
What Is the Interplay Between the Gut Microbiome and Arsenolipids in In Vitro Mucosal Simulator of the Human Intestinal Microbial Ecosystem?
C. Xiong, Graz University / Chemistry
Seafood intake is a source of arsenic exposure, especially in the chemical form of arsenolipids, which can pose a risk for human health. However, little is known about the bioconversion of arsenolipids in the gastrointestinal tract and how could affect the human gut microbiome. Information about toxicity and toxicokinetic of inorganic arsenic is wide, being one of the toxicants of higher concern and a well-recognized carcinogen compound, but much less is known about the fate of arsenolipids on the human body. To describe the interplay between the gut microbiome and arsenolipids could help us to manage their potential health risk. An in vitro mucosal simulator of the human intestinal microbial ecosystem (M-SHIME), with three sequential gastrointestinal reactors (gastric, small intestinal, and colonic) was used in our experiment, the M-SHIME vessels were inoculated with fecal slurries during incubation for 70 h from 4 individual healthy donors. To evaluate the interplay between two typical arsenolipid standards (arsenic-containing fatty acid 362, AsFA 362 and arsenic-containing hydrocarbon 332, AsHC 332) and the colonic microbial digestion process, a single dose of 100 µg arsenolipids (AsHC 332 or AsFA 362) were added to the gastric reactor and pre-digested by the SHIME system, then maintained for 70 hours. The metabolism of arsenic and the effect of the toxicant on the structure and function of the gut microbiome were characterized. The total arsenic uptake and arsenic species in mucus, bacteria, and supernatant from colonic compartment were analyzed by HPLC-ICP-MS/ESI-HR-MS-MS. The short-chain fatty acids (SCFA) used as benchmarks of community activity in luminal and mucosal from the colonic compartment were analyzed by gas chromatography. The microbial community of luminal and mucosal samples was taxonomically characterized by 16S rRNA gene amplicon sequencing. This study demonstrated that arsenic uptake in mucus, bacteria, and supernatant samples from the colonic compartment are donor-dependent; for all four donors, arsenic uptake in mucus samples are more sensitive with AsFA 362 treatment, while bacteria samples are more sensitive with AsHC 332 treatment; colonic incubation metabolized both arsenolipids to their thiolate analogs, while AsFAs additionally metabolized to a group of AsFEs and a group of unknown compounds (the structures not confirmed yet, but the accurate mass data suggest they could be a group of arsenic-containing sterol compounds); arsenolipids modified the bacterial activity in the luminal compartment; discriminant analysis of principal component (DAPC) analysis and Adonis analysis reported that donor, time and compartment were the main contributors to the differences observed in microbiota profiles, while treatment (of different arsenolipids) was not significant. Concretely, OTU95 was significantly reduced by AsHC 332 and AsFA 362 exposure.

Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment

2.04.03
Toxicity Effects of Synthetic- and Bio-Microfibers on Lemna minor
R. Cui, Konkuk University / Department of Environmental Health Science; Y. An, Konkuk University / Department of Environmental Heath Science
With the increase of attention to microplastics, the research on the impact of microplastics on aquatic organisms is in progress. According to monitoring, fibers are the microplastics generally found in the aquatic system. Bioplastics products are being developed and used in our lives to protect the environment. In this study, the toxicity effects of two kinds of synthetic microfibers (polypropylene and polyethylene terephthalate) and two kinds of bio-microfibers (cellulose and lyocell) with similar size were evaluated and compared by 3-d acute test and 10-generation test of *L. minor*. As a result, frond number and root length were not affected by microfibers in a acute test. However, the photosynthetic activities in all microfiber exposed groups showed significant effects compared with the control group. In the generation test, although the growth and photosynthetic effects of different generations were different, the overall trend was PET > CL > LY > PP. Overall, both synthetic- and bio-microfiber have acute and general effects on *L. minor*. In our life, There are many types of microfibers, and they will flow into the aquatic system, and have adverse effects on aquatic organisms. Therefore, other types of microfibers research should also be conducted.
Acknowledgement. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2020R1A2B5B02001734). This study was also funded by the Ministry of Environment (MOE) through the Graduate School of Specialization for Safe Management of Chemicals.

2.04.08
Direct and Indirect Effects of Agricultural Run-Off and Climate Warming on Regime Shifts in Shallow Aquatic Systems
E.M. Gross, CNRS UMR 7360 / Université de Lorraine / Laboratoire Interdisciplinaire des Ecosystemes Continentaux; J. Allen, University of Lorraine / LIEC, CNRS UMR 7360; V. Vijayaraj, Universite de Lorraine / Laboratoire Interdisciplinaire des Ecosystemes Continentaux LIEC UMR CNRS; N.L. Amoussou, University of Lorraine / LIEC UMR 7360 CNRS; N. Kipferler, Ludwig-Maximilians-University Munich / Department of Biology; C. Courcoul, University of Toulouse / ECOLAB UMR 5245 CNRS; B.H. Polst, Helmholtz centre for environmental research - UFZ / Department of Bioanalytical Ecotoxicology; M. Laviale, University of Lorraine / LIEC UMR 7360 CNRS; G.A. López Moreira Mazacotte, Institute of Freshwater Ecology and Inland Fisheries (IGB); F. Hölker, Leibniz Institute of Freshwater Ecology and Inland Fisheries / Ecohydrology; M. Schmitt-Jansen, Helmholtz Centre for Environmental Research UFZ / Bioanalytical Ecotoxicology; S. Hilt, Leibniz Institute of Freshwater Ecology and Inland Fisheries / Ecosystem Research; H. Stibor, Ludwig-Maximilians-University Munich / Department of Biology; J. Leflaive, University of Toulouse / ECOLAB UMR 5245 CNRS
Shallow aquatic systems are frequent in agricultural regions. They often suffer from enhanced anthropogenic eutrophication and from organic or inorganic pesticides. A good ecological status exists with clear water and submerged macrophyte dominance, while phytoplankton blooms represent a bad ecological state. However, regime shifts between both states occur depending on the level of disturbance. Our French-German project CLIMSHIFT investigates how agricultural run-off (ARO) composed of nitrates, copper and organic pesticides in combination with climate warming affects such systems. We use indoor microcosms to investigate the impact of different exposure scenario to ARO on primary producers alone or in combination with primary consumers. Primary producers consisted of selected strains of planktonic and benthic algae and three different species of macrophytes. Primary consumers were *Daphnia magna, Dreissena polymorpha* and *Lymnaea stagnalis,* reflecting different feeding modes. Microcosms ran for 3 or 4 weeks after ARO addition, temperature was 22 and 26°C. We performed 4 microcosm experiments. Exp-1 was based on only the three groups of primary producers, and Exp-2 included all primary consumers. Exp-1 and -2 were exposed to individual or multiple pulses of ARO. Exp-3 investigated if nitrate would alleviate negative effects of pesticides on primary producers and consumers. In a separate experiment, negative effects of ARO on *L. stagnalis* were evaluated. ARO with or without nitrate negatively affected *L. stagnalis* and *D. magna*. In Exp-2, positive effects on periphyton were likely due to indirect negative effects of ARO on snails. In Exp-3, *Daphnia* developed well in all treatments without pesticides, suppressing phytoplankton and enhancing macrophyte growth. Several pulses instead of one, or nitrate additionally to pesticides increased ARO effects of on periphyton or phytoplankton, respectively. ARO had an indirect positive effect on the development of periphyton or phytoplankton through trophic interactions. Our results underline the necessity to understand the mechanisms behind the observed effect, determined here using structure equation modelling (SEM). Such studies are needed for a realistic risk assessment of these complex yet important aquatic ecosystems.

2.04.10
Assessing Suitability of Non-Crop Species for the Potential Use in Guideline Plant Protection Product Testing for the Risk Assessment of Non-Target Terrestrial PLANTS

S. Fellmann, A. Elischer, P. Köhler, Bayer AG, Crop Science Division / Environmental Safety - Environmental Effects; R.J. Isemer, Bayer Ag / Environmental Safety - Environmental Effects; C. Mihan, Bayer CropScience AG / Ecotoxicology; D. Jans, Bayer AG, Crop Science Division / Environmental Safety - Environmental Effects; V. Ducrot, Bayer Ag / Environmental Safety - Environmental Effects

For assessing the risk of plant protection products (PPP) to non-target terrestrial plants (NTTP), official guidelines provide test designs. To fulfill the validity criteria specified in the guideline, germination rates of at least 70% and a rate of 90% for the survival of emerged plants are required. A homogeneous germination and seedling growth are additionally of high importance to allow for statistically reliable and reproducible results. Crops are preferred over non-crop species (ncs) for testing as they typically show the desired properties. This often causes discussions, whether ncs are more or less sensitive than crop species and whether the ncs are adequately covered within the current PPP testing and risk assessment. Research articles indicate higher, lower and similar sensitivity for ncs compared to crop species, depending on the used PPP, the species and the test conditions. However, the validity criteria mandatory for regulatory guideline studies are often not reported. This work aims to identify ncs which might be suitable to fulfill the guideline requirements. Germination and BBCH-stages (to assess the development) of different ncs from different suppliers were compared. Species were selected based on frequently used species from the literature and availability at suppliers. It has to be carefully considered when selecting ncs for testing whether the selected species are among the target species (weeds) of the tested PPP. To identify optimum conditions, two different sowing depths (1 cm of soil and a shallow layer of sand) were compared. Depending on the supplier and the sowing depth, 12 species out of 28 reached the required germination rate of 70 %. Considering both germination and homogeneity of BBCH stages, 5 species were identified to be suitable for guideline testing. Challenges were seen regarding seed quality and the need for specific growth conditions for ncs. As an example, for M. recutita seeds reached a germination rate of 90 % from supplier A compared to 63 % from supplier B. For this species, the sand cover promoted the germination rate (90%) while 1 cm soil cover decreased it (33%). In conclusion, guideline testing using ncs is possible in general but extreme care has to be taken when choosing the species, the growth conditions and the quality of seeds. Whether cumbersome testing of ncs is needed to increase protectiveness of the NTTP risk assessment has to be further investigated and discussed.

2.04.12

Assessing Effects of Herbicides on Reproduction of Non-Target Terrestrial Plants in the Field: DOs and DON´Ts

R.J. Isemer, Bayer Ag / Environmental Safety - Environmental Effects; V. Ovcharova, University of Koblenz Landau / Institute for Environmental Sciences; P. Köhler, D. Jans, Bayer AG, Crop Science Division / Environmental Safety - Environmental Effects; V. Ducrot, Bayer Ag / Environmental Safety - Environmental Effects

To assess potential risks of herbicide use for non-target terrestrial plants (NTTPs), routine testing in form of standardized lower tier studies is conducted. In these studies, effects on vegetative parameters (e.g. shoot weight, emergence, survival) are measured in the greenhouse. Higher tier testing may be needed to assess whether the identified risks may occur under more realistic conditions in the field and whether the herbicide potentially affects reproduction of plants. We developed a method for conducting higher tier field studies with NTTPs (Isemer et al., 2020. A Field Study Method as a Potential Higher Tier Option to Refine Herbicide Risk Assessment for Nontarget Terrestrial Plants IEAM 16, pp 691-705) which allows for assessing and comparing effects on reproductive and vegetative parameters. Throughout the growing season 2017 (May to September), the sulfonyl-urea herbicide iofensulfuron-sodium was tested on 11 plant species sown as a seed mixture for establishing artificial plant communities on a test acre in Germany. Three herbicide rates were applied, and vegetative parameters (vegetation cover, dry biomass of total vegetation, phytotoxicity, BBCH stage) were assessed. In order to measure effects on reproduction, fully ripened seeds were collected from the plants in the field during the last two months of the study and germination of the F1 generation was assessed in the greenhouse. Other reproduction measurements (e.g. number of flowers, yield) were regarded as less suitable because recording of these parameters in the field was proven to be infeasible in our study design. Furthermore, F1 seed germination is identified as an important parameter for plant species population dynamics. Suitability of the used test species for reproduction testing was judged by their seed´s availability in the test plots, collectability, seed handling and ability to germinate in the lab. In order to compare effects of different growing conditions, the germination tests were done in petri dishes on filter papers, in pots filled with soil as well as in the dark or in a dark/light regime, respectively. Results indicated that growing conditions need to be carefully considered when conducting germination experiments in order to not tamper effects of the herbicide treatment. In conclusion, vegetative endpoints were lower than reproductive endpoints confirming that reproduction is not generally the more sensitive parameter.

2.04.13

Developing a Predictive Tool for Plant Communities in the Vicinity of Agricultural Fields/Orchards in Europe to Refine Risk Assessment for Non-Target-Terrestrial Plants

G. Arts, Wageningen Environmental Research - WUR / Environmental Risk Assessment; S.M. Hennekens, P.J. Verweij, M. van Eupen, Wageningen Environmental Research WUR

The terrestrial plant risk assessment of pesticides is currently based on standardized greenhouse tests with a limited number of plant species, mostly crop species. Higher tier tests of any kind (e.g. field, semi-field) are not standardized. As in Europe landscape level risk assessment is in development, the question arises how such a risk assessment can be performed for terrestrial plants. When considering the landscape level, the level of biological organization is not the species, but rather the plant community. Therefore, at the request of European Crop Protection Association (ECPA), NTP ad-hoc group, a project was initiated to deduce an appropriate number (presumably 5 – 10) of representative plant communities in terms of habitats, functionalities and structures at the European level to be used e.g. as reference tier in the risk assessment and/or to inform testing for herbicides. As a first step the year 2019 was dedicated to exploring data that could be used to identify plant communities and plants species and their predictors at the landscape level that are characteristic for the vicinity of agricultural areas in Europe. Currently further work is performed on linking the available databases in the QUICKSCAN tool (http://www.QUICKScan.pro). It is a spatial modelling environment that combines expert knowledge with spatial and statistical data. The EUNIS (the European Nature Information System; https://eunis.eea.europa.eu/) habitat classification has been used as a basis to identify the 6 man-made habitats characteristic of agricultural cropped areas. These habitats include 143 characteristic species, belonging to 31 plant families and refer to 40,000 vegetation plot observations in the European Vegetation Archive (http://euroveg.org/eva-database). The modelled suitability maps of these EUNIS habitats were combined with crop distribution maps for wheat to generate potential occurrence maps of EUNIS habitats in agricultural land surrounding wheat crops. Plant traits including leaf type, monocotyl / dicotyl, plant functional type, plant life span (longevity) and seed longevity were requested from the TRY-database and used to collate a characteristic trait spectrum per EUNIS habitat. Further work is currently performed on linking the traits to the EUNIS habitats, the EUNIS suitability maps, the wheat distribution maps and underlying predictors, e.g. soil and climate. Study performed for ECPA (European Crop Protection Association) Supervised by the ECPA monitoring group: Rena Isemer (Chair), Joanna Davies, Eileen Patterson, Stefania Loutseti, Christoph Julian Mayer, Tiffany Kung

Emerging and Legacy Contaminants in Wildlife: Biomonitoring, Exposure, and Effects

2.05.01

Maternal Transfer and Occurrence of Siloxanes, Chlorinated Paraffins and Legacy POPs in Herring Gulls (Larus argentatus) of Different Urban Influence

N. Knudtzon, University of Oslo / Department of Biosciences; H.S. Thorstensen, Department of Bioscences, University of Oslo / Department of Bioscences; A. Ruus, Norwegian Institute for Water Research; M. Helberg, Østfold University College; K. Bæk, NIVA; E. Enge, Norwegian Institute for Air Research; K. Borgå, University of Oslo / Department of Biosciences

Herring gulls (*Larus argentatus*) are generalist predators. Urban gulls are exposed to contaminants from aquatic, terrestrial and anthropogenic sources, including legacy contaminants known to accumulate in aquatic food webs, and contaminants of emerging concern (CECs), currently in use. We assess if differences in urbanisation between the inner and outer Oslofjord affect ecological niche and contaminant concentrations in female herring gulls, and maternal transfer to eggs. Blood from 15 females and one corresponding egg were collected in two Oslofjord colonies. The Oslofjord is an urban area; however, urbanisation is higher around the inner than the outer fjord. Ecological nice was assessed using stable isotope (SI) ratios of carbon and nitrogen (δ13C and δ15N). The measured contaminants include persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), CECs like the lipophilic chlorinated paraffins (CPs), cyclic volatile methyl siloxanes (cVMSs), dechloranes, emerging brominated flame retardants (BRFs), non-lipophilic per- and polyfluorinated substances (PFASs) and metals. The stable isotopes indicated a more marine ecological niche in the outer than the inner fjord colony, although overlapping. POP concentrations were similar in the colonies, indicating similar exposure. The CECs varied, with higher concentrations of a cVMS and short-chained CPs in blood and eggs of the inner fjord colony, showing that the gulls' exposure to CECs differs from POP exposure. PFAS concentrations were higher in the outer fjord colony, likely linked to point-source releases from an airport situated nearby. Dechloranes and emerging BFRs were detected in few samples. Maternal transfer efficiencies of cVMSs were similar to the POPs, while it was

lower for the CPs, which agrees with studies on other species. To our knowledge, this is the first report of maternal transfer ratios of CPs in seabirds. Combined, these results indicate that individual specialisation and local sources are more important than between-colony feeding habits for contaminant occurrence in these gulls.

2.05.02

Occurrence of Bisphenols and Benzophenone UV Filters in White-Tailed Eagles (Haliaeetus albicilla) From Smøla, Norway

B. Oró-Nolla, S. Lacorte, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Environmental Chemistry; K. Vike-Jonas, S.V. Gonzalez, Norwegian University of Science and Technology (NTNU) / Department of Chemistry; T. Nygård, Norwegian Institute for Nature Research NINA; A.G. Asimakopoulos, Norwegian University of Science and Technology (NTNU) / Department of Chemistry; V. Jaspers, Norwegian University of Science & Technology / Biology

There is a growing concern about the occurrence of bisphenols and benzophenone UV filters in the environment, while data is limited regarding their actual occurrence in wildlife species. In this study, concentrations of bisphenol and benzophenone UV filter analogues such as bisphenol A (BPA), bisphenol AF (BPAF), bisphenol B (BPB), bisphenol F (BPF), bisphenol S (BPS), bisphenol M (BPM), bisphenol P (BPP), benzophenone-1 (BzP-1), benzophenone-2 (BzP-2), benzophenone-8 (BzP-8) and 4-hydroxybenzophenone (4-OH-BzP) were determined in liver tissue samples (N=38) from white-tailed eagles (*Haliaeetus albicilla*) that were found dead in Smøla (2006-2018). Smøla is a Norwegian municipality that holds one of the largest and densest breeding populations of white-tailed eagles in Europe. Liver samples (0.1–0.2 g) were extracted using established methods, which involved the incubation of the samples in ammonium acetate (1.0M) with β-glucuronidase at 37°C for 12 h to establish total concentrations (free and conjugated chemical species), followed by solid-liquid extraction with ethyl acetate. Among the detected compounds, BPAF (a fluorinated analogue) was the most ubiquitous contaminant since it was detected in 32 out of 38 liver samples at concentrations ranging from 1.08 to 6.68 ng/g wet weight (w.w.), followed by BPA (mean 10.4 ng/g w.w.), BzP-1 (mean 3.24 ng/g w.w.) and 4-OH-BzP (mean 0.62 ng/g w.w.). The concentrations found in livers suggested that white-tailed eagles seem to accumulate bisphenols and benzophenone UV filters, which raises concern, as these plastic and personal care product related emerging contaminants show potential endocrine disrupting properties. The high detection frequency of the fluorinated BPAF warrants further attention as other fluorinated compounds have proven to be extremely persistent and potentially harmful to wildlife. Furthermore, the presence of BPs and BzPs in the livers of white-tailed eagles suggests that these species can accumulate these contaminants, and therefore could be promising biomonitoring species for these compounds.

2.05.08

Feces Are Effective Biological Samples for Measuring Pesticides and Flame Retardants in Primates

S. Wang, Indiana University, Bloomington / School of Public and Environmental Affairs; T. Steiniche, M. Wasserman, Indiana University / Department of Anthropology; M. Venier, Indiana University / SPEA

The habitats of wild primates are increasingly threatened by surrounding anthropogenic pressures, but little is known about primate exposure to frequently used chemicals. We applied a novel method to simultaneously measure 21 legacy pesticides (OCPs), 29 current use pesticides (CUPs), 47 halogenated flame retardants (HFRs), and 19 organophosphate flame retardants (OPFRs) in feces from baboons in the USA, howler monkeys in Costa Rica, and baboons, chimpanzees, red-tailed monkeys, and red colobus in Uganda. The most abundant chemicals were alpha-HCH, beta-HCH, and hexachlorobenzene among OCPs across all sites, chlorpyrifos among CUPs in Costa Rica and Indiana, DBDPE in Costa Rica and Indiana and BDE-47 in Uganda as HFRs, and tris(2-butoxyethyl) phosphate (TBOEP) as OPFRs across all sites. The detected chemical concentrations were generally higher in red-tailed monkeys and red colobus than in chimpanzees and baboons. Our methods can be used to examine the threat of chemical pollutants to wildlife, which is critical for endangered species where only noninvasive methods can be used. Authors: Shaorui Wang, Indiana University, USA; Tessa Steiniche, Indiana University, USA; Jessica M. Rothman, Hunter College of the City University of New York, USA; Richard W. Wrangham, Harvard University, USA; Colin A. Chapman, George Washington University, USA; Richard Mutegeki, Kibale National Park, Uganda; Rodolfo Quirós, Organization for Tropical Studies, Costa Rica; Michael D. Wasserman, Indiana Universiy, USA; Marta Venier, Indiana University, USA.

2.05.09

High Levels of Emerging and Legacy Contaminants in Stranded Whales From Norway

C.M. Andvik, University of Oslo / Department of Biosciences; E. Jourdain, Norwegian Orca Survey; J.L. Lyche, Norwegian University of Life Sciences (NMBU) / Centre for Environmental Radioactivity (CERAD); K. Borgå, University of Oslo / Department of Biosciences

Marine mammals are considered sentinel species for the marine ecosystem health due to the bioaccumulation of legacy and emerging contaminants in their tissues. Killer whales in Norway have recently been found to be of higher risk of contaminant-mediated health effects than previously assumed, however contaminant levels for many other marine mammal species in Norway is not known. The aim of this study was to conduct the first thorough screening of legacy and emerging contaminants in marine mammals from Norway, and investigate inter-species differences. We collected samples from 33 individuals of nine different species 2015-2020, including one neonate killer whale (*Orcinus orca)* of approximately 10 days of age. Legacy organohalogen contaminants (OHCs) and emerging brominated flame retardants were quantified in blubber (n=32). Total mercury (Hg) levels were quantified in the skin (n=27), muscle (n=22) and liver (n=3) and PFAS in muscle and liver. We additionally quantified the stable isotope ratios of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$, respectively) in skin and muscle.We screened for 4 unregulated brominated flame retardants, and found pentabromotoluene (PBT) and hexabromobenzene (HBB) in the blubber all killer whale individuals (median PBT: 0.091 ng/g lw; HBB: 1.4 ng/g lw). Perfluoroalkyl substances (PFAS) and total mercury levels were lower in the killer whale neonate than adults, suggesting less efficient maternal transfer of these substances. ΣPCB levels exceeded the threshold for a risk of health effects (10 µg/g lw) in 75% of the killer whales, and levels in the neonate exceeded thresholds for immune effects (9 µg/g lw). The presence of PBT and HBB in the neonate is the first evidence of maternal transfer of these unregulated contaminants in marine mammals.We found Hg levels in skin to be positively correlated with $\delta 1^5N$ values in skin of the other stranded marine mammal species (Spearman's rank correlation, S = 2,572, p = 0.006, rho = 0.48), with the highest levels in sperm whales (*Physeter macrocephalus*; n=6; 13.1-15.5 ‰) and the lowest in the humpback whales (*Megaptera novaeangliae;* n=3; 12.0-12.6 ‰). Our results are relevant for an understanding of the prevalence and persistence of these contaminants in the Arctic to aid in possible future regulation.

2.05.14

Effect Agricultural Management on the Female Reproductive Parameters of Iberian Hare (Lepus granatensis)

J.C. Cañaveras, Instituto de Investigación en Recursos Cinegéticos (IREC) CSIC-UCLM-JCCM; M. Risalde, Universidad de Córdoba, España / Departamento de Anatomía y Anatomía Patológica Comparadas y Toxicología; V. Alzaga, Gestión Ambiental de Navarra, S.A.; P. Camarero, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM) / Toxicology in wildlife; F. Talavera, Instituto de Investigación en Recursos Cinegéticos (IREC) CSIC-UCLM-JCCM; J. Viñuelas, Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla La Mancha (IRIAF) / CIAPA-Marchamalo; P. Acevedo, IREC-Instituto de Investigación en Recursos Cinegéticos; R. Mateo Soria, IREC (CSIC- UCLM) / Wildlife Toxicology; M. Martinez-Haro, IREC-Instituto de Investigación en Recursos Cinegéticos / Department of Life Sciences

During the 20th century agriculture underwent important changes based on the search for maximum productivity, which has led to an important homogenization of the landscape due to the concentration of land and to the use of a large amount of pesticides and fertilizers in the environment. This change in agricultural management has been linked to the loss of plant and wildlife biodiversity. In the specific case of pesticides, there are significant gaps in the knowledge of their secondary or sub-lethal effects on wildlife. Under this context, the aim of this study was to evaluate the impact of different agricultural management models, based on the use or not of pesticides, on the reproductive success of the Iberian hare (Lepus granatensis), as a sentinel species of the potential effects that the use of pesticides can have on the wildlife of the Iberian agroecosystems. Females of Iberian hare were collected during the 2018/2019 hunting season from four hunting grounds located in two different types of agricultural areas: one based on the use of pesticides (treated, n=35), and other pesticides-free (n=18). For all animals collected, a detailed necropsy was performed after noted the main biometric data for body condition assessment. Then, the ovaries and crystalline lens were stored in a 10% formalin solution for subsequent analysis. Ovaries were histologically prepared for follicle and corpora lutea counting, and crystalline lens were dried and weighted for relative age determination. This is the first study carried out in which the potential reproductive effect of the agricultural management for wild females of Iberian hares is addressed under real field conditions. Our study did not detect significant differences in the biometric parameters of the females collected. However, the agricultural management based on the use of pesticides, seem to exert an effect on the biometric parameters of the ovaries in the Iberian hare, observing a lower weight, size and width in animals from treated areas than in those pesticides-free. Furthermore, females from treated areas presented higher number of secondary and atretic follicles than those areas free of pesticides. A similar negative effect was detected in males collected in these same areas, suggesting that agricultural management based on the uses of pesticides may exert a negative effect on the reproduction of the Iberian hare inhabit agroecosystems.

2.05.15

Ecotoxicity of a Highly Brominated Organophosphate Flame Retardant in Amphibians

S.A. Robinson, S.D. Young, Environment and Climate Change Canada / Wildlife and Landscape Science Directorate; C. Brinovcar, Environment and Climate Change Canada / Water Science and Technology Directorate; A. McFee, Carleton University / Biology; A.O. De Silva, Environment and Climate Change Canada / Water Science and Technology Directorate

Highly toxic and persistent flame retardants are being restricted in their production and use. However, their replacement products, such as, the brominated organophosphate ester flame retardant, tris(tribromoneopentyl) phosphate (CAS 19186-97-1, molecular formula $C_{15}H_{24}Br_9O_4P$, acronym TTBrNP) are less-well characterized, but have potential to bioaccumulate and disrupt normal hormone processes in vertebrates. Such changes to endocrine functions can affect biological development and thus the production of healthy, reproductively capable individuals. We evaluated the sex-steroid and thyroid disrupting potential of TTBrNP in aquatic wildlife. Specifically, we assessed the acute (embryo and Gosner stage 25 (GS25)) or sub-chronic (GS25-41 tadpoles) toxicity and bioconcentration of TTBrNP in two North American native species, *Lithobates sylvaticus* and *L. pipiens*. Nominal exposure concentrations of TTBrNP included 0 (water and solvent controls), 30.6, 61.3, 122.5 and 245.0 µg/L. We found after 96 to 120 h of exposure, no effects of TTBrNP on wood or leopard frog embryo or tadpole survival, occurrence of abnormalities or hatchling size up to our highest concentration of 245 µg/L. We also found no effects on size, development or sex ratio of leopard frogs after the 30-d sub-chronic exposures. We found measurable concentrations of TTBrNP in tadpole whole bodies showing that TTBrNP bioconcentrates in tadpoles, but bioconcentration factors were low at 26 ± 3.1 L/kg wet weight. In conclusion, up to 245 µg/L TTBrNP had no overt detrimental effects on wood frog or leopard frog tadpoles. Our study provides essential information for risk assessment of an emerging flame retardant by providing some of the first aquatic vertebrate toxicity data for this compound.

2.05.19

Development of an Untargeted Metabolomics Approach Based on LC-(ESI)-HRMS, Applied to Three-Spined Sticklebacks Exposed to Diclofenac

E. Lebeau, URCA / 51; G. Daniele, CNRS UMR 5280; A. Fildier, CNRS UMR 5280 / TRACES Team; C. Turiès, INERIS / UMR I-02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); A. Bado-Nilles, INERIS / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); J. Porcher, INERIS / Ecotoxicology; O. Dedourge-Geffard, University of Reims Champagne-Ardenne / SEBIO UMR-I 02; A. Geffard, Université de Reims Champagne Ardenne / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); E. Vulliet, CNRS UMR280 / TRACES Team

Introduction The aquatic systems are the ultimate repository of a large part of the compounds due to human activities. In response to these chemical stresses which aquatic environment may suffer, most studies have focused on the use of biomarkers as early responses induced by these pressures. In recent years, several studies showed that metabolomics can be used to compare and determine the causal link between exposure and effects for predicting toxic effects of contaminants in aquatic organisms. Especially, untargeted approaches based on high resolution mass spectrometry has gained more and more popularity due to its benefit of analysing the whole metabolome in a global view. This approach is a promising tool to provide biomarkers of effect and information on exposure ways in organisms in view of aquatic biomonitoring and environmental risk assessment. The aims of this study were : - To develop an exhaustive untargeted approach able to cover a huge range of metabolites within three-spined sticklebacks (*Gasterosteus aculeatus*) - To identify impacted metabolites after a sub-chronic exposure of this species to diclofenac. **Materials and methods** A solid liquid extraction followed by liquid chromatography (RSLC U3000 from Thermo Scientific®) coupled to QtoF (Maxis Plus from Bruker Daltonics®) were used. The data were processed using Metaboscape 4.0 (Bruker Daltonics®). The extraction process was performed on 25 or 50 mg of whole fish, liver, gills or brain with 2.5 mL of MeOH/H_2O/heptane. Two complementary analytical methods were then combined : a reverse phase separation on HSS T3 C18 column for positive ionization mode, and an HILIC separation on Nucleodur column for negative ionization mode. This development was done to identify finer and then more relevant biomarkers by a comparative strategy between control and exposed individuals to diclofenac. **Results and discussion** Two LC-HRMS methods have been developed based on the responses of analytical standards representative of different metabolite groups (amino acids, sugars...). The extraction protocol has been optimized in all matrices, taking into account the number of features (m/z, tr) observed in LC-HRMS. The study shows the influence of the nature of the solvents during the extraction stage as well as the choice of chromatographic conditions. These methods were applied to adult fish exposed to diclofenac, a non-steroidal anti-inflammatory drug ubiquitous in the environment. Individuals were exposed for 21 days at 1 and 100 µg/L. The results of the untargeted metabolomic approach will be presented during the speech. **Conclusions** This study provides a complete reliable analytical method for the characterization of metabolites in three-spined sticklebacks. Thus, it enables to identify biomarkers through a comparative strategy, for biomonitoring of water bodies quality. **Keywords** Three-spined stickleback, fish, diclofenac, LC-HRMS, metabolomics

2.05.23

Monitoring and Impact Assessment of Organic Micropollutants in Important Bird and Biodiversity Areas From Spain

M. Dulsat-Masvidal, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); C. Ciudad, O. Infante, SEO/BirdLife, Melquiades Biencinto, 34, 28053 Madrid, Spain; R. Mateo Soria, IREC (CSIC- UCLM) / Wildlife Toxicology; S. Lacorte, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Research Council (CSIC) / Environmental Chemistry

Important Bird and Biodiversity Areas (IBAs) are sites identified by BirdLife International as being globally crucial for the conservation of bird populations based on an internationally agreed set of criteria. In this study, we have developed a monitoring scheme to determine the presence of organic micropollutants in water, soil and sediment from 140 IBAs from Spain. In water, 59 compounds were analysed, including 21 pharmaceuticals, 17 pesticides, 16 perfluorinated alkyl substances (PFAS), caffeine, nicotine and 3 organophosphorus flame retardants (OPFR). In soils and sediments another set of 59 different contaminants were analysed including 16 polycyclic aromatic hydrocarbons (PAH), nicotine, 17 organochloride pesticides, 3 organophosphorus pesticides, 7 polychlorinated biphenyl (PCB), and 6 phthalates, octyl- and nonyl-phenol and bisphenol A. Water samples were solid phase extracted and analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Soil and sediments samples were solid-liquid extracted and analysed by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). The most detected compound in water samples was caffeine, present at 73% of the samples and was used as a marker of urban contamination. Pharmaceuticals were widespread along all IBAs water bodies studied. The most ubiquitous pharmaceuticals were valsartan (to treat blood pressure), metformin (to treat diabetes), paracetamol (an analgesic drug) and ibuprofen (an anti-inflammatory drug). Among pesticides, chlortoluron (an herbicide), tebuconazole (a fungicide) and chlorpyrifos (an insecticide) were widely detected in most IBAs, whereas OFPR and PFAS presented lower concentrations. 4-4' DDE was ubiquitous in soils and sediments, but detected at trace concentrations. Among studied contaminants in soils and sediments, plasticizers were detected at the highest concentrations while PAHs showed a profile suggesting a pyrolytic origin associated to combustion processes. Overall, this study is intended to generate information on the occurrence and impact of organic pollution in IBAs that can help adopting correct management and conservational measures against chemical pollution. *Acknowledgement* - The authors thank financial support from SEO/BirdLife and Ecoembes through LIBERA project. The Ministry of Science and Innovation of Spain under the project PID2019-105732GB-C21 is also acknowledged. The authors also gratefully acknowledge funding from Severo Ochoa Program.

2.05.27

Internal Bioaccumulation and Maternal Transfer of Organic Pollutants in Sea Turtles, Long-Lived Marine Sentinels

C. Munoz, J. Hendriks, Radboud University / Environmental Science; A.M. Ragas, Radbound University / Environmental Science; P. Vermeiren, Radboud University / Environmental Science

Knowledge on the bioaccumulation of organic pollutants in sea turtles is highly fragmented across studies. Nonetheless, organic pollutants can accumulate in long-lived species such as sea turtles, which, combined with their large migrations, makes sea turtles suitable sentinels for biomonitoring of organic pollutants in the marine environment. Alternatively, organic pollutants also negatively impact sea turtle health and survival, underlying the importance of increased understanding of bioaccumulation. To improve the use of sea turtles as sentinels for organic pollution, we aimed to synthesize and harmonize current scattered data, and identify factors driving differences in bioaccumulation among internal tissues, and maternal transfer.A systematic review for data on organic pollutant concentrations within tissues of sea turtles was conducted, and a homogenized database developed. Lipid normalized concentrations of OCPs and PCBs in *Caretta caretta* were close to equilibrium in lung and muscle, decreased slightly in the heart and kidney, and decreased up to an order of magnitude in fat and brain relative to the liver. Log bioaccumulation ratios of PCBs, OCPs, and PBDEs in whole blood were about one order of magnitude larger than in whole egg for both *Dermochelys coriacea* and *Chelonia mydas*, while they were around equilibrium in maternal and hatchling blood. Differences in the bioaccumulation of legacy and emerging organic pollutants could be explained by observed differences between species, life stages, and sexes. Species-specific differences related mainly to energy storage, while intraspecific differences were most notable between juvenile and adult stages. Our analyses demonstrate that the concentration of pollutants in tissues can be predicted based on their lipophilicity, although some pollutant–tissue combinations deviate up to an order of magnitude

from equilibrium. Consequently, the physiology of sea turtles and the chemical properties of pollutants need to be accounted for. These results improve the synthesis of patterns in pollution levels needed for assessment and management of sea turtle populations facing legacy and emerging organic pollution.

Impact of Chemical Pollutants in Food Webs within and across Ecosystem Boundaries

2.06.01

Seabird-Mediated Transport of Organohalogen Compounds to Remote Sites

J. Grimalt, IDAEA-CSIC / Environmental Chemistry; N. Nagar, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); M. Bartrons, University of Vic, Spain; S. Brucet, Catalan Institution for Research and Advanced Studies (ICREA) / Biosciences; T. Davidson, Aarhus University

here is evidence that birds may act as carriers of pollutants over long distances. In the present study, the role of seabirds for the transport of organochlorine compounds to very remote sites, e.g. western Greenland, is evaluated. Three lakes were selected for this purpose, one, NOW5, with a little auk (*Alle alle*) bird colony and the other two, NOW14 and Q5, devoid of these seabirds. NOW14 has a historic albeit inconsistent human presence record, and Q5 is away from either bird or human presence. Samples were collected from the freshwater lakes on the coast of NOW polynya in the summers of 2015, NOW5D and NOW14, and 2016, Q5. Lake coring was carried out using an HTH gravity corer. The cores were sliced and stored frozen until analysis. The waters of NOW5 had a strong acidic pH, 3.4, whereas the pH values of NOW14 and Q5 were close to 8. This difference can be attributed to the influence of *A. ale* guano depositions. In Spitzbergen, guano depositions of these birds also resulted in acidic lake water. Other differences between the waters of NOW5D lake and the others were related to the fertilizing guano effects. Thus, NOW5D showed high chlorophyll concentrations (74 µg/L vs. 1.6-3.4 µg/L, respectively), higher content of total phosphorous (0.34 mg/L vs. 0.007-0.01 mg/L, respectively) and total nitrogen (3.75 mg/L vs. 0.21-0.75 mg/L, respectively). These differences reflected that the waters of NOW5D received more nutrients than in the other two lakes. The concentrations of all organohalogen compounds found above the limit of detecion were much higher in the lake under the influence of *A. ale* than in the other lakes showing the strong influence of these seabirds in the transport and deposition of these hydrophobic compounds to remote sites. However, not all compounds showed the same relative increases. The most volatile, hexachlorobenzene and the hexachlorocyclohexanes, were about 20 time higher in NOW5 whereas the enrichment of the chlordanes, PBDEs and PCBs was between 4 and 6 times and DDTs three times. These differences evidence a selective effect in the seabird accumulation and transport of organohalogen to remote sites.Seabirds play a significant role in the tranport of oranohalogen compounds to remote sites but their effect is selective depending on the chemical composition of these pollutants.

2.06.02

Diet Related Effects of the Pesticides Terbuthylazine and Tebuconazole on Scraper and Shredder Freshwater Invertebrates

I. Muñoz, University of Barcelona / Ecology; N. De Castro, X. Herbera, M. Menéndez, Universitat de Barcelona / Evolutionary Biology, Ecology and Environmental Sciences; M. Solis, Instituto de Limnología “Dr. Raúl Ringuelet”; A. Viza, Universitat de Barcelona / Evolutionary Biology, Ecology and Environmental Sciences

Leaf litter or algae are the most important basal resources for invertebrate primary consumers in freshwater ecosystems.This first level in the food chain is essential for nutrient and energy transfer to the entire food web. These basal source-consumer links can be influenced by chemical pollutants such as pesticides widely used in agriculture activities. Herbicides can affect algae growth in stream biofilms and fungicides in turn, may alter leaf-associated fungal community; both finally affecting food quantity and quality for invertebrate consumers. In this study we focus on the effects of the herbicide terbuthylazine and the fungicide tebuconazole, both frequently detected in streams and rivers. Our aim is to evaluate the diet-related effects of these two pesticides on the consumption and growth rates of the snail *Physella acuta* (scraper) and the crustacean *Echinogammarus* sp (shredder). We conducted a 14-day experiment using artificial channels where biofilm, leaf-litter and invertebrates were exposed to control conditions and to both pesticides, separately and in mixture (12 treaments with 3 replicates each one). The herbicide had a negative effect on chlorophyll concentration resulting in a lower growth rate of snails regarding to the control. Fungicide also reduced, but less, the chlorophyll in the biofilm, and the snail growth rate in this treatment was significantly higher than the growth rate in the herbicide treatment. We also observed lower effects on chlorophyll and growth rate in the mixture treatment comparing with the herbicide treatment. The observed changes in the biofilm and ultimately in the snail growth may be driven by a competitive advantage of algae in front of fungi in the fungicide treatments (alone and in the mixture). We did not observe significant effects of the pesticides on leaf litter quality (CN ratios), however the relative growth rate of the gammarid was slightly lower when was exposed to the herbicide. The present study shows that the effects of both pesticides via the dietary pathway seem to affect the growth rate of the consumers. The reduction of fungi in biofilm favours algae biomass and the growth rate of the snail. The mechanisms of the herbicide affecting gammarid growth rate are still unclear. These preliminary results point to the need of a better understanding of the effects of pesticides on microbial community to assess the indirect effects on food chains.

2.06.03

Trophic Interactions in Marine Zooplankton Communities Exposed to Closed Loop Scrubber Water

C. Jonander, J. Egardt, P. Tiselius, University of Gothenburg / Department of Biological & Environmental Sciences; I. Hassellöv, Chalmers University of Technology / Mechanics and Maritime Sciences; M. Rasmussen, University of Gothenburg / Department of Marine Sciences; I. Dahllöf, University of Gothenburg / Department of Biological & Environmental Sciences

Stricter global limits regarding sulphur content in marine fuels apply since January 2020, with the purpose of decreasing sulphur oxide emissions from shipping. To comply with these new regulations an increasing number of ships are installing an exhaust gas cleaning system, also known as a scrubber. A closed loop scrubber recirculates the washwater used to remove the sulphur oxides, yet most systems have a bleed off of between 1.8-10.8 m^3 washwater per hour for a medium sized ship (operating at 15 MW). Water from closed loop scrubbers contain high concentrations of metals and organic pollutants and has been shown toxic to zooplankton. Mortality in mesozooplankton (>200 µm), such as copepods, will likely lead to changes in community structure as species can have different tolerances. Such structural changes has the potential to trigger trophic cascades, which could ultimately lead to effects on microzooplankton (< 200 µm) or even primary producers. In order to test the effects of closed loop scrubber emissions on trophic interactions within the zooplankton community, we exposed a field-sampled mesozooplankton community to two concentrations of closed loop scrubber water or a control (1.5%, 3%, Control, *n=5*). We further tested how this affected their reproduction, and their predation on a natural microzooplankton community. The mesozooplankton were exposed for 72 h, and then transferred to bottles with microzooplankton for another 20 h, and copepod eggs were collected at 72 h and at 96 h. The results show that copepod reproduction is affected moderately at 1.5 %, and severely at 3 %. The results from the mesozooplankton and microzooplankton community structures are still under analysis, but will be assembled by the end of 2020. However, the results of the reproductive endpoints can already tell that zooplankton in heavily trafficked areas could be affected by these discharges, but more studies are needed in order to characterise environmental concentrations of contaminants related to scrubber use.

2.06.06

Effects of Metal Contaminated Sediments: Delayed Metamorphosis and Sex-Specific Accumulation in Aquatic Insects

. Berglund, Umea University / Ecology and Environmental Science; J. Lidman, Umea University / Department of Ecology and Environmental Science

Aquatic ecosystems have generally been regarded as sinks for contaminants, receiving for example metals from the terrestrial ecosystem through runoff, direct discharge or deposition into the aquatic environment. However more recently, the interest in the opposite feedback-loop, from water to land, have increased, where aquatic insects may serve as biovectors of contaminants when they emerge as adults and become part of the terrestrial food web. Metals may also hamper sensitive life-stages such as metamorphosis, hence reducing prey availability for riparian consumers. In the present study we aimed to evaluate the impact of metal contamination on aquatic insect emergence and to assess the transfer of metals from aquatic to terrestrial ecosystems. We exposed chironomid larvae for a gradient of Pb/Zn contaminated sediment and assessed the timing and success of adult insect emergence. We were also interested in potential sex-differences in survival, emergence and transport of metals. Fewer adults emerged from treatments with high metal exposure and the emergence was also delayed for both males and females. However, metal contamination primarily affected the survival of insect larvae, and had limited effect on metamorphosis. A reduction and delay in emergence may result in reduced food availability and ecological mismatch for terrestrial consumers relying on aquatic subsidies. Male chironomids accumulated slightly higher concentrations of Pb than females, but due to their smaller sizes the individual contribution of Pb, but also Zn, was still higher from females. Thus, terrestrial insectivores will be subjected to different pollution regimes depending on preference for smaller (males) or larger (females) prey items.

2.06.11

Cross-Ecosystem Links in Polluted Areas: Effects on Breeding Success and Nestling Health in Pied Flycatchers (Ficedula hypoleuca) Under Lead Exposure

L.E. Malm, J. Lidman, Umea University / Department of Ecology and Environmental Science; D.C. Lau, Umea University / Department of Ecology and Environmental Sciences; M. Jonsson, Umea University / Department of Ecology

and Environmental Science; . Berglund, Umea University / Ecology and Environmental Science
Aquatic subsidies may serve as pathways for contaminant transfer across ecosystems but other factors may be of importance for the fitness of predators in polluted riparian environments. Prey from aquatic sources may have nutritional properties which can counteract the negative effects of ingesting polluted prey and the health effects of exposure to pollution may therefore be hard to disentangle from the nutritional benefits. We investigated lead uptake, breeding success and nestling health in an insectivorous passerine, the pied flycatcher *Ficedula hypoleuca* near a closed lead and zink mine using both lake (aquatic) and terrestrial breeding sites which were either polluted or unpolluted (reference sites). Previous results from the same study site has shown that, while the mine was active, and 3-5 years after mining had stopped, lead concentration was higher while hemoglobin was lower in nestlings from polluted sites compared to reference sites and the breeding success was also reduced. 15-19 years after mining stopped, lead levels in nestling blood were still similar to the concentration during active mining. However, breeding success (i.e. nestling survival) in both aquatic and terrestrial polluted sites were no longer affected in polluted sites. There was no difference in breeding success, hemoglobin levels or nestling weight between lake and terrestrial sites, but nestling weight was, surprisingly, significantly higher in polluted sites across both lake and terrestrial sites. Measures of arthropod abundances indicated an increased availability of potential prey in polluted aquatic and terrestrial sites, and an increase of aquatic prey in aquatic sites, which may compensate for higher lead exposure. This highlights the importance of prey availability to counteract toxic effects of metal exposure and we will further investigate whether the quality of prey (polyunsaturated fatty acids) are important for nestling health and survival.

Insect Decline - the Contribution of Multiple Stressors on Landscape Level

2.07.01
Insect Decline - Evaluation of Potential Drivers of a Complex Phenomenon
M.E. Grevé, Bayer AG, Division Bayer CropScience; T. Schad, Bayer AG Crop Science Division / Environmental Modelling; C. Baden, Bayer AG Crop Science Division / Pest Control; S. Eilmus, Bayer AG Crop Science Division / Agricultural Solutions; M.T. Marx, Bayer AG Crop Science Division / Ecotoxicology; C. Maus, Bayer AG Crop Science Division / Regulatory Scientific Affairs
Insect decline is considered a complex phenomenon, resulting from a range of causal factors, which act at different spatial and temporal scales, with different impacts on different insect groups. There is a variety of data sets which describe changes in insect populations and biocenoses over time, however, only few long-term monitoring data are available which were generated according to a consistent scheme. In the absence of a broad data basis which could provide direct insights into factors causing insect decline, correlation analyses can help to identify potential factors which are associated with declines. Here, we are presenting a detailed analysis of the development of potential drivers of insect decline over the last decades at locations in Germany where a decline of insect biomass has been measured in previous studies, and of changes in the states of these potential drivers. Potential factors investigated included landscape structure, land use change, grassland management, cropping practices, pesticide use, climate, and light pollution. Data about the developments of these parameters over the last decades were obtained from statistical data such as cropping statistics and market research, and long-term sequences of satellite imagery. The results of the correlation analysis suggest that habitat loss caused by land use and land cover change, as well as intensification of land use are factors frequently correlating with declining tendencies in insects in Germany. This is to our knowledge the first study, where all of these factors got intensively investigated, from a retrospective point of view. With this extensive dataset we will be able to correlate the often-mentioned habitat drivers (landscape change, monotonicity of agricultural structures, loss of fragmented structures) for insect decline tendencies combined with several disturbance factors (pesticides, urbanization, night light).

2.07.02
Grassland Management Change Over 25 Years: A Landscape Analysis Using Remote Sensing
C.M. Holmes, Applied Analysis Solutions, LLC; J. Pritsolas, R. Pearson, Southern Illinois University Edwardsville / Laboratory for Applied Spatial Analysis; C. Butts-Wilmsmeyer, Southern Illinois University Edwardsville / Center for Predictive Analytics; T. Schad, Bayer AG Crop Science Division / Environmental Modelling
Decline in insect biomass is an emerging issue of concern. The complexity of natural biosystems over such long periods of time make a comprehensive understanding of all the relevant factors difficult to achieve. Characterizing change in landscape over time as it relates to insect habitat can provide one piece to the larger puzzle of insect decline. We applied image processing methods to capture landscape-level changes in grassland management over a 25-year period near Krefeld and Wahnbachtal, Germany. For each site, over 150 satellite images were acquired from 1989 to 2013 across all seasons. Images within each year were normalized for atmospheric and illumination variation to focus on vegetation changes due to plant structure and vigor. Similar groups of grassland fields were identified using a k-means clustering algorithm using spectral information within and across years. The distinctness of the clusters was assessed using Tansformed Divergence and confirmed using an independent statistical analysis. K-means clustering was performed with three vegetation indices to assess consistency in clustering. In Krefeld, four clusters of grassland parcels (818 parcels) were identified with separate vegetation and temporal characteristics, and in Wahnbachtal five distinct clusters (1367 parcels) were separated. In Krefeld 3 distinct patterns emerge in grassland biomass. Two clusters have a constant high biomass over 25 years, while parcels in another cluster start lower and increase in biomass in the mid-1990's, reaching the levels of the consistent clusters around year 2000. The final cluster starts lower and remains constant until the early 2000's and then increases to almost the level of the other clusters by 2013. Similar patterns of changes in biomass were observed in Wahnbachtal. Analysis also demonstrated that biomass variability within clusters decreased over time. Periods of distinct increase in vegetation biomass (defined by vegetation indices) may indicate changes in grassland management practices over that time to include more intensive management activities (e.g., fertilization, more frequent cutting, silage production). Other groups showed relative consistency over time. Decrease of variability of biomass within clusters also indicates less diversity of grassland habitats, and likely reduction in insect biodiversity, over time.

2.07.03
Drivers and Pressures of Carabidae and Lepidoptera Population and Biodiversity Trends in Europe - a Review
A. Toschki, gaiac Research Institute; C. Baden, Bayer AG Crop Science Division / Pest Control; M. Bergtold, BASF SE; M.T. Marx, Bayer AG Crop Science Division / Ecotoxicology; C. Maus, Bayer AG Crop Science Division / Regulatory Scientific Affairs; J. Oellers, Q. Rumohr, gaiac Research Institute; M. Schade, Syngenta
Insect declines have been discussed intensively among experts, policymakers and the public. Many of the recent publications aim to identify the amplitude and potential drivers of this phenomenon. For the latter, it is often difficult to identify their impact, as ecosystems are a complex network of interdependent biotic and abiotic factors as well as anthropogenic activities that influence habitats, biocenoses, populations and individual organisms. We conducted a literature evaluation of publications with Carabidae and Lepidoptera population and biodiversity trends with data of at least 15+ years in Europe. From the resulting 82 publications, we extracted all reported trends and classified the environmental stressors discussed with these trends according to the DPSIR model (drivers, pressures, state, impact and responses). Further, we analysed the level of scientific verification behind the discussed stressors. Our classification based on the DPSIR model brings more clarity to the complex of stressors interacting even after simplifying and aggregating them to more general classes. In particular, our classification enables the precise allocation of a particular stressor with its corresponding driver. The total number of extracted trends for both species groups shows that declines are more frequent than increases, which underlines the overall declining trend. Whether negative or positive trend, our semi-quantitative analysis shows that changes in insect populations today are primarily anthropogenically driven. The drivers seem to be diverse: agriculture, climate change, nature conservation, urbanisation and anthropogenic activities in general. We could only attribute a fraction of discussed stressors to intrinsic changes in insects species or nature. The scientific verification level is different between trend types and exposes areas with research needs as well as drivers, like climate change, that have been discussed on a more profound basis over the last decade.

2.07.07
DNA Metabarcoding - Applying a Powerful Digital Green Technology Tool to Monitor Insect Decline
J. Moriniere, AIM - Advanced Identification Methods GmbH / CEO; K. Moriniere, AIM - Advanced Identification Methods GmbH
Over the past years, numerous studies have reported a decrease in insect diversity [1-6], species richness and abundance [7], biomass [8], as well as shifts in species composition [9]. Climate change and the anthropogenic degradation of the environment due to a steadily growing demand for food and biological raw materials have led to an increasingly rapid decline in beneficial insect species that carry out essential ecosystem services, and a rapid increase in pests in agriculture and forestry. In order to stop this development without neglecting contemporary needs, a sound management of natural habitats is necessary. To make informed decisions, we need to identify the driving forces of insect decline, their effects and interactions, and establish the extent to which they orchestrate the loss of biological diversity. This applies to the use of pesticides and fertilizers, as well as land-use-relevant measures in agricultural, forest and urban systems. Comparing industrial or other areas which are aimed to be designed with sustainable goals to

protected areas, a biological “status quo ante” can be determined, which can be used as a benchmark for changes in biodiversity. To capture the complex spatiotemporal picture of these changes, high-throughput and cost-effective biodiversity monitoring methods are essential. DNA metabarcoding is an innovative molecular method in DNA-based species identification and a powerful digital tool for surveying changes in species compositions in response to changing environmental conditions. By scanning a small region of the genome (DNA barcode), it is able to comprehensively identify species compositions (e.g. from traps) by comparing the barcode with existing entries in a global sequence database. Due to decades of hard work by taxonomic experts, in Germany alone, reference databases have recorded more than 26,000 native animal species (www.boldsystems.org [10]). However, traditional biodiversity surveys are burdened by high workload and restricted to individual focus groups and can only cover small areas and time periods. The decisive advantage of metabarcoding over traditional methods is therefore the high proportion of taxonomic coverage in the biodiversity survey and the resulting significantly higher informative added value. Although the genetic makeup of the native fauna has not yet been fully recorded in databases, DNA metabarcoding can already assess the majority of the spatially expected species and a large number of rare species. The knowledge gained in this way can be used to plan, assess and implement projects in many areas, and to use financial and human resources efficiently. Here, we aim to present some applications of DNA metabarcoding-based biodiversity monitoring in order to demonstrate its usefulness for illuminating species diversity – in particular, its ability to process hundreds to thousands of arthropod bulk samples in an automatized, high-throughput, and digitally optimized manner.

Multiple Stressor Effects in Aquatic Organisms and Ecosystems under a Changing Climate

2.08.04

The Exposure Order Strongly Modifies How a Heat Spike Increases Pesticide Toxicity

S. Meng, Faculty of science, KU Leuven / Biology; V. Delnat, R. Stoks, KU Leuven / Biology

The exposure order may strongly affect the impact of stressors, yet is largely ignored for the frequently occurring combinations of toxicants with natural stressors. We tested how exposure order shaped the interactive effects of serial exposure to the pesticide chlorpyrifos and to a heat spike in the larvae of the mosquito *Culex pipiens*. Notably, the chlorpyrifos-induced mortality was much more magnified by the heat spike and a synergism was already detected at the low concentration when exposure to chlorpyrifos followed the heat spike. This suggests that the preceding heat spike weakened the larvae as reflected in their lower net energy budget, moreover the chlorpyrifos-induced inhibition of its target enzyme (acetylcholinesterase) was only magnified by the heat spike when it was the first stressor. Also the chlorpyrifos-induced reduction in heat tolerance was stronger when the pesticide pulse followed the heat spike, and was buffered by the heat spike when this was the second stressor. Our results provide the first evidence that the exposure order can strongly change the magnifying effect of an important climate change factor on the toxicity of a pesticide. This highlights the importance of exposure order in ecological risk assessment of toxicants under realistic combinations with natural stressors.

2.08.05

Reduced Gene Expression of Stress Defence Responses Contributes to the Higher Toxicity of a Pesticide Under Warming

V. Delnat, KU Leuven / Biology; J. Swaegers, KU Leuven / Evolutionary Stress Ecology and Ecotoxicology; J. Asselman, Ghent University / Blue Growth Research Lab; R. Stoks, KU Leuven / Biology - Evolutionary Stress Ecology and Ecotoxicology

There is a pressing need to identify the molecular mechanisms underlying the, often magnifying, interactive effects between contaminants and natural stressors. Here we test our hypothesis that lower general stress defence responses contribute to synergistic interactions between stressors. We focus on the widespread pattern that many contaminants are more toxic at higher temperatures. Specifically, we tested the effects of an environmentally realistic low-effect and high-effect concentration of the pesticide chlorpyrifos under warming at the gene expression level in the mosquito *Culex pipiens*. By applying the independent action model for combined stressors on RNA-sequencing data, we identified interactive gene expression patterns under combined exposure to chlorpyrifos and warming for general stress defence responses: protection of macromolecules, antioxidant processes, detoxification and energy metabolism/allocation. Most of these general stress defence response genes showed upregulated antagonistic interactions (i.e., were less upregulated than expected under the independent action model). This indicates that when pesticide exposure was combined with warming, the general stress defence responses were no longer buffering increased stress levels, which may contribute to a higher sensitivity to toxicants under warming. These upregulated antagonistic interactions were stronger for the high-effect chlorpyrifos concentration, indicating that exposure to this concentration under warming was most stressful. Our results highlight that quantitative analysis of the frequency and strength of the interaction types of general stress defence response genes, specifically focusing on antagonistic upregulations and synergistic downregulations, may advance our understanding of how natural stressors modify the toxicity of contaminants.

2.08.06

Higher Mean and Fluctuating Temperatures Jointly Determine the Impact of the Pesticide Chlorpyrifos on the Growth Rate and Leaf Consumption of a Freshwater Isopod

C. Theys, KU Leuven; J. Verheyen, KU Leuven / Biology; N. Tüzün, R. Stoks, KU Leuven / Biology - Evolutionary Stress Ecology and Ecotoxicology

There is growing evidence that both increases in mean temperature and the widespread daily temperature fluctuations (DTF) may increase pesticide toxicity. Nevertheless, the likely more stressful, realistic combination of the two warming-related stressors has rarely been considered in ecotoxicology. Moreover, we have little knowledge on whether these stressor combinations could impair ecosystem functioning. We examined the effect of the pesticide chlorpyrifos under an increased mean temperature (+4 °C, from 18 °C to 22 °C) and in the presence of DTF (constant and 8 °C) on two life-history traits (mortality and growth rate) and one ecologically important behavioural trait (feeding rate) in the freshwater isopod *Asellus aquaticus*. The chlorpyrifos concentration used, 0.2 µg/L, did not cause mortality in any thermal condition, nor did it cause sublethal effects at the mean temperature of 18 °C. A key finding was that growth rate was strongly reduced by the pesticide only under the combination of both a higher mean temperature and DTF, highlighting the importance of testing toxicity under this realistic thermal scenario. The leaf consumption of chlorpyrifos-exposed isopods increased at the higher mean temperature when this was kept constant, however, it lowered again towards control levels when DTF was induced, thereby contributing to the growth reduction at this most stressful condition. These alterations of growth and leaf degradation rates may impact nutrient recycling, a key ecosystem function. Our results highlight the importance of integrating both increases in mean temperature and in DTF to improve current and future ecological risk assessment of pesticides.

2.08.07

Facing Future Temperature Extremes and Changes: How DO Freshwater Ecosystems Under Chemical Stress Respond to Reoccurring Heatwaves and Increased Temperatures?

M. Hermann, E. Peeters, Wageningen University & Research / Department of Environmental Sciences; P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences

Environmental alterations caused by a global changing climate is projected to push freshwater ecosystems and the various inhabiting forms of life outside of their thermal tolerance range. With an ongoing increase in global mean temperatures and more severe and frequent extreme events (i.e. heatwaves) expected until the end of the 21st century, aquatic ecosystems will face unprecedented environmental conditions. A strong decline in freshwater species documented over the past years indicate potential impacts of these natural pressures with, yet, unforeseen consequences due to unknown interactions with other anthropogenic stressors (i.e. chemicals). Most multiple stressor studies conduct experiments with single species and constant elevated temperature regimes. Population or community-level approaches including elevated, daily temperature fluctuations and thus natural variability are scarce. However, ecosystem complexity by considering intra- and interspecific interactions of species at the same or adjoining trophic level plus the variability factor in temperature are crucial aspects if we want to study chemical effects under environmentally relevant climate change scenarios.

The study objective was to investigate the single and combined effects of future climate change scenarios on zooplankton-dominated freshwater ecosystems under chemical stress. Temperature variability and extreme events were operated by the Transportable temperature and heatwave control engine (TENTACLE). All temperature scenarios were replicated five times with or without the fungicide carbendazim in indoor microcosms. We studied ten endpoints to comprehend changes in the abundance of benthic and planktonic species, community structure and ecosystem functioning.

Results revealed a significant decline in the abundance of macro- and micro-zooplankton species after a heatwave under chemical stress. Within the community, copepods seem to be less sensitive towards thermal and chemical stress than crustaceans, while rotifers indicated high sensitivity for the combined stressors. Indirect effects through changes in physico-chemical variables (e.g. dissolved oxygen concentrations) may explain significant changes in the abundances of the benthic species Gammarus pulex, Asellus aquaticus, Lymnaea stagnalis. Further significant effects on primary producers were detrimental and differences in microbial degradation highlight the need for novel experimental approaches in the context of climate change.

2.08.11

Pyrethroid Effects on Behavior of Early Larvae of an Endangered Fish Species Across a Salinity Gradient

A.E. Segarra, University of California Davis / Anatomy, Physiology & Cell Biology; F. Mauduit, R.E. Connon, University of California, Davis / School of Veterinary Medicine Anatomy, Physiology and Cell Biology; S.M. Brander, Oregon State University / Environmental and Molecular Toxicology

Climate change impacts are predicted to alter abiotic conditions which may potentially increase environmental stress on native species. Climate-related global sea level rise and increased duration of drought periods results in salinity intrusions in coastal areas. Salinity interacts with chemical properties of organic compounds and modulates their toxicity. Studies have shown that the fraction of pyrethroid pesticides in the aqueous phase increases with increasing salinity, potentially increasing the risk of exposure at higher salinities. Pyrethroid pesticides are commonly detected in waterbodies worldwide. In the San Francisco Bay Delta (SFBD) estuary, pyrethroid concentrations increase during the rainy season coinciding with the spawning season of Delta smelt (DS, *Hypomesus transpacificus*), an endangered, endemic fish. Detections of permethrin and bifenthrin are of concern as they relate to their early larval development. Given the lipophilic nature of pyrethroids, these insecticides are readily absorbed and bioaccumulated in tissues with a high lipid content such as larval yolk-sacs. Therefore, examining the salinity effect on the toxicity of pyrethroids is essential for risk assessments, especially on early life stages of estuarine fish. To evaluate this, we investigated behavioral effects of the permethrin and bifenthrin at three environmentally relevant concentrations (0.1 - 100 ng/L) across a salinity gradient (0.5, 2, and 6 PSU) on DS yolk-sac larvae. Behavioral alterations were studied by using a light and dark locomotion test. Our data demonstrate significant hypoactivity for larvae exposed to permethrin at low concentrations and low salinity while those exposed at high concentrations and high salinity exhibited hyperactivity. In parallel, bifenthrin caused a hyperactivity at all concentrations and salinities, and anti-thigmotaxis (center preference) positively correlated with the salinity. These results suggest that environmentally relevant pyrethroids concentrations can perturb DS larvae behaviour even at the lowest concentrations (sub ng/L) and that salinity can change the dynamic of pyrethroids toxicity in terms of behavioral effects. Understanding the biological consequences of interactions between salinity and contaminants in estuarine systems provides essential management information for the conservation of species of concern.

2.08.12

Salinity Influences Toxicity and Toxic Effects of Biocides in a Model, Estuarine Fish Species (Menidia beryllina)

S. Hutton, Oregon State University / Environmental and Molecular Toxicology; S. Siddiqui, E.I. Pederson, Oregon State University / Department of Fisheries and Wildlife; S.J. St. Romain, Louisiana State University; A.E. Segarra, University of California Davis / Anatomy, Physiology & Cell Biology; K. Armbrust, Louisiana State University / Environmental Sciences; M.L. Hladik, U.S. Geological Survey / Water; R.E. Connon, University of California, Davis / School of Veterinary Medicine Anatomy, Physiology and Cell Biology; S.M. Brander, Oregon State University / Environmental and Molecular Toxicology

Increasing salinity in estuaries due to sea level rise as a result of climate change has the potential to influence organisms' interactions with aquatic pollutants and alter their toxicity. As ionic concentration increases, the hydrophobicity and water solubility of some compounds have been shown to increase and decrease respectively. Findings from recent studies in our group show that LC_{50} and LC_{10} values in euryhaline Inland silverside (Menidia beryllina) exposed to biocides decreased, or became more toxic, with increased salinity (5 PSU to 15 PSU). This highlights the need to understand the influence of salinity on biocide toxicity when assessing risk to fish species that inhabit estuaries for part or all of their lives, particularly in sensitive early life stages. For example, early life exposures to pyrethroid pesticides can cause toxicity at environmentally relevant concentrations (1 ng/L), such as reduced hatching and survival as well as increased morphological deformities and alterations in behaviour. Endpoints such as behavior and gene expression are sensitive and can detect the effects of environmentally relevant concentrations of pollutants. As such, we have exposed the Inland silverside during the first few day's post-hatch to environmentally relevant concentrations of pyrethroid pesticides across a salinity gradient, followed by an assessment of potential changes in behaviour and gene expression. Here we present results from behavioral analysis of Inland silverside parent generations exposed to the pyrethroids insecticides. Preliminary results indicated significant behavioural changes when fish were exposed to light with increasing salinity and pyrethroid exposure. Additionally, we will rear Inland silverside in clean water to spawn and further evaluate the effects of early life exposure in parent (F0) generations on their offspring (F1). These data will provide knowledge to managers and environmental planners to help further protect threatened and endangered fishes in estuaries and bay regions threatened by climate change.

2.08.13

Effects of Environmentally Relevant Micro and Nano Plastics (Polypropylene and Polylactic Acid) Particles on Estuarine Indicator Species

S. Siddiqui, Oregon State University / Environmental & Molecular Toxicology; J.M. Dickens, Oregon State University / CEOAS; B. Cunningham, S. Hutton, Oregon State University / Environmental and Molecular Toxicology; E.I. Pederson, Oregon State University / Department of Fisheries and Wildlife; S.L. Harper, Oregon State University / EMT; B.J. Harper, Oregon State University / Department of Environmental and Molecular Toxicology; S.M. Brander, Oregon State University / Environmental and Molecular Toxicology

With increasing use of plastics since 1950s plastics overtook the global market causing increased concentration in estuaries and coastal waters that may cause potential adverse health impacts on aquatic biota. With time plastics may undergo various physical changes and wear down resulting in micro and nano size plastic particles and mix into marine debris and could potentially take up by marine biota and potentially cause adverse health impacts. In this study polypropylene (PP) and polylactic acids (PLA) have been used to represent marine debris at micro and nano size. PLA is a newer "bio-plastic" that is suggested to be safer for use. The estuarine indicator fish *Menidia beryllina* and invertebrate *Americanmysis bahia* were exposed at three salinities (5, 15, 25 ppt for fish / 15, 20, 25 ppt for mysid shrimp). Results indicates significant differences in behaviour in highest and lowest salinities for *Menidia beryllina*. Here we present partial results from fish behaviour study. This work in its entirety, to be completed in both organisms by the end of 2020, utilizes behavioral responses, which are shown to be highly sensitive to micro and nanoplastic exposure8 to fill gaps in potential impacts of plastic debris in estuarine organisms. These data will be used in a risk assessment framework for aquatic organisms' exposure to environmentally relevant concentrations across a wide range of salinities and other conditions.

2.08.14

Biochemical Effects of Organic and Inorganic Compounds, at Different Temperature Scenarios, on the Diatom Thalassiosira weissflogii

A. Mesquita, Department of Biology & CESAM - University of Aveiro / Department of Biology and CESAM; F.J. Gonçalves, University of Aveiro / Department of Biology and CESAM; J.C. Marques, University of Coimbra, MARE - Marine and Environmental Sciences Centre / Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal; A. Goncalves, MARE - Marine and Environmental Sciences Centre, University of Coimbra / Life Sciences

The exponential increase of human population demands an over usage of fertilizers and pesticides in agriculture practices to supress the food production needs, with dangerous effects to the ecosystems (e.g. biodiversity losses) and damage to the public health. Oxyfluorfen is a fluorine-based herbicide, non-selective and of broad-spectrum. In the last years, its application has increased since it is seen as an alternative to control the glyphosate resistant weeds. Copper sulphate is an inorganic compound used in pesticide products, being noted that it is the 2nd main constituent of fungicides. The chemicals discharge on aquatic systems have impacts to these ecosystems and its communities, from primary producers until to top consumers. *Thalassiosira weissflogii* is a marine diatom, with a key ecological role, greatly used as food source to zooplankton. Alterations on its populations and nutritional value may lead to consequences along the trophic chain. Few studies have evaluated biochemical impacts on the diatom species when exposed to these chemicals. So, this study intends to evaluate the effects of oxyfluorfen and copper sulphate on the growth rate of *T. weissflogii* , at three temperatures (15ºC, 20ºC and 25ºC), considering the actual scenario of climatic changes, and to access the biochemical changes through the analysis of fatty acids (FA) and carbohydrate profiles. *T. weissflogii* was exposed to copper sulphate and to oxyfluorfen at three distinct temperatures, for 96 hours, to determine the effects on the growth rate. Considering the ECs values, sub-lethal bioassays were performed by 7 days to analyse the changes at the biochemical level. An increase of growth rate was observed to both chemicals with the temperature raise . Moreover, considering the ECs values, the oxyfluorfen showed to be more toxic than copper sulphate to the marine diatom. On the other hand, at the biochemical level, the microalga was greatest affected when exposed to oxyfluorfen at 20ºC and 25ºC, exhibiting a decrease on the FA content and when exposed to copper sulphate at 15ºC, showing a decrease of the unsaturated fatty acids, including the essential FA (e.g. EPA and DHA) and an increase of the saturated fatty acids. Carbohydrate profile was also assessed, with calculations being ongoing to further understand the biochemical impacts in the diatom species and at its nutritive value when exposed to the tested conditions.

2.08.16

Using Definitive Screening Design to Conduct Complex Experimental Testing of Chemical and Non-Chemical Mixtures

C. Eastabrook, Newcastle University / School of Natural and Environmental Sciences; K. Petersen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; E. Jarosz, Norwegian University of Life Sciences NMBU; L. Xie, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; Y. Song, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; R. Wolf, NIVA - Norwegian Institute for Water Research / Section for Ecotoxicology and Risk

Assessment; G.S. Caldwell, Newcastle University / School of Natural and Environmental Sciences; H. Teien, Norwegian University of Life Sciences NMBU / Centre for Environmental Radioactivity (CERAD); K. Tollefsen, Norwegian Institute for Water Research (NIVA) / Computational Toxicology Program
Marine organisms in coastal environments experience strong impacts from chemical pollution, further exacerbated by climate change stressors. The potential impacts of chemical mixtures and non-chemical stressors such as UV are largely unknown. As mixture experiments are complex and time-consuming, regulatory guidelines can often be based on single effect data and concentration addition (CA) modelling assuming an additive response. However, assumed additivity could cause synergistic or antagonistic effects to be overlooked. Using a widely studied and ecologically important marine copepod *Tisbe battagliai*, the present study aimed to: (1) individually characterise and develop toxicity pathways for the effects of three trace metals (copper, zinc and nickel chlorides) and UVB radiation at different levels of biological organisation; (2) conduct a combined toxicity assessment of trace metals; and (3) conduct a combined toxicity assessment of trace metals with UVB radiation. We used a Design of Experiments technique (Definitive Screening Design; DSD) to conduct a combined toxicity assessment using the least number of experimental runs, thereby saving time and resources, notably reducing chemical and animal use. To quantify the toxicity pathway, endpoints including *in situ* reactive oxygen species (ROS) formation, cyclobutene pyrimidine dimers (DNA lesions), changes in gene transcription (RT-qPCR) and survival were conducted. Predicted combined effects were made using CA and independent action models, using data from single stressors. These predictions were compared to observed effects from combined experiments. This experimental mixture design used LC_{10}, LC_{50}, and LC_{80} values from single stressors. The observed effects from the combined toxicity study identified zinc as having the significant main effect ($P < 0.05$) on survival. ROS increased 10-fold after 48-hours when all metals were in solution at their highest concentrations. The joint effect of the metals on ROS formation was higher than the individual metal effects (copper 5-fold, zinc 2-fold and nickel showed little change). The results show that the use of DSD experimental design for combined toxicity assessment can inform on the nature of the combined effects (additivity, synergy, antagonism) without the need for additivity modelling. The resource and time saving aspect of DSDs, especially reducing the number of animals required, makes them a noteworthy route for effect assessments of multiple stressors.

2.08.17
Drivers of Copper Sensitivity in Copepods - a Meta-Analysis of LC50s
J. Heuschele, T. Lode, University of Oslo / Department of Biosciences; H. Konestabo, Department of Bioscences, University of Oslo / Department of Biosciences; T. Jager, DEBtox Research; J. Titelman, T. Andersen, K. Borgå, University of Oslo / Department of Biosciences
Copper is both an essential trace element and a potent pesticide, which in aquatic environments also affects non-target species, such as copepods that constitute one of the critical links in marine food webs.The Lethal Concentration 50 (LC50) is the exposure concentration where 50% of the test organisms are dead after a specific exposure time. The LC50 is a traditional endpoint for toxicity testing, to rank chemicals, and to compare species sensitivity. It is used in standardised toxicity testing, but is also an endpoint in many ecological studies. Here,we combine literature data from 32 peer-reviewed studies recording LC50 for copper in copepods and the respective environmental, developmental, and taxonomic parameters during the experiments. The variability in the metadata allowed for a general analysis of the drivers of copper sensitivity in copepods.Using a generalised additive modelling (GAM) approach, we found that temperature affected copper sensitivity only at higher temperatures by increasing toxicity. As expected, increasing salinity reduces copper sensitivity, but only to a threshold level beyond which a further rise in salinity led to increased sensitivity. Unsurprisingly, nauplii are more susceptible to copper exposure than adult copepods. The resulting model can be used to predict copper concentration ranges for future experiments, but also to generalise findings from one species or compare copper sensitivity between phylogenetic orders of copepods. Our approach can easily be applied to other toxicants and taxa, and might reveal underlying patterns that are otherwise obscured by taxonomic and experimental variability. This way we can have an informed analytical approach to range testing in future dose-response experiments.

Soil Ecotoxicology: New Methods and Novel Applications in Environmental Risk Assessment

2.09.01
Closing the Gaps in the Pesticide Risk Assessment for Soil Organisms
S. Pieper, German Federal Environment Agency (UBA) / Plant Protection Products; P. Kotschik, German Federal Environment Agency UBA / Plant Protection Products
In the daily work of risk assessors, decisions have to be taken in order to conclude on whether intended uses of plant protection products (PPP) can be authorized or not. The task is to implement General Protection Goals as laid down in Regulation EC 1107/2009. Protection Goals are the same for every PPP and at every step of the risk assessment: no unacceptable effects on the environment, taking particularly the impact on non-target species, biodiversity and the ecosystem into account. The risk assessment for soil organisms exposed to PPP is performed in a tiered approach, which follows a set of agreed criteria. The question is whether the identified gaps in the available data have been closed in the last years and if the outcome of the risk assessment for soil organisms leaves the risk assessor well confident to have implemented the General Protection Goals set by legislation or not. Until Specific Protection Goal options are newly agreed, the guidance in place dates back to 2002. To further complicate the issue, data requirements for pesticides have been updated in 2013. The consequences of the missing alignment of tools and provisions for the assessment of PPP under current requirements have been analysed. Several issues have been identified as problematic, i.e. as possibly leading to higher risk in the field than concluded in the risk assessment: the use of standard artificial soils that do not resemble agricultural field soils; the assumptions on the ecotoxcologically relevant type of concentration; the detection power of field tests with soil organisms and the respective consequences for the protection goal; missing to adress indirect effects on soil organism communities. Decisions on chemical authorization regarding the protection of soil organisms are therefore currently accompanied by major uncertainties. Since the assessment/uncertainty factor for the first tier risk assessment step is not too large (AF = 5), we might often conclude on low risks following PPP intended uses due to drawbacks in the risk assessment. Correct hazard identification and risk assessment would possibly identify higher risks for soil organisms. The contribution of scientific work to better regulatory decisions cannot be stressed enough. If PPP impacts results from intended uses of pesticides (e.g. in-field), then acceptability of risks needs to be agreed also with public stakeholders, based on transparent and effective risk communication efforts.

2.09.02
Uncertainties in the HIGHER Tier Risk Assessment for Soil Mesofauna
P. Kotschik, Umweltbundesamt / Federal Agency of Environment / Risk assessment for plant protection products; S. Pieper, German Federal Environment Agency (UBA) / Plant Protection Products
The first tier risk assessment for in soil organisms exposed to plant protection products is based on standardized laboratory tests with earthworms [1], collembolans [2] and mites [3] as well as predicted environmental concentrations in combination with an assessmentfactor. The scale of the the assessment factor is regulated in EU Nr. 546/2017 [4]. For some substances, collembolans and mites do show a higher senstivity than earthworms, resulting in an unacceptable risk at the first step of the risk assessment. Based on the current guidance document for terrestrial organisms [5] an unacceptable risk for collembolans or mites can be relieved in the higher tier risk assessment by a litter bag test. However, EFSA as well as the scientific committee do question the suitabilty of functional tests in order to adress risks on structural level as stated in the Scientific opinion for in soil organisms [6]. Consequently, field tests on collembolans and mites are conducted for substances, indicating an unacceptable risk in the first step of the risk assessment in the regulatory praxis. At the moment, guidance is given for the sampling of in soil mesofauna [7] but no official guidance is available for the conduction of field studies on soil mesofauna, exposed to chemicals. Therefore, these field studies are performed similarly to earthworm field studies where guidance is given on test site selection, setup and performance [8,9]. However, no requirenments on the statistical power of field tests with soil meso-organisms are currently in place. Based on the existing guidance on earthworm field studies, appropriate advice can be given for the conduction of field studies with soil mesofauna. However, some amendments are necessary and some open points have been identified. Therefore, we recommend future research regarding - the detection of natural abundance and community composition of soil mesofauna, - the statistical evaluation as well as - the current study set up. Last but not least, a new OECD guideline for field studies with soil mesofauna needs to be developed and finally, the prediction of observed effects on soil mesofauna at one special site towards other sites / fields has to be tackled in the upcoming guidance document.

2.09.04
From Standard to Alternative Tests for Hazard Assessment: Lessons Learned From Novel Materials Testing in Soil - a Case Study With E. crypticus
S.I. Gomes, CESAM & DeBio, University of Aveiro / CESAM & Department of Biology; J.J. Scott-Fordsmand, Aarhus University / Biosciences Department; M. Amorim, Universidade de Aveiro / Biology &CESAM
Over the last 20 years, with the emergence of novel technologies and the mass production of materials other than chemical substances, e.g. nanomaterials (NMs), plastics, etc., it has become clear that previously developed (ISO/OECD) standard guidelines either need adaptations or new ones are required. There has been much progress, proposals range from extensions to the OECD standard tests to alternative tests, e.g. test where omics provide mechanistic understanding,

additional endpoints and knowledge. There is strong regulatory support for New Approach Methodologies (NAMs), and its added-value is well recognized. In the present we have collected the available literature [using the search engine Web of Science (WoS), the database WoS Core Collection, in the Basic Research mode, with the keywords: nano*, soil, toxicity, ecotox*, gene, and biomarkers in different combinations] on NAMs to assess hazards of materials. We focus on the terrestrial environment and discuss the advantages, challenges and gaps, while outlining recommendations and way forward. Even though omics are not yet applied or meet standard information requirements for regulatory hazard and risk assessment, their integration within a system toxicology approach has potential to improve several layers of the process. We here use a case study to identify the status and advantages, using the soil standard species *Enchytraeus crypticus*, exposed to Cu/CuO NMs, for which endpoints covering the different levels of biological organization are available. The results are presented in integrated way. Overall, it was clear that even though standard tests have an unquestionable place, progress could be made promoting chronic type tests (in detriment of acute survival). Standard extensions represent clearly show-cases of the importance of longer-term exposure, and should benefit from facilitated implementation (via addition to current guidelines as annexes). Alternative tests, such as omics endpoints give in-depth mechanistic understanding, and the potential to predict effects at an earlier stage. The selection of time points at which effects are monitored is a challenge that needs to be addressed to ensure the capture of the key initiating events. One of the major benefits lays on the integration of the information from the various levels is the ability to develop the Adverse Outcome Pathways (AOPs) concept as outlined and recommended by OECD.

2.09.05

Alive and Kicking? - Earthworm Cocoon Test for TKTD Modelling in Soil Risk Assessment of Chemicals

K. Rakel, Research Institute gaiac / gaiac- Research Institute for Ecosystem Analysis und Assessment; G. Ernst, Bayer Ag / Ecotoxicology; A. Gergs, Bayer Ag / Research & Development, Crop Science Effect Modelling

In soil risk assessment, toxicokinetic-toxicodynamic (TKTD) modelling is considered to be not yet ready for use. Reasons include the heterogeneous environment, the more complex sampling methods which make it challenging to measure endpoints over time and unclear exposure. Especially data on biological data like growth and reproduction observed for multiple time points is scarce and chemical measurements are not required in the standard tests. However, TKTD modelling may provide a meaningful refinement also in soil risk assessments. Currently used in risk assessment for earthworms is the earthworm reproduction test OECD 222. We developed a test design which is aligned with the existing test setup of OECD 222 and, with small adaptions, provides data which are needed for TKTD modelling. In this study we show an example of how this new data with carbendazim and *Eisenia fetida* can feed a Dynamic Energy Budget Theory (DEB)-TKTD model and its possible future use in risk assessment. On basis of the existing entry for *E. fetida* in the Add-my-Pet database, different physiological modes of actions were implemented to allow for parameterisation of the sublethal effects. The new cocoon test showed a dose-response relationship for growth and reproductive performance of *E. fetida*. The parameterized model was able to describe the patterns observed in the experiments. Increased somatic and maturity maintenance costs were assumed to be the primary mode of action resulting in reduced growth and production of neonates. As the number of juveniles per cocoon decreased with increasing exposure concentrations, hazard during oogenesis was employed as a secondary mode of action. The proposed earthworm cocoon test with its temporally resolved biological data (growth and reproduction) enables the use of DEB-TKTD modelling in soil risk assessment and can serve as possible refinement step. With this kind of modelling that modes of action of a substance can be discovered and well described. The existing OECD 222 provides the information for the model validation.

2.09.07

Effect-Based Tools in ERA of Hydrochar: The Application of the DR CALUX® Bioassay in Screening Dioxin and Dioxin-Like Compounds

G. Liberatori, University of Siena / Department of Physical, Earth and Environmental Sciences; F. Ferraro, pH TÜV Italia srl; C. Mazzoli, University of Siena / Physical, Earth and Environmental Sciences; L. Sturba, University of Siena / Department of Physical Earth and Environmental Sciences; M. Vannuccini, D. Baroni, University of Siena / Physical, Earth and Environmental Sciences; P.A. Behnisch, BioDetection Systems; S. Vitolo, M. Puccini, University of Pisa / Department of Civil and Industrial Engineering; R. Gori, University of Florence / Department of Civil & Environmental Engineering; I. Corsi, University of Siena / Physical, Earth and Environmental Sciences

Hydrothermal carbonization (HTC) represents an eco-friendly thermochemical process by which what is consider a “waste” as sewage sludge (SL) can be converted and re-used as a precious carbon resource, the hydrochar (HC). However, the presence of unwanted toxic chemicals in HC, could determine an interference in their potential future usage as a soil amendment. With the aim to define suitable tools for ecological risk assessment (ERA) of HC which will include also environmental scenarios of their application, bioanalytical tools could be promoted being largely used in environmental monitoring and assessment of complex environmental samples. In the present study the suitability of the DR CALUX® (Dioxin Responsive, Chemically Activated LUciferase gene eXpression) bioassay to screen the occurrence of Polychlorinated dibenzo-*p*-dioxins (PCDDs), Polychlorinated dibenzofurans (PCDFs), dioxin-like Polychlorinated biphenyls (dl-PCBs) in HC and related SL sample, was investigated. Although DR CALUX® has been extensively used to screen a large variety of environmental samples including wastewaters, to date, no studies have been conducted so far on HC samples. At first a new method for the extraction and purification of this complex matrix was developed. Then, aliquots of urban SL, collected from (n=6) wastewater treatment plants (WWTPs) were thermochemically processed by HTC, under controlled laboratory conditions. DR CALUX® Bioanalytical Toxicity Equivalents (BEQ) values found in HC samples resulted one order of magnitude higher in the HC matrix with respect to the SL ones. For both matrices, BEQ values detected in the *dl*-PCBs fraction, resulted one order of magnitude higher than PCDD/Fs one. Bioassay results were compared with chemical analysis performed with Gas Chromatography-Mass Spectrometry and a high correlation (R_S= 0.8252, $p < 0.001$; R_P=0.8029, $p < 0.01$) between BEQ and TEQ (Toxic Equivalency) values was found. BEQ and TEQ values showed the same trend in both matrices, however, BEQ resulted one order of magnitude higher with respect to TEQ for all the WWTPs analysed. Moreover, the different aerobic or anaerobic biological treatment of the SL feedstock, influenced the BEQ values in both HC and SL matrices. DR CALUX® has been proven to be an integrated state-of-the-art tool, capable of characterizing the real toxicity of complex mixtures of contaminants that occur in environmental samples.

2.09.13

Clay Type Matters: Toxicity and Bioaccumulation of Copper Oxide Nanoparticles to Springtails in Artificial Soils

J. Fischer, G.A. Talal, J. Filser, University of Bremen / Center for Environmental Research and Sustainable Technology UFT

Copper oxide nanoparticles (CuO-NP) are applied as an efficient alternative to conventional Cu in agriculture and are expected to increase substantially, ending up in the soil environment. Most studies indicate no toxicity of CuO-NP towards soil invertebrates even at very high concentrations in sandy soils. However, we recently found medium to strong effects on the springtail *Folsomia candida* at field-realistic concentrations, but only in loamy field soils. In the current study, we tried to ascribe the impact on CuO-NP toxicity to single clay types. We produced four different artificial OECD test soils which contained either 20 or 30% of clay and either kaolin or montmorillonite as clay type. We spiked these soils with uncoated CuO-NP (> 50 nm) or $CuCl_2$ at 1-30 mg Cu/kg dry soil, respectively. We performed 28-day reproduction tests and additionally recorded survival, dry weight and Cu content of the individuals introduced at test start. Negative effects were only observed in montmorillonite soils and only for CuO-NP. In the 30% montmorillonite soil, survival was reduced by 33% at 3 mg/kg and by 52% at 1 mg/kg. In all kaolin containing soils, dry weight was increased by 20-90% for most treatments. Cu body content of springtails was nearly similar for CuO-NP and $CuCl_2$, but Cu levels in kaolin soils were generally higher than in montmorillonite soils and they differed significantly from the control at lower concentrations (3 vs. 10 mg/kg). The discrepancy between observed toxic effects and measured Cu body content may be explained by moulting, which is an important detoxification mechanism in springtails. However, elevated moulting would also consume energy and resources that cannot be invested in reproduction and growth. We propose to focus on clay-NP interactions for a comprehensive risk assessment of CuO-NP in the soil environment.

2.09.16

Assessment and Management of Transfer of Persistent Organic Pollutants From Soil to Outdoor Reared Animals

F. Amutova, al-Farabi Kazakh National University; N. El Wanny, Université de Lorraine - UdL; C. Feidt, University of Lorraine - UdL / INRA URAFPA; G. Konuspayeva, al-Farabi Kazakh National University / Faculty of biology and biotechnology; S. Jurjanz, Université de Lorraine UL / URAFPA INRA; M. Delannoy, Université de Lorraine - UdL / INRA URAFPA

Foods of animal origin are well-known contributors of human exposure to POPs due to their lipophilicity. Soil ingestion represents in this context one of the main exposure pathways of outdoor reared animals as it acts as a POPs reservoir. That would raise the question to quantify their transfer into the food these animals would produce. Therefore, the aim of the current study was to estimate the transfer of POPs into food of animal origin using i) summarizing published knowledge on POP transfer by a meta-analysis; ii) evaluate the transfer of the emerging pesticide chlordecone (CLD) using new data of an *in vivo* study. Finally, these outcomes are used to assess the risk and, if necessary, to manage using a remediation strategy to limit their transfer.Transfer was quantified using transfer rate (TR) for excreted milk and eggs and bioconcentration factor (BCF) for edible tissues.The most toxic compounds such as 2,3,7,8-TCDD (34% and 39%), 1,2,3,7,8-PeCDD (27% and

36%), 2,3,4,7,8-PeCDF (36% and 40%) were highly transferred into milk and eggs respectively. Dioxin-like PCBs were also shown as extremely transferred with up to 50% of TR for congeners 105, 114, 118, 153, 156, 157, 167, 180, 189 and up to 40% of TR for congeners 126, 148 and 169 in both milk and eggs.TRs of DDTs and HCHs to chicken eggs were up to 58% and up to 32% respectively. These two groups of pesticides could also accumulate in similar extent in the chicken gizzard (BCF 17 and 5), liver (BCF 15 and 16) and muscle (BCF 3 and 2).*In vivo* study with laying hens showed very high TRs to eggs when exposed to CLD contaminated andosol or nitisol with respectively 78% and 65% as well as BCFs in liver of 6 and 2. Amendment of contaminated soil with AC allow to decrease the transfer of CLD to chicken eggs from 65% to 45% (nitisol) and from 78% to 52% (andosol). In addition, BCF of CLD to liver reduced also from 2,4 to 1,5 (nitisol) and from 6 to 4 (andosol). POPs are generally highly transferred in food producing animals when they are raised on contaminated soils. Specific methodologies as TRs and BCFs allow to quantify and ranking risk focusing on food safety providing a relevant assessment. In this frame, it allows to derive safe levels in soil in order to sustain animal rearing practices in contaminated areas. When a management strategy is needed, an amendment of contaminated soils with sequestration materials like ACs may be investigated to reduce this POP transfer.

2.09.24

Lipids and Life Stage Influence in the Reproductive Decline in C. Elegans Exposed to Ionizing Radiation

E. Dufourcq Sekatcheff, IRSN-Institute for radioprotection and nuclear safety; S. Cuine, CEA- Commissariat à l'Energie Atomique et aux energies Alternatives / Department of Biotechnologies - BIAM - EBM; Y. Li-Beisson, CEA / Department of Biotechnologies - BIAM - EBM; L. Quevarec, IRSN-Institute for radioprotection and nuclear safety / Department of Biology and Ecology Laboratory of Ecotoxicology LECO; C. Lecomte-Pradines, Institut de Radioprotection et de Sûreté Nucléaire / Department of Biology and Ecology Laboratory of Ecotoxicology LECO; S. Galas, Université de Montpellier; S. Frelon, IRSN / Department of Biology and Ecology Laboratory of Ecotoxicology LECO

Natural and artificial ionizing radiations represent a continuous stress for the environment. Improving the environmental risk assessment of exposure to such radiations relies on the comprehension of radioinduced biological effects. To date, among the ecologically relevant parameters directly influencing population dynamics, the reproduction endpoint seems to be the most radiosensitive for many invertebrate species. Especially, chronic exposure from egg to young adult (YA) stage of *Caenorhabditis elegans*, an hermaphroditic round worm of soil, has shown a significant loss in their reproductive ability, *i.e.* decreased broodsize associated with effects on the germline such as cell cycle arrest, decrease in spermatocyte number and a misregulation of some proteins and genes involved in the reproduction process and lipid metabolism. Among these, the vitellogenins which are lipid transporters involved in the maturation of oocytes were found to be overexpressed. Therefore, to go further and elucidate the underlying mechanisms of such radioinduced reprotoxicity, this study aims at determining which developmental stage, *e.g.* embryogenesis, gonadogenesis, and whole development, is the most radiosensitive regarding the reproductive endpoints. In addition, due to previous evidence and the known relationship between reproduction and fat metabolism, a particular focus was made on lipid modulation after irradiation. Nematodes from the N2 hermaphrodite strain were chronically exposed to a ^{137}Cs source at 50 $mGy.h^{-1}$ throughout three different life stages *i.e.* embryogenesis, gonadogenesis preceding meiosis, and full development, respectively. All of them were analyzed at L4-YA stage. The broodsize and hatching success were measured during 8 days following irradiation and a lipid analysis was conducted to characterize the amount of neutral and polar lipids (HPTLC) and fatty acids (GCMS/FID). Our results showed more or less severe response in reproduction and opposite responses in lipid metabolism, suggesting different mechanisms occurring at different irradiated stages. Fatty acids revealed new hypothesis regarding possible effects on nutrient assimilation, food intake and longevity. We concluded that these different responses could be due to opposite signaling from the germline and the soma of nematodes, contributing to maintain lipid homeostasis and more globally to maintain its physiological functions linked to fitness (reproduction, lifespan and growth).

Soil Function and Biodiversity: Impacts and Resilience under Stressed Environments

2.10.03

Toxicological Effects of Single and Binary Mixtures of Nanoparticles on Enchytraeus crypticus

J. Santos, University of Aveiro / Biology Department & CESAM; .A. Barreto, University of Aveiro; M. Amorim, Universidade de Aveiro / Biology &CESAM; V. Maria, Biology Department of Aveiro University / Biology & CESAM

Nanoparticles (NPs) have a worldwide use for generating various consumer goods. Studies have reported NPs (e.g. silver and copper) toxicological effects on soil invertebrates, but for boron (B NPs) and vanadium (V NPs) the information is unknown. This work aimed to apply an ecologic-biochemical approach to investigate the effects of B and V NPs (80 to 100 nm) on the ecotoxicological model, *Enchytraeus crypticus*. Two single concentrations of 10 and 50 mg/kg (for both NPs) and the four possible binary mixtures were tested to evaluate the reproduction and survival (as ecological level) after 28 days of exposure in LUFA 2.2 soil. Physicochemical characterization of the B and V NPs, at the selected concentrations, was also performed. Afterward, sub-lethal concentrations of each NP (10 mg/kg) and the respective binary mixture was also applied to assess biochemical biomarkers responses related to oxidative damage (lipid peroxidation-LPO), antioxidant system (catalase-CAT, glutathione S-transferase-GST and glutathione reductase-GR activities) and neurotransmission (acetyl-cholinesterase-AChE activity). This multi-biomarker assay was performed after 3, 7, and 14 days of exposure in LUFA 2.2 soil. Single exposures of B and V NPs did not affect the organismsâ_ survival, except the highest tested concentration of V NPs. When in mixture with B NPs, 50 mg/kg of V NPs was also lethal. Therefore, the juveniles' number was also affected. In addition, the number of juveniles decreased at binary mixture with equal nominal concentrations, i.e., 10 mg/L of each NPs. B NPs earlier (3 days) induced oxidative damage, but a delay (7 days) was found for the binary mixture. B NPs also enhanced the antioxidant system, in general, increasing CAT and GR activities. V NPs were also determinants (i.e., inducers) for the reposition and consumption of glutathione by GR (as reduced glutathione) and GST (as oxidized glutathione), respectively. In the combined exposure, the activities of GR and GST also increased after 7 days. However, GR activity decreased over time (14 days), i.e., compared to single NPs and a mixture of 7 days exposure. Considering the neurotransmitter activity, the mixture earlier inhibited AChE, contrasting with V NPs single exposure that delayed this effect. The observed apical and downstream effects may reflect future perturbations in the soil ecosystems in terms of population dynamics and biodiversity, which must require our careful attention.

2.10.07

The Effects of Different Temperature in Mercury Toxicity to the Terrestrial Isopod Porcellionides pruinosus

R. Morgado, University of Aveiro / CESAM & Department of Biology; D. Nunes Cardoso, CESAM, University of Aveiro / CESAM & Department of Biology; M. Prodana, University of Aveiro / CESAM & Departament of Biology; A. Pereira, University of Aveiro / Department of Biology; A.R. Silva, University of Aveiro / Dept.of Biology & CESAM; A. Vinhas, University of Aveiro / Department of Biology; C.C. Malheiro, Department of Biology, University of Aveiro / Biology; A.M. Soares, Universidade de Aveiro / CESAM & Department of Biology; S. Loureiro, Universidade de Aveiro / Biology

Climate changes and contamination are two of the most pervasive stressors affecting soil ecosystems. The cumulative exposure to multiple stressors, such as xenobiotics under extreme or sub-optimal temperatures, is increasingly likely to occur. Mercury (Hg) is one of the most toxic heavy metals present in the environment, and its effects have often been reported to interact with temperature. Studies on the interaction between temperature and Hg toxicity or bioaccumulation are mostly limited on the aquatic ecosystems, while little is known about how temperature affects the toxicity of Hg contaminated soils. We performed a comprehensive array of experiments to find whether temperature influences the effects of Hg-contaminated soil on the terrestrial isopod *Porcellionides pruinosus*, as well as to unravel the processes involved. Toxicity and bioaccumulation experiments were replicated at 15ºC, 20 ºC, and 25 ºC to understand how sub-optimal temperatures affect the toxicokinetics and toxicodynamics of Hg via soil exposures. Genotoxicity and energy reserves were assessed using the same testing strategy to identify early warnings of impairments and alterations in life-history traits. Overall, our results did not denote a significant effect of temperature on the toxicity of Hg to *P. pruinosus*. Time-course survival revealed high mortality within the first few days of the experiment at the highest concentrations, followed by stabilization of mortality henceforth. This might be partly related to the toxicokinetics of Hg, which were also similar across temperatures. To cope with a very high uptake rate, organisms showed a fast elimination rate, reaching the steady-state within the 21 days of uptake, but mostly resorted to the storage of Hg in an inert form. The ability to cope with the initial uptake seems to be a critical determinant. Regarding comet assays, there seemed to be higher DNA damage caused by Hg exposures at 25 ºC. Although temperature itself increased the percentage of DNA damage in control isopods, an evident increase in genotoxicity was found in combination with Hg, even at the 6 mg Hg kg^{-1} soil. There seemed to be little temperature-mediated effects on *P. pruinosus* survival to Hg exposures. Similar toxicokinetics patterns, relying on fast elimination plus Hg storage in inert form, may be partly responsible for that. Although, genotoxicity by Hg seemed to increase with temperature. Temperature was the main factor causing changes in the energy reserves.

2.10.10

Ecotoxicity of Historically Metal(Loid)-Contaminated Soils to Folsomia Candida Under the Influence of Climate Alterations

A.R. Silva, University of Aveiro / Dept.of Biology & CESAM; C.C. Malheiro, Department of Biology, University of Aveiro / Biology; M. Prodana, University of Aveiro / CESAM & Departament of Biology; S. Loureiro, Universidade de Aveiro / Biology; M. Gonzalez-Alcaraz, Technical University of Cartagena / Agronomic Engineering

Global warming is drastically altering the climate conditions of our planet. Anthropogenic activities have led to a huge increase of greenhouse gas emissions, especially CO_2, leading to increasing global surface temperature and changes in the precipitation patterns. Soils will be among the most affected components of terrestrial ecosystems, especially in anthropogenic-contaminated areas where living organisms must deal with multiple stressors. This study aimed to assess if changes in single climate factors (air temperature, soil moisture and atmospheric CO_2) affect the toxicity of historically metal(loid)-contaminated soils to the soil invertebrate *Folsomia candida*. For this, ecotoxicity tests (survival and reproduction) with *F. candida* were performed in field soils from central-northern Portugal affected by anthropogenic metal(loid) contamination (mining soil: soil from a former mining district; agricultural soil: soil from a former agricultural area near an industrial chemical complex) under different climate change scenarios. The climate scenarios tested were: standard – conditions recommended by OECD guidelines (20 °C + 50% soil water holding capacity - WHC); increased daily air temperature (20-30°C + 50% WHC); increased (20 °C + 75% WHC) and decreased (20°C + 25% WHC) soil moisture content; increased atmospheric CO_2 levels (20°C + 50% WHC + 600/800/1000 ppm of CO_2, still in progress). When atmospheric CO_2 was not modulated, the concentrations inside the acclimatized chambers were ~400-500 ppm. Moreover, the aim was also to assess the recovery capacity of *F. candida* to the possible stress(es) induced by soil contaminations and/or climate alterations. For this, after the exposure phase, collembolans were transferred to a clean LUFA 2.2 soil (also used as a control soil in the exposure phase), incubated under standard conditions, and survival and reproduction evaluated. So far (atmospheric CO_2 tests in progress), increased daily air temperature was the major climate condition negatively affecting *F. candida* performance (decreased survival and reproduction), regardless of the metal(loid) soil contamination. Less pronounced effects on *F. candida* performance were observed when altering soil moisture content. When it was possible to move to the recovery phase, *F. candida* was apparently able to recover from the exposure to metal(loid) soil contamination and/or climate alterations (air temperature and soil moisture), especially in terms of reproduction.

2.10.15

Can Bioplastics Help Combat Plastic Pollution in Soil? A Degradability Study in Soil Ruled by the Microbial Community

C.C. Malheiro, Department of Biology, University of Aveiro / Biology; M. Prodana, University of Aveiro / CESAM & Departament of Biology; J. Lopes, G. Oliveira, University of Aveiro / CICECO & LAQV-REQUIMTE & Department of Materials and Ceramic Engineering & Department of Chemistry; I. Gonçalves, P. Ferreira, University of Aveiro / CICECO & Department of Materials and Ceramic Engineering; M.A. Coimbra, University of Aveiro / LAQV-REQUIMTE & Department of Chemistry; A. Alves, University of Aveiro / CESAM & Department of Biology; M. Gonçalves, University of Aveiro / CESAM & Deparment of Biology; S. Hilário, University of Aveiro / CESAM & Department of Biology; A.M. Soares, Universidade de Aveiro / CESAM & Department of Biology; S. Loureiro, Universidade de Aveiro / Biology

The UN Food and Agriculture Organization predicts that the global population will reach 9.1 billion by 2050. To keep up, enhanced food production will be required to compensate food demand. To ensure safe food, adequate packaging is essential. Nowadays, several plastic-based food packing materials are produced and, at a final stage, used in an unsustainable manner. Plastic contamination from improper use and disposal, and its impact on terrestrial ecosystems, has been gaining more research attention lately. Moreover, food systems are facing other sustainability challenges, such as food waste. In recent years, technological innovations for the use of these residues are growing, particularly as an integrated part of industrial processing chains, thus promoting circular bioeconomy. The development of new packaging solutions, such as bio-based biodegradable materials, can significantly reduce the reliance of food industry on conventional plastics. Also, reuse of by-products, which would be otherwise discarded, represents an added value. Still, little is known about the degradability, behaviour and environmental fate of bio-based polymers. The present study aimed to understand the biodegradability of two different types of bio-based plastics in soil – produced of potato starch and locust bean gum by-products, in comparison with a synthetic plastic. Biodegradation in Lufa 2.2. natural soil was evaluated by looking at bioplastics weight, along with the microbial community structure (fungi species) and function, and soil enzymatic activity measurements. Overall, the results revealed that the quality and position of the plastic sample in soil determined the initial fragmentation of the bioplastics, especially in the case of the locust bean gum-based one. Besides, we also observed enzyme-specific responses when the bioplastics and synthetic plastic were placed to the soil. Peaks of enzymatic activities of DHA and urease were somewhat in agreement with the fragmentation, although different trends were observed for the other enzymes. During the four weeks neither bioplastics nor synthetic plastic substantially affected the soil enzymes. A total of 8 fungal species were isolated and detected, with Zygomycota dominating and Ascomycota showing higher fungal diversity. Long-term assessment is ongoing and will be compared with the present results.

Unravelling the Occurrence and Impact of Multiple Stressors of Natural and Antropogenic Origin Pollutants in Polar Regions

2.11.01

Can Plastic Pollution Counteract the Ability of the Ocean to Store carbon?- CUPIDO Project

C. Manno, British Antarctic Survey

The ocean is absorbing almost 35% of CO_2 from the atmosphere which contributing to climate change mitigation. This process is supported by the Biological Carbon Pump (BCP) which promote the sequestration of carbon from the surface to the deep ocean. Despite the recognised and fundamental role of the ocean in the earth system, 8 million tonnes of plastic end up into the ocean each year. As this plastic debris degrades, it fragments in smaller pieces. Due to their small size, microplastic (plastic debris < 1 mm) can get ingested by zooplankton which play a pivotal role in regulating the efficiency of the BCP. Here we present CUPIDO (CalcUlating the strength of the Plastic pump In counteracting the Deep export of Oceanic carbon), a project funded by UKRI Future Leadership Fellowship. The project developed a new concept of the "Zooplankton Plastic Pump (ZZP)", to collectively describe the transport of plastic from the surface to the deep ocean when incorporated into zooplankton dynamic. The overall goal of CUPIDO is parametrizing the strength of the Zooplankton Plastic Pump and assess how this mechanism may decrease the ability of the marine ecosystem to transfer carbon to the deep ocean and in turn counteracting the efficiency of the Biological Carbon Pump. CUPIDO focus on two regions located in the Southern Ocean and the Mediterranean Sea. The contrasting conditions of the two selected regions (relatively pristine vs. highly polluted) allow for a comparative analysis of the impact of the Zooplankton Plastic Pump on the ocean's ability to export and sequester carbon within a low (Southern Ocean) and high (Mediterranean Sea) plastic input regime. The project involve two intense multidisciplinary field campaigns, in which both shipboard instrumentation and moored platforms will be deployed, providing data for subsequent analytical modelling. CUPIDO will provide an additional mechanistic to help the understanding of ocean carbon uptake processes and will deliver new insight on the influence of plastic on climate change particularly with regards the interaction between microplastics and the marine ecosystem service provided by the Biological Carbon Pump. This outcome will be especially relevant for the Southern Ocean which absorb a billion tons of carbon each year (about 40% of the entire ocean uptake).

2.11.02

Antarctic Octopod Beaks As Proxy for Mercury Concentrations in Soft Tissues

R.S. Matias, MARE - Marine and Environmental Sciences Centre, University of Coimbra / Department of Life Sciences; J. Seco, University of Aveiro & CESAM / Department of Chemistry; S. Gregory, Government of South Georgia and South Sandwich Islands; M. Belchier, British Antarctic Survey, Natural Environment Research Council; M.E. Pereira, University of Aveiro & CESAM / Department of Chemistry; P. Bustamante, LIENSs CNRS & Université de la Rochelle UMR 7266 / LIENSs; j. xavier, MARE - Marine and Environmental Sciences Centre, University of Coimbra / Department of Life Sciences, University of Coimbra

As the role of mercury is poorly known in Southern Ocean biota, the total mercury (T-Hg) concentrations were evaluated in upper/lower beaks, digestive gland, gills and mantle muscle of *Adelieledone polymorpha* and *Pareledone turqueti*, two of the most abundant octopod species around South Georgia. Both species are also the main food source for some top Antarctic predators breeding in the region thus understanding how Hg accumulates in the different tissues of *A. polymorpha* and *P. turqueti* can provide crucial information regarding Hg pathways in that region's ecosystems. Beaks had the lowest T-Hg concentrations (*A. polymorpha*: [T-Hg]Upper = 27.2 ± 12.9 ng·g−1 and [T-Hg]Lower = 27.5 ± 20.0 ng·g−1; *P. turqueti*: [T-Hg]Upper = 34.6 ± 13.9 ng·g−1 and [T-Hg]Lower = 56.8 ± 42.0 ng·g−1), followed by gills and muscle. The highest values were recorded in the digestive gland (*A. polymorpha*: 251.6±69.7 ng·g−1; *P. turqueti*: 347.0 ± 177.0 ng·g−1). Significant relationships were found between the concentrations of T-Hg in the beaks and muscle of A. polymorpha (T-Hg in muscle is 10 times higher than in beaks). This study shows that beaks can be used as proxy for T-Hg in muscle for some octopod species, and a helpful tool for estimating total Hg body burden from beaks.

2.11.03

Amplification of Persistent Organic Pollutants at Costal Antarctica

G. Casas, P. Casal, A. Martínez-Varela, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Environmental Chemistry; J. Roscales,

Institute of Organic Chemistry of the Spanish National Research Council (IQOG-CSIC) / Environmental Chemistry; M. Vila-Costa, IDAEA CSIC / Environmental Chemistry; B. Jimenez, IQOG-CSIC / Department of Instrumental Analysis and Environmental Chemistry; J. Dachs, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Environmental Chemistry
Many legacy and emerging persistent organic pollutants (POPs) have been reported in polar regions which serve as sentinels of global pollution. Maritime Antarctica is recipient of abundant snow precipitation. Snow scavenges air pollutants, and after snow melting, it can induce an unquantified and poorly understood amplification of concentrations of POPs. Amplification of concentrations of surface-active and hydrophobic POPs can also occur in the marine surface microlayer (SML). Air, snow, the fugacity in soils and snow, seawater and the SML were sampled in three sampling campaigns at Livingston and Deception Islands (South Shetlands, Antarctica), and analyzed for polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and perfluoroalkyl substances (PFAS). Coastal seawater mirrored the pollutant profile in snow, consistent with the amplification of concentrations in snow and the snowpack releasing POPs to seawater during the austral summer. The influence of snowpack and glacier inputs was further evidenced by the correlation between net volatilization fluxes of semi-volatile POPs and seawater salinity. In addition, there was an amplification of PFAS in the SML and aerosols, supporting the role of sea-spray aerosols as a vector for long-range atmospheric transport of PFAS. These results further indicate that amplification of concentrations in snow and the SML contribute to the generalized occurrence in Antarctica of legacy and emerging organic pollutants with a wide range of physical chemical properties and confirms the role of polar regions as sentinels of global pollution.

2.11.04
Plastics in Polar Environments: Main Findings and Ongoing Studies of the 4 Years PLANET Project in Antarctica
I. Corsi, E. Bergami, University of Siena / Physical, Earth and Environmental Sciences
Plastic pollution has been largely documented in the more populated regions worldwide, however still very little is known about the Polar Regions. Although at low level compared to more anthropized regions, records of plastic litter on Arctic and Antarctic islands date back to 1970-80s and now is documented in surface waters, sea ice, sediments and biota. Impacts on the polar wildlife are manifold, not only due to plastic intrinsic properties but also as a consequence of extreme polar environmental conditions which could significantly affect their fate and properties. Plastic pollution, as a further anthropogenic stressor, could influence the ability of polar species to cope with those already documented to pose a threat on local population, communities and ecosystems. The project PLANET funded in 2016 by the Italian National Program on Antarctic Research aimed to fill such knowledge gaps by addressing the risk posed by plastic, from macro to nano, in the Antarctic environment. The impact of unlabeled polystyrene nanoparticles (PS-NPs, 50nm) as proxy for nanoplastics were investigated through *in vitro* acute short-term cultures on the immune cells (coelomocytes) of the Antarctic sea urchin *Sterechinus neumayeri* and on the Antarctic krill *Euphasia superba* juvelines upon acute *in vivo* exposure. A large piece of PS foam (34x31x5cm) covered by microalgae, moss, lichens and microfauna was retrieved along the shores of the Fildes Peninsula (King George Island) and on several sub-samples, isolation and identification of microbial communities, characterization of their structure and functions including antibiotic resistance were investigated as well as interactions with specimens of Antarctic collembolan *Cryptogyrus.* The PLANET project revealed how nanoplastics can seriuosly affect both benthic and planktonic Antarctic species. Without urgent efforts to understand sources and origin of nanoscale plastic particles in polar areas, they will like become a major anthropogenic threat for such delicate ecosystems. The project further demonstrated that plastic can serve as vector and potential reservoir of antibiotics resistance bacteria across Antarctic terrestrial and marine environments and it showed for the first time that microplastics has entered the Antarctic soil food web. The current lack of knowledge on potential consequences on polar terrestrial ecosystems calls for urgent future studies addressing the ecological risk posed by plastic debris.

2.11.05
In Situ Microplastics Ingestion by Antarctic Marine Benthic Invertebrates
J. Hurley, The University of Hull / Energy and Environment Institute; J. Hardege, The University of Hull / Biological Sciences; K.C. Wollenberg Valero, The University of Hull / Biological and Marine Sciences; S.A. Morley, British Antarctic Survey, Natural Environment Research Council
Microplastics have been documented as persistent contaminants, pervasive throughout the marine environment. Furthermore, mounting evidence supports the designation of microplastics as anthropogenic stressors to marine organisms and consequently, via biological connectivity, to the wider ecosystem. Despite its remote location, studies have reported microplastics contamination in every Antarctic environment investigated to date. Due to ocean currents and frontal systems, polar regions may represent the final destination of marine microplastics. This increases bioavailability to inhabiting fauna which are sensitive to environmental change, highlighting the need to prioritise research on Antarctic species. In addition, microplastics accumulate in surface waters and sediments which are the habitats of marine benthic invertebrates throughout their lifecycle. Such taxa occupy low levels of food webs and are key contributors to ecological functioning, necessitating their candidature for microplastics research. The current study aimed to quantify and characterise microplastics ingestion by two Antarctic marine benthic invertebrates, encompassing different feeding strategies. The epifaunal, carnivorous polychaete Barrukia cristata and the infaunal, filter-feeding bivalve, Laternula elliptica were sampled from coves adjacent to Rothera research station, Adelaide Island. Animals were individually digested in 10% sodium hydroxide at room temperature for 96 hours and then vacuum filtered prior to microscopic examination. Ingested microplastics by individual organisms of both species were counted and characterised by shape, colour, size and polymer type by Micro-Fourier transform Infrared spectroscopy. Despite microplastics fibres representing the dominant type of contamination in this area, ingestion of fragments was reported here. Polyethylene terephalate was the most abundant polymer type however, polyacrylonitrile and ethylene-vinyl acetate were also identified. Results determined the current level of microplastics ingestion by the study species in the sampling locations and indicated bioavailability of microplastics as well as the potential for trophic transfer throughout the Antarctic marine food web.

Wildlife Poisoning Incidence: Impacts on Biodiversity, Ecosystems, Human Health and Legislative Regulations

2.12.01
Interpreting Anticoagulant Rodenticide Residues and Potential Toxicity in Predatory and Scavenging Birds
B.A. Rattner, U.S. Geological Survey, Eastern Ecological Science Center / Patuxent Wildlife Research Center
Anticoagulant rodenticides (ARs) are one of the principal vertebrate pesticides used to control commensal and invasive rodents. Non-target AR exposure is well documented in raptorial birds, and can result in toxicosis with mortality incidents ranging from anecdotal (death of an individual) to long-term low-level mortality (barn owls in rural settings) to large-scale events following remote island rodent eradication efforts (death of 46 bald eagles at Rat Island, Alaska). The AR potency, dose and frequency of exposure make the poison; low level infrequent exposure can result in subclinical effects (reversible, drug-like), while greater dose or frequency of exposure can result in overt toxicosis and lethality. AR toxicity data are limited to a few avian species, and some studies suggest that raptors may be more sensitive than commonly tested granivorous species. With the global trend to replace animal toxicity tests with alternative methods, avian physiologically-based pharmacokinetic modeling and *in vitro* hepatic microsomal or cell-based vitamin K epoxide reductase assays may be useful in more fully characterizing interspecific differences in sensitivity. Signs of toxicosis and post-mortem presence of AR residues in liver are key to classifying individuals as presumably or definitively poisoned, however AR concentrations associated with poisoning are highly variable. The summing of multiple AR liver residues on a mass basis is commonplace; perhaps molar mass in conjunction with *in vitro* derived AR potency data might be useful in developing a toxic equivalence approach to facilitate diagnosis and determination of cause of death. Age, physiological condition, vitamin K status and the exposome may also affect AR sensitivity, responses and onset of toxicosis. Further understanding of these factors could enable pesticide regulators and natural resource managers to better predict and even mitigate AR risk to non-target individuals, and forecast effects in wildlife from the level of the individual to the population.

2.12.02
Could Exposure to Second-Generation Anticoagulant Rodenticides Explain the Absence of Marsh Harrier in an Optimal Habitat?
A. Lopez Antia, Universiteit Antwerpen / Biology; J. Feliu, J.J. López-Perea, IREC (CSIC -UCLM) / Grupo de Toxicología de Fauna Silvestre; R. Mateo Soria, IREC (CSIC- UCLM) / Wildlife Toxicology
The reason why the Ebro delta, one of the most important wetlands in the western Mediterranean, was deserted by the marsh harrier (*Circus aeruginosus*) for breeding in the mid-80s remains an open question. In this study, we explored the role of second-generation anticoagulant rodenticides (SGARs), as a possible factor that caused this local extinction and disrupted the subsequent re-colonization. For this we, i)monitored, in the wintering population in the Ebro delta (2015/2016), exposure to SGARs and its association with the diet through pellet analysis and by studying exposure in prey (rats), ii) analyzed SGARs concentrations in the liver of nocturnal raptors from the Ebro delta and, iii)measured SGARs concentrations in the livers of marsh harriers from different regions of Spain. Residues of SGARs were found in 4 pellets (4.4%, n=91), all these pellets were from the same sampling time (February), a period when marsh harrier rely more on mammal preys (i.e. 54% of pellets contained mammal remains) comparing to November

and April (40 and 39% of pellets contained mammal remains respectively), when birds were most abundant prey. Moreover, by the end of winter, channels and rice fields are dried and this has facilitated large-scale treatments with SGAR in the last decades. Residues of SGARs were found in 62% of rat liver samples (n=24), mean concentration of ∑SGARS (± SD) was 17±25 ng/g. SGARs residues were detected in 67% of nocturnal raptors livers (n=43), mean concentration (±SD) of ∑SGARS was 149±194 ng/g. Per species, prevalence and mean (±SD) concentrations of ∑SGARS (ng/g) found were: barn owl (n=17, 65%; 276± 258), eagle owl (n=9, 89%; 128±77), Eurasian scops owl (n=7, 50%; 158±101) and little owl (n=6, 84%; 11±10; p<0.0001). Residues of brodifacoum, bromadiolone, difenacoum, and flocoumafen were found in these species. The toxicity threshold (> 200ng/g) was surpassed by 5 barn owls and 1 eagle owl. The prevalence of SGARs in marsh harrier liver was 15.9% (n=44). Concentrations found were below the toxicity threshold in all samples but one from Aragon. Marsh harrier was less exposed to SGARs than nocturnal raptors in the Ebro delta. However, intensive treatment campaigns with SGARs in this area ceased in 2014, and two pairs successfully bred in 2019. Our study reveals the important presence of SGARs in the Ebro delta and highlights the necessity of monitoring SGARs exposure and health parameters in sensitive populations.

2.12.03

VKORC1 Single Nucleotide Polymorphism in Wild Rattus Norvegicus in Madrid

A. Bermejo-Nogales, INIA-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria / Departamento de Medio Ambiente (Lab. DETC); J. Navas, INIA - National Institute for Agricultural and Food Research and Technology / Department of the Environment and Agronomy

Wild rodents are a potential cause of disease for humans, domestic animals and wildlife. Strategies based on an Integrated Pest Management (IPM) are associated with the management of these animals implementing pesticide reduction and minimal pesticide resistance. Anticoagulant rodenticides (AR) have been widely used to control rodent populations. AR inhibit the enzyme vitamin K 2,3-epoxide reductase (VKORC1) producing the animal death due to internal haemorrhages. Resistance to AR is mainly associated with mutations or single nucleotide polymorphism (SNP) in the *vkorc1* gene. The aim of this study, as part of the IPM of Madrid City, is to monitor the presence of SNP that can favour resistance in brown rat (*Rattus norvegicus*) by means of PCR in the exon 3 of the *vkorc1* gene. Additionally, we aim to characterize binding properties of AR to VKORC1 with new SNP by *in silico* analysis. We found a specific genotypic variation in Madrid City at codon S149I. Indeed, AutoDock Vina virtual screening analysis showed that this mutation confers less binding affinity and therefore resistance to brodifacoum and difenacoum. Whereas binding is increased to flocoumafen and make individuals more susceptible. These results have direct implications in the change and use of AR.

2.12.06

The African Wildlife Poisoning Database - a Valuable Tool in Conservation Decision-Making to Reduce the Impact of a Significant Threat to Wildlife

A.J. Botha, Endangered Wildlife Trust / Vultures for Africa Programme; D. Ogada, The Peregrine Fund / East African Region; L. Roxburgh, Endangered Wildlife Trust / Conservation Science Unit; C. Murn, Hawk Conservancy Trust / Research and Conservation

Africa's vultures are currently facing a crisis with seven of the 10 resident and breeding species on the continent classified as either endangered or critically endangered according to the IUCN Red List of Threatened Species (2020). The most significant threat that drives the decline of vulture populations across the continent is poisoning in various forms that causes large-scale losses exacerbated by the feeding biology and slow reproductive rate of these birds. The Multi-species Action Plan for the Conservation of African-Eurasian Vultures (Botha *et al* 2017) recommends a range of actions that can be implemented by range states to combat wildlife poisoning and to reduce its impact on vulture populations. This includes the establishment and management of relevant databases across the range and focused activities to reduce the impact of poisoning ion vultures and other wildlife. The African Wildlife Poisoning Database was established in 2012 and is jointly managed by the Endangered Wildlife Trust and The Peregrine Fund under the auspices of the Vulture Specialist Group of the Species Survival Commission of the IUCN. It aims to capture available data of all wildlife poisoning events that happen in Africa, from current and past events, through engagement with an extensive network of organisations and individuals throughout the continent and also by means of a user-friendly App that simplifies electronic capture of such data. We will reflect the findings of an initial analysis of various aspects of this threat to Africa's wildlife, such as the drivers of wildlife poisoning and the methods and substances used by the perpetrators of these acts. We also aim to share insights in terms of the challenges of the management of this database as well as the value of access to such data in terms of informed decision-making and the measuring of impact with regard to appropriate conservation interventions in identified target areas across the continent.

2.12.07

Accidental and Deliberate Poisoning of Wildlife in Spain With Anticholinesterase Pesticides

R. Mateo Soria, IREC (CSIC- UCLM) / Wildlife Toxicology; I.S. Sanchez-Barbudo, Institute for Game and Wildlife Research, IREC (CSIC-UCLM) / Wildlife Toxicology; M. Martinez-Haro, IREC-Instituto de Investigación en Recursos Cinegéticos / Department of Life Sciences; M. Herrero Villar, University of Castilla La Mancha / Ecotoxicology; P. Camarero, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM) / Toxicology in wildlife

The chemical monitoring schemes must be more integrated within other monitoring programs of disciplines like population demographics or diseases in which contaminants may have a silent role. Here we present the example of a monitoring program of wildlife incidences in Spain, in which the main goal was to diagnose intentional and illegal poisonings. This forensic monitoring has also permitted to detect the presence of other accidental poisonings or just to monitor some contaminants at sublethal levels in many different species. During a 16-year period (from April 2004 to July 2020), a total of 3210 animals and 876 suspected baits were analysed at this service for the detection of anticholinesterase (Anti-ChE) pesticides among other types of poisons by GC-EI-MS and LC-ESI-MS. Poisoning was confirmed as the cause of the death in 1334 (41.6%) of the 3210 animals analysed. The most common chemicals involved in wildlife poisoning were carbamates (n=715, 53.6%), organophosphates (n=245, 18.4%), coumarins (n=169, 12.7%), strychnine (n=74, 5.5%) and barbiturates (n=45; 3.4%). In the case of suspected poisoned baits or food involved in animal poisonings, 448 (51.1%) of them (n=876) contained a poison. The most frequently used chemicals in baits were carbamates (n=292, 65.2%), organophosphates (n=71, 15.8%), arsenic (n=19, 4.2%), strychnine (n=13, 2.9%), metaldehyde (n=11, 2.5%), organochlorines (n=10, 2.2%) and coumarins (n=10, 2.2%). Most of the mortality events in wildlife caused by anti-ChE pesticides were deliberate poisonings to kill predators (87.0%) and granivorous birds (10.5%). The most frequently used anti-ChE pesticides used for the deliberate poisoning of wildlife were carbofuran (28.0%), aldicarb (20.2%) and fenthion (5.2%). In the case of aldicarb and carbofuran, the used formulations were microgranulates, which despite being unregistered as plant protection products since 2003-2007, they were still frequently used in 2020 (26.4 and 32.1%, respectively). The frequent misuse of anti-ChE pesticides for the deliberate poisoning of wildlife highlights the importance of an adequate regulation of highly toxic substances, especially when the commercial formulations have a high content of the active ingredient (5-10%). Moreover, the recurrent detection of unregistered anti-ChE pesticides (mainly aldicarb and carbofuran) shows the need of a better control of old stocks and the illegal distribution of these highly toxic products.

2.12.08

A Review of Convicting Prosecutions for Wildlife Poisoning in Spain 1994-2020

A. Garcia-Fernandez, University of Murcia / Health Sciences; I. Navas, University of Murcia / Toxicology and Forensic Veterinary Service, Faculty of Veterinary, Department of Health Sciences.; P. María-Mojica, University of Murcia / Wildlife Rehabilitation Center of Alicante, Generalitat Valenciana, Alicante, Spain; I. Valverde, Universidad de Murcia (Q-3018001-B) / Toxicology and Forensic Veterinary Service, Faculty of Veterinary, Department of Health Sciences.; P. Gomez-Ramirez, University of Murcia (Q-3018001-B) / Sociosanitary Sciences; P. Jiménez, S. Espín, P. Sánchez-Virosta, University of Murcia / Department of Health Sciences, Area of Toxicology

Poisoned baits are the tools most commonly used to kill predators around the world and poisoning is one of the types of wildlife crime recognized by the EU. Consequently, predators and scavengers are threatened globally due to these actions involving conflicts between humans and wildlife, which poses a significant threat for biodiversity. In 2004, the Spanish Ministry for the Environment approved the Spain's National Strategy against the illegal use of poisoned baits in the natural environment. This strategy is built on three pillars: prosecution of crime, prevention and deterrence, and improving knowledge and information. Despite the efforts made, few cases of ilegal wildlife poisoning reach the courts and end up being resolved with convictions. A total of 116 court sentences for wildlife poisoning passed in Spain in the period between 1994 and 2020 have been reviewed. The sentences were collected from the database of the Public Prosecutor's Environmental and from the compilation carried out by WWF/España and SEO/Birdlife available on www.venenono.org. Diverse information was compiled from each sentence, such as toxic compounds, location of the poisoning incident, animals and species affected, etc. The chemical compounds were reviewed according to the legal status for manufacturing, commercialization, use and measures about restrictions or prohibitions to be used. The main conclusions were: i)Poisoning of endangered species of raptors and mammals in Spain have been subject of criminal proceedings. Species include red kite, cinereous vulture, Egyptian vulture, bearded vulture, Iberian imperial eagle and Iberian lynx, among others. ii) The majority of the sentences in Spain resulted in verdict of guilty, and a third part included imprisonment penalties. iii) The

majority of the poisoned animals had necrophagous dietary habits. iv) Carbamates are the pesticides more frequently used to poison wildlife and domestic animals (mainly dogs). Four of the top 5 poisons are carbamates, being specially relevant aldicarb and carbofuran. v) Further increase is expected in the future in court proceedings of poisoning by anticoagulant rodenticides. vi) It would desirable to reduce the delay time to obtain a sentence and to improve collaboration between the interested actors in order to improve the effectiveness in the prosecution of this crime. *Acknowledgement*: The authors thank the Seneca Foundation, CARM (20945/PI/18). In loving memory of Prof. Richard F. Shore.

2.12.13

Barbiturate Poisoning in Avian Scavengers and Other Predators in Spain

M. Herrero Villar, University of Castilla La Mancha / Ecotoxicology; I.S. Sanchez-Barbudo, Institute for Game and Wildlife Research, IREC (CSIC-UCLM) / Wildlife Toxicology; P. Camarero, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM) / Toxicology in wildlife; R. Mateo Soria, IREC (CSIC- UCLM) / Wildlife Toxicology

Pharmaceuticals are considered as emerging contaminants for wildlife, and one of these concerning chemical groups are euthanasia agents used in veterinary medicine. Here we present the occurrence of barbiturate intoxication in a long-term (2004-2020) monitoring study of wildlife and domestic animal poisonings in Spain (n=3210). Barbiturate intoxications in animals represented 3.4% (45/1334) of the total confirmed poisoned animals. This places barbiturates in fifth place in the scale of frequently detected contaminants in poisoning events. Prevalence by regions was estimated in up to 34.5% in Navarra. Barbiturates were detected in 0.2% (1/448) of baits containing detectable poisons. The most frequently detected barbiturate was pentobarbital (42/45, 93.3%), but we also detected phenobarbital, barbital, and thiopental (2.2%, each). Avian scavengers were the most frequently affected by barbiturate intoxications (n=36), especially griffon vultures (*Gyps fulvus*) (n=28). Median pentobarbital concentrations detected in intoxicated griffon vultures was 27.3 µg/g in the gastric content and 38.1 µg/g in the liver. At least two poisoning events affecting griffon vultures were related to the consumption of carcasses from euthanized livestock. One of these events affected 8 griffon vultures that were found dead next to a foal carcass that contained pentobarbital with a median concentration of 80.9 mg/kg. However, we also found phenobarbital in a prepared bait linked to the intoxication of one common buzzard (*Buteo buteo*). Moreover, although circumstances of the death were unclear in 7 mammals, deliberate poisoning was the most possible diagnosis (15.6%). This study concludes in the need of a stronger regulation on barbiturate use to avoid secondary poisonings due to the unproper disposal of euthanized livestock and to eradicate primary poisonings due to their deliberate misuse in baits to kill predators.

2.12.16

Oxidative Stress in the Seaside Sparrow (Ammospiza maritima) Following the Deepwater Horizon Oil Spill

A.N. Angel, California State Polytechnic University, Pomona / Department of Biological Sciences; S.S. Taylor, Louisiana State University AgCenter / School of Renewable Natural Resources; P.C. Stouffer, Louisiana State University Agricultural Center / School of Renewable Natural Resources; J.K. Jellyman, A. Bonisoli-Alquati, California State Polytechnic University, Pomona / Department of Biological Sciences

The Deepwater Horizon (DWH) oil spill introduced massive amounts of oil into Louisiana saltmarshes. Seaside Sparrows (*Ammospiza maritima*) incorporated petrogenic carbon into their tissue and showed extensive changes in gene expression, including *cyp1a*, a gene involved in metabolizing polycyclic aromatic hydrocarbons (PAHs), toxic components of oil. PAH metabolism generates reactive oxygen species, inducing oxidative stress when the antioxidant capacity of an organism is overwhelmed. Our study aims to determine whether DWH oil exposure increased oxidative stress in Seaside Sparrows. We measured plasma reactive oxygen metabolites (ROMs) and antioxidant capacity in sparrows from oiled and control sites (2013-2014). Analysis of total protein damage in cardiac tissue was accomplished using Western blot (2013-2015). Antioxidant capacity of sparrows from oiled sites was ~50% higher than sparrows from control sites in 2013, but not 2014. Additionally, sparrows from oiled sites had 95% higher ROMs in 2014 than in 2013, with no difference in antioxidant capacity. In 2013, oil-exposed sparrows demonstrated a varied effect of oiling on protein damage; a 26% decrease and a 104% increase in oil-exposed sparrows from separate comparisons. The variation in response may be attributed to site differences within the oil and control groups, ultimately a result of heterogenous PAH contamination. Interestingly, oil-exposed sparrows in 2014 and 2015, show 17% and 9% decreases in protein damage, respectively. The data suggest that exposed sparrows upregulated or mobilized antioxidants from storage tissues to combat oxidative damage in 2013 in a site-specific manner. Further, the reduction of oxidative damage in later years may suggest sparrows improved their antioxidant regulation over time. This is supported by an increase in circulating ROMs and a reduction in oxidative damage to protein from 2013-2014. Ongoing analyses of antioxidant concentrations in the heart will provide additional insight into oxidative balance. Specifically, we are measuring glutathione (GSH), a key intracellular antioxidant which scavenges ROS and supports GSH peroxidase activity in the reduction of ROMs. We will address the variation in stress response attributed to ecological variation by relating our findings to measurements of exposure via integrating sediment and liver PAH concentrations. Our results will provide crucial insight into the toxicity of sublethal DWH oil exposure in terrestrial birds.

2.12.17

Occurrence of Persistent Organic Pollutants in Relation to Gender, Age and Breeding Success in the Globally Endangered Bermuda Petrel From the NW Atlantic and Implication for Its Conservation

B. Oró-Nolla, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Environmental Chemistry; L. Campioni, MARE - Marine and Environmental Sciences Centre; J. Madeiros, Ministry of the Environment / Department of Conservation Services; M. Silva, Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculty of Sciences of the University of Lisboa; J. Granadeiro, CESAM Centre for Environmental and Marine Studies, Faculdade de Ciências, Universidade de Lisboa / Department of Animal Biology; S. Lacorte, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Environmental Chemistry

Persistent organic pollutants (POPs) are contaminants characterized for being lipophilic, semi-volatile and resistant to chemical and biological degradation. POPs are bioaccumulated and biomagnified in lipid-rich food webs affecting high level consumers as avian wildlife. Bermuda petrel (Pterodroma cahow) is a pelagic seabird endemic to the Bermuda Islands and classified as endangered. In 1968, it was reported for the first time the presence of DDT, DDE and DDD (mean levels of 31,000, 62,000 and 7,000 ng/g wet weight (w.w.)) in eggs and chicks suggesting the vulnerability of the species to these organochlorine pesticides (OCPs). Although the population is recovering (134 breeding pairs, 2020), breeding failure due to unhatched and broken eggs has been increasing over the last decade (25% to 44%). Considering the critical conservation status of the species and its vulnerability, here we aimed to determine whether the Bermuda petrel is exposed to legacy POPs possibly present in their pelagic foraging grounds which may hamper its population recovery. During nest monitoring in January-February 2019 that is, Cahow's incubation period, birds of known age were captured, weighed with a spring balance and 1.5 mL of blood was sampled from the brachial vein for sex determination. Blood samples (N=58) were analysed by gas chromatography (GC) coupled to high-resolution/accurate-mass Orbitrap™ mass spectrometry (MS) in a target/untargeted approach. In a first step, target analysis was performed to quantitatively determine the presence of 25 OCPs, 7 polychlorinated biphenyls (PCBs) and 8 polybrominated diphenyl ethers (PBDEs). In a second step, deconvolution software was used to identify other POPs. As main results, DDE was detected in all birds at levels from 0.21 to 9.34 ng/g w.w., while DDT was found in 29% of the samples at lower concentration indicating its past use. PCBs were detected at concentrations of 0.62 – 1.28 ng/g w.w., and the flame retardant PBDE 209 was found in one sample at 5.25 ng/g w.w. In addition, using the non-target approach we detected some POP degradation products and other contaminants such as short chain chlorinated paraffins. Overall, the distribution of POPs will be assessed in terms of trophic levels, gender, breeding success and implications of anthropogenic pressures on species conservation and health of open ocean environments will also be discussed.

Extended submission 2 - Ecotoxicology becomes stress ecology: from populations to ecosystems and landscapes

2.13.01

Holo- and Hemimetabolism of Aquatic Insects: Implications for a Differential Cross-Ecosystem Flux of Metals

K. Cetinic, Ruder Boskovic Institute / Department of Physical Chemistry; A. Previsic, University of Zagreb Faculty of Science / Department of Biology; M. Rozman, Ruder Boskovic Institute / Department of Physical Chemistry

Increased levels of chemical pollutants in aquatic habitats come as a result of both anthropogenic and natural sources. Emerging aquatic insects that play an indispensable role in these environments, transferring resources and energy to higher trophic levels in both aquatic and terrestrial habitats, may inadvertently also act as biovectors for metals and other contaminants. This study measured levels of 22 different metals detected in biofilm, aquatic and terrestrial life stages of Trichoptera and Odonata, as well as riparian spiders, to examine the uptake and transfer from freshwater to terrestrial ecosystems. We show that emerging insects transfer metals from aquatic to terrestrial ecosystems, however with large losses observed on the boundary of these two environments. Significantly lower concentrations of most metals in adult insects were observed in both hemimetabolous (Odonata) and holometabolous insect orders (Trichoptera). In holometabolous Trichoptera, however, this difference was greater between aquatic life stages (larvae to pupae) compared to that between pupae and adults. Trophic

transfer may have also played a role in decreasing metal concentrations, as metal concentrations generally adhered to the following pattern: biofilm > aquatic insects > terrestrial invertebrates. Exceptions to this observation were detected with a handful of essential (Cu, Zn, Se) and non-essential metals (Cd, Ag), which measured higher concentrations in adult aquatic insects compared to their larval counterparts, as well as in aquatic and terrestrial predators compared to their prey. Overall, all metals were found to be bioavailable and biotransferred from contaminated waters to terrestrial invertebrates to some degree, suggesting that risks associated with metal-contaminated freshwaters could extend to terrestrial systems through the emergence of these potential invertebrate biovectors.

2.13.02

Exploring the Interplay Between Selenium and Mice Gut Microbiota Combining Metallomics, Metabolomics and Microbiome Analysis

T. García-Barrera, University of Huelva / Chemistry; B. Callejón-Leblic, S. Ramírez Acosta, Research Center for Natural Resources, Health and the Environment (RENSMA). University of Huelva / Chemistry. Faculty of Experimental Sciences; M.P. Selma-Royo, Institute of Agrochemistry and Food Technology (IATA-CSIC), Food Biotechnology, Agustin Escardino 7. Paterna (Valencia) / Food Biotechnology / Chemistry. Faculty of Experimental Sciences; N.P. Abril, University of Córdoba, Campus de Rabanales, Edificio Severo Ochoa / Department of Biochemistry and Molecular Biology; M.P. Collado, Institute of Agrochemistry and Food Technology (IATA-CSIC), Food Biotechnology, Agustin Escardino 7. Paterna (Valencia) / Food Biotechnology

Selenium (Se) is an essential element which plays important roles in medicine [1]. Selenoproteins develop key functions in mammals such as redox signalling, antioxidant activity, thyroid metabolism, transport/storage or protein folding [1]. The impact of Se on gut microbiota has been recently pointed out, but the absolute quantification of selenoproteins have not been performed before. To this end, metallomic analytical approaches aimed the *in vivo* screening for the native metal-protein and metal-metabolite complexes by hyphenated techniques that combine a high-resolution separation techniques with sensitive elemental (inductively coupled plasma, ICP) or molecular (electrospray or MALDI) mass spectrometric detection[2]. In this work, an experimental desing with *Mus musculus* mice has been developed including conventional mice and microbiota depleted mice after antibiotics treatment that were supplemented with Se. We combined a metallomic analytical approach for the absolute quantification of selenoproteins in mice (plasma, brain, liver, kidney and testicles) with metataxonomics for characterizing gut microbiota. The analytical methodology was based on the crhomatographic separation of selenoproteins by size exclusion chromatography (SEC) and affinity chromatography (AFC) both coupled to inductively coupled plasma mass spectrometer (ICP-MS). Microbiome analysis was performed by 16S rDNA gene sequencing. Moreover, these two omics were integrated with metabolomics using an analytical platform combining gas chromatography coupled to mass spectrometry (GC-MS) and ultra high performence liquid chromatography coupled to quadrupole time of fligh mass spectrometry (UHPLC-QTOF). Our results demonstrate a novel interplay between Se and microbiota. We have found that Se supplementation shapes the composition, diversity and richness of microbiota depleted mice and some bacterial groups correlated with selenoproteins including the potential beneficial genus (*e.g. Lactobacillus*). Moreover, we determined that metal homeostasis was more affected by Se-supplementation in microbiota depleted mice than in conventional ones, suggesting potential role of microbes. **Keywords:Selenium, metallomics, metataxonomics, ICP-MS, gut microbiota References** [1] Rayman, MP. 2012. Selenium and human health. The Lancet. 379: 1256-1268. [2] R. Lobinski, JS. Edmonds, KT. Suzuki, PC. Uden. 2000. Pure and Applied Chemistry 72: 447-461.

2.13.05

Life History Traits of Aquatic Insects Determine Bioaccumulation and Transfer of Emerging Contaminants From Aquatic to Terrestrial Ecosystems

A. Previsic, Universtity of Zagreb, Faculty of Science / Department of Biology; M. Veseli, University of Zagreb, Faculty of Science / Department of Biology; M. Vilenica, University of Zagreb, Faculty of Teacher Education / Department in Petrinja; M. Petrovic, ICRA; M. Rozman, Ruder Boskovic Institute / Department of Physical Chemistry

A wide range of emerging contaminants (ECs) such as pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) enter freshwaters globally. These can be transported from aquatic to terrestrial ecosystems through food webs and emergence of aquatic insects. Thus, understanding their impacts on both aquatic and terrestrial ecosystems remains a major challenge. Accordingly, the aim of the current study was to provide novel insights into bioaccumulation of ECs in freshwaters, as well as aquatic-terrestrial food web coupling. We examine differences of bioaccumulation and bioamplification of PhACs and EDCs in aquatic insects with different life history traits, namely, the holometabolous Trichoptera and hemimetabolous Odonata. We show that the type of insect metamorphosis and feeding behaviour determine the bioaccumulation patterns of ECs. Adult Trichoptera, an important food source for riparian predators, showed an increased body burden of both, PhACs and EDCs. Moreover, we further examine differences at finer taxonomic resolution, i.e. between the two suborders of Odonata, Anisoptera and Zygoptera. Results show similar trends in total ECs concentrations and of individual compounds for both suborders, being mostly higher in nymphs compared to adults. However, some compounds show significantly higher bioaccumulation factors in Zygoptera. Our results suggest that ecological traits of aquatic insects have an important contribution on bioaccumulation and bioamplification of ECs, thus highlighting the necessity for conducting taxon- and trait-specific research.

2.13.06

Toxicity Effects of Cigarette Butt Leachates on Marine Sentinel Species

G. Lucia, Università Politecnica delle Marche / DISVA; S. Gorbi, F. Regoli, M. Giuliani, Polytechnic University of Marche / Department of Life and Environmental Sciences; G. D'Errico, Polytechnic University of Marche / Department of Life and Environmental Science; M. Di Carlo, D. Fattorini, Polytechnic University of Marche / Department of Life and Environmental Sciences

Cigarette butts (CBs: intact filters, covering paper, unsmoked tobacco and ash) are one of the most common litter items found in the beaches. They are considered as environmental hazards for aquatic organisms since they contain over 5000 chemical compounds (e.g. nicotine, trace metals, polycyclic aromatic hydrocarbons), among which 150 highly toxic, mainly because of their carcinogenic and mutagenic potential. Most of these compounds are leached when CBs finished in seawater.The main objective of this study was to assess the biological effects exerted by CBs leachates obtained with the two concentrations of 100 CBs/L and 25 CBs/L (in triplicate) and used for chemicals determination, ecotoxicological bioassays and *ex vivo* exposure.Chemical analyses revealed that CBs release different concentrations of trace metals, aliphatic hydrocarbons and polycyclic aromatic hydrocarbons in ASW after 24 hours. The amount and type of these substances could depend either on quantity of CBs or on different brands of cigarettes utilized during leachates preparation. Bioassays results showed that leachates of different concentrations induced a significant reduction of bioluminescence in *Aliivibrio fischeri* (bacterium), alteration of larvae development of *Crassostrea gigas* (oyster) and growth inhibition of the microalgae *Phaeodactylum tricornutum, Dunaliella tertiolecta* and *Skeletonema costatum*. Finally, 100 CBs/L leachate determined a significant reduction of *acox1*, *gst-pi, cat* and *Cu/Zn-sod* gene expression in *Mytilus galloprovincialis* digestive gland PCTS (Precision-Cut Tissue Slices). Thus revealing a significant modulation of peroxisomal proliferation, biotransformation and oxidative stress, despite viability remains unchanged during time exposure (72 hours). These results revealed that cigarette butts can release different chemical compounds in seawater, causing toxicity effects on some marine sentinel species. An ecotoxicological approach based on the integration of chemical analyses, bioassays, molecular and cellular biomarkers is useful to clarify the impact of cigarette butts leachate on marine ecosystems.

Advances in Methods to Evaluate Environmental Degradation and Persistence

3.01.01

Biodegradation Kinetics of Personal Care Chemicals in a Mixture - Changing Test Concentrations 5 Orders of Magnitude

H. Birch, DTU Environment / DTU Environment; K. Knudsmark Sjoholm, Technical University of Denmark (DTU) / DTU Environment; C. Sparham, Unilever; R. van Egmond, Unilever / SEAC; P. Mayer, Technical University of Denmark / DTU Environment

Biodegradation of a chemical in the environment occurs typically in the presence of other chemicals and at low concentrations, whereas laboratory biodegradation tests are often conducted at high concentrations with a single substance at a time. The aim of this study was to investigate the effect of different test mixture concentrations on the biodegradation kinetics of individual chemicals and on the bacterial community development during incubation. Six parallel biodegradation experiments were conducted with concentrations spaced a factor 10 apart – thus covering 5 orders of magnitude of mixture concentrations. Abiotic and biotic test systems were prepared in 20 mL headspace vials. Effluent from a Danish wastewater treatment plant (WWTP) served as inoculum and ultrapure water was used for abiotic test systems. A stock solution of 19 chemicals in ultrapure water was prepared using passive dosing and diluted to vary compound concentrations: 1-8000 µg/L in the highest concentration experiment, down to 0.6-80 ng/L in the lowest concentration experiment. Background concentrations of test chemicals in the WWTP effluent ranged from not detected to the third highest test concentration. Test systems were incubated at 12°C for 1-30 days, and triplicate biotic and abiotic test systems analyzed at seven time-points using immersion Arrow-SPME GCMS/MS. DNA extraction and microbial community analysis were performed by an external company using 16S V1-3 rRNA gene amplicon sequencing and Illumina

MiSeq reagent kit. Samples for DNA extraction included original inoculum, inoculum incubated 14 d. without test substances, and inoculum incubated 14 d. with three different mixture concentrations. Most of the chemicals that fully degraded at all test concentrations showed similar degradation rate constants, but increasing lag-phases with increasing concentrations (linalool, geraniol, citronellol, 4-*tert*-butylcyclohexyl acetate, 2-ethylhexyl-4-methoxycinnamate, *tert*-butyl-4-methoxyphenol). Naphthalene and Phenanthrene (reference chemicals) on the other hand, had slower degradation at the lowest two test concentrations, and dicyclohexyl phthalate were only degraded at the highest two concentrations. Microbial populations after 14 days of incubations deviated clearly from the original inoculum for all treatments. The microbial community at the highest test concentration developed markedly different compared to the lower test concentrations and the inoculum without test chemicals.

3.01.04

Biodegradation of Phenanthrene in an Improved Water-Sediment Systems (OECD 308) - Effect of Test Setup

P. Shrestha, Fraunhofer- IME / Ecological chemistry; C. Hughes, Ricardo Energy & Environment / Chemical Risk; L. Camenzuli, ExxonMobil; D. Lyon, CONCAWE; B. Meisterjahn, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry; A. Martin-Aparicio, CONCAWE; M. Griffiths, Ricardo Energy & Environment / NCEC; D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry

OECD 308 is a higher tiered standard test used to simulate biodegradation of chemicals in water-sediment systems. Volatile chemicals are generally recommended to be tested using closed setup (OECD 308). Significant depletion of oxygen conditions within the closed setup due to the use of higher co-solvent concentration (0.25% V/V) and stagnant water-sediment systems has been reported in previous studies. Our own studies suggested, improvement in oxygen conditions in the closed setups with the use of larger test system geometries, agitation of the water sediment sample (shaking /overhead stirring) and lower co-solvent (0.01% V/V) concentratiotn. The present study, aims 1) to investigate the influence of different test setups and agitation techniques on the degradation, distribution and non extractable residue (NER) formation of ^{14}C labelled phenanthrene 2) to determine an appropriate closed setup for conducting OECD 308 tests with phenanthrene. 500mL cylindrical flasks were used to prepare different closed setups (Setup 1: diameter Ø = 5.5cm and Setup 2: Ø=7.5cm). The water sediment samples were prepared using sediment:water (S:W) ratio of 1:3 (V/V) in both the setups. Setup 1 were kept stagnant whereas the setup 2 was agitated using different approaches (shaking at 80rpm/40rpm and overhead stirring of water phase). The samples prepared in this way were applied with ^{14}C labelled phenanthrene with a starting test substance and co-solvent (acetone) concentration of 0.07mg/L and 0.01% (V/V) respectively. In parallel, parameter control samples prepared for oxygen, DOC and turbidity measurements. When the oxygen saturation measured were < 15%, the samples were aereated for 20sec. Additional, abiotic control samples were also prepared. The results showed stable oxygen saturation in the water phase throughout the test with improved biodegradation and faster partitioning processes in samples under setup 2. Although the degradation and the oxygen conditions in samples with overhead stirring and shaken samples were quite comparable, the shaking approach was more robust and reproducible, and easier to establish. The degradation and distribution varied considerbly with different shaking speed (80 and 40rpm). However, shaking with 80rpm resulted in more stable oxygen concentration in the water phase and showed no influence in abiotic NER formation. Thus, these results suggest a promising approach for testing volatile hydrophobic chemicals using OECD 308 guideline.

3.01.06

Analysis of Non-Extractable Residues (NER) for Use in Chemical Persistency Assessment - Proposal for a Standardised Testing Procedure

D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry; A. Schäffer, RWTH Aachen University / Institute for Environmental Research; M. Kästner, Helmholtz Centre for Environmental Research UFZ / Dept Environmental Biotechnology; S. Trapp, Technical University of Denmark DTU / Department of Environmental Engineering; U. Jöhncke, German Environment Agency UBA / Chemicals; A. Wiemann, German Environment Agency UBA / IV Pharmaceuticals; D. Classen, German Environment Agency / Institute for Environmental Research; J. Schmidt, G. Speichert, German Environment Agency (Umweltbundesamt); A. Brock, DTU Environment / DTU Environment; C. Gaertner, Fraunhofer Institute for Molecular Biology and Applied Ecology IME

The formation of NER in degradation testing are an issue in regulation of chemicals in Europe. However, NER are treated incoherently considering NER as non-degraded substance if not proven otherwise for chemicals under the REACH regulation while other, e.g. plant protection product regulation, up to now consider NER as degraded. As this might have significant impact on the degradation half-lives determined, the German Environment Agency (UBA) is working on a harmonized approach. However, a standardized method for further characterization of NER is still lacking. In the last years, efforts have been made to differentiate NER into different categories of concern. A first approach was started on behalf of the UBA in 2013. In September 2018 a follow up project was initiated by UBA in order to harmonise the results with the procedures described in the ECHA discussion paper on NER [1] in which experimental approaches to distinguish sequestered (type 1 NER), covalently bound (type 2 NER) and biogenic NER (type 3 NER) are discussed. In the present UBA project we have evaluated experimentally the applicability of a harmonised approach. We tested a set of three different substances in a standard OECD 307 approach. Different procedures for exhaustive extraction were applied in order to compare current NER determination approaches. We conducted these experiments using test substances labelled with either ^{13}C or ^{14}C in parallel. In case of a positive correlation, data from literature using either label can be reconciled. We showed that NER characterization methods are reproducible and applicable for routine analyses (see [2]). Experiments were accompanied by the modelling tool Microbial Turnover to Biomass (MTB) [3]. This tool relies on the CO_2 measured from the mineralization of test substance as indication of microbial degradation in order to estimate the formation of biogenic NER. Experimental data determined in the current project were used to verify the outcome of this approach. The results of the project will be presented in an international workshop in February 2021. The aim of the workshop is to present and discuss the proposal to achieve a standardized and harmonised concept for NER to be used in regulatory assessment of the environmental persistence of REACH chemicals, pesticides, biocides and pharmaceuticals. Beside the results of the project we will wrap up the outcome of the workshop discussions regarding the proposed harmonised concept.

3.01.07

Characterization of NER in Aerobic Soil Degradation of 14c-Phenanthrene

B. Meisterjahn, D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry; N. Wang, TOTAL France; C. Hughes, Ricardo Energy & Environment / Chemical Risk; A. Martin-Aparicio, European Petroleum Refiners Association / REMG

Regulation of chemicals in Europe is subject to the REACh (EC 1907/2006), Biocidal Products Regulation (EC 528/2012) or plant protection products regulation (EC 1107/2009). In these regulations, the degradation of chemicals is tested by application of simulation tests such as soil degradation according to OECD test guideline 307. During degradation in the soil many chemicals form non-extractable residues (NER) which can be determined only if the test has been performed with isotopic labelled chemicals. NER may contain potentially remobilizable fractions. NER can be differentiated in type I NER (entrapped parent compound and/or transformation products, potentially releasable), type II NER (covalently bound residues) and type III NER (bioNER). Up to now, a standardized method for characterizing and quantifying these different fractions is lacking. The aim of this project was to evaluate methods for differentiation among different types of NER. Therefore, we tested the degradation of ^{14}C-labelled phenanthrene in two different soils according to OECD 307. The soil samples were exhaustively extracted by aqueous and solvent extraction followed by harsh extraction using accelerated solvent extraction (ASE). The remaining NERs were quantified by combustion analysis. These NERs were subjected to different further extraction procedures in order to quantify the different types. For release of type I NER after ASE the soil residues were subjected to a silylation procedure and alternatively extracted with 0.1M EDTA solution. For determination of the bioNER the soil residues were extracted by 6N HCl for hydrolysis of peptides and other biological macromolecules. These hydrolysates were analysed for amino acids by radio-TLC after clean-up. In order to compare different extraction procedures on the NER content additional soil samples were directly extracted only by ASE, using a proposed standard solvent mixture and the solvent applied for exhaustive extraction of the other samples The experimental procedure is accompanied by a modelling tool. The Microbial Turnover to Biomass (MTB) approach uses released CO_2 as indicator of microbial activity and theoretical biomass yield to estimate the biogenic NER formation. The experimental data determined in the current project should be used to further validate the model.

3.01.10

Ready Biodegradability Testing of (Alkylated) Polycyclic Aromatic Hydrocarbons

J. Woutersen, N. Timmer, Charles River Laboratories / Environmental Sciences; D.M. Saunders, Shell International / Toxicology; A. Martin-Aparicio, CONCAWE

Polycyclic aromatic hydrocarbons (PAHs) are organic substances containing at least two fused aromatic ring structures. Sources of PAHs include coal, crude oil, gasoline, forest fires and food preparation with excessive heat. Many PAHs can be toxic, mutagenic and/or carcinogenic. PAHs low water solubility lowers their bioavailabiltiy, impacting the results of toxicity and biodegradability studies. Due to their intrinsic toxicity, environmental concern for PAHs is driven by their (apparent) persistence. To accurately assess biodegradability of PAHs, we

investigated optimizing bioavailibility of PAHs in studies based on the OECD 301F design. Phenanthrene (PHE), anthracene, pyrene and several alkylated homologs were studied. We used two inoculum sources to study the biodegradability of PAHs: activated sludge (AS) and river water (RW). AS was obtained from a municipal sewage treatment plant receiving predominantly domestic sewage. RW was sampled directly from the Meuse river. Several AS treatments were applied to maximize microbial diversity in the inoculum while simultaneously increasing homogeneity. Experiment were in accordance with ECHA Guidance (R11 and R7b). Silicon dioxide (SiO_2) and silicone oil (SO) were used as bioavailability optimizers. A solvent pre-treatment with dichloromethane (DCM) was used in parallel with direct addition, where DCM was allowed to evaporate in a laminar air-flow cabinet. DCM stock solution was applied directly to the SiO_2; SO was added to vessels after DCM was evaporated. Sodium benzoate was used as procedural control; naphthalene was included as additional reference item. Bioavailability of PHE was clearly an issue with AS and RW.Biodegradability improved significantly with addition of SO and SiO_2 (>60 % biodegradation in 28 d), with SiO_2 reaching the highest plateau (76 %). Anthraquinone (AQ) was used as recommended in R7b to evaluate efficacy of SiO_2 and SO. The suitability of AQ for this purpose is debatable, with no effect on lag-time (~14 d in all treatments) and failure to reach 60 % biodegradation within 28 d on multiple occassions, while PHE reached >60 % using the same batch of AS. Biodegradation of >60 % in 28 d was also observed for several other PAHs with AS and, in some instances, RW. Overall, our data clearly shows that the majority of the PAHs tested in this work should be regarded as biodegradable and, due to their bioavailability limitations, are prone to false negatives within the 301 test.

3.01.13

Strategy to Identify of Transformation Products of Anthropogenic Organic Compounds in Complex ENvironmental Matrices

C. Gardia Parege, EPOC-LPTC / UMR EPOC 5805, LPTC; M. Dévier, V. Dupraz, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC; C. Albasi, Université de Toulouse INPT / UPS Laboratoire de Génie Chimique; J. Choubert, INRAE REVERSAAL; H. Budzinski, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC

Although treatment processes applied in wastewater treatment plant (WWTP) have improved in the last decades, it was recently demonstrated that it could also generate transformation products (TPs). Because target chemical analyses rely on commercially available analytical standards, the search for TPs using such methodologies is limited to known TPs. In order to improve the identification of unknown TPs in complex matrices, it is essential to develop a screening strategy. Non-target screening (NTS) using high-resolution mass spectrometry (HRMS) has demonstrated a high potential to highlight the presence of unknown compounds, and among them, TPs. In this study, an approach combining suspect screening and NTS was developed in order to highlight and identify TPs in wastewater samples from a French urban WWTP. The NTS was first performed on WWTP influents and effluents using LC-QTOF. A full-scan MS analysis was performed to acquire the chemical fingerprints. A statistical comparison of chemical profiles was then carried out to highlight the presence of newly detected TPs. These analyses allowed detecting about 2500 features in the WWTP influent and about 630 features in the effluent. About 14% of the features detected in the influent remained unchanged after the WWTP treatment process, while the remaining features were eliminated. In the effluent, 53% were persistent features, while 47% were newly detected features. In order to identify TPs in WWTP samples, influent and effluent extracts were analysed using data-dependent acquisition mode (DDA). A DDA based on abundance allowed the identification of several compounds using spectral databases. The majority of identified chemicals in WWTP influent and effluent extracts were drugs. Additionally, the presence of two TPs (human metabolites), 5-hydroxypropafenone and N-desalkylverapamil, was suspected in effluent extracts. To quickly investigate unknown TPs, we purposed to use an internal suspect list. Starting with 6 psychotropic drugs, we obtained a list of 294 TPs from theoretical processes and the literature. Focusing on newly detected features, 3 unknown TPs were suspected in the WWTP effluent. These TPs were derived from citalopram (2 TPs, CT45, CT44) and venlafaxine (1 TP, V23b). After a thorough observation of the MS/MS spectra, the presence of CT44 was invalidated. The structural elucidation of two other TPs in still in progress using an *in silico* software such as Metfrag and Molecular Structure Correlator.

3.01.14

Effect of Water Extractable Organic Matter From Sediment of Lake Pamvotis of Psychoactive Drugs Under Simulated Solar Light

C.J. Holgado, University of Ioannina / Department of Chemistry; C. Richard, CNRS-Université Blaise Pascal / Department of photochemistry; V. Sakkas, University of Ioannina

Psychoactive drugs are classified as contaminants of emerging concern but there is limited information on their fate in surface waters. Here, we studied the fate of three drugs (sertraline, clozapine and citalopram) upon irradiation in the presence of the water extractable organic matter (WEOM) of the Lake Pamvotis sediment. The three drugs showed affinity with the sediment in the dark in the order: sertraline > clozapine > citalopram. Adsorption data were well fitted with a Freundlich model with K_F = 1210, 302, 62, respectively and $0.84 < n < 1.43$. Characterization of WEOM by spectral techniques revealed a percentage of aromaticity of 6.5, and an average molecular weight of 1.7 kDa. WEOM (5 $mgC.L^{-1}$) enhanced the drugs phototransformation under simulated solar light by a factor of 2, 4.2 and 16 for sertraline, clozapine and citalopram, respectively. The drastic inhibiting effect of 2-propanol (5×10^{-3} M) demonstrated that hydroxyl radical was the key intermediate in these reactions. A series of photoproducts were identified by combining ultra-high performance liquid chromatography system (UHPLC) and high resolution mass spectrometry (HRMS). The photodegradation of the three drugs proceeded through oxidation of the N-containing aliphatic ring with or without O atom inclusion, oxidation of the aromatic ring with phenols formation, N elimination, and substitution of the halogen by OH. Sertraline and citalopram also underwent elimination of the halogenated ring while for clozapine opening of the N-containing aliphatic ring was observed. These results show that natural organic matter from the lake sediments greatly affects the fate of the three antidepressant drugs.Jiménez-Holgado Cristina, Richard Claire, Sakkas VasiliosUniversity of Ioannina, Department of Chemistry, Ioannina, GreeceLaboratoire de Photochimie moléculaire et Macromoléculaire, CNRS-Université Blaise Pascal, Aubiere Cedex, France

3.01.15

Determination of Fungicides and Insecticides in Soil Samples: Occurrence and Enantiomeric Distribution

L. Pérez-Mayán, Universidade de Santiago de Compostela / IAQBUS - Institute of Research on Chemical and Biological Analysis; M. Ramil, University of Santiago de Compostela / IAQBUS- Institute of Research on Chemical and Biological Analysis; R. Cela, Universidade de Santiago de Compostela / Department of Analytical Chemistry- Institute of Research on Chemical and Biological Analysis; I. Rodríguez, University of Santiago de Compostela / Department of Analytical Chemistry - Institute of Research on Chemical and Biological Analysis

Viticulture is one of the agriculture areas with the highest application rate of organic synthetic pesticides, particularly fungicides. Thus, concern about the missuse of those compounds has increased abruptly, as far as the possibility of migration to foodstuff (grapes, wine, must,etc). Lately, research has been focused on the development of accurate and robust methodologies to detect low concentration of pesticides in environmental matrixes. However, little has been studied about their presence and persistence in vineyard soils.In this research, we developed an accurate methodology for the determination of 51 pesticides (fungicides and insecticides belonging to different families and employed in viticulture) in soils from vineyards located in Northwest Spain, a region with a high consumption rate of these phytochemicals. To this end, a combination of pressure liquid extraction (PLE) and liquid cromatography (LC-MS/MS) was optimized. Moreover, pesticides were monitored during a 2-year period in different geographical areas in the Northwest of Spain. This procedure was applied to samples collected at different times during consecutive campaings to asses the occurrence and persistence of pesticides in this matrix. The majority of componds detected at the end of the application campaing (end of summer), remained in soil in the spring of the following year, which points out to potential concentration in this environmental compartment. Many of the fungicides employed in viticulture are chiral molecules and, in most cases, they are sprayed on vineyards as racemates. This practice reduces production costs but rises contamination of agricultural areas, since it requires increasing the total concentration of a given compound for the effective control of fungal diseases affecting vines. Thereafer, their dissipation in soil might be an enantiomeric selective process. The second part of this presentation investigates the enantiomeric fractions for a selection of five fungicides, previously identified as moderately persistent in soil, in samples from vineyards with different soil features.

3.01.17

Modelling Removal Rate Constants of Chemicals in Activated Sludge Based on QSARs and Field Data Lead to Qualitative Insights but Not Quantitative Predictions

D. Mooij, Radboud University; T. van Bergen, J.A. Hendriks, Radboud University / Environmental Science; T. Nolte, Radboud University Nijmegen / Department of Environmental Science; A.M. Ragas, Radbound University / Environmental Science; M. Huijbregts, Radboud University Nijmegen / Department of Environmental Sciences; R. van Zelm, Radboud University / Environmental Science

The incomplete removal of chemicals in wastewater treatment plants (WWTPs) has led to growing concerns about their environmental fate and impact. Additionally, an in-depth mechanistic understanding of the removal processes that do occur in WWTPs is currently lacking. To improve the qualitative understanding of removal processes and make accurate quantitative fate

estimations, general and class-specific quantitative structure-activity relationship (QSAR) models were developed. Pseudo-first order rate constants of chemicals were calculated using WWTP data of influent and effluent concentrations from literature and water authorities. By means of partial least squares regression, the removal rate constants were related to the chemicals physicochemical properties. General QSAR models, including all structurally diverse chemicals from the dataset and class-specific QSAR models were developed for chemicals that undergo cleavage of a C-N bond as expected primary biodegradation step. The goodness of fit (R^2) of the general models (n=10) varied in the range of 0.42-0.60 for the general models and in the range of 0.49-0.71 for the class-specific models (n=10). The goodness of prediction based on internal validation (R^2 CV) showed that the models were stable, with R^2CV in the range of 0.26-0.51 for the general models and R^2 CV in the range of 0.28-0.61 for the class-specific models. The predictive power on the test sets was highly variable and generally low, $-2.69 < Q^2 < 0.16$ for the general models and $-1.48 < Q^2 < 0.39$ for the class-specific models. The class-specific models outperformed the general models and the constructed QSAR models could qualitatively differentiate between chemicals but could not make quantitative predictions of removal rate constants. The identified descriptors were interpreted mechanistically and indicated that electrostatic properties, molecular size, and shape play an important role in the removal of chemicals in WWTPs and were mostly related to biodegradation processes. Future research should focus on the development of models including parameters related to the WWTP operational conditions and microbial community to improve the mechanistic understanding and prediction of the removal of chemicals in WWTPs.

Air Pollution, Exposure Assessment, and Health Effects

3.02.01

Oxidative Potential, Cytotoxicity, and Intracellular Oxidative Stress Generating Capacity of PM10

M. Lionetto, M. Guascito, M. Giordano, R. Caricato, A. De Bartolomeo, M. Romano, University of Salento / Dept. Biological and Environmental Sciences and Technologies; M. Conte, A. Dinoi, Institute of Atmospheric Sciences and Climate, CNR / Division of Lecce; D. Contini, Istituto di Scienze dellAtmosfera e del Clima, CNR / Division of Lecce

Exposure to atmospheric particulate matter (PM) induces negative effects on human health, in particular on pulmonary and cardiovascular systems. Several effects of PM on human health may be mediated by the induction of oxidative stress. The measurement of the oxidative potential (OP) is considered as a possible metric of PM, however, the relationships between the measurement of OP and the effects at the cellular level and health effects are not completely known. The present study aimed to study the correlation among the OP assessed by the acellular dithiothreitol assay (DTT test), with cellular endpoints such as cytotoxicity and intracellular oxidative stress. The study was carried out on PM10 samples collected between 19/02/2019 and 29/04/2019 at the Environmental-Climate Observatory of ISAC-CNR in Lecce (Southern Italy, 40°20'08" N—18°07'028" E, 37 m asl), a regional station of the Global Atmosphere Watch (GAW) network, characterized as an urban background site. Cytotoxicity was evaluated by the MTT assay (based on a colorimetric reaction dependent on mitochondrial respiration of the cells), on the cell line A549, representative of the alveolar type II pneumocytes of the human lung, exposed to aqueous extracts of PM10 samples for 24h. The intracellular oxidative stress was assessed on the same cell line and in the same experimental exposure conditions using the ROS-sensitive fluorescent probe CM-H2DCFDA. OP was evaluated using the dithiothreitol assay (DTT). The toxicological endpoints were integrated with the measure of the PM10 concentration and carbon content (OC, EC, and TC).Results showed that OP was statistically correlated with cytotoxicity in the exposed cells, which is a cellular endpoint integrating all the potentially toxic effects that PM10 can exert in the cell. Moreover, the intracellular oxidative stress induced in A549 cells was statistically correlated to OP, suggesting that the ability of PM10 to generate intracellularly reactive species is in a certain way related to its intrinsic oxidative potential, assessed by the acellular test. Moreover, cytotoxicity was related to the PM oxidative stress inducing capacity, suggesting that the intracellular oxidative stress plays a key role in the multiple mechanisms underlying PM10 cytotoxicity. This work was supported by the Regione Puglia through the project PAPER under grant PH3B166.

3.02.02

CoDo - a Combined Dosimetry Model Allowing Linking In Vitro Doses to Exposure Levels: A Case Study About Titanium Dioxide

D. Romeo, ETH Zürich/Empa; P. Wick, Empa Swiss Federal Laboratories for Materials Science and Technology / Particles-Biology Interactions Laboratory; B. Salieri, R. Hischier, B. Nowack, EMPA / Technology and Society Lab

Traditional Human Health Risk Assessment and Life Cycle Assessment rely on animal studies to provide information about nanomaterials toxicity. The reduction of such studies due to their costs, ethical issues, and reproducibility concerns represents a challenge for these methodologies. In a previous work [1], we identified dosimetry as a mature in silico tool that could support the use of in vitro toxicity data in place of in vivo studies. In vitro dosimetry simulates the deposition of particles on the cells in submerged systems [2], while lung dosimetry simulates the deposition of particles in the respiratory tract [3]. While such models already exist, they are stand-alone and time-consuming to apply to big data sets.We developed a Combined Dosimetry model (CoDo) that estimates the exposure levels corresponding to the concentrations used in vitro in submerged systems, accounting both for in vitro and lung deposition processes. CoDo includes multiple exposure scenarios and allows batch processing. Using the model, we evaluated the last five years of publications about Titanium dioxide toxicity on lung cells in vitro and compared the estimated exposure levels to the Occupational Exposure Limits. The results show that the doses used in vitro are representative of longer exposure times, e.g. one year of exposure in the workplace. Instead, when considering the same exposure times as in vitro, only a third of the doses used are realistic. Moreover, we determined the Lowest Observed Adverse Effect Concentration (LOAEC) based on cytotoxic effects. Interestingly, significant cytotoxic effects (viability < 80%) are sometimes observed at realistic doses. Identifying which in vitro doses are realistic can help to select the data that provides relevant information for Risk Assessment and LCA, and avoid overload conditions. Moreover, CoDo can be used to link in vitro toxicity to human exposure, by estimating the exposure levels corresponding to in vitro doses.1. Romeo, D., Salieri, B., Hischier, R., Nowack, B. & Wick, P. An integrated pathway based on in vitro data for the human hazard assessment of nanomaterials. *Environment International* 137, (2020).2. Cohen, J. M., DeLoid, G. M. & Demokritou, P. A critical review of *in vitro* dosimetry for engineered nanomaterials. *Nanomedicine* 10, (2015).3. Miller, F. J., Asgharian, B., Schroeter, J. D. & Price, O. Improvements and additions to the Multiple Path Particle Dosimetry model. *J. Aerosol Sci.* 99, (2016).

3.02.03

Bioaccessibility of Carcinogens, Endocrine Disrupting Chemicals and Reactive Oxygen Species Precursors in Ambient Fine Particulate Matter

G. Lammel, Masaryk University, RECETOX; Z. Novakova, Masaryk University / Research centre for toxic compounds in the environment; Z. Kitanovski, Max Planck Institute for Chemistry / Multiphase Chemistry Department; P. Kukucka, Masaryk University, Faculty of Science, RECETOX / RECETOX Research Centre for Toxic Compounds in the Environment; J. Novak, P. Pribylova, Masaryk University, Faculty of Science / RECETOX; P. Shahpoury, Environment and Climate Change Canada / Air Quality Research Division; M. Wietzoreck, U. Pöschl, T. Berkemeier, Max Planck Institute for Chemistry, Multiphase Chemistry Department; K. Hilscherova, Masaryk University, Faculty of Science, RECETOX / RECETOX

Various adverse health effects are caused by long-term exposure to elevated levels of particulate matter (PM). Carcinogenicity and arylhydrocarbon (AhR) activation potency of PM are dominated by aromatic compounds. Endocrine disruptive potencies are carried by mostly unidentified organic PM components. Quinones, by producing reactive oxygen species, contribute significantly to the oxidative stress potential (OP) of PM. Total and inhalation-bioaccessible gas and particulate phase concentrations of aromatic compounds i.e., polycyclic aromatic hydrocarbons (PAHs) and their nitrated and oxygenated derivatives (NPAHs, OPAHs), were determined in the gas and particulate phases at a polluted urban and a central European background site in the Czech Republic. The mass size distributions peaked in the PM_1 size fraction. Nevertheless and notably, high mass fractions of the targeted substances were found in the gas-phase, even in winter. The bioaccessible fraction of particulate organic matter in ambient air, $f_{bio\ p}$, was determined by leaching filter samples with two simulated epithelial lung fluids, a modified Gamble's solution (SLF) and artificial lysosomal fluid (ALF). Using human cell lines, various PM size and polarity fractions were biotested for (anti-)estrogenicity (ER), (anti-)androgenicity (AR), AhR and thyroid receptor (TR)-mediated activities, as well as cytotoxicity to bronchial cells. Highest potencies were found in the PM_1 size fraction and mostly in winter. While only small fractions of parent PAHs were found bioaccessible (0.1-0.8 % on a mass basis, < 0.1-5 % on a toxic equivalent (TEQ_{BaP}) basis; PM_1 samples, using SLF), these were slightly higher for the more polar OPAHs (0.1-8.0 %). The PAHs' limited leachability is also reflected in the low fraction of bioaccessible AhR activating compounds in PM_1 (1-8 % of TEQ_{BaP}). BaP accounted for 7-20 % of TEQ_{BaP}. The bioaccessible fractions of quinones were 1.6 (< 0.1-2.4) % in winter and 2.6 (0.6-3.3) % in summer. The bioaccessible fractions of ER and TR potencies were 4-10 % and ≥ 90 % of the total, respectively. Results from reconstituted mixtures testing suggest that ER and TR activation are almost unrelated to PAHs and derivatives. The bioaccessible fraction of lipophilic organics through partitioning from the gas phase is significantly higher than from dissolution of PM. Accordingly, a model simulation including SLF-air partitioning (gas-phase), $f_{bio\ p}$ (particulate phase) and kinetic constraints of lung and downstream body uptake reveals that PAH inhalation dose mostly originates from the gas-phase, even for PAHs which are transported and inhaled predominantly as PM.

3.02.05

Involvement of Calcium Signaling and Mitochondria Alteration in Nickel

Oxide Nanoparticles - Induced Alterations in Human Pulmonary Artery Endothelial Cells

O. Germande, UMR EPOC 5805 UNIVERSITE BORDEAUX / UMR EPOC 5805; J. Deweirdt, University of Bordeaux / CRCTB - INSERM U1045; C. Guibert, INSERM U1045 / CRCTB; I. Baudrimont, University of Bordeaux / CRCTB - INSERM U1045; M. Baudrimont, Université de Bordeaux / UMR EPOC 5805

Keywords: human pulmonary endothelial cells, nickel oxide nanoparticles, calcium signaling, mitochondria

Anthropic activities such as mining amplifies the natural erosion of nickel mines leading to atmospheric emission of nickel oxide nanoparticles (NiONPs). New Caledonia is particularly affected by nickel mining activities. Workers and population living nears could be exposed to NiONPs. After inhalation, NPs penetrate deeply into the airways and can cross the capillo-alveolar barrier to reach the pulmonary circulation and exert deleterious effects on cardiovascular system. Pulmonary artery endothelial cells (HPAEC) can be a direct target of inhaled particles. Alteration calcium homeostasis is critical event involved in the physiopathology of vascular diseases, such as mitochondria alteration. Only a few studies have investigated the effect of NiONPs on pulmonary vascular endothelial cells and the cellular mechanisms remain unclear. The aim of this study was to assess the cytotoxic effects of NiONPs on HPAEC, especially calcium signaling and mitochondria function. HPAEC were exposed for 4 or 24 h to NiONPs (0.5 to 5 µg/cm²). Different endpoints were studied (i) calcium signaling and TRPV4 activity respectively by confocal microscopy (Fluo-4 AM probe) and qPCR, (ii) mitochondrial superoxide anion production by confocal microscopy (MitoSOX probe) and (ii) mitochondrial dysfunction by confocal microscopy (TMRM and MitoTracker probes). In HPAEC, NiONPs altered calcium homeostasis with a calcium cytosolic level ($[Ca^{2+}]_i$) increased. The extracellular calcium chelator (EGTA) and TRPV4 channels inhibitor (HC-067047) significantly reduced this $[Ca^{2+}]_i$ rise. These results suggest that NiONPs-induced $[Ca^{2+}]_i$ rise involve mostly the extracellular source of calcium. Furthermore, our results provides evidence that NiONPs exposure increased the intracellular level of mitochondrial superoxide anion and $[Ca^{2+}]_m$ level leading to mitochondrial dysfunction. Thus, workers and population exposure to NiONPs in New Caledonia may be a risk factor for the exacerbation of pre-existent vascular diseases.

3.02.06

High Resolution Exposure Assessment of Air Pollution and the Related Impacts on Health in the Nordic Countries

C. Geels, Aarhus University / Department of Environmental Science

The negative health impacts related to air pollution compounds such as ozone (O_3), nitrogen dioxide (NO_2) and fine particulate matter ($PM_{2.5}$) are well established (as reviewed in e.g. by WHO[1]). The potential social inequalities in the exposure to air pollution has also attracted attention[2]. In the interdisciplinary project NordicWelfAir we take advantage of the unique detailed databases and health registers in order to increase our understanding of the link between air pollution impacts and the distribution of welfare in the Nordic countries. A detailed common Nordic emission inventory down to 1 km x 1 km resolution has been constructed and used as input to state-of-the-art advanced air pollution model systems. The DEHM/UBM system has been run for a 40-year period with a spatial resolution of 1 km x 1 km for the Nordic area. This new high resolution and multi-year air pollution dataset have made it possible to take advantage of the comprehensive cohort and nation-wide health registers in the Nordic countries. Health studies and exposure assessments confirms that air pollution is associated with numerous health impacts in an area were the air pollution levels are relatively low[2]. Overall, the premature deaths attributable to $PM_{2.5}$ in Nordic area is on the order of 8,500 to 11,400 per year[3]. Our results also indicates that inequalities in both the exposure to PM and the related risks across different population groups due to underlying differences in health status, needs to be understood. This can serve as input to the development of future mitigation strategies that will benefit all population groups. [1] WHO. 2013. Health risks of air pollution in Europe – HRAPIE project. Recommendations for concentration–response functions for cost–benefit analysis of particulate matter, ozone and nitrogen dioxide. Pp 60. [2] Raaschou-Nielsen, O., Thorsteinson, E., Antonsen, S., Holst, G. J., Sigsgaard, T., Geels, C., Frohn, L. M., Christensen, J. H., Brandt, J., Pedersen, C. B., and Hvidtfeldt, U. A. 2020. Long-term exposure to air pollution and mortality in the Danish population a nationwide study, EClinicalMedicine, 28, 100605, https://doi.org/10.1016/j.eclinm.2020.100605. [3] Lehtomaki, H., Geels, C., Brandt, J., Rao, S., Yaramenka, K., Astrom, S., Andersen, M. S., Frohn, L. M., Im, U., and Hanninen, O. 2020. Deaths Attributable to Air Pollution in Nordic Countries: Disparities in the Estimates, Atmosphere-Basel, 11, ARTN 467 10.3390/atmos11050467.

3.02.10

Exposure of E-Waste Dismantlers in Catalonia (Spain) to Inhalable Organophosphate and Halogenated Flame Retardants

A. Balasch Garcia, M. López, M. Viana, IDAEA CSIC; C. Reche, IDAEA CSIC Barcelona; T. Moreno, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Research Council (CSIC) / Geosciences; E. Eljarrat, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Research Council (CSIC) / Department of Environmental Chemistry

The recycling of electronic waste (e-waste) is becoming a concern for global environmental and human health as the amount of e-waste is increasing. This process is performed in e-waste recycling facilities, which can be considered hot spots of air pollution due to expected significant emissions of particulate material (PM) during crushing processes. The emitted PM can contain hazardous substances as heavy metals and flame retardants (FRs), which potentially presents a risk for the employees working in this industry.The current study describes the results of a sampling campaign performed in an e-waste recycling facility located in Catalonia, Spain, in order to assess indoor air quality and the occupational exposure of e-waste dismantlers. Two different working areas were monitored for three days each. In the first area, the only activity was the dismantling of cathode-ray tube TVs. The second area corresponded to the manual selection and trituration area, where they treated residues such as plastic, cables, circuits and metals. PM2.5 and PM10 samplers (PEM, SKC) were deployed during working hours (6-8 h), using quartz microfiber filters as sample media. Control samples were also collected outdoors to correct for background concentrations.PM2.5 and PM10 samples collected on quartz microfiber filters will be analyzed to characterize two FR families, organophosphorus flame retardants (OPFRs) and halogenated flame retardants (HFRs), as well as heavy metals. Finally, human exposure to FRs via ambient inhalation will be assessed, and an estimation made of the non-carcinogenic and carcinogenic risks of FR exposure via airborne particle inhalation during the time expended in the factory.

Assessment of the Exposure and Effects of Contaminants of Emerging Concern in Drinking and Wastewater Systems and Assays to Evaluate their Removal

3.03.01

Pharmaceuticals and Personal Care Products (PPCPs) in Field Sampled Marine Fauna in Relation to Sewage Release From Arctic Settlements

A. Sarno, SINTEF Ocean; L. Sørensen, SINTEF Ocean / Climate and Environment; R. Kallenborn, Norwegian University of Life Sciences / Chemistry, Biotechnology and Food Sciences; G. Gabrielsen, The Norwegian Polar Institute; I. Overjordet, SINTEF Ocean / Climate and Environment

One group of emerging pollutants that has received little attention in the Arctic is pharmaceuticals and personal care products (PPCPs). The knowledge of levels and effects of PPCPs in Arctic marine fauna is limited, in particular in lower trophic levels. We have investigated the presence of contaminants in a variety of low trophic benthic and pelagic marine species collected close to a sewage outlet in Kongsfjorden in Svalbard. Pelagic invertebrates were collected by net hauls, while benthic species were collected in baited traps. The bulk samples were sorted to species or genus before transfer to precleaned glass vials for storage at -20 °C. Samples of sewage effluent (4oo mL, 3 replicates) were collected monthly between June and September to assess seasonal variation in PPCP releases to the fjord. In addition, PPCPs in sewage effluent and seawater was sampled using ChemCatcher passive samplers with HLB membranes to obtain time integrated concentrations. All the samples were subject to solvent extraction and highly sensitive tandem mass spectrometry techniques for target analysis of persistent compounds (GC-MS/MS) and pharmaceuticals (LC-MS/MS). Targeted and non-targeted screening for a wide range of pharmaceuticals including anti-depressants, antibiotics and analgesics were performed. Seven pharmaceuticals were quantified in several of the invertebrate species, including ibuprofen, diclofenac, ciprofloxacin and citalopram, despite very low population density in the area. In addition, screening techniques revealed several other PPCP compounds in the same samples. At the time of sampling, raw sewage was released directly to the marine environment from the small research settlement in the area, while a sewage treatment was installed later that year. The results from the initial sampling will be compared to samples collected two years after the sewage treatment was installed to investigate potential reductions in the concentrations of PPCP compounds in the marine fauna. In addition, seasonal concentrations of PPCPs in sewage outlet water and seawater will be presented for exposure assessment.

3.03.02

Contaminants of Emerging Concern in Groundwater Under the Influence of Onsite Septic Systems

S.T. Glassmeyer, U.S. Environmental Protection Agency / Office of Research and Development; T.D. McCobb, US Geological Survey / New England Water Science Center; E.T. Furlong, U.S. Geological Survey / Laboratory & Analytical Services Division; D.W. Kolpin, US Geological Survey / Central Midwest Water Science Center; M. Mills, U.S. Environmental Protection Agency / Office of Research & Development; J.A. Shoemaker, U.S. Environmental Protection Agency / Office of Research and Development; D.R. LeBlanc, J.R. Barbaro, US Geological Survey / New England Water Science Center; M. Belaval, US Environmental Protection Agency / Region 1, Water Division

Contaminants of Emerging Concern (CECs), as a class of chemicals and potential biological stressors, are difficult to measure, and can be linked to many ongoing and historic sources. One challenge with source identification and mitigation is that in many instances multiple sources contribute to contaminant loading to the aquatic system. Research has identified municipal wastewater-treatment discharges (e.g. aqueous effluents, biosolids) as important sources of CECs to the environment. Although more than 20% of households across the United States use private onsite septic systems, yet understanding and characterizing environmental exposures and corresponding environmental effects derived from such sources are lacking. Groundwater is an important resource to the Cape Cod region of Massachusetts. Cape Cod's fifteen towns rely on a sole-source aquifer for drinking water and groundwater is the primary receiver of wastewater disposal for both on-site and municipal wastewater treatment. To better manage wastewater input to the aquifer and to improve groundwater quality, over 1,400 homes in the town of Falmouth were connected to the municipal sewer system beginning in 2016. This conversion from onsite septic to a municipal sewer system provided a unique opportunity to observe potential changes occurring in groundwater quality in response to the change in the CEC source term. The results of this study will provide important information to other areas where aquifers are overlain by high densities of onsite septic systems. Groundwater samples were analyzed for over 100 chemical and microbial CECs. Maximum observed concentrations of target CECs were often found at similar (e.g. buproprion, cotinine, tramadol) or even higher (e.g., carbamazepine, fluconazole, sulfamethoxazole, sucralose) concentrations compared to that found in municipal wastewater effluent. These results clearly document onsite septic systems as another important environmental pathway for CECs.

3.03.08

Combining Laboratory and Field Studies for the Identification of Priority Pharmaceuticals and Photo-Transformation Products in Surface Water

O. Gómez Navarro, IDAEA- CSIC / Environmental Chemistry; N. Montemurro, Jordi Girona 18-26, null / Dipartimento di scienze agro-ambientali e territoriali; L. Bertrans Tubau, BETA Technological Center - UVic; M. Abril, L. Proia, BETA Technological Center - UVic / Aquatic Ecology and Ecotoxicology; S. Pérez, IDAEA CSIC / Environmental Chemistry

Pharmaceuticals are worldwide used for prevention and treatment of diseases for both humans and veterinary uses. However, they are ubiquitous in the environmental with recognized recalcitrance and potential bioaccumulation. Their presence in the aquatic system is mainly derived by the low removal rate of pharmaceuticals by municipal wastewater treatment plants (WWTPs) as actual wastewater treatments are not effective in treating these. Hence, a natural abiotic phenomena as solar photolysis arises importance as a possible degradation process for pharmaceuticals and its human metabolites discharged by WWTPs. Exposure to sunlight activates photo-transformation reactions by either direct or indirect photolysis. As a consequence, photo-transformation products (photo-TPs) are produced which could accumulate in surface water and have a greater ecotoxicity than their precursors. Surface water samples from six different WWTPs in Osona (Catalunya, Spain) with different sample points: upstream, discharge point of the effluent and downstream (20, 40, 60, 80, 100, 150, 250m) were analysed in order to study and identify the presence of pharmaceuticals and its photo-TPs. Laboratory photo degradation experiments were performed for a cocktail of the most abundant pharmaceuticals found, under simulated solar radiation (Suntest CPS, Heraeus) in order to study the kinetic degradation of pharmaceuticals and the generation of photo-TPs. All samples were extracted using a home-made multi-layer solid phase extraction cartridge and samples were analysed through a liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Sporadic detection of pharmaceuticals along the investigated Catalan rivers was observed, showing a clear impact of the WWTP discharges on pharmaceutical contamination downstream WWTP outlet. The suspect screening analysis of water samples allowed the detection of more than ten different pharmaceuticals including losartan and its identified photo-TP 335. Only one photo-TP was detected compared to the higher number of photo-TPs produced at laboratory scale. This low detection frequency of TPs in surface water can be related to their low concentration or unfavourable conditions in the environment. Thus, this approach combining laboratory and field studies highlights that LC–HRMS is a powerful tool for simultaneous quantitative and qualitative analysis, allowing the search for suspected compounds.

3.03.09

Non-target protein identification in wastewater using the regions of interest multivariate-curve resolution (ROIMCR) chemometrics method

C. Pérez López, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Department of Environmental Chemistry; A. Ginebreda, CSIC - Spanish National Research Council / Environmental Chemistry; D. Barcelo, ICRA- G17920851; M. Carrascal, J. Abian, Institute of Biomedical Research of Barcelona, Spanish National Research Council (IIBB-CSIC/IDIBAPS); R. Tauler, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Research Council (CSIC) / Environmental Chemistry

A method based on Regions of Interest followed by Multivariate Curve Resolution (ROIMCR), is proposed as a new methodology for non-target protein analysis in environmental proteomic studies. Proteins were captured from wastewater using polymeric (polycaprolactonediol) devices placed during 11 days in 3 different sites of a wastewater treatment plant (Gavà-Viladecans, Barcelona, Spain): influent water, anoxic reactor (denitrification), and effluent water; the polymeric probes were processed and analyzed by LC-MS and LC-MS/MS.Regions of Interest (ROI) methodology was used for data filtering and compression, generating a data matrix with 17158 retention times (in the rows) and 2838 ROI values (in the columns) with the information of all the analyzed samples. Multivariate Curve Resolution-Alternating Least Square (MCR-ALS) applied to this ROI compressed data matrix resolved 162 components explaining 95.15% of the variance, most of them associated with peptide signals (apart from a few background and solvent signal contributions). A Partial Least Squares-Discriminant Analysis (PLS-DA) was used to study which of these ROIMCR components are the most significant, selecting 85 of them when a Selectivity Ratio threshold of 9.12 (F-test 95%) was employed. Principal Component Analysis (PCA), after ROI compression, MCR analysis and PLS-DA Selectivity Ratio analysis was applied to the peak heights of the analyzed samples, after Standard Normal Variate (SNV) sample normalization to study the similarity of the proteomic profiles of the samples from the same sampling site and the differences between the samples at the different sampling sites of the WWTP. An improvement in the description of the different WWTP sampling sites and protein sources was observed when PCA was applied to the data from MCR and PLS-DA results, much better than when PCA was applied directly after ROI compression. Finally, signals resulting from ROIMCR, and those selected by PLS-DA, were analyzed and compared using Proteome Discoverer, with the identification of 281, 314, and 58 possible peptides, respectively for each sample site in the case of ROIMCR results, and with the identification of 137,123 and 31 possible peptides in the case of the PLS-DA results. These peptides represented proteins from a variety of species, e.g human, chicken, rat, and mouse, and they were represented in different amounts in the different WWTP sites.

3.03.11

Assessing the Presence of >2000 Contaminants of Emerging Concern in the Wastewater-Soil-Plant Continuum Under Real-Field Conditions During Irrigation With Treated Wastewater

I.C. Iakovides, Nireas-International Water Research Center, University of Cyprus / Department of Civil and Envrironmental Enginnering , School of Engineering, University of Cyprus; V. Beretsou, Nireas-International Water Research Center, University of Cyprus / Civil and Environmental Engineering; A. Christou, Agricultural Research Institute / Ministry of Agriculture, Rural Development and Natural Recourses; G. Gkotsis, National and Kapodistrian University of Athens / Laboratory of Analytical Chemistry, Department of Chemistry; M. Nika, National and Kapodistrian University of Athens / Laboratory of Analytical Chemistry, Department of Chemistry; N. Thomaidis, National and Kapodistrian University of Athens / Department of Chemistry; D. Fatta-Kassinos, University of Cyprus / Nireas International Water Research Center

Climate change and global warming have been widely acknowledged during the last few decades. The ever-increasing shortage of water, the increasing needs for food due to the expanding world population, and for crop irrigation water, both in respect to good quality and quantity, render reuse a '*sine qua non*' condition. According to the principles of the circular economy, the use of treated wastewater (TWW) has been recognized as one of the main measures of mitigation of the water crisis, as it can be a suitable alternative to water supply for the irrigation of crops. Whilst TWW reuse for irrigation is recognized as one of the indispensable practices for the mitigation of water scarcity, challenges such as the existence of contaminants of emerging concern (CECs) act as a limiting factor in effectively presenting the reuse practices. The contamination of the environment and the possible uptake and bioaccumulation of CECs in the edible parts of food crops and fodders during TWW irrigation, and their subsequent entry into the food chain have attracted great scientific attention over the last several years. Within this context, this study aimed at assessing such challenges mediated from TWW irrigation of lettuce (*Lactuca sativa L.*) plants grown in lysimeters under real-field conditions, and irrigated with treated effluent from conventional activated sludge (CAS) and membrane bioreactor (MBR) treatment facilities. Different matrices such as tap water, TWW, leachate, soil and lettuce were collected and analysed for >2000 of CECs using wide–scope target screening methodologies in order to identify chemical compounds in the different matrices. In total, 187 CECs were detected in all the examined samples. Detected compounds included pesticides, antibiotics, cardiovascular drugs, analgesics, antidepressants, antidiabetics, antiepileptic, etc. It is worth noting that 25 transformation products/metabolites of the 8 corresponding parent compounds detected were also identified. The majority of the detected CECs were found in TWW. In total, 114 and 99 compounds were detected in the CAS- and MBR-treated effluent, respectively. Moreover, 43 compounds were detected in the leaves sampled from plants irrigated with CAS- and MBR-treated effluents, respectively. Lower number of CECs were detected in soil and leachate samples. Information on the quantified findings will be presented

during the oral presentation.

3.03.12

The Effect of Carrier Gas Change During Pyrolysis on the Polycyclic Aromatic Hydrocarbons Persistence and Bioavailability in Biochar-Amended Soil

P. Godlewska, Maria Curie-Sklodowska University / Department of Radiochemistry and Environmental Chemistry; P. Oleszczuk, Maria Curie Skłodowska University / Department of Radiochemistry and Environmental Chemistry

Sewage sludge (SSL), as a by-product of wastewater treatment, can contain certain amounts of contaminants such as heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs) but also may also contain valuable nutrients. Very often SSL is used in agriculture as a fertilizer. However, due to toxic substances (e.g PAHs), SSL may generate a threat when is mixed with soil. One of the ways of SSL utilization can be converting it to biochar during pyrolysis and then use it in agriculture as a soil additive. During the process of pyrolysis the undesirable components of SSL i.e. inorganic and organic contaminants as well as pathogenic elements can be transformed into less mobile forms or eliminated from the material. Unfortunately, biochar produced from SSL may still contain HMs (even concentrated compared to raw SSL) and PAHs (that can be formed during pyrolysis). For decrease of the amount of toxic substances occurring in SSL-derived biochar, it was proposed to use carbon dioxide (CO_2) instead of the commonly used nitrogen (N_2) as a carrier gas during pyrolysis. This solution besides of contaminants reduction, may also improve other biochar properties i.e. increase carbon content and specific surface area and decrease ash content compared to biochar produced in N_2 atmosphere. Because of the biochar is planned to be applied to the soil system the research should focus on the contaminants content in biochar-amended soil. The aim of the study was to determine the persistence of the total (C_{tot}) and bioavailable (C_{free}) PAHs in SSL-derived biochar-amended soil prepared in N_2 or CO_2 carrier gas. The total content of PAHs (C_{tot}) in biochar- amended soil was higher than in the clean soil and similar to SSL-amended soil. However, the content of these pollutants in bioavailable and mobile fraction (C_{free}) was similar to clean soil and lower than in SSL- amended soil. Since the bioavailable fraction of contaminants is crucial in direct evaluation of the threat to the environment, it is expected that biochar-amended soil will pose less risk than SSL-amended soil. The project was funded by the National Science Centre granted on the basis of the decision number DEC-2018/31/N/ST10/01588.

3.03.16

Biological Activity in US Food Processing Plant Effluent

E.K. Medlock Kakaley, M.C. Cardon, N. Evans, C. Lambright, U.S. Environmental Protection Agency / Office of Research and Development/Center for Public Health and Environmental Assessment; B.R. Blackwell, R.N. Hofer, U.S. Environmental Protection Agency / Office of Research and Development/Center for Computational Toxicology and Exposure; L.E. Hubbard, US Geological Survey / Upper Midwest Water Science Center; P. Bradley, U.S. Geological Survey / South Atlantic Water Science Center; J.M. Conley, L.E. Gray, U.S. Environmental Protection Agency / Office of Research and Development/Center for Public Health and Environmental Assessment; D.W. Kolpin, US Geological Survey / Central Midwest Water Science Center; D. Villenueve, US Environmental Protection Agency / Office of Research and Development/Center for Computational Toxicology and Exposure

Complex mixtures of anthropogenic chemicals in freshwater environments have long been established. We previously evaluated agricultural, municipal, and industrial impacted surface and source waters for hundreds of chemical contaminants as well as biological activity indicative of potential adverse physiological outcomes. Commercial food processing facilities also produce wastewater with complex chemical mixtures. However, current monitoring of these wastewaters is primarily focused on basic constituents including, pH, dissolved oxygen, and suspended solids. Therefore we measured approximately 580 organic chemicals and biological activity in a two-phase study of wastewater effluent from 23 food, beverage, and feedstock processing facilities across the United States. In Phase I, extracted effluent samples were assessed for estrogenic (T47D-KBluc), androgenic (CV1-chAR), and glucocorticoid (CV1-hGR) activity, as well as activation of 26 additional nuclear receptors and 52 transcription factor signatures using the multi-endpoint Attagene FACTORIAL^{TM} bioassays. All Phase I effluent samples contained estrogenic activity above the method detection limit (MDL: 0.0017 ng E2Eq/L) and ranged 0.019 – 1.62 ng E2Eq/L. Androgenic activity was detected in 9 of 23 sites above the MDL, (0.055 ng DHTEq/L) and ranged 0.19 – 8.41 ng DHTEq/L. No facility discharges were found to contain glucocorticoid activity, and no field blanks contained biological activity above bioassay MDLs. Attagene results corroborated the single endpoint bioassay results indicating contaminants causing estrogenic activity existed in all screened effluent extracts. We compared estrogenic activity and detected known estrogen concentrations from Phase I (9 estrogens detected using HPLC/MS-MS; converted to total E2Eq) through linear regression which resulted in $R^2 = 0.46$. Although nearly half the samples contained cumulative bioactivity (estrogenic) in concentrations high enough to potentially elicit adverse physiological effects in aquatic organisms, the chemical concentrations quantified using targeted analytical methods did not completely explain all measured biological activity. These results support the application of bioassays in water quality screening applications and their continued use in Phase II sample analysis. Phase II sampling, expanded to include stream water, bed sediment and aquatic organisms collected above and below the outfall of a subset of 7 facilities (in addition to effluent sample) will provide additional insight regarding potential effects of exposure to these biologically active contaminants. Abstract does not necessarily reflect USEPA views or policy.

3.03.17

Comprehensive Assessment of Wastewater by a Direct Coupling of Thin-Layer Chromatography and Bioassays

S. Buchinger, Federal Institute of Hydrology / Department G Biochemistry Ecotoxicology; C. Riegraf, Ecotox Centre CH; A.M. Bell, Federal Institute of Hydrology / Biochemistry and Ecotoxicology; B. Becker, German Federal Institute of Hydrology; U. Feiler, Federal Institute of Hydrology; S. Schaefer, Federal Institute of Hydrology / Biochemistry and Ecotoxicology; D. Shakibay, The Hebrew University of Jerusalem; L. Moscovici, The Hebrew University of Jerusalem / Institute of Life Sciences, Department of Plant and Environmental Science; S. Belkin, The Hebrew University of Jerusalem; G. Reifferscheid, Federal Institute of Hydrology / Biochemistry and Ecotoxicology

Due to an expected rise in water demand, paralleled with water shortage in e.g. arid regions, the intensity of water reuse is likely to increase. Therefore, there is a need for improved water treatment technologies and efficient methods to evaluate removal of contaminants of emerging concern. In this respect, effect based methods are highly relevant to investigate the overall toxicity of treated wastewater, because they cover all substances contributing to an observed effect. However, detected biological activity by means of a bioassay alone might not be directly linked to the responsible compounds. To combine the advantages of chemical analysis and bioassays, effect directed analysis can be applied. A variety of methods was recently developed to combine chromatographic separation of compounds by thin layer chromatography (TLC) with effect based detection directly on the surface of the thin-layer plate. Examples include hormone-like and genotoxic compounds, or inhibitors of photosystem II. This approach can be used to assess wastewater treatment quality and as a screening tool to identify and prioritize pressures and sources of chemical pollution in the context of the EU-Water framework directive. Furthermore, it can serve as a starting point for Toxicity Identification Evaluation. As one example influent and effluent samples were collected from six different wastewater treatment plants enriched by solid phase extraction and analysed by the p-YES for agonists of the estrogen receptor. In this way, estrogenic effect profiles of the samples were generated. These profiles allow the identification of compound- and treatment-specific differences in elimination efficiencies that might guide the optimization of treatment processes. Detected active sample components can be extracted from the surface of the thin-layer plate and directed to chemical analysis by mass spectrometry for a compound identification. By the use of this method, bisphenol A was identified as the main driver for estrogenicity in leachates from landfill sites. Due to its high robustness and sensitivity, the method can not only be applied to influent samples but also to ground water samples allowing a harmonized assessment of water quality across different types of water. A battery of effects combined with TLC is available to address a range of specific molecular effects. This work is ongoing by the development of assays for the detection of specific antagonistic effects such as anti-estrogenicity and anti-androgenicity.

3.03.22

Assessing Tobacco and Nicotine Use by Wastewater-Based Epidemiology

Q. Zheng, QAEHS - The University of Queensland; C. Gartner, The University of Queensland / School of Public Health; B.J. Tscharke, University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); J.W. O'Brien, QAEHS - The University of Queensland / QAEHS; K. Thomas, The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); J. Mueller, UQ / Queensland Alliance for Environmental Health Sciences; P.K. Thai, Entox / National Research Centre for Environmental Toxicology Entox

Measurement of population tobacco use and non-tobacco nicotine use are essential to evaluate the efficacy of tobacco control strategies and to monitor aspects of public health. Wastewater analysis is a valuable complement to conventional survey methods (e.g. questionnaires, interviews), providing non-invasive information on tobacco and non-tobacco nicotine use. In this study, we measured tobacco-specific biomarkers, anabasine and anatabine, in wastewater samples to analyse trends in tobacco use over six years (2012-2017) in a catchment of 100,000 people and compared this with the trends of total nicotine use by nicotine biomarkers, cotinine and hydroxycotinine. In addition, seven consecutive daily urine samples were collected from smokers to drive a suitable factor to accurately convert wastewater data to estimates of population tobacco use. The results shows a significant annual declines of tobacco use (-3.0%) and nicotine use (-2.4%). A

significant annual increase in the ratios of cotinine to anabsine (1.2%) suggested a relative increase in the use of non-tobacco nicotine products at the same time that tobacco use was declining. In smokers' urine samples analysis, the daily mass load of anabasine and anatabine was estimated 458 ±114 and 254 ± 96 ng/cigarette, respectively. This study demonstrates that tobacco monitoring using anabasine and anatabine are likely to reveal more realistic trends on tobacco use, compared to the standard biomarkers for nicotine, cotinine and hydroxycotinine, which are widely used. Daily mass load of anabasine and anatabine in smokers' urine was estimated in this study and could be used to provide a more realistic picture of tobacco use in population.

3.03.23
National Gout Prevalence Estimates Through Wastewater-Based Epidemiology in Australia
F. Ahmed, Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland; B.J. Tscharke, University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); J.W. O'Brien, QAEHS - The University of Queensland / QAEHS; J. Mueller, UQ / Queensland Alliance for Environmental Health Sciences; K. Thomas, The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS)
A method to assess the prevalence of gout in the Australian population using wastewater-based epidemiology (WBE) was established. We used oxypurinol, the urinary metabolite of allopurinol as a biomarker of gout disease for calculating gout prevalence from wastewater which has been used as a first-line gout preventive medication in Australia. Wastewater collected from 75 wastewater treatment plants (WWTPS) across Australia that covered approximately 52% population on the 2016 Australian census day. Oxypurinol loads were also compared with a large set of wastewater-based epidemiology (WBE) biomarkers, socio-economic index for areas (SEIFA), and disease factors to reveal the drivers of the gout disease at the population level. Allopurinol consumption ranged from 1.9 to 32 g/day/1000 people equating to 4.8 to 80 DDD/day/1000 people. We assumed one defined daily dose consumed per patient each day and converted the allopurinol consumed DDD/day/1000 to gout prevalence. Results showed that the prevalence of gout ranged from 0.5 % to 8 % in different areas with a median prevalence of 2.9% nationally. No significant positive associations were found between allopurinol consumption and alcohol consumption, mean age of catchment population, remoteness, or higher socioeconomic status. We found a significant positive correlation with naproxen (analgesic drug) consumption. Our study demonstrates that WBE can be used to estimate gout prevalence and can provide further information when triangulated with other biomarkers to evaluate disease-specific risk factors at the population level.

Biodegradation of Organic Trace Pollutants in the Environment

3.04.02
Methyltins Distribution and Behaviour in Marine Environment; In Situ Study of Tin Methylation in Sediment and Porewater
M. Furdek Turk, Ruder Boskovic Institute / Department for Marine and Environmental Research; M. Monperrus, Université de Pau et des Pays de l'Adour, IPREM, CNRS / Laboratoire de Chimie Analytique BioInorganique et Environnement; N. Mikac, Rudjer Boskovic Institute / Division for Marine and Environmental Research
The speciation of Sn plays a crucial role in its ecotoxicological behaviour; while inorganic Sn is considered as non-toxic, the organotin compounds (OTCs), especially trisubstituted species, are highly toxic to living beings. Among all OTCs, only methyltins (MeTs) are not exclusively of anthropogenic origin because they can be formed in nature by the methylation process. Although it is known that some microorganisms successfully perform biomethylation of Sn, this process and its significance in the environment have not been fully elucidated so far. The aim of this study was to evaluate the behaviour of MeTs in the marine environment. This was accomplished by *in situ* investigation of methylation of Sn in surface sediments and corresponding porewaters, both in oxic and anoxic conditions. In addition, the distribution of MeTs in surface sediment and seawater collected at 59 locations along the Croatian Adriatic coast was determined. **1. 2. 3.** To access the Sn methylation activity in marine sediments, the MeTs transformations were determined in incubated sediment and porewater samples, isolated from surface oxic (0-2 cm) and anoxic (10-12 cm) sediment layers of two different cores. The results obtained after three days of incubation demonstrated that methylation of Sn in marine sediments occurred under both oxic and anoxic conditions, being particularly efficient in porewaters, while the methylation activity decrease with increasing number of methyl groups bound to Sn atom. The methylation rate constants, calculated following a first-order kinetic model, demonstrated that the methylation of inorganic Sn to MMT is 5-15 times faster than the methylation of MMT to DMT, while the methylation process is more successful in oxic than in anoxic conditions. The determination of MeTs concentrations in surface sediments and seawater samples collected at various coastal areas revealed that MeTs, mostly MMT and DMT, were present only in seawater, in the concentrations ranging from 0.38 to 14.3 ng(Sn) L^{-1}, but not in sediments (they were detected in sediments at only two locations). This finding strongly suggests that MeTs, due to their solubility and low affinity for adsorption onto particulate matter, are transferred into the water column shortly after being formed in sediments.

3.04.03
Flow Cytometry-Based Monitoring of Microbial Communities During Biotransformation of Munition Compounds in the Marine Environment
K. Kundu, Ghent University (UGent) / Center for Microbial Ecology and Technology; J.W. Van Landuyt, Ghent University (UGent) / BW25 Biotechnology; V.C. Mattelin, B. Martin, R. Herweyers, Ghent University (UGent); M. Neyts, K.F. Parmentier, RBINS / OD Nature; N. Boon, Ghent University - LabMET
Organic contaminants, even in diluted concentrations, can interact with native flora and fauna to change the microbial diversity and the functioning of the natural ecosystem. Hence, accurate measurements of the bacterial concentration, composition, and physiology can provide excellent insight into the process to assess pollution. In this study, we used flow cytometry (FC) approach to understand the influence of organic contaminants at the microbial community level and determine indicator sub-population for pollution. The rapid assessment of microbial communities is possible as FC allows analyzing as much as 50,000 cells s^{-1} at high accuracy. Marine sediment samples were incubated in the presence of model munition compounds - tri-nitro-toluene (TNT) and thiodiglycol (TDG) (mg L^{-1}- µg L^{-1} concentrations). The incubation was performed both under aerobic and anaerobic conditions. Biotransformation of TNT was observed, whereas the analysis of transformation products suggested a specific degradation pathway for aerobic and anaerobic conditions. FC analysis indicated the emergence of sub-populations when exposed to high TNT concentration, whereas less toxic TDG induced minimal effect on populations. Besides visual observation on flow cytogram, "phenotypic fingerprinting" was performed with Phenoflow R-package developed at CMET. Alpha and beta diversity analysis demonstrated that flow cytometry monitoring of microbial samples could capture the shift in microbial community structure when exposed to toxic pollutants.

3.04.04
Particle Associated Bacterioneuston Is the Major Player in the Degradation of PAH in Coastal Antarctic Seawater Microlayer
A.M. Varela, CID-IDAEA / Environmental Chemistry Department; G. Casas, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Environmental Chemistry; N. Berrojalbiz, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Department of Environmental Chemistry; J. Dachs, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Environmental Chemistry; M. Vila-Costa, IDAEA CSIC / Environmental Chemistry
The active exchange and mixing dynamics between the Sea Surface microlayer (SML), the atmosphere and the Subsurface layer (SSL) contribute to the enrichment of amphiphilic, hydrophobic, surfactant-like compounds at the SML, including those from anthropogenic sources such as the toxic and semi-volatile Polycyclic Aromatic Hydrocarbons (PAH). The entry of PAH into the global oceans by atmospheric diffusive deposition is estimated to exceed that of any marine oil spill to date. Marine bacteria are known to mediate PAH degradation processes after oil spills, but little is known of the interaction of bacteria inhabiting the SML, known as bacterioneuston, and PAH chronic environmental pollution. In this study we investigated how the structure and metabolic functioning of size-fractionated free living (FL) and particle associated (PA) microbial cohorts from SML and SSL respond to background concentrations of PAH after 24 h exposure in Coastal Livingston Island (Antarctica). Higher changes between PAH exposed and non-exposed microbial compositions were observed in SML PA than in SSL after 24h. SML-PA PAH exposed communities showed an exponential growth of "rare biosphere" taxa *Pseudoaltermonas*, reported to be a PAH early stage degrader. Metatranscriptomes showed down-regulation of PAH degrading genes at the PAH exposed community of the SML PA. Oppositely, at the SSL PA, PAH degrading genes were up-regulated, while the FL fraction of both layers was in an intermediate stage. Altogether, our data suggest different rates of biodegradation in each fraction and layer. Consistently, SML turnover rates of individual high molecular weight (HMW) PAH compounds, expectedly more abundant at the SML, increased along with their octanol-water partition coefficient. HMW hydrophobicity facilitates the sorption to suspended particles, susceptibly becoming growth substrate to PA bacterial cohorts. Our results suggest that PA bacterioneuston is a hotspot of PAH removal at the SML of coastal Antarctic seawaters, specially of refractory HMW PAH compounds, which are the most bioaccumulative and toxic ones. PA bacterioneuston communities showed the quickest responses to background PAH concentrations at both structural and functional level. This study incites to distinguish between PA and FL fractions of microbial communities in future biological studies devoted to improve our understanding of biodegradation and

bioremediation of pollutants in our oceans.

3.04.05

Determining the Temperature Dependency of Biodegradation Kinetics for 45 Hydrocarbons While Avoiding Chemical and Microbial Confounding Factors

K. Knudsmark Sjoholm, Technical University of Denmark (DTU) / DTU Environment; H. Birch, DTU Environment / DTU Environment; R. Hammershoj, Technical University of Denmark (DTU) / DTU Environment; D.M. Saunders, Shell International / Toxicology; A. Dechesne, Technical University of Denmark / DTU Environment; A.P. Loibner, BOKU - University of Natural Resources and Life Sciences Vienna / IFA-Tulln; P. Mayer, Technical University of Denmark / DTU Environment

Biodegradation kinetics data are keystone for the environmental persistency and risk assessment of chemicals. Biodegradation rates depend on the prevailing temperature, which influences microbial community structures, metabolic rates and chemical availability. There is a lack of high quality comparative biodegradation kinetics data that are determined at different test temperatures, but with the same microbial inoculum and chemical availability. The present study aimed at determining the effect of test temperature on the biodegradation kinetics of hydrocarbons using inocula from a Northern (2.7°C) and a Central European (12.5°C) river. Aqueous stock solutions containing 45 individual hydrocarbons were generated by passive dosing and added to river water containing the native microorganisms. Compound specific biodegradation kinetics were then determined at 2.7, 12 and 20°C based on substrate depletion. Main findings comprise: (1) Degradation half times ($DegT_{50}$) determined at different test temperatures were largely consistent with the Arrhenius equation (activation energy 65.4 kJ/mol). (2) $DegT_{50}$ determined at higher test temperature were largely consistent with low temperature testing and Arrhenius conversions, but yielded data for more chemicals. (3) Within the European Union under REACH, the standard test temperature was recently reduced from 20°C to 12°C, which typically will lead to a doubling of degradation half-times ($DegT_{50}$ values). The experimental data of the present study clearly demonstrate that this reduction of standard test temperatures can lead to a considerably stricter PBT and vPvB assessment, unless persistency criteria also are adjusted.

3.04.06

Do Initial Concentration and Activated Sludge Seasonality Affect Pharmaceutical Biodegradation Rate Constants?

T. van Bergen, Radboud University / Environmental Science; A.B. Rios Miguel, Radboud University / Department of Microbiology; T. Nolte, Radboud University Nijmegen / Department of Environmental Science; A.M. Ragas, Radbound University / Environmental Science; R. van Zelm, Radboud University / Environmental Science; M. Graumans, Radboud UMC / Risk assessment and molecular epidemiology; P. Scheepers, Radboud UMC / Risk Assessment and Epidemiology; M.S. Jetten, Radboud University / Department of Microbiology; J.A. Hendriks, Radboud University / Environmental Science; C. Welte, Radboud University / Department of Microbiology

Pharmaceuticals find their way to the aquatic environment via wastewater treatment plants (WWTP). Biodegradation plays an important role in mitigating environmental risks of these pharmaceuticals, however a mechanistic understanding of involved processes is limited. The aim of this study was to evaluate potential relationships between first-order biodegradation rate constants of nine pharmaceuticals and initial concentration of the selected compounds, and sampling season of the used activated sludge inocula. Four-day bottle experiments were performed with activated sludge from WWTP Groesbeek (The Netherlands) of two different seasons, summer and winter, spiked with two environmentally relevant concentrations (3 and 30 nM) of pharmaceuticals. Concentrations of the compounds were measured by LC-MS/MS and microbial community composition was assessed by 16S rRNA gene amplicon sequencing. Pseudo-first order biodegradation rate constants (kb) were calculated. The biodegradable pharmaceuticals, ranked from high to low biodegradation rates, were metformin, acetaminophen, metoprolol, terbutaline, and phenazone. Carbamazepine, diatrizoic acid, diclofenac and fluoxetine were not converted. Summer and winter inocula did not show significantly different microbial community composition, but they produced slightly different kb values for individual compounds at the same initial pharmaceutical concentration. Likely microbial activity was responsible instead of community composition. Our test also showed that even similar biodegradation assays with the same inoculum may lead to different results, depending on pharmaceutical concentration (in case of metformin and metoprolol). In general, biodegradable compounds had a higher kb when the initial concentration was higher. We can conclude that our test shows environmental realism, as the microbial community did not change during the experiment and can therefore be translated to WWTP conditions. Based on Michaelis-Menten kinetics, kb should be constant when the substrate concentration is far below km (substrate concentration at half the maximum reaction rate). Our results show that Michealis-Menten kinetics has shortcomings for some pharmaceuticals at low, environmentally relevant concentrations (metformin and metoprolol). Therefore, the pharmaceutical concentration should be taken into account when predicting/measuring the kb in order to reliably predict the fate of pharmaceuticals in the WWTP. The dependence of kb on pharmaceutical concentrations may even be used to more accurately the fate in the WWTP without knowing exact mechanisms. In addition, more research is needed to understand and implement the complex microbial factors influencing kb.

3.04.07

Relating Biotransformation Kinetics of Selected Micropollutants in Laboratory and Field

C. Seller, Eawag - Swiss federal Institute of Aquatic Science and Technology / Environmental Chemistry; M. Honti, Budapest University of Technology and Economics; K. Fenner, Eawag & University of Zurich / Department of Chemistry

Actual levels of water pollution with chemicals depend on their persistence in the aquatic environment. In recent years, international regulatory frameworks require the assessment of a substance's environmental persistence in tiered biotransformation simulation studies prior to its market authorization. For the evaluation of chemicals that may enter surface waters, two OECD test guidelines are relevant: the OECD 308 guideline, which targets biotransformation in aquatic sediments, and the OECD 309 guideline, which assesses biotransformation in the pelagic water body. Since their introduction, several shortcomings of OECD 308/ 309 studies have been discussed; generally, study outcomes are often highly variable and, in many cases, not reproducible. A thorough understanding of transformation processes, system-specific differences, and a clear guidance on how to translate outcomes of biotransformation simulation studies to compound persistence in the environment is still missing. The approach of our research was therefore three-fold. First, we further explored transformation and phase transfer processes in experimental setups within and beyond standards of OECD 308/ 309 studies. Second, we determined environmental fate of chemicals emitted into the catchment of the river Rhine through wastewater treatment plants. Third, we search for a robust metric, which could be used to compare and predict compound persistence in a river catchment based on laboratory experiments. We thereby found that a bioavailability-corrected and biomass-normalized second-order biotransformation rate constant (k'bio) may be a generally valid parameter for quantifying biotransformation across different water-sediment systems and a robust indicator for aerobic biotransformation. Derived k'_{bio} values from laboratory and field data allowed to consistently identify atenolol, bezafibrate, clopidogrel carboxylic acid, cyclamate, diclofenac, levetiracetam, metoprolol, saccharin, sulfamethoxazole, trimethoprim, and valsartan as fast transformed compounds and acesulfame, carbamazepine, citalopram, gabapentin, lamotrigine, sitagliptin, and venlafaxine as rather persistent compounds in both laboratory and field.

3.04.09

Bentazone Degradation in Water by Trametes Versicolor and Identification of Formed Transformation Products

M.G. Vara, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); K. Hu, Universitat Autònoma de Barcelona; C. Postigo, IDAEA, CID-CSIC / Environmental Chemistry; L. Olmo, Universitat Autònoma de Barcelona; G. Caminal, IQAC (CSIC-UAB) / Biocatàlisi Aplicada; M. Sarrà, Universitat Autònoma de Barcelona; M. López de Alda, Institute of Environmental Assessment and Water Research (IDAEA CSIC) / Environmental and Food Chemistry (ENFOCHEM)

Bentazone, an herbicide widely applied in rice and cereal crops, is widespread in the aquatic environment. This study evaluated the capacity of *Trametes versicolor* to remove bentazone from water. The fungus was able to completely remove bentazone after three days at Erlenmeyer-scale incubation. Both laccase and cytochrome P450 enzymatic systems were involved in bentazone degradation. A total of 19 transformation products (TPs) were identified to be formed during the process. The reactions involved in their formation included hydroxylations, oxidations, methylations, N-nitrosation, and dimerization. A laccase mediated radical mechanism was proposed for TP formation. In light of the results obtained at the Erlenmeyer scale, a trickle-bed reactor with *T. versicolor* immobilized on pine wood chips was set up to evaluate its stability during bentazone removal under non-sterile conditions. After 30 days of sequencing batch operation, an average bentazone removal of 48% was obtained, with a considerable contribution of adsorption onto the lignocellulosic support material. Bacterial contamination, which is the bottleneck in the implementation of fungal bioreactors, was successfully addressed by this particular system according to its maintained performance. This research is a pioneering step forward to the implementation of fungal bioremediation on a real scale.

3.04.10

Review on PPCP Transformation Products in the Environment: Towards a Classification of Transformation Pathways Based on the Chemical Structures of Parent Molecules

K. Bonnot, INRA - UMR EcoSys; P. BENOIT, INRA, UMR1402 ECOSYS, 78580 Thiverval Grignon, France; L. Mamy, INRA; D. Patureau, Laboratoire de Biotechnologie de l'Environnement, INRA

Describing the transformation pathways of PPCPs is essential for understanding and predicting their environmental fate and impacts. Thus, the aims of this work were to perform a literature review on PPCP transformation products (TP) identified in different environmental matrices, to describe the main chemical reactions producing these TPs and the media where these reactions occur, and, finally, to establish a first correlation between the parent molecule structures and their respective transformation pathways. The available data on the detection and identification of the TPs of 116 selected PPCPs were reviewed and analyzed with respect to the transformation pathways responsible of their formation. In total, 1373 TPs were identified from biotic (633), abiotic (565), both biotic and abiotic (61), and unidentified (114) origins. Eleven reactions or main types of reactions emerged from this analysis, with hydroxylation being the predominant reaction. Then, the most observed reactions were dehydrogenation, cleavage, "other eliminations", hydrolysis, "other substitutions", dealkylation, oxygenation, addition, dehalogenation, and rearrangement. The relationship between PPCP chemical structures (described through fingerprints and molecular descriptors) and transformation pathways was studied using Hierarchical Clustering on Principal Components. The classification allowed us to distinguish nine clusters which were separated into two main groups: the first one with clusters 1,3 and 4 which were poorly discriminated; the second one with clusters 2, 5, 6, 7, 8 and 9 which was better discriminated and made it possible to highlight structure/reaction relationships. However, the reaction/structure trends need to be further investigated with a larger number of PPCP molecules.

Environmental Chemistry and Exposure Assessment: Analysis, Monitoring, Fate and Modeling

3.05.02

Online Prioritization of Toxic Compounds in Water Samples Through Intelligent HRMS Data Acquisition

N. Meekel, KWR Water Research Institute; D. Vughs, KWR Water Research Institute / Chemical Laboratory; F.M. Been, KWR Water Research Institute / Chemical Water Quality & Health; A.M. Brunner, KWR Water Research Institute

LC-HRMS-based non-target screening (NTS) has become the method of choice to monitor organic micropollutants (OMPs) in drinking water and its sources. OMPs are identified by matching experimental fragmentation (MS2) spectra with library or *in silico* predicted spectra. This requires informative experimental spectra and prioritization to reduce feature numbers, currently performed post data acquisition.Here, we propose a different prioritization strategy to ensure high-quality MS2 spectra for OMPs that pose an environmental or human health risk. This online prioritization triggers MS2 events based on characteristics in the full scan, or an additional MS2 event based on patterns detected in a first MS2 spectrum. Triggers were determined using cheminformatics. After MS acquisition parameter optimization, performance of the online prioritization was experimentally examined. Triggered methods led to increased percentages of MS2 spectra, and additional MS2 spectra for potentially toxic compounds. Application to surface water samples resulted in additional MS2 spectra of potentially toxic compounds and more confident identification, emphasizing the method's potential to improve monitoring studies.

3.05.03

Passive Samplers As Innovative Tools for Atmospheric Assessment of Pesticide Contamination

S. Martin, LPTC-EPOC, University of Bordeaux / EPOC UMR 5805; E. Barron, University of Bordeaux, CNRS / EPOC UMR 5805; M. Devier, University of Bordeaux / UMR CNRS EPOCLPTC; K. LE MENACH, University of Bordeaux / EPOC UMR 5805; P. Pardon, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC; E. Villenave, Université Bordeaux / EPOCCNRS; H. Budzinski, Université de Bordeaux / UMR EPOC LPTC

The massive use of pesticides in agricultural and urban areas leads to widespread contamination of the environment, especially with regard to ambient air. Through various transfer processes that take place during and after pesticide application (atmospheric drift, volatilization, wind erosion), 25 to 90% of applied pesticides would be transferred to the atmosphere depending on their physicochemical properties, application methods and weather conditions. In this context, the project aimed to implement passive samplers to characterize levels and seasonal variations of atmospheric pesticides in wine-growing, urban and peri-urban environments. Polyurethane foams based passive air samplers (PUF-PAS) were used as adsorbents to accumulate pesticides in Bordeaux area (southwest of France) between June and October 2020. 59 molecules were monitored at 6 study sites with rural, urban, wine-growing or mixed environments. Passive air samplers were changed every three weeks. An experimental strategy has been developed combining pressurized liquid extraction and different analytical pathways, including analyses by liquid chromatography coupled to tandem mass spectrometry, gas chromatography coupled to tandem mass spectrometry associated to solid phase micro extraction system, and gas chromatography coupled to high resolution tandem mass spectrometry. The optimised protocol offers the hability to monitor a wide variety of molecules belonging to various chemical families with heterogeneous physicochemical properties, for example conazoles (fenbuconazole, tebuconazole, tetraconazole,...), neonicotinoids (imidacloprid, thiamethoxam,…) or phthalimides (captan, folpel), with limits of quantification (LoQs) ranging from 0.02 to 2.38 ng/PUF-PAS. The protocol is thus adequate to be used for envionmental monitoring, adressing trace and ultra-trace pesticide contamination and was applied to the samples collected during the summer of 2020. Preliminary results have shown pesticide contamination for all the sampling areas. Many pollutants were found in a concentration range from 0.048 to 758.6 ng/PUF-PAS. Cymoxanil, cyprodinil, metolachlor, pyrimethanil, tetraconazole and trifloxystrobine were compounds detected at the highest quantities in PUF-PAS. Several banned molecules were also identified, as well as some molecules not used in the studied areas, sign of the ubiquity of these products and the overall environmental impregnation. PUF-PAS are tools easy to use and deploy, small, inexpensive, that can be deployed anywhere and do not require a power source. They enable an average concentration to be obtained over a defined time by accumulating a large number of organic compounds by molecular diffusion and adsorption. Experiments and developments carried out on the extraction and analytical protocols have resulted in very good performances, particularly in terms of LoQs, thus offering efficient, repeatable, reproducible and reliable monitoring.

3.05.04

Comparison of Sample Preparation by Vacuum Evaporation Concentration (VEC) and Solid-Phase Extraction (SPE) for the Chemical and Toxicological Screening of Wastewater

S. Tisler, University of Copenhagen / Department of Plant and Environmental Sciences; M. Castro, University of Copenhagen / PLEN - Department of Plant and Environmental Sciences; J. Lundqvist, Swedish University of Agricultural Sciences / Department of Biomedical Sciences and Veterinary Public Health; N. Cedergreen, University of Copenhagen / PLEN - Department of Plant and Environmental Sciences; J.H. Christensen, University of Copenhagen / Department of Plant and Environmental Sciences

In this study, we show the feasibility and limitation of the vacuum evaporation concentration (VEC) and the multilayer solid-phase extraction (SPE) for a combined chemical and toxicological fingerprint. Wastewater effluent was obtained from Copenhagen, Denmark. The mean recovery for the target compounds (log D between -6 and 5) was slightly higher for VEC (83±7%) than for the SPE (79±10%) in ultrapure water. However, changing the matrix from ultrapure water to wastewater effluent led to a decrease of the mean recovery for VEC to 35% (±9%) and for SPE to 77% (±13%). In SPE, the recovery drop was caused by the most polar compounds (49±26%). In a non-target screening, also more polar compounds could be detected in VEC and more unpolar compounds in the SPE. We could confirm that the high salt concentration was responsible for the salting-out effect of the organic compounds in VEC. Some of the ions precipitated > 90% (e.g. Ca^{2+}) during evaporation, whereas for other around 50 % precipitated (e.g. Cl^- and SO_4^{2-}). This results in a salt concentration of more than 20 g/L in the 200 times up-concentrated extract. Flushing the extract with methanol in the last evaporation step can reduce the ion strength, i.e. to 10 g/L in a H2O:MeOH (40:60) extract. However, this is reducing the advantage of a solvent-free extract of VEC over SPE from a toxicological perspective. For evaluating the feasibility of the salty VEC for toxicity tests, artificial wastewater was compared with real wastewater. The salt caused no initial problems in the *in vitro* tests: no cytotoxicity was detected at a relative enrichment factor (REF) of two. In the cell lines, both artificial and real effluent extract showed minor effects. The AhR and Anti-AR showed no activity in either of the samples. The AR showed a concentration-dependent increase for some of the real samples, but not for the artificial. The ER and Nrf2 showed some effects for real and artificial wastewater.The final SPE the extract was obtained in methanol or ethyl acetate. For algae and nematodes, a 10% decrease (EC10) in growth or reproduction was obtained at 0.072% and 0.45% (v/v) methanol in test media, respectively (EC10). Ethyl acetate was relatively less toxic for algae with an EC10 at 0.6% (v/v), but for nematode reproduction, the EC10 was found to be at 0.46%. As a conclusion, the final extract needs to be evaporated to dryness to reconstitute in pure water for conclusive effect analysis. This additional process step could lead to a loss of more volatile and hydrophobic compounds.

Environmental Forensics - State of the Science and Global Applications

3.06.01

Integrated Environmental Forensics and Applications

i. Rhodes, GSI Environmental, Inc

Chemical fingerprinting is typically thought to be for the determination of what has been released to the environment and who might be responsible and support to potential litigation. The investigation can include analysis of petroleum, fuels and chemicals ranging from reference materials, LNAPL, soil, water and air. The

ability to identify potential sources becomes more challenging in environmental media as compound types and of different sizes partition and degrade at different rates even within the same spill depending on spatial location from source areas and site conditions. Comingled and chronic releases make the job of the forensic scientist more challenging. Furthermore, determination of time of release is not possible based on compositional changes alone. Numerical estimation of residual material in the environment are obtained from variable analytical methods that report "total petroleum hydrocarbons" (TPH). In reality, the results are defined by the method used and may not be "total" or "petroleum hydrocarbons" as anything that is detected by the method get labeled as "TPH", thus it is essential that "TPH" be investigated the same way as LNAPLs and not simply defined by the label as TPH-diesel, for instance. Other important applications of environmental forensics include· use of regulatory limits regarding sulfur, lead, additives in fuels can be useful in "dating" releases· assistance in conceptual site model development using multiple lines of evidence in relation to LNAPL source and mobility· selection of remedial system and evaluation of remediation effectiveness, timing and end points· provide input to regulatory processes This presentation is intended to highlight challenges and misconception of environmental forensics and to raise awareness of other useful applications.

3.06.02

PAH Source Differentiation Between Historical MGP and Significant Urban Influences for Sediments in San Francisco Bay

R. Jordan, Natural Spectrum; M.J. Cejas, ChemQuants; H. Costa, T. Sauer, L. McWilliams, Haley & Aldrich, Inc

A forensic source evaluation of polycyclic aromatic hydrocarbons (PAHs) in nearshore sediments in San Francisco Bay examined total PAH greater than ambient concentrations in sediments, and potential pyrogenic source relationships with respect to PAH compounds typically associated with point and nonpoint pyrogenic source types, including PAHs potentially associated with historical manufactured gas plant (MGP) operations. Diagnostic source ratio analysis was employed for determination of potential PAH source relationships. A two-model approach indicated distinct potential source signatures, as identified from the distributions of higher PAH concentrations in some sediments. Source characterization was aided by Polytopic Vector Analysis (PVA) and data visualization with t-Distributed Stochastic Neighbor Embedding (t-SNE). Two signatures exhibited pyrogenic character likely consistent with historical MGP sources, and one signature was related to creosote. A distinct and significant source of PAHs to the investigation area sediment consisted of ubiquitous nonpoint and potential unidentified point sources is termed "urban influence".

3.06.03

Extended Analysis of More PACs Than PAH16 Combined With Multivariate Methods Allowed Determination of Additional Sources to PACs in Sediment Near an Aluminium Smelter in Sweden

M. Grung, NIVA / Ecotoxicology and Risk Assessment; P. Holmlund, WSP Environmental Sweden; A. Kringstad, NIVA Norwegian Institute for Water Research; S. Meland, NIVA Norwegian Institute of Water Research / Ecotoxicology and Risk Assessment; A. Macken, NIVA / marine pollution

In 2008, Söderberg techniqe was faced out at the aluminium smelter in Sundsvall (Sweden), and production of aluminium continued with prebaked anodes. Analyses of polyaromatic compounds (PACs) and metals was conducted in 2019 and levels in both sediments (19 stations) and mussels (*Limecola balthica*, 5 stations) were investigated. More than 50 different PACs (including alkylated PACs) and 8 metals were analysed. Concentrations of US EPA PAH16 and metals in sediment compared with historical data from 2002 revealed an overall reduction in concentrations for PAH16. A mean of 62% reduction of PAH16 was observed, and a reduction was observed for 75% of the stations investigated both years (n=12). For metals, increasing concentrations were found for As, Mn and Hg, while Cu, Cr and Vn concentrations were decreased. The stations associated with the greatest increase in metal concentrations were not close to the discharge points of the aluminium smelter. Many PACs exceeded the environmental quality standards (EQS) near the outlet of the aluminium smelter, while exceedances of metals were more seldom. A Norwegian classification system based on EQS (acute and chronic effects) were used to prioritise PACs and metals based on environmental risk. Also, the stations were classified according to environmental risk. The concentrations of most PACs correlated negatively with distance from the aluminium smelter, but this pattern was not observed for metals. The extended analysis of PACs allowed for a hierarchical clustering method to identify 4-6 distinct clusters of stations, where the PAC pattern in sediments indicated different sources to the PACs. The clustering explained the PACs concentrations that did not correlate negatively with distance to the smelter, and therefore probably had another source. The PAC pattern in sediment correlated well with the pattern observed in mussels, indicating an uptake of PAC from sediment. Our conclusion is that an extended analysis of PACs combined with multivariate statistical methods allowed for identification of additional sources to PACs in sediment near an aluminium smelter. Without the extended PACs analyses, the additional sources would otherwise have been difficult. We believe that the method described here also be employed at more sites with PAC-contamination, and we have employed the same technique in analyses of e.g. road runoff.

3.06.04

Evaluation of PVA Performance for Forensic Source Identification at Multi-Source Sites

N. Rose, T. Negley, C. Monti, TIG Environmental

Introduction. Unmixing models, including polytopic vector analysis (PVA), are commonly used in environmental forensics to determine the source composition and the proportion of each source in a sample. To our knowledge, the sensitivity of these models has not been tested for environmental samples. The goal of this work is to utilize artificially generated datasets representative of real-world environmental conditions to determine the sensitivity of PVA to various environmental sampling conditions including the seeding method, number of samples, noise in the dataset, presence of non-detects, and number of potential sources. **Materials and Methods.** We developed an R-based implementation of PVA that was validated against the MATLAB version developed previously. We also developed an algorithm that generates a synthetic population of samples with sources of polychlorinated biphenyls (PCBs) taken from real-world environmental contaminant sources (such as Aroclor standards) and sampled this population. This algorithm was used to generate many different potential sampling events and introduce variability commonly encountered in environmental datasets (including small to large sample sizes and noise due to variation in sampling and analytical methods). These sampling events were then evaluated using PVA with four different seeding methods (EXRAWC, Fuzzy C-means clustering [FuzzyQ], nonnegative singular value decomposition [NNSVD], and externally provided sources) to estimate the composition of each source and the proportion of each source contributing to each sample. The results obtained from the PVA analysis were compared to the actual source composition and sample proportion used to generate the synthetic dataset to assess the sensitivity. **Results and Discussion.** Our analysis of the data found that the choice of seeding method can influence whether the PVA algorithm converges, with EXRAWC and NNDSVD converging more often than Fuzzy Q or the external method. In addition, the FuzzyQ method had higher sample and source MSEs than the other methods in certain circumstances. This analysis also showed that increasing noise resulted in an increase of Sample MSE and Source MSE and that the lowest Sample MSE and Source MSE occur when 50 to 100 samples were collected. Based on this analysis, we recommend running PVA using EXRAWC or NNDSVD on between 50 to 100 samples with relatively low noise.

3.06.05

PFAS Fingerprinting: A Multivariate Forensic Analysis to Detect the Origin and Extent of PFAS Contamination in Northern Italy

C. Monti, T. Negley, N. Rose, TIG Environmental

PFAS are omnipresent in environmental media and are distributed globally. Their potential to enter the food chain is high, thanks to their ability to migrate from water to soils and be taken up by plants. These substances can enter the human body through food, drinking water, and direct skin contact; moreover, they are not metabolized and can bio-accumulate. Contact with PFAS-containing materials (e.g., water-resistant materials, detergents, paints, fabrics) can also increase human exposure. The scope of this project is to demonstrate an approach to identify original PFAS sources using a multivariate method (PCA and cluster analysis) and PVA (polytopic vector analysis) algorithm developed in R by the authors. This case study is in the Veneto Region of Northern Italy. The chronology of the case is: 2013: PFAS was found in groundwater, surface water and drinking-water supply for 127,000 people; 2013 – 2018: Water and bio-monitoring studies conducted; 2014: PFAS thresholds established for Veneto Region (PFOS: ≤ 30 ng/L; PFOA: ≤ 500 ng/L; Other PFAS: ≤ 500 ng/L); 2017: Legal case developed against a nearby chemical plant manufacturing PFAS; 2018: Chemical plant filed for bankruptcy and released all 121 employees.Data were obtained from a public water monitoring database[1] containing more than 8,000 samples analyzed for PFAS in groundwater, surface water, springs, and treated industrial wastewater discharged to surface water. After database review, nearly 1,400 samples and eight PFAS out of 18 (PFBA, PFPeA, PFBS, PFHxA, PFHpA, PFHxS, PFOA, PFOS) were deemed useable for fingerprinting. The fingerprint of a nearby chemical plant manufacturing PFAS was also derived from public data. The PCA, cluster analysis, and PVA results indicate that in certain parts of the Veneto region groundwater and surface water contamination is associated with the nearby PFAS manufacturer and industrial sources, while other sources are responsible for PFAS contamination elsewhere in the Region. The results highlight the ability of the approach to differentiate original sources of PFAS contamination, particularly when source fingerprints can be derived. [1] https://www.arpa.veneto.it/dati-ambientali/open-data/idrosfera/concentrazione-di-sostanze-perfluoroalchiliche-pfas-nelle-acque-prelevate-da-arpav

3.06.07

Why We Should Be Using Environmental Forensic Procedures in Microplastic Pollution Studies

C. Gwinnett, Staffordshire University / Criminal Justice and Forensic Science; R.Z. Miller, Rozalia Project for a Clean Ocean

Microplastics (MPs) are acknowledged as a global problem that are present in all environments including water, air and soil. The study of microplastics (described as polymers smaller than 5mm in size) has increased dramatically in the last 10 years and with this increase in studies has come improved methods for sampling and analyzing MPs. Despite this increase in studies, there has been limited use of the extensive knowledge within the domain of environmental forensics, particularly in the processes used to recover, analyse and interpret these contaminants. The field of environmental forensics is diverse and multidisciplinary and as such, it has much to offer microplastic pollution work. Environmental forensic scene sampling approaches and the use of sequential analysis of samples for the full characterisation of morphological, optical and chemical properties of samples have many benefits for MP studies. This paper will provide an overview of how environmental forensic approaches can be applied to microplastic contaminant studies and demonstrate the benefits of doing so. The methods used in environmental crime scene analysis for the prevention and detection of procedural contamination is particularly important as many extant MP studies have failed to utilize strict protocols during field sampling and sample processing. This paper will highlight the need for forensic science principles for procedural contamination prevention to be applied in MP studies via a pilot study conducted as part of a microplastic pollution expedition along the Hudson river (USA). Five sampling events were carried out; a Waste Water Treatment Plant (WTTP) study where strict forensic protocols were in place and 4 sampling events where these were strategically removed from the sampling or processing activities. Control samples were taken using environmental forensic principles and used to identify the extent of any procedural contamination. The study found that without using the types of protocols implemented in environmental forensic work, significant amounts of procedural contamination occurs. This paper will make recommendations for the implementation of environmental forensic approaches for future microplastic pollution studies with the aim to improve the quality and accuracy of data. The presentation will be conducted by both authors to fully present the two sides of the study – one from a marine expert and one from an environmental forensics expert; thus showing its applicability to the field.

Fate and Metabolism of Wastewater-Derived Pollutants

3.07.01

Emerging Contaminants in Two Saudi Arabia Urban Areas: Environmental and Human Health Risk Assessment

Y. Pico, University of Valencia / Environmental Quality and Soil; V. Soursou, Desertification Research Centre / Environmental and Food Safety Research Group, CIDE (UV, GV, CSIC); J. Campo, CIDE-CSIC / Degradación y Conservación de Suelos; A.H. Alfarhan, King Saud University; M.A. El-Sheikh, King Saud University / Department of Botany and Microbiology, College of Science; D. Barcelo, ICRA- G17920851

Emerging contaminants (ECs) are one of the most important indicators of the anthropic influence on the environment, and comprised pharmaceutical and personal care products (PPCPs), pesticides, perfluoroalkyl substances (PFASs), flame retardants (FRs) among others. These ECs have been found in all environmental compartments including water, soils, sediments, and biota. Nowadays, the water pollution by ECs is facing a new challenge due to the added effect of climate change. Some studies reveal that water scarcity caused by climate change can concentrate ECs, and the increasing use of wastewater to alleviate this scarcity can be a source of them. In these circumstances, hyper-arid zones have become an example to follow and the Middle East is one of the areas most affected by climate change. Accordingly, the main objective of this study was to assess the environmental risk due to occurrence of ECs in urban areas of Saudi Arabia as well as their implications to human health. This study reported the occurrence 12 organophosphorus flame retardants (OPFRs), 64 pesticides, 21 PFASs and 34 pharmaceuticals and PPCPs in surface water, sediments and vegetation collected from 7 locations along the South Riyadh and 6 locationsat the Al-Jubail industrial city (Saudi Arabia). Of the 131 target ECs analyzed, 70 were detected in water at average concentrations from 0.01 to 1.84 µg/L, 60 in sediment ranging from 0.01 to 9.8 µg/g d.w., 63 in crops intended for human consumption (market or farm) between 0.04 and 507 ng/g w.w., and 36 in wild vegetation from 0.04 to 893 ng/g d.w. Predominant compounds in all matrices were tris(1,3-dichloro-2-propyl)phosphate, acetamiprid, imidacloprid, caffeine, bisphenol A, diclofenac, ibuprofen. Furthermore, tris(2-butoxyethyl) phosphate, tris-(2-ethylhexyl)phosphate, perfluoroctanoic acid, perfluoroalkyl sulfonate and paracetamol were in almost all samples but at low concentrations. The levels found in the samples showing similar values in both cities, except in wild vegetation that present higher concentration of OPFRs in Al-Jubail and of pesticides in Riyadh. The risk assessment performed for the aquatic biota pointed out that risk quotients of abamectin, diazinon, bisphenol A and caffeine had the highest risk levels. However, a study of the cumulative risk assessment showed that the contaminant mixture in all water samples is of concern. Regarding risk for human health, individual compounds does not show important hazard to the population. However, OPFRs and pesticide requires a closed monitoring since % of admissible daily intakes or reference doses are high. Despite being one of the most comprehensive study covering environmental and human risk assessment of ECs carried out in Saudi Arabia, further research on their occurrence, behavior, fate and potential risks in water, sediment and vegetables is needed.

Acknowledgement - The authors thank the financial support from the project number (RSP-2020/11) King Saud University, Riyadh, Saudi Arabia.

3.07.05

Distribution of Pharmaceuticals in Fish Tissues From Llobregat River Basin

D.M. López, IDAEA CSIC Barcelona / Environmental Chemistry; J.M. Peña Herrera, Universidad del Valle / Vicerrectoría de investigaciones; L. Benejam Vidal, University of Vic - Central University of Catalonia / Aquatic Ecology Group; N. Montemurro, Jordi Girona 18-26, null / Dipartimento di scienze agro-ambientali e territoriali; S. Pérez, IDAEA CSIC / Environmental Chemistry

In recent years, the consumption of some pharmaceutical products (PhACs) has increased. This fact has generated that more and more this kind of compounds are detected in environmental matrices, such as aquatic ecosystems, because some PhACs are not eliminated in wastewater treatments plants (WWTP). The PhACs present in the water can come into contact with aquatic organisms such as fish, through the gills, epithelial tissues and mainly through ingestion according to the diet of each species. In order for PhACs to accumulate in fish they must be able to cross the biological membrane, either through the gills, the intestine or the skin. Once the drug is absorbed, it can reach the target organ through blood circulation These compounds can have a high potential for bioaccumulation in fish and affect their behavior and hunting abilities. In this study, four different species of fish *(Chelon ramada, Luciobarbus graellsi, Cyprinus carpio, Anguilla Anguilla)* collected in the Llobregat river basin (Catalonia, Spain) were analyzed for PhACs bioaccumulation in different organs and tissues (brain, kidney, skin, liver, pancreas, hearth, gillis, muscle, blood an billis) according a previous validated method was employed. 47 PhACs were analyzed by UPLC-HRMS using QToF system and SWATH acquisition technology. Of the 27 compounds detected in the water, 48% were also found in the fish, showing that there is a relationship between the presence of these compounds in the water and their possible bioaccumulation in the fish. The antidepressant Sertraline was the compound that was detected in the largest number of samples (C.máx 1604 ng g^{-1} in the pancreas from *Chelon ramada*). In the 12 individuals captured 16 PhACs were detected and quantified: acetaminophen, acridone, caffeine, carbamazepine, cllarithromycin, codeine, diazepam, diltiazem, fluoxetine, ketoprofe, loratadine, metoprolol, sertraline, sotalol, trimethoprim, venlafaxine. The accumulation of PhACs seems to be oriented towards the kidney, the skin, the liver, the brain and the pancreas, since they were the organs where more PhACs were detected.

3.07.07

Occurrence of Pharmaceutically Active Compounds and Their Metabolites in Radishes Grown Under Real Soil Conditions by Lc-Hrms/Ms

F.L. Roig, CSIC - Spanish National Research Council / Department of Environmental and Food Chemistry (ENFOCHEM); N. Montemurro, Jordi Girona 18-26, null / Dipartimento di scienze agro-ambientali e territoriali; S. Pérez, IDAEA CSIC / Environmental Chemistry

Nowadays obtaining quantity of water is a challenge, specially in arid regions. Due to this reason, using treated wastewater for agriculture irrigation is a common practice in many countries. However, the conventional treatments of wastewater treatment plants (WWTPs) are not able to remove different types of contaminants, specially organic contaminants like pharmaceutically active compounds (PhACs), and could result into soil contamination. As this practice is gaining more relevance globally, several studies performed in different crops such as lettuce, spinach or tomatoes demostrated the capability of plants to uptake PhACs via root uptake and translocate them into aerial parts [1]. This studies have been raised the interest of studying crops irrigated with reclaimed wastewater. In this study, radish crops were selected as the target crop due to its fast grown and its raw consumption. Besides the crops ability to uptake PhACs, similar to animals and humans, they are able to metabolize contaminants through two main mecanisms [2]: a) Phase I: oxidations, reductions and hydrolisis of the parent compound drug or b) Phase II: parent drug or/and phase I metabolites are conjugated to sugars, amino acids, malonic acid or glutathione. However, metabolism pathways in crops is not well undestood, therefore there is the need to evaluate the formation of metabolites. Since, no standards for the plant metabolites are commercially available hence, the identification of the unknown compounds is performed using liquid cromatography high resolution mass spectrometry LC-HRMS/MS, usually by means of suspect and non-target analysis. The extraction of PhACs, belonging to various families as antibiotics, non-steroidal antiinflamatories and diuretic drugs, from radish tissues were performed by QuEChERS (quick, easy, cheap, effective, rugged and safe). Different salts for the extraction of the target compounds were used depending on the matrix, for radish root Original salts (non-buffered salts) were emplyed whereas for radish leaves CEN 15662 salts (buffered salts) were the salts demonstrating greater performance. Detection of pharmaceuticals compounds and their metabolites was performed by means of SCIEX X500R

QTOF demonstrating QqQ and TOF advantadges. High resolution multiple reaction monitoring (MRM^{HR}) acquisition for accurate detection and quantification was performed. Whereas, Sequential Window Acquisition of all Theoretical Mass Spectra (SWATH) acquisition was used as a tool for robust identification unknown compounds as well as metabolites screening. *Key Words* – Pharmaceuticals, Uptake, Metabolism, QuEChERS, LC-HRMS/MS.

3.07.09

Accumulation and Uptake of Wastewater-Borne Organic Micropollutants in Soil and Lettuce Leaves: A Field Study

R. Manasfi, HydroSciences Montpellier; M. Brienza, IRD; N. Ait-mouheb, Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE); N. Montemurro, Jordi Girona 18-26, null / Dipartimento di scienze agro-ambientali e territoriali; S. Perez, CSIC / Department of Environmental and Food Chemistry (ENFOCHEM); S. CHIRON, Université de Montpellier / Depatment of Public Health and Environmental Sciences 36

Water scarcity for agricultural purposes is a serious issue of today. The reuse of treated wastewater (TWW) for irrigation practices mainly in arid and semi-arid area is an important way to overcome this issue. However, these practices have some limitations, mainly the introduction of organic micropollutants to the agroecosystems, with potential uptake by plants, posing potential risks for human health. In consequence, field studies are needed to evaluate the actual risks to consumers. For this purpose, this study aimed at developing and validating QuEChERS extraction methods and LC-QTOF analysis methods to follow wastewater-borne organic micropollutants in soil and lettuce, grown under real greenhouse cultivation conditions and irrigated with TWW. Lettuces were grown in tanks during 5 consecutive cycles and were irrigated with different kind of water, mainly secondary TWW and spiked secondary TWW with 14 selected organic micropollutants at 10 µg/L concentration level (each), covering not only different physico-chemical properties but also showing contrasting behavior with respect to photo- and bio-transformation rates. Surface drip irrigation system was used, making root uptake the only relevant uptake pathway. Finally, lettuce leaves were collected at mature stage, as well as the soil was sampled near the lettuce roots for pollutants analysis. Field results with fortified TWW showed that 8 and 13 out of 14 compounds were detected in lettuce leaves and soil irrigated with spiked TWW respectively, at concentration levels ranging from 1 to 30 ng/g (d.w.). This study confirmed that the accumulation of 14 compounds in soil and in lettuce leaves irrigated with fortified TWW was very limited. The main reason for these low accumulation rates was an intensive degradation in soil and metabolic transformations in plant. Metabolites/TPs concentrations of selected compounds were always estimated below those of the parent compounds. Unexpected pharmaceuticals such as clarithromycin and hydrochlorothiazide were yet detected in lettuce leave, which might be linked to active transport processes for these compounds. The food additive sucralose was also encountered at high concentration in lettuce leaves for the first time. As a whole, this study confirmed *a de minimis* human health risk related to the consumption of raw green vegetable (e.g. lettuce) irrigated with TWW.

3.07.11

Evidence for the Metabolism of Antiretroviral Pharmaceuticals in PLANTS

P. Akenga, University of Plymouth / SoGEES; S. Comber, Plymouth University / Environmental Science; M. Fitzsimons, Plymouth University; A. Gachanja, Jomo Kenyatta University of Agriculture and Technology / Chemistry

Plants can take up pharmaceuticals from contaminated agroecosystems. However, little is known about their metabolism in plants. In this hydroponic study, lettuce (*Lactuca sativa*) was used to investigate uptake and transformation of 3 antiretroviral (nevirapine, efavirenz, and lamivudine) and one antiviral (oseltamivir) pharmaceuticals. The plant was exposed to a concentration range of 1 – 100 µg L^{-1} of the pharmaceutical mixture. Eight suspect metabolites were identified for screening, and lettuce extracts were analyzed using liquid chromatography – high-resolution mass spectrometry. Under full scan mass screening, the exact mass of the protonated and sodium and ammonium adducts of two predicted metabolites, lamivudine sulfoxide and 8- hydroxyl-nevirapine glucuronide, respectively, were detected in both the root and leaf tissues. However, metabolites were only detected in samples exposed to the highest concentration solution. A semi-quantitative analysis revealed that each metabolite was uniformly concentrated in the leaf and root tissue. This study advances the understanding of natural biodegradation pathways for pharmaceutical compounds in the environment.

3.07.12

Soil-Root-Lettuce Distribution and Metabolism of Wastewater-Borne Drugs

S. Perez, IDAEA-CSIC / Department of Environmental and Food Chemistry (ENFOCHEM); N. Montemurro, Jordi Girona 18-26, null / Dipartimento di scienze agro-ambientali e territoriali

Concentrations of human pharmaceuticals and their metabolites are consistently detected in wastewaters. Since the use of treated wastewater to irrigate crops is becoming widespread in regions where freshwater is limited, drugs are released into agrosystems by wastewater irrigation. Consequently, soils are exposed to drugs and plants, such as lettuce, can uptake and translocate them eventually becoming a food safety concern. Over the past few years, many studies have focused on the uptake and metabolism of human pharmaceuticals by edible plants with respect to their potential risks related to the consumption of contaminated food as a result of irrigation with treated wastewater. However, many of the reported plant uptake studies have been performed in hydroponic culture setups, which is why information on the fate of these compounds in more representative soil-lettuce systems is not generated. Importantly, the uptake of those xenobiotics by plant roots in soil-root-leaves systems depends largely on their sorption and desorption behavior in soil, which in turn is governed by their physicochemical properties. Pharmaceuticals are a broad group with diverse physico-chemical properties. These properties influence drug absorption, their interaction with the metabolizing enzymes, they control also their mode of action in the human body and plants. They affect their environmental fate (degradation, persistence) which is a relation between its intrinsic physicochemical properties and the system with which they are interacting. Lettuce samples were grown in pots in a controlled environment and irrigated during the entire growing period (60 days for lettuce and 25 days for radish) with spiked tap water containing the target compounds at low and high concentrations (10 and 100 ng mL-1, respectively).. At the end of each experiment, leaves, roots, and soil samples were extracted using a modified QuEChERS-based method followed by detection with the new SCIEX X500R QTOF-MS, a compact hybrid QToF-MS combining advantages of TOF and QqQ systems with accurate mass. Different QuEChERS extraction salts (Original and CEN) were tested and compared to evaluate their extraction efficiencies. In this study we investigated the use of MRMHR and MS/MSAll with SWATH® acquisition performed for the targeted analysis (using reference standards). Following irrigation with spiked water, neutral PhACs were detected in high concentrations in lettuce while acidic compounds were taken up to a minor extent. Unexpectedly high concentrations of the basic drug methadone were detected in the plant; its low HBD + HBA count of 2 is hypothesized to favor its uptake and translocation. Some of the ionizable compounds are readily taken up and translocated in lettuce but their logP does not correlate with the uptake, it seems that HBD+ HBA≤3 is a key factor for their uptake. ***Acknowledgements*** – This study has been financially supported through the Spanish national project (CICLIC, RTI2018-097158-B-C33). Authors also acknowledge SCIEX for providing the loan instrument LC/HRMS QTOF X500R.

Harmonized Data Reporting and Analyses in Micro- and Nanoplastics Research

3.08.01

Fifteen Years and Counting: Is Ongoing Method Diversification a Barrier to a Harmonized Approach in Microplastic Research?

S. Rist, DTU (Technical University of Denmark) / DTU Environment; N.B. Hartmann, Technical University of Denmark (DTU) / DTU Environment; N.A. Welden, University of Glasgow / School of Interdisciplinary Studies

Since 2004, there has been a marked diversification in the methods used to determine aquatic microplastic (MP) concentrations; however, the proliferation of these new methods has accelerated in recent years. Both minor method adaptations and entirely novel approaches have been introduced to overcome barriers to reliable MP sampling, extraction and quantification. This increasing specialisation has resulted in a variety of complimentary but also competing approaches. As our focus has developed from one of research to also encompass routine monitoring, the comparability of the data arising from these apparently disparate methods has been called into question. Furthermore, the extent to which new methods are acknowledged and adopted, and the apparent drivers of adoption are currently unclear. To explore these issues, the rate of method diversification and its implications for both monitoring and research were examined in a systematic review. Of particular interest were the sampling of water for MP, MP extraction from water samples, MP extraction from sediment samples, MP extraction from biota, and MP characterisation. The rate and degree of diversification was determined by scoring each method by its "degree of novelty": highly novel methods, secondary adaptations of existing methods and smaller, tertiary adaptations of existing methods. The first use of a method was determined by approaching the literature in chronological order, and the apparent interest in new methods was determined by normalising the number of citations by the number of months since publication. This analysis revealed that the rate of method diversification has been greatest since 2011, driven by the experience or facilities available to the research group, the need to improve MP recovery in complex matrices, to push the lower size limit for MP analysis, or to reduce the cost or increase the sustainability of sampling programmes. The adoption of novel methods was seen to be inconsistent across the field, with many researchers falling back on methods that are well established in the existing literature. Perhaps most importantly, there is little consistency in the reporting of MP concentrations, however, these differences are seldom driven by method selection and are rather the result of discrepancies between researchers.

3.08.02

Quality of Nanoplastics and Microplastics Ecotoxicity Studies: Refining Nanomaterial-Related Quality Criteria

A. Jemec, University of Ljubljana, Biotechnical Fac. / Department of Biology; N.B. Hartmann, Technical University of Denmark (DTU) / DTU Environment; A. Potthoff, Fraunhofer Institute for Ceramic Technologies and Systems IKTS; D. Drobne, University of Ljubljana, Biotechnical Faculty / Department of Biology; D. Kuehnel, UFZ - Helmholtz Centre for Environmental Research

The number of ecotoxicity studies on nanoplastics and microplastics has been increasing steadily. It is thus becoming critical to develop assessment criteria for the quality of studies to be used for regulatory purposes or for reporting in a public knowledge base. In this study, we evaluated whether existing criteria, developed for engineered nanomaterials mainly composed of metals, metal oxides or carbon, apply to nanoplastics and whether additional, nanoplastic specific criteria, are needed to cover specific peculiarities occurring for this material group. We applied the GUIDEnano and the DaNa criteria to PS NPs studies (n=47; published until the end of 2019) which currently represent the majority of nanoplastics studies. Most of these studies complied to questions which address the development/reporting of the study, but many criteria regarding the particle properties were not meet. We identified some criteria that were not entirely relevant for nanoplastics and these are: stability of substance in terms of dissolution, surface area of pristine nanoplastics, and some specific material properties (magnetic properties, acidity/basicity, KoW). The GUIDEnano scoring resulted in stricter assessment for PS NPs studies; roughly 40% of studies were unacceptable according to GUIDEnano and 19% by DaNa. The reason for different outcome is due to ability to involve the expert opinion in DaNa assessment. When the questions, irrelevant for nanoplastics, were removed from GUIDEnano list more studies were found acceptable which implies a need for a redefinition of existing nanomaterial criteria for nanoplastics. A list of additional nanoplastic specific criteria was also suggested, among most important being: source and protocol for nanoplastics production, impurities/chemical additives, polymer identity, surface topography, density, hydrophobicity, colour and leaching of chemicals from nanoplastics in test medium. We conclude that by few refinements, existing study quality evaluations approaches originally developed for nano(eco)toxicology studies can be adopted to the evaluation of studies dealing with nanoplastics. So far, the modified study quality evaluation approach is valid for primary nanoplastics, and could be implemented as well for primary and secondary microplastics. The list provided here is intended as a starting point for further elaboration, taking different purposes for an evaluation into account.

3.08.03

Mp-Tox-Rob Risk of Bias Tool for Assessing Microplastic (MP) In Vitro and In Vivo Toxicology Studies

E. Danopoulos, The University of Hull / Hull York Medical School; M. Twiddy, University of Hull / Hull York Medical School; J. Rotchell, University of Hull / Biological and Marine Sciences

Systematic review and meta-analysis methodology are increasingly used in the field of microplastic (MP) research to collate and appraise existing evidence in a standardised manner. Quality assessment, termed risk of bias (RoB) assessment, is a key part of this methodology. RoB refers to the possible introduction of systematic error (bias) in any part of research from inception to publication.MP systematic reviews have focused mainly on environmental studies, but they can also be executed for MP toxicology studies, providing vital information for the risk assessment process. A number of RoB tools exist, but a bespoke tool is needed to address the particularities of the fast-moving MP scientific research.The MP-tox-RoB tool was developed using widely accepted and validated systematic review methodology recommended by the Cochrane collaboration and current guidelines from scientific and governmental bodies. The tool focuses on two study designs: experimental *in vitro* (human and animal) and *in vivo* (animal) toxicology studies. It is structured by overarching domains of study design, execution and reporting, each comprising a set of signalling questions. The responses to these questions are either yes/probably yes/ no/ probably no/ no information or are numeric. Decisions have to be accompanied by a justification cited from the published paper, thus providing transparency in the rating. Each domain is rated individually and subsequently an overall rating is assigned. The RoB rating can be low, moderate, serious, critical or unclear.The rating is used to judge whether the study will be included in the synthesis of the data and the weight given to the evidence. The current quality of scientific research is the standard by which studies are measured against, not a fixed ‘gold standard’. MP-tox-RoB does not employ fixed scales for the rating process but scientific judgement. As such, MP-tox-RoB can continue to evolve as more data are added to the body of evidence and MP research advances.MP-tox-RoB can play an important role in the systematic review process and meta-analysis, as well as in the wider setting of a MPs risk assessment in the processes of hazard identification and dose-response assessment. MP-tox-RoB can also be used as a set of guidelines for the design, execution and reporting of MP toxicology research, promoting harmonization and standardization in different aspects of MPs research.

3.08.04

Reporting Guidelines for Increasing the Comparability and Reproducibility of Microplastic Research

W. Cowger, A.B. Gray, University of California Riverside / Environmental Science

Microplastics research is at a turning point where improving comparability and reproducibility of research is critical for the advancement of monitoring, modeling, and synthesis to effectively inform management. Reporting guidelines describe the minimum information that should be reported at the time of publication to make research comparable and reproducible. The incorporation of reporting guidelines into new research methods is particularly important during this period of rapid development in the field. Here we present reporting guidelines for microplastic research methods developed using an open science framework. A diverse group of coauthors and collaborators, from graduate students to tenured professors around the world, contributed to the reporting guidelines development. To increase the utility and adoption of these guidelines, we produced a suite of tools for the development of reporting procedures and reference during the reporting or review process. Funding and research agencies have already begun implementing the reporting guidelines into their workflow.

3.08.05

How Can Risk Assessment Data for Micro- and NanoPlastics Contaminations Be Generated in a Way That Is Useful for the Development of LCIA Models?

C.A. Askham, NORSUS AS; V. Pauna, Parthenope University of Naples / Department of Science and Technology; A. Boulay, CIRAIG - École Polytechnique de Montréal / Chemical engineering department; P. Fantke, Technical University of Denmark / Quantitative Sustainability Assessment, Department of Technology, Management and Economics; O. Jolliet, University of Michigan / Environmental Health Sciences, School of Public Health; J. Lavoie, CIRAIG, UQÀM / ISE - Institut des sciences de l'environnement; A. Booth, SINTEF Ocean / Environmental Technology; C. Coutris, Bioforsk / Dpt. for Soil Quality and Climate; F. Verones, NTNU / Department of Energy and Process Engineering; M. Weber, HYDRA Marine Sciences GmbH; M.G. Vijver, CML Leiden University / Environmental Biology; A. Lusher, NIVA Norwegian Institute of Water Research / Environmental Contaminants; C. Hajjar, CIRAIG; N. Casagrande, MARE - Faculty of Sciences and Technology, Universidade Nova de Lisboa

Life Cycle Impact Assessment (LCIA) for micro- and nanoplastics is in its infancy. To reduce the LCIA gap related to micro- and nanoplastics (MNPs), the international working group MarILCA was launched in 2018. The group aims to foster the development of impact assessment methods for marine impacts in LCA. It brings together international experts working on the development of impact pathway modelling for different plastic emissions to the environment. LCIA uses environmental sampling and laboratory toxicity data to calculate characterisation factors (CFs) for impacts arising from emissions to the environment. The authors want to address the creators of this exposure and toxicity data to aid understanding and provision of data that is as useful as it can be across these disciplines. LCIA relies on results from sampling and laboratory studies in order to derive fate and effect factors. It is vital that the data LCIA experts mine from literature is of relevant detail and quality for the derivation of CFs. Much of the available data has to be rejected. The systems engineering approach required for LCA differs from risk assessment. Environmental sampling data for MNPs contributes to both effect and fate model development, and thus the CFs used in LCIA. Therefore, LCIA experts are dependent on the data generated from environmental sampling and lab experiments in order to allow for the mining and translating of these data into effect and fate models suitable for LCIA. While there are different LCIA models used for the calculation of (human and ecosystem) toxicity, the work presented draws upon the USEtox approach. With USEtox, ecotoxicity of a substance, in the form of $HC50_{EC50S}$, is used to quantify the effect. This represents the toxicity of a hazardous substance in an ecosystem. Due to the need for interdisciplinary work to encourage the development of higher quality models which include the many and varied effects caused by nano- and microplastics pollution in LCA, the authors will present a checklist of metadata that could facilitate easier use of environmental sampling data, discuss effective experimental design for the investigation of MNPs and elaborate on species sensitivity curves. The check lists and guidance are presented with the goal of facilitating this truly interdisciplinary work and enable more data from scientists and researchers working in the risk assessment field to be used in the systems engineering world of LCA.

3.08.11

The Road to Microplastic Sampling Validation

G. Bordós, WESSLING Hungary Ltd.; S. Gergely, Budapest University of Technology and Economics / Department of Applied Biotechnology and Food Science; J. Háhn, Szent István University / Department of Environmental Protection and Environmental Safety; Z. Palotai, WESSLING Hungary Ltd; . SzabÃ, G. BesenyJĮ, A. Salgy, Budapest University of Technology and

Economics / Department of Applied Biotechnology and Food Science; P. Harkai, B. Kriszt, S. Szoboszlay, Szent IstvЂn University / Department of Environmental Protection and Environmental Safety

In the field of microplastic (MP) research in the environment, a significant amount of the currently reported results is uncertain because of the inappropriate methods of sampling, detection and quantification of MPs. Fortunately, many research groups are aware of these challenges, but validated methods, which are the prerequisite of standardized measurements, are scarce. Recovery tests are especially rare in the field of MP sampling. The aim of our research was to collect data on cascade filtration recoveries by modelling different turbulence conditions and sampling depth applying environmentally relevant MP concentrations while obtaining large sample volumes. As reference materials, different polymer types (polyethylene b_ PE; polypropylene – PP; poly[ethylene terephthalate] – PET; poly[vinyl chloride] – PVC; polyamide – PA) and shapes (sphere, fragment, fiber) were used, and for detection near-infrared spectroscopy/microscopy was applied. The developed method provides information not only on system based MP losses, but on sampling efficiency in a model environment as well. Based on the results, the highest recovery rate of all polymers was 31.4% on average, sampled from the water surface during continuous stirring. In these conditions, 92.4% of the PE spheres and 31.9% of the PE fragments were recovered. This indicates, particles reported in environmental monitoring studies might be less than the real environmental concentration. We can conclude, that surface sampling is more efficient than sampling in a deeper layer of the water column. Our research revealed, that the widespread application of microspheres as reference materials might lead to a too optimistic recovery values. The application of reference particles (fragments, fibers) with higher environmental relevance shows much lower recovery rates. Our results highlight, that validating the efficiency of the whole sampling process in the environment is more important than measuring only the filtration device's recovery. This study helps us to better understand the relationship and the possible gaps between the reported MP results and the real-life concentrations in the environment. This work was supported by the National Research, Development and Innovation Fund of Hungary, under the "Vállalati KFI_16" funding scheme (project no. KFI_16-1-2017-0477) and by the Thematic Excellence Programme (NKFIH-831-10/2019 - Szent István University, 2019) awarded by the Ministry for Innovation and Technology.

3.08.14

Some Advances in the Use of Mass Spectrometry for the Quantification of Micro(Nano)Plastics in Environmental Samples

A. ter halle, CNRS / Chemistry; M. Albignac, University of Toulouse, CNRS / IMRCP laboratory; E. Leroy, Université Paul Sabatier CNRS / Institut de Chimie de Toulouse ICT; C. Labrune, Laboratoire d'Ecogéochimie des Environnements Benthiques, LECOB UMR 8222; J. Ghiglione, LOMIC Laboratoire d'Océanographie Microbienne, LOMIC UMR 7621,

With the realization that microplastics are found throughout the planet (in water, air and animals), the scientists in the field of analytical chemistry provided an important effort. Consequently, there was important recent improvements of the methods for plastic particles characterization. These recent advances even allowed to reach their detection at the nanoscale. In parallel to the development of these new methods for micro(nano)plastic analysis, there is a need for coordinated and harmonized methods in the view of risk assessment evaluations. The use of mass spectrometry for micro(nano)plastics quantification seems to emerge. We will discuss the option of developing a very selective method of detection and the implication in the simplification of sample preparation. This option will be discussed regarding time and costs reduction as well as the evaluation of the robustness and reliability of the proposed method. Their will be an illustration with the detection of 6 polymers in benthic organisms collected on the sea floor in the Mediterranean sea. The use of targeted mass spectra analysis provides mass concentration data for a list of selected polymers. This type of output will be discussed regarding expected harmonized data reporting in the perspective of use in risk assessments.

3.08.15

Influence of Simulated Weathering on the Quantification of Microplastic Polypropylene by Pyrolysis Gas Chromatography Mass Spectrometry

T. Alajo, University of Queensland; E.D. Okoffo, The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); S. OBrien, The University of Queensland; S.D. Burrows, The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); F. Ribeiro, Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); M. Gallen, UQ / Queensland Alliance for Environmental Health Sciences; S. Ede, J. Colwell, Queensland University of Technology; A. Whittaker, University of Queensland; K. Thomas, The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); S. Kaserzon, University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS)

Degraded or weathered microplastics possess characteristic properties different from its virgin counterparts. Comprenhensive assessments on how these properties influence variability when quantitative analysis is performed by Pyrolysis Gas Chromatography/Mass Spectrometry (Pyr-GC/MS) have yet to be performed. This study assessed the accelerated laboratory weathering (0.68 W/m2) of micro-sized isotactic polypropylene of two different forms; beads (size: ~5 mm; shape: oval) over an exposure time series of up to 80 days and particles (size: 250-500 µm and 500-1000 µm, shape: irregular) over an exposure time series of up to 37 days). The photo-oxidation of the microplastics was confirmed via Fourier-transform Infrared - Attenuated Total Reflection (FTIR-ATR), scanning electron microscopy (SEM), and differential scanning calorimetry (DSC). We examined whether photo-oxidation affects quantitative measurements using a double shot Pyr-GC/MS method. We further assessed the differences in degree of degradation between the two forms of microplastics and identify weathering markers at a lower pyrolysis temperature. The degree of degradation occurred 2-fold faster for particles compared to beads, possibly due to higher surface area. Comparison of chromatograms between virgin and weathered polypropylene showed no evident qualitative changes in the oligomer mixture, yet there were visible quantitative changes. Specifically the pyrolysis product, 2,4-dimethyl-1-heptene (trimer), showed peak areas decreasing by 50% for beads and 58% for particles (after 80 and 37 days, respectively). Six weathering markers (oxidation products) were observed to overall increase with higher exposure days. These study suggest that weathering processes are affecting quantitation of isotactic polypropyl microplastics by Pyr-GC/MS and highlights the need for further analytical comparisons between virgin and weathered microplastics in order to avoid underestimation of microplastic expsoure studies. Keywords Pyrolysis Gas Chromatography Mass Spectrometry (Pyr-GC/MS), photo degradation, accelerated laboratory weathering, carbonyl index

3.08.16

Fourier-Transform Infrared Spectroscopy of Environmentally Weathered Textile Fabrics for Enhanced Microplastic Identification

S. Patankar, E. Vassilenko, M. Watkins, Ocean Wise Conservation Association / Plastics Lab; A. Posacka, P.S. Ross, Ocean Wise Conservation Association / Coastal Ocean Research Institute

Microplastic pollution in oceans is among the global environmental concerns of our time. Emerging research on ocean environments indicates that microfibers, such as those originating from textiles, are some of the most commonly occurring type of microplastic contaminants. While Fourier-transform infrared spectroscopy (FTIR) is commonly used to identify and characterize pollutant samples obtained from the environment, this identification is challenging because infrared spectra of materials can be modified by exposure to the ocean, air, UV light, and other ambient conditions, in a process referred to as "weathering". We report preliminary efforts in improving FTIR characterization of microplastics by building a library of infrared spectra of common textile fibers weathered under a selection of ambient conditions. Consumer textile materials including polyester, nylon, cotton, and other, were exposed to a selection of ambient conditions: ocean, air, and wastewater treatment stages, in a controlled weathering experiment. Infrared spectra were monitored for up to 52 weeks, with the resulting data illuminating on the environmental fate and longevity of synthetic and natural fibers. Spectral changes caused by weathering were found to depend strongly on both the composition of the material and the specific ambient conditions. This library of weathered material spectra is useful not only in easier identification of environmental microfibers, but also in helping us estimate the duration and manner of weathering that a given environmental microfiber may have experienced.

3.08.17

How to Achieve Solid Data Sets for Monitoring and Risk Assessment With Quantum Cascade Laser Ir-Imaging

M. Godejohann, MG Optical Solutions GmbH; S. Primpke, Alfred Wegener Institute / Shelf Sea System Ecology; G. Gerdts, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research / Shelf Sea System Ecology

1. Abstract Monitoring and risk assessment of micro- and nanoplastics are based on statistics and hence demand a huge data set in order to ensure reliable conclusions. Actually a lot of efforts are put into harmonizing procedures, analytical techniques and data reporting in order to increase the quality and accessability of data. This is essential for getting a complete overview of the actual situation and to benefit from already existing information. However, the confidence level of conclusions depend as well from quantitity of data. Quantum cascade laser based IR microscopy (QCL-IR) offers multiple times higher throughput than conventional techniques such as Fourier Transform-Infrared microscopy (µ-FT-IR) or the even more time consuming method of Raman-microscopy. In order to benefit from the high throughput of QCL-IR measurements the results have to be harmonized with established techniques. Primpke et alt. [1] published in November 2020 the results of comparing the measurement results of identical samples on Anodisc-filters taken with QCL-IR and µFT-IR. Regarding the harmonization of different measurement principles it turned out that for particles down to the size of >50µm both techniques deliver

similar results but at significantly different measurement speeds. For smaller particles the quantum cascade laser based system results are more similar to the results taken with Raman-microscopes. Due to harmonizable results and the high throughput QCL-IR-microscopes are a promising way to install enough measurement capacity for routinely monitoring environmental samples. 2. References [1] Sebastian Primpke, Matthias Godejohann, Gunnar Gerdts,. 2020. Rapid identification and quantification of microplastics in the environment by quantum cascade laser based hyperspectral infra-red imaging. Environmental Science & Technology 2020 *Acknowledgement* - The authors thank DRS Daylight Solutions for the technical support ans use of SPERO 'QT QCL-IR microscope for the measurements for this study. We thank Marcus Bach for performing the measurements. S.P. and G. G. thank the German Federal Minister of education and Research for financial support (Project BASEMAN-Defining the base linse and standards for microplastic analyses in European waters; BMBF grant 03F07434A

3.08.18

Automated Ultra-Fast Analysis of Microplastics in Large µFTIR Imaging Datasets From ENvironmental Samples Via RDF Classifiers

B. Hufnagl, Purency GmbH; H. Lohninger, Vienna University of Technology / Institute of Chemical Technologies and Analytics; M.G. Löder, C. Laforsch, University of Bayreuth / Animal Ecology I and BayCEER

Modern micro Fourier-transform infrared (µFTIR) microscopy in combination with focal plane array (FPA) detectors, allows for real chemical imaging (FPA-based µFTIR Imaging) and for measuring even small MP down to 10 µm from whole environmental samples concentrated on filters after plastic-conserving enzymatic-oxidative purification (Löder et al. 2015, Löder et. al 2017). However, the analysis of the large resulting hyperspectral image (HSI) data is still challenging. A typical HSI of one sample may consist of up to 4.3 Mio. IR spectra. Even with a small number of samples a Big Data problem evolves with respect to the analysis of this data. Currently available tools for data analysis of HSI from MP samples are based on spectral comparison and are relatively time consuming (Primpke et al. 2017), which prevents a real high-throughput analysis. Machine learning approaches are a solution and Hufnagl et al. (2019) developed a first Random Decision Forest (RDF)-based approach, which already allowed a fast processing of large MP datasets (1 Mio. spectra) within minutes. In our presentation we will give a short overview of the Bayreuth Microplastics Finder (BMF), an analysis tool that combines polymer type identification and particle size characterization to facilitate a fast computer-assisted analysis of MP concentrations from different environmental matrices such as air, water and soil. The original version is based on a random decision forest (RDF) classifier for MPs that can distinguish between five different polymer types and at the point of publication it remained an open question whether the method can be scaled to cover a larger variety of plastics. In the here presented expanded version of the BMF the number of supported polymer types has been increased to 20 plastic types including the 10 most important polymers. References: Hufnagl et al. 2019, Analytical Methods 11 (17), 2277-2285, https://doi.org/10.1039/c9ay00252a Löder et al. (2015) Environmental Chemistry , 12, (5), 563-581. https://doi.org/10.1071/EN14205 Löder et al. (2017) Environmental Science & Technology 51 (24), 14283–14292, https://doi.org/10.1021/acs.est.7b03055 Primpke et al. (2017) Analytical Methods 9 (9), 1499–1511, https://doi.org/10.1039/c6ay02476a

3.08.19

Practical Comparison of Microplastic Identification Methods: Manual, Semi-Auto and Automated FTIR and Raman Spectroscopy

Y. Song, Korea Institute of Ocean Science & Technology; S. Eo, Korea Institutue of Ocean Science and Technology (KIOST); S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; W. Shim, Korea Institute of Ocean Science and Technology (KIOST) / Oil and POPs Research Group (OPRG)

The vibrational spectroscopy such as Fourier transform infrared (FTIR) and Raman microscope is popular microplastic analytical method for their quality and quantity. The spectroscopy can confirm the polymer composition, however, still it is unavoidable to human bias by manually selecting plastic-like particles for FTIR analysis using microscope. We tried to find best practice for microplastic analysis by reducing time demand, human bias (false negative) and automatic identification bias (false positive and false negative) using spectroscopy (FTIR and Raman microscopy). The manual analysis under transmission mode, semi-automated method using ultrafast mapping and spectrum profiling, and automated method using ultrafast mapping, spectrum profiling and fully automatic identification were compapred. In the automated method, to check false positive rate during identifcation, all spectra were manually duble-checked after automated method have done. The automated method took the shortest time (3.2±0.5 h, which is occupied time by operator) to analyze whole filterpaper (Ø25 mm), but the polymer types were limited to the number of profile spectrums, fiber could not be detected, and the rate of false positive was 80±15%. While, semi-auto analytical method using spectrum profiling was suitable for microplastic analysis in all aspects. It took shorter time than those of manual analysis (manual: 6.1±0.8 h and semi-auto: 4.0±0.6 h), fiber could be distinguished by chemical and mapping image. And 22±12% (fasle negative) more microplastic particles were detected using semi-auto than using maual analysis. Two types of spectroscopic analysis (FTIR and Raman) for microplastics were compared in four different methods, manual identification in ATR and transmission mode and semi-auto method using FTIR, and manual using Raman. The analysis duration was overwhelmingly taken for long time (34 ± 1.5 h) in Raman followed by transmission (5.6 ± 0.3 h), ATR (4.8 ± 0.9 h) and semiauto (3.6 ± 0.5 h). However, the detected number of microplastics using Raman were 1.4, 1.9 and 3 times higher than using semiauto, transmission and ATR mode, respectively. And the range of 0-50 µm was peaked in size distribution of Raman, but the range of 50-100 µm was peaked in size distribution of the others. Depending on the microplastic size range of interest, it could be appropriate identification method.

3.08.20

Quantifying Microplastics in Complex ENvironmental Samples: Data Comparison of Py-Gc/Ms and Hyperspectral FTIR Imaging - Potential and Limitations

S. Primpke, Alfred Wegener Institute / Shelf Sea System Ecology; M. Fischer, Carl von Ossietzky University Oldenburg / Institute for Chemistry and Biology of the Marine Environment ICBM; C. Lorenz, Aalborg University / Department of the Built Environment; G. Gerdts, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research / Shelf Sea System Ecology; B. Scholz-Boettcher, University of Oldenburg, ICBM / Institute for Chemistry and Biology of the Marine Environment ICBM

Microplastics (MPs) analysis in environmental samples is performed with various methods and instruments. The most common ones to identify the chemical nature of the particles are often based either on FTIR/Raman spectroscopy or on thermal degradation coupled to gas chromatography-mass spectrometry (GC/MS). In general, both approaches result in two different types of data sets that are either particle number or mass related. Each gives important information for assessing the environmental impact of MP. Although a complementary use of both methods is ideal, an often-asked question is the transferability of particle related data, which are by far more represented in literature, into masses. As a proof of principle samples of waste water treatment plants (WWTP) were pretreated and concentrated on filters. The same samples were analyzed first with hyperspectral Fourier-Transform infrared (FTIR) [1] and subsequently with pyrolysis (Py)-GC/MS [2]. The MPs composition was determined in terms of types/particle using FTIR and types/masses using Py-GC/MS, respectively following a comprehensive comparison. Resulting similar trends for the general MP contamination underlined the applicability of both analytical approaches in MP analysis. Different relative polymer compositions resulted from the size-dependent impact to the respective method and deviated relative calculations. Here, it was found that a preceding clustering process of polymers is necessary for any data harmonization between the different detection principles. This is of general importance but might distort the resulting polymer pattern of the respective method putatively due to different polymer detection principles of the respective methods. Mass conversion from particle data was found to be highly dependent on the applied approach and might lead to serious overestimation. In conclusion, the complementary use of both methods for reliable data generation is highly recommended [3]. [1] Primpke, S., Lorenz, C., Rascher-Friesenhausen, R. and Gerdts, G. 2017. Anal. Methods 9, 1499-1511. doi: 10.1039/c6ay02476a[2] Fischer M, Scholz-Böttcher B.M. 2019. Anal. Methods, 11, 2489-2497. doi: 10.1039/c9ay00600a.[3] Primpke, S., Fischer, M., Lorenz, C., Gerdts, G., Scholz-Böttcher, B.M., 2020. Anal. Bioanal. Chem. 412: 8283-8298. doi.org/10.1007/s00216-020-02979-w.

3.08.24

In Search of the Quality of Micro and Nano Plastics Analysis and Reference Materials

B. Van Bavel, NIVA / Environmental Pollutants; A. Lusher, NIVA Norwegian Institute of Water Research / Environmental Contaminants; J. de Boer, VU University / Environment & Health; W. Cofino, Wageningen University; L. van Mourik, Vrije Universiteit Amsterdam / Department Environment and Health; H.A. Leslie, Institute for Environmental Studies VU Amsterdam; S. Crum, Wageningen Environmental Research - WUR / Environmental Risk Assessment; E. Martinez-Frances, Norwegian Institute for Water Research (NIVA) / Environmental Chemistry; J. Johansen, Chiron AS / Administration

The environmental monitoring of plastics is positioned high on the agenda of countries and international organisations worldwide. Large scale national and regional monitoring programs are however hampered by the lack of urgently needed harmonised analytical methods and reference materials for QA/QC of these methods. Data on the presence of plastics in the environment varies largely regarding quality and comparability. Robust and validated harmonised methods and QA/QC tools still missing. Interlaboratory studies are an efficient way to achieve this and both gather information on the state of the art, can be used to validate the methods in terms of uncertainty and to select candidate reference materials. Here we will present the result of two ILS studies of which one is

currently on-going and the approach of the new EU project EUROqCHARM which will focus on the harmonisation of analytical methods for nano-, micro- and macro plastic in environmental samples and the production of reference material.

Importance of Chemical Speciation and Bioavailability in Hazard Assessment, Risk Assessment and Regulation of Metals in Multi-Stressor Environmental Conditions

3.09.05

Enhanced Olivine Weathering Through Physical Stress: Implications for CO2 Sequestration and Possible Ecosystem Impacts

G. Flipkens, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp / Biology; R.M. Town, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp / Department of Biology (SPHERE Research Group); R. Blust, University of Antwerp / Biology

Active carbon dioxide (CO_2) removal from the atmosphere by negative emission technologies (NET) is needed to limit global warming to well below 2°C. Increasing the CO_2-consuming weathering of silicate minerals by distributing micron-sized grains along the world's coastline, so-called coastal enhanced silicate weathering (CESW), has been proposed as a NET. The abundant, fast weathering mineral olivine is a prime candidate for this application. In addition to direct CO_2 sequestration, CESW has the possible benefit of counteracting ocean acidification and indirect CO_2 sequestration through silicon (Si) fertilization. However, accurate predictions on the in-situ CO_2 sequestration rate are lacking and possible adverse ecosystem impacts of trace metals released during olivine weathering, of which nickel (Ni) and chromium (Cr) are most concerning, remain to be assessed. We investigated the effects of long term, continuous grain-grain collisions on olivine weathering and associated trace metal release, to obtain a first simplified estimation of the effects currents and waves could have in CESW. We hypothesized that increased grain-grain collisions would result in enhanced olivine weathering through increased particle fragmentation. A 70-day experiment was conducted in which a tumbling device was used to simulate grain-grain collisions by continuous rotation of olivine sand in natural seawater at a speed of 0, 18, or 54 rotations per minute (RPM). Concentrations of olivine dissolution products were measured on a weekly basis. Results showed that olivine weathered 5 to 7 times faster when rotating at 18 or 54 RPM, respectively compared to the static situation. Both continuous grain-grain collisions and flushing of reaction products at the grain surface likely explain this observation. Nickel release was higher than expected at the end of the experiment and Cr was only released at the start of the experiment in highly variable amounts. Based on these results, we can infer that in a large scale CESW scenario, Ni could accumulate above the European environmental quality standard (8.6 µg/l) in shallow coastal waters with a water residence time of several weeks. However, a more rigorous risk assessment is needed, taking into account metal bioavailability and potential mixture toxicity. This study provides the first evidence on how physical stress could enhance olivine weathering and associated CO_2 sequestration and metal release in a CESW application.

3.09.06

Effects of Wildfires on Mercury Mobilisation in Eucalypt and Pine Forests

I. Campos, Universidade de Aveiro / Environment; J. Keizer, University of Aveiro-CESAM / Department of Environment and Planning; P. Pereira, University of Aveiro / Department of Biology and CESAM; N. Abrantes, University of Aveiro-CESAM / CESAM/DAO

Wildfires and subsequent rainfall have a major impact on forest ecosystems and on the redistribution of trace elements, which are mobilized from soil and ashes. In particular, the fire-induced release of mercury (Hg) into the environment is relevant due to its volatilisation and toxicity. The present study addresses this concerning issue by comparing the concentrations of Hg in topsoil (0-2 cm) and ashes in burnt and long-unburnt forest plantations (Ermida and São Pedro do Sul, North-centre of Portugal). Fire was classified as moderate in Ermida and moderate to high severe in S. Pedro do Sul. Sampling collection took place immediately and 4 months after the fire, the latter following an episode of heavy rainfall. Mercury analysis was performed in an Hg analyser (AMA 254 LECO Instrument), in which samples were thermally decomposed by controlled heating. The final decomposition products were passed through an Hg amalgamator heated to 700 °C and Hg^0 was released and detected by absorption spectrometry at 254 nm. This technique allows eliminating the digestion procedure prior to analysis and is free from matrix interferences. The principal results obtained in this study were the following: (1) 30% of the Hg retained in eucalypt soils was released by the fire, corresponding to a loss of 1.0-1.1 g Hg per hectare of burnt soil; (2) levels of Hg in burnt eucalypt soils doubled the values registered in burnt pine soils for both areas; the Hg concentrations in ash revealed a similar pattern; (3) Hg concentrations in both soils and ashes differed significantly between the two burnt areas, possibly due to differences in fire severity; (4) rainfall caused a loss of 1.0 g Hg per hectare from ashes and a slight enhancement of 0.5 g Hg per hectare in the soil washed out. The difference between the previous estimations, 0.5 g Hg per hectare, corresponds to the quantity of Hg transported to the surrounding areas or eventually introduced into aquatic systems. Our results highlight that wildfires and subsequent rainfall play a key role in the mobilisation of mercury in the environment and point out the importance of further studies to assess the impact of Hg in aquatic systems downstream to burnt areas.

3.09.07

Mercury Bioavailability and Effects to Green Alga Chlamydomonas Reinhardtii During Combined Exposure With nanoTiO2

V. Slaveykova, University of Geneva / Department F.A. Forel for Environmental and Aquatic Sciences; M. Li, University of Geneva; W. Liu, University of Geneva / Department F.A. Forel for Environmental and Aquatic Sciences

Phytoplankton are typically exposed to a combination of trace metals and other environmental stressors, including engineered nanoparticles (ENPs). Dispite a significant progress achieved in the understanding of the toxicity during a combined exposure to metals and ENPs, the underlaing interactions and relationship between metal speciation, bioavailability and effects are not fully understood. The overall goal of the present study is to explore the interactions of Hg(II), nanoTiO2 and green alga Chlamydomonas reinhardtii. Research questions to address are: (i) How does presence of nanoTiO2 of different primary size influence the speciation of Hg in the exposure environment? (ii) What are consequences for Hg bioavailability and effects to algae?. Mercury was chosen as a hazardous pollutant of global importance and nanoTiO2 as one of the most extensively used ENPs. The effects of three nanoTiO2 materials with different compositions (anatase (A) and rutile/anatase (AR) and primary particle size of 5 (A5), 15 (A15), and 20 nm (AR20) were explored. Results demonstraed that nanoTiO2 materials adsorbed significant amount of Hg(II) and underwent a fast agglomeration and sedimentation in the medium in a manner depending mostly on nanoTiO2 initial concentration and primary particle size. Presence of Hg(II) contributed to the enhancement of the aggregation and sedimentation. The addition of nanoTiO2 reduced significantly Hg bioavailability as compared with Hg(II) treatment alone. This alleviation effect was more pronounced for A5 and higher concentration. It decreased in the order A5 > A15 > AR20 showing the importance of the primary particle size of nanoTiO2. This observation is in line with the observed relationship between the amount of intracellular Hg and the concentrations of dissolved Hg(II) in the medium that is not adsorbed to the aggregates of nanoTiO2. Combined exposure to Hg(II) and nanoTiO2 resulted in reduction of the toxicity (growth rate inhibition, oxidative stress, membrane damage), which was more important at higher nanoTiO2 concentrations. The protective role was more pronounced for the nanoTiO2 with smaller primary size. Overall, the obtained results contribute to the enlargement of the understanding of behaviour of cocktails of trace metals and ENPs in freshwater environment, which are highly relevant in the context of metal risk assessment in contaminated environment.

3.09.08

Derivation and Validation of Bioavailability Models to Derive a Maximum Allowable Concentration Environmental Quality Standard for Nickel

C. Nys, Arche consulting; A. Peters, wca; C.E. Schlekat, NiPERA Inc - test; E.R. Garman, E.T. Middleton, NiPERA Inc / Ecotoxicologist

Recent scientific developments on the behaviour, fate and chronic ecotoxicology of nickel have lead to the development of biotic ligand models (BLMs) for nickel. These models have also been validated to ensure they deliver predictions to within acceptable ranges. These principles have been applied in the derivation and application of the annual average environmental quality standard (AA EQS) for nickel under the European Union's Water Framework Directive for some years. However, these bioavailability principles have not yet been applied for the derivation of an EQS for acute, or short term, effects on ecosystems due to nickel toxicity. Bioavailability models for the acute effects of nickel toxicity have been developed previously, but because the principal regulatory focus at the time was a continental scale risk assessment and the derivation of the AA EQS these models have not previously been validated against data for dissimilar species in order to demonstrate that they can be reliably applied in EQS derivation. This study identifies a number of potential bioavailability models for the acute toxicity of nickel and demonstrates their validity against independent datasets for dissimilar species. A variety of acute bioavailability models have previously been developed for nickel toxicity. This study takes each of these models individually and evaluates their appropriateness for applying bioavailability corrections over relatively broad ranges of water chemistry conditions. Independent validation datasets that include a minimum of two acute toxicity tests on nickel performed in different water chemistry conditions. A validation dataset is then compiled against which the relevant bioavailability models can be tested, depending upon the trophic level of the validation species and the coverage of the model. All of the tested bioavailability models are able to perform relatively well in terms of their ability to normalise the acute toxicity data for the validation species, although the fish bioavailability model does generally tend to provide less reliable predictions for some of the tests conducted. Bioavailability models for acute nickel toxicity have been validated against independent datasets for a variety of different species,

and are applied to normalise the bioavailability for all ecotoxicity test data that are included in the SSD for acute toxicity in order to enable a MAC EQS to be derived.

3.09.09

European-Wide Compliance Assessment With the Freshwater Bioavailability-Based Nickel Environmental Quality Standard

I.A. Wilson, A. Peters, G. Merrington, wca; C.E. Schlekat, NiPERA Inc - test; E.R. Garman, E.T. Middleton, NiPERA Inc / Ecotoxicologist

Nickel in currently classified as a Priority Substance under the European Union's Water Framework Directive (WFD). This means that an Environmental Quality Standard (EQS) for nickel is derived and applied across all European Member States. In 2011, the annual average EQS derived for nickel was 4 μg L^{-1}, accounting for bioavailability. Therefore, in order to appropriately assess compliance with the nickel EQS, bioavailability must be considered. In order to assess compliance across Europe regulatory freshwater monitoring data are required. Much of these data for Member States are publicly available. In this assessment 11,497 sites were used for a tiered compliance assessment of nickel: · Tier 1, annual mean measured dissolved concentration at the site are direclty compared to the EQS, and if the mean exceeds the EQS, move the to the next Tier; · Tier 2 a simplified bioavailability screening tool is used, again if the EQS is exceeded then move to the next Tier; · Tier 3 full bioavailability are accounted for, using Nickel Biotic Ligand Models, importantly, failure of the EQS at the site can only be determined once all three tiers have been undertaken. At Tier 2, accounting for bioavailability, using the simplified tool, just 172 sites of the 11497 showed an exceedance. Taking these sites, to Tier 3 and using the Biotic Ligand Models, is likely to result in a further reduction in exceedances. Appropriate implementation of the nickel EQS by different member states appears highly variable. Some member states have fully developed systems for accounting for nickel bioavailability, many others take a much more simplistic approach and perform the compliance assessment only at the first Tier. Most importantly failures that are identified in this way are not a robust basis upon which to consider management approaches and are only indicative of a need for more detailed information for the site, and a higher tier assessment should be performed. Although the EQS for nickel was derived almost a decade ago there is still a great deal of work required to enable all the member states to properly implement it. This compliance assessment, demonstrates the low levels of potential risk of aquatic risks of nickel (< 1%) Importantly, assessing compliance through the appropriate implementation of the bioavailability EQS ensures regulatory focus and programmes of measures efforts fall on those sites of potential ecological impact.

3.09.10

A Practical Comparison of Two Nickel BLMs

E.T. Middleton, NiPERA Inc / Ecotoxicologist; A. Peters, wca; R.C. Santore, Windward Environmental LLC; C.E. Schlekat, NiPERA Inc - test; E.R. Garman, NiPERA Inc / Ecotoxicologist

Bioavailability models are gaining regulatory acceptance around the globe. Among these models, the biotic ligand model (BLM) is a popular template based on a mechanistic understanding of how water chemistry parameters interact with organisms. The goal of this study is to explore the similarities and differences between two nickel BLMs: the European model which is currently used in the Water Framework Directive and the North American model which is currently being considered for Water Quality Criteria development by the US Environmental Protection Agency. The aim of this work is not to identify a "best" model, but rather to explore the variations between the two models and enhance the user's understanding of how these models are developed. This study will examine the conceptual factors that underpin the BLMs and explore how both models were developed with particular emphasis on jurisdictional requirements. Regulatory constraints on the development and application of the models will be considered as these can alter many stages of the process, from model development, data applicability, and ultimately in the outputs that they produce. Examples of jurisdictional judgements include toxicity test duration, taxa included in the assessment, and methods of data normalization. We will explore the model interfaces, input fields and predominant toxicity modifying factors. Finally, we will use an independent dataset to evaluate the performance of the European BLM and the North American BLM, respectively. The results predicted by the models will first be compared against the measured toxicity threshold values. Given that both the European and North American nickel BLMs have been validated using multiple datasets, we hypothesize that both models will perform well with an independent dataset. Following this verification of model performance, a comparison between model results will be performed. Communication to enhance the understanding of bioavailability models is a limiting factor towards the acceptance and implementation of BLMs into the regulatory arena. By presenting a comprehensive, yet digestible, comparison of the development, use, and outputs of two nickel BLMs we aim to enhance the transparency and remove the veil of perceived complexity that surrounds BLMs to give users confidence and clarity in the tool. Ultimately, this study will allow the audience to understand how models can be developed differently, use different criteria, and yet maintain confidence in the accuracy of the toxicity predictions.

3.09.12

Accounting for Bioavailability in the Derivation and Use of a Maximum Allowable Concentration Environmental Quality Standard for Nickel

A. Peters, wca; G. Merrington, I. Wilson, WCA Environment Limited; C. Nys, Arche consulting; C.E. Schlekat, NiPERA Inc - test; E.R. Garman, E.T. Middleton, NiPERA Inc / Ecotoxicologist

Recent scientific developments on the behaviour, fate and acute ecotoxicology of nickel have lead to the developments of biotic ligand models (BLMs) for nickel. These models have also been validated to ensure they deliver predictions to within acceptable ranges. The key water chemistry parameters influencing both acute and chronic nickel bioavailability in freshwaters are pH, Dissolved Organic Carbon (DOC), and calcium. This means that the same additional supporting parameters are required for implementation of a bioavailability based Maximum Acceptable Concentration Environmental Quality Standard (MAC EQS) for nickel as they are for the existing annual average EQS under the European Union's Water Framework Directive. An acute BLM for nickel toxicity has been developed and validated, and this model has been applied to normalise an acute toxicity database to the local water chemistry conditions at each site in order to provide an SSD for acute nickel toxicity that is specific to each site. This approach follows essentially the same approach as is used to perform bioavailability normalisations for the annual average EQS to specific local sites. The MAC EQS was applied to the available data for water chemistry and nickel exposures in order to perform an indicative compliance assessment for short term exposures to nickel in European freshwaters. This compliance assessment was performed in a number of different ways with each approach using a slightly different metric for the exposure, including the maximum, the 99th percentile, and the 95th percentile. The overall assessment aims to take account of the uncertainty associated with each different measure of exposure based upon the total number of monitoring samples that the exposure assessment was based on. Regulatory monitoring data from a several different countries, representing different water chemistry conditions have been selected in order to demonstrate the influence of accounting for bioavailability upon the potential exceedances of the MAC EQS. It is important when using a MAC that it is compared to a high percentile concentration of the exposure data, normally a 99.5th, and not to the maximum concentration measured and included a confidence of failure statement (e.g. 95%). This is because a MAC is an 'absolute limit', but compliance is rarely assessed through continuous monitoring, but rather with a number of discrete samples which are often collected over a relatively wide time frame.

3.09.13

Validation of Bioavailability Models Nickel for Algae, Invertebrates, and Fish in Chinese Surface Waters

A. Peters, wca; G. Merrington, WCA Environment Limited; J. He, State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences; F. Wu, Chinese Research Academy of Environmental Sciences; C.E. Schlekat, NiPERA Inc - test; E.R. Garman, E.T. Middleton, NiPERA Inc / Ecotoxicologist

Recent scientific developments on the behaviour, fate and ecotoxicology of nickel have lead to the developments of biotic ligand models (BLMs) for nickel. These models have been applied in the derivation and application of the annual average environmental quality standard (AA EQS) for nickel under the European Union's Water Framework Directive for some years. Regulatory authorities in several regions have considered it necessary to demonstrate the applicability of the bioavailability normalisation approach either for their local surface waters, local ecotoxicity test species, or both. The consideration of bioavailability-based approaches for understanding and regulating nickel emissions in China also requires information to demonstrate the validity of these approaches for the local water chemistry conditions. Toxicity tests were conducted on *Pseudokirchneriella subcapitata*, *Daphnia magna*, and *Brachydanio rerio*. Nineteen toxicity tests were conducted with *P. subcapitata*, seven tests were conducted with *D. magna*, and ten tests were conducted with *B. rerio*. All experimental data are within a factor of three of the BLM predicted values for all tests with all species. Bioavailability models for nickel toxicity have been validated for three standard test species in field collected natural water samples from a range of different Chinese surface waters. Comparison of experimental data against BLM predictions shows that the existing nickel bioavailability models are able to explain the differences in toxicity that result from water chemistry conditions. Replicate tests performed on algae in the same test medium demonstrate that there is a very low level of inherent variability in the test system for this species.

3.09.16

Sensitivity of Lymnaea stagnalis to Trace Metals

A. Peters, wca; I. Wilson, WCA Environment Limited; D. Leverett, WCA-Environment Ltd; K. Viaene, ARCHE; C. Nys, Arche consulting; M.J. Chowdhury, International Lead Association / Senior Scientist -Environment; C.E.

Schlekat, NiPERA Inc - test; E.R. Garman, E.T. Middleton, NiPERA Inc / Ecotoxicologist; S. Baken, European Copper Institute; C. Claytor, Copper Development Association Inc. / HESD; D. Boyle, P. Marsh, Cobalt Institute; C. Cooper, International Zinc Association / Environment & Sustainability; A.C. Ryan, International Zinc Association; A. Boullemant, Rio Tinto Aluminium Pechiney; W.A. Stubblefield, Oregon State University / Environmental and Molecular Toxicology

The freshwater snail *Lymnaea stagnalis* has been demonstrated to be sensitive to metals, including copper, cobalt, lead, and nickel in various laboratory studies. These studies use larval stages as test organisms and the most sensitive endpoint is consistently growth. However, there is clear evidence of uncertainties related to the observed high sensitivity of this species in laboratory studies. *L. stagnalis* is widely distributed in freshwaters across the globe with no clear evidence of population-level impacts despite its apparently high sensitivity to metals under laboratory conditions. Within Europe the species has a widespread distribution in freshwaters of all countries. Thus, the high sensitivity of this species to trace metals is not expected based on the fact that they occur widely in field observations, although this could potentially be due to reduced metal bioavailability in the field. This study has focused on the collection of regulatory monitoring data reporting either the presence or absence, or the abundance, of *L. stagnalis*, and the closely related snail species *Radix balthica*, in freshwater aquatic ecosystems. Databases from both The Netherlands and England contain species specific information, which for some sites can be matched to chemistry monitoring data which allows an assessment of the potential bioavailability of trace metals to be considered. Variability in the source of snail cultures, the culture conditons in which snails are maintained prior to testing, and the ecotoxicity testing conditions could all lead to differences in toxicity between studies. Evidence from tests on distinct cultures from different geographical regions suggests that sensitivity among cultures can vary, although the water chemistry in which the different populations have adapted may play a role. For example, populations from hard water and soft water ecosystems appear to show differences that need to be considered in the broader weight of evidence.

3.09.17

Positively Correlated Single Metal Sensitivities Among Freshwater Algal Species Lead to Stronger Synergisms of Ni, Cu and Zn Mixtures at Community Level

A. Fettweis, KULeuven / Department of Earth and Environmental Sciences; S.E. Hansul, Ghent University (UGent) / Laboratory for Environmental Toxicology and Aquatic Ecology; K. De Schamphelaere, Ghent University (UGent) / Environmental Toxicology; E.E. Smolders, KULeuven / Earth & Environmental Sciences

Predicting chronic metal mixture interactions at environmentally realistic concentrations is vital to advance risk assessment of metals. Currently, metal mixture effects are mostly predicted from single metal data on single species. A key question here is whether single species mixture data can be extrapolated to community level where different ecological interactions take place. In that respect it might be relevant to understand how single metal sensitivities correlate among the species in a community. To test this hypothesis, environmentally realistic metal mixtures of Ni, Cu and Zn (alongside the single metals) were tested using two fixed ray designs (1:1:3 and 5:1:12 Ni:Cu:Zn ratio based on weight) on 14 algal communities of 4 species each, with communities that either had positive, no or negative correlation of metal sensitivities (ranging between -0.2 and +0.9). In general, single metal and metal mixture sensitivities on communities could only be partially predicted based on single species sensitivities, and biomass was on average a more sensitive endpoint than biodiversity (factor 1.8 based on EC50's). Indicating that the insurance hypothesis could not fully explain the effects at community level and that interspecies interactions, other than resource competition, might play a vital role. Communities rarely exhibited more synergistic mixture interactions than what was observed in any of the single species in that community. Communities with positively correlated sensitivities had more species for which synergistic interactions were found on the single species. The extent of interactions were assessed as the observed-predicted mixture effect, and showed a negative correlation (R^2=0.4, $p < 0.001$) with the correlation of metal sensitivities among the single species for the 14 communities. Showing that algal communities consisting of species with positively correlated sensitivities to the different metals are more prone to synergism than communities with contrasting or negatively correlated sensitivities to metals. A clear explanation is not yet found, and modelling is required for further understanding.

3.09.20

Evaluation of Seasonality and Metal Contamination in the Performance of the Polychaete Diopatra Neapolitana Collected in Different Locations of Ria Aveiro

V. Giménez, University of Aveiro / Biology; P. Cardoso, C. Sá, University of Aveiro / Department of Biology; E. Figueira, University of Aveiro / Biology CESAM; A. Pires, University of Aveiro / CESAM & Department of Biology

Coastal zones usually serve as sinks for toxic elements due to the discharge of effluents resulting from anthropogenic activity. These elements can alter pH, temperature and salinity of the waters, endangering the organisms that live there. Benthic organisms are at higher risk of exposure *via* pore water and sediments. Previous studies demonstrated that metals and metalloids accumulate in sediments in higher concentrations than in the water column and have been shown to affect polychaetes. Polychaetes are usually the most abundant group in marine ecosystems becoming themselves key elements on estuarine and coastal food webs. As they inhabit most of the time in the sediment, this causes them to accumulate more contaminants because their feeding strategy involves ingesting sediments thus leading to maximum exposure to contaminants from both sediment and water. This study aimed to analyze whether biochemical changes occur in organisms throughout the seasons and in different locations and whether the accumulation of metals is affecting these changes in biochemical parameters. Polychaetes of *Diopatra neapolitana* species were collected from five different sites of Ria de Aveiro during the Fall of 2018 and the Winter, Spring and Summer of 2019. They were brought to the laboratory and then biochemical parameters were tested to check for differences between the seasons and sites in biochemical responses: indicators of cell damage, antioxidant, biotransformation enzymes and energy-related parameters enzymes. Metal quantification was done by ICP-MS. Cu, Pb and Cr were accumulated both in soluble and insoluble parts of the organisms in Summer and Autumn. The results obtained in this study showed that it is in summer and spring that the highest accumulation of trace metals occurs. It was also observed that the activity of antioxidant enzymes such as catalase and non-protein thiols was higher, while lipid damage increased in winter. Moreover, obtained results also demonstrated that organisms accumulated more trace metals like Nickel, Zinc, Cadmium and Arsenic, in the soluble fraction, in Summer and Spring, indicating that these metals are more available during these seasons. The presence of trace metals seems to be associated with these biochemical changes. Overall, this study demonstrated that seasonality and metals strongly affect polychaetes biochemical responses.

3.09.22

Evaluation of the Release of Particles and Metals During the In-Water Hull Cleaning Process and Its Environmental Risk

Z. Soon, Korea Institute of Ocean Science & Technology / Risk Assessment Research Center; J. Jung, Korea Institutue of Ocean Science and Technology (KIOST) / Risk Assessment Research Center; D. Shin, M. Kim, Korea University of Science and Technology/Korea Institute of Ocean Science and Technology / Risk Assessment Research Center

Ship in-water cleaning is encouraged by certain countries before entering local ports to clear off foulants from ship hulls for preventing the introduction of non-indigenous species to the local marine environment. This cleaning process is expected to be done with a capturing system on board to prevent the release of foulants and paint particles into the water column. However, not all in-water cleaning and capture (IWCC) systems can effectively treat the effluents before releasing back to the water, thus posing risks to the environment. In this study, effluents were collected during in-water cleaning (manual cleaning by divers and automated cleaning by ROV) and analyzed for TSS, particle size distribution, and metal concentrations. A high abundance of particles was observed in 8 to 10 µm size fraction, with a range of 23 to 80% of total particles. It also suggests that it is possible to remove up to 93.7% of particles from effluents by using 8 µm of filter size. Manual cleaning was observed to generate a similar amount of TSS compared to ROV cleaning, with average concentrations of 173.4 and 229.9 mg/L, respectively. After treating the effluent by filtering system, the average concentration of TSS was reduced to 64.4 mg/L. The reduction was also observed in metals concentration, where average Cu concentration reduced from 161.8 to 24.5 µg/L, and average Zn concentration reduced from 1140.2 to 776.5 µg/L. Manual cleaning, on the other hand, produced average Cu concentration of 209.2 µg/L and average Zn concentration of 1513 µg/L. The metal concentrations were converted to release rate and used for predicting the risk in a local port. The predicted concentrations of Cu and Zn showed manual cleaning by divers has higher impact on the local marine environment compared to ROV cleaning. Despite that these concentrations were subjected to vary with the surface area of ships cleaned and cleaning location, in-water cleaning should only be encouraged when capture systems are available to prevent the release of contaminants back to the local marine environment.

3.09.26

First Evidence of Metal Contamination in Glacial Melt Waters As Possible Legacy of World War One in the Italian Alps

V. Lencioni, MUSE-Museo delle Scienze / Invertebrate Zoology and Hydrobiology; A. Franceschini, F. Paoli, MUSE-Museo delle Scienze Trento / Dept. of Invertebrate Zoology and Hydrobiology; A. Lutton, The Ohio State University, Columbus OH / School of Earth Sciences; J. Olesik, The Ohio State University, Columbus OH / School of Public Health; P. Gabrielli, The Ohio State University, Columbus OH / School of Public Health; Byrd Polar and Climate Research Center

Relicts of the Great War in the Alps (1915-1918, "White War", WW1) were

buried in the ice and are now reemerging due to glacier retreat. When bombs, bullets, cannon parts and barbed wires are brought to the surface, they release metals that enter glacier-fed streams with unknown effects on biodiversity and ecosystem functions. To address this emergent contamination, we selected three glacier-fed streams originating from glaciers that were differently affected by WW1 operations on the Italian- Austro-Hungarian front: Lares, Presena and Amola (Adamello-Presanella mountain range, Trentino, North Eastearn Italy, 46°N, 10°E). We hypothesized that the highest metal contamination is linked to the frequent battlegrounds (Lares), and the lowest, to locations that were farer from the active front (Amola). We quantified the occurrence of trace elements in water and animal samples collected in icemelt water within 1 km from the glacier fronts in August 2016 and 2017 (six sites between 2421 and 2685 m a.s.l). Fauna was represented by Diptera Chironomidae (*Diamesa* spp.) that was collected as larval stage. Metal concentration was determined by Inductively Coupled Plasma Sector Field Mass Spectrometry (ICP-SFMS). Trace elements (TE) were interpreted using the crustal enrichment factor calculated as EFc = (TE/Al) sample/ (TE/Al) crustal. The concentration of metals in the water from all sites was very low and ranged from 1 ppm (Al, Fe, Na, Mg, Ti) to 1 ppt (Ag, Bi, Cd, Sb, Ta), in accordance with the expected levels of these elements in the aquatic environment. Only moderate enrichments were observed for Ag, As, Bi, Cd, Li, Mo, Pb, Sb and U in Lares and for Sb and U in Presena. The concentrations of trace elements in chironomid larvae (per unit of dry weighted mass) ranged from 1000 ppm (Al, Fe, Na, Mg) to 10 ppb (Bi, Sb, Ta, Tl), in each sites higher than in the water. Low to moderate enrichments of Ag, As, Cd, Cu, Mo, Sb and Zn were observed in larvae from all sites, and also Ni at the Presena and Lares sites. As expected, these latter two sites resulted the most contaminated. However, only Ni in chironomids from Lares were found higher than prescribed by the United States Environmental Protection Agency (EPA) limits for acute and chronic toxicity. Further experiments will i) clarify whether this pollution is only due to the WW1 legacy and ii) define the ecotoxicological risk associated to the presence of these contaminants at ecosystem level in downstream reaches.

Measuring, Monitoring and Modelling of Pesticide Fate and Mitigation in a Regulatory Context

3.10.01

Estimation of Photochemical Degradation Rates of Pesticides in Surface Water

P.I. Adriaanse, J. Boesten, Wageningen University and Research / Wageningen Environmental Research, Environmental Risk Assessment; M. ter Horst, Wageningen Environmental Research / Wageningen Environmental Research, Environmental Risk Assessment; C. Jacobs, Wageningen University and Research / Wageningen Environmental Research; C.v. Griethuysen, Board for the Authorisation of Plant Protection Products and Biocides

Estimation of the degradation rate of plant protection products in water under realistic conditions may be important for correct estimation of exposure concentrations for regulatory purposes. Standardised tests for degradation in water and in water-sediment systems in the laboratory exist, but these do not reflect degradation under field conditions. This is especially true for studies on photolytic degradation; therefore, photolytic degradation is generally not accounted for in the lower tiers of the exposure assessment. The aim of this study is to develop a procedure for the estimation of photochemical degradation rates from outdoor cosm experiments for use in the higher tiers of the exposure assessment. Observations in outdoor ponds or cosms are regularly used as a higher-tier risk assessment to evaluate the ecotoxicological effects on the aquatic ecosystem in a more realistic way. By means of inverse modelling of the behaviour of the compound in the cosm we determined the degradation rate in water, $DegT_{50}$, in outdoor cosms for three compounds which are known to degrade photolytically. We did so by coupling the fate model TOXSWA to the optimisation tool PEST and determined the $DegT_{50}$ for a reference daily UV radiation, weighed with a vitamin-D action spectrum, assuming that the degradation rate was directly proportional to the amount of weighed UV radiation. The UV radiation data were derived from satellite-based observations accounting for the effects of the thickness of the ozone layer and the cloud cover. For cosm studies with metribuzin, imidacloprid and metamitron we obtained satisfactory estimates of $DegT_{50}$ values. After correcting these for effects of water depth, coverage of water surface by plants and the skyview factor on the UV radiation in the water, the variation between the $DegT_{50}$ values of metribuzin and imidacloprid was smaller than the variation between $DegT_{50}$ values derived (in an earlier study) by assuming that the degradation rate depended on water temperature and not on UV radiation. For metamitron this comparison was not possible, but the variation between the radiation-based $DegT_{50}$ values of metamitron was considerably smaller than the variation between the radiation-based $DegT_{50}$ values of either metribuzin or imidacloprid. This indicates that for photolabile compounds assuming a radiation-dependent degradation rate will probably lead to a more realistic exposure estimation in the regulatory surface water scenarios. Keywords: photolysis, outdoor cosm, TOXSWA model.

3.10.07

Development of the Pesticide Dissipation From Agricultural Land (PeDAL) Model for Improved Pollinator and Beneficial Insect Risk Assessment

K.J. Hageman, Utah State University / Chemistry & Biochemistry; S. Lyons, Utah State University / Chemistry and Biochemistry; A.D. Kinross, Utah State University / Chemistry & Biochemistry

A pesticide's risk to pollinators and beneficial insects depends on both pesticide toxicity and the concentration of pesticide encountered. The pesticide concentration in plants decreases after application at a rate that depends heavily on field and weather conditions (plant properties, intercept fraction, temperature, wind speed, etc) and several processes (e.g. volatilization, photodegradation). Current pesticide risk assessment approaches for pollinators and beneficial insects could be improved if we were able to better predict scenario-specific pesticide concentrations in plants at different times after pesticide application. A number of models for predicting pesticide fate, or certain aspects of pesticide fate, in planted fields have already been described; however, the photodegradation process has not been fully developed in such models. Modeling foliar photodegradation is complex because photodegradation rates vary tremendously among chemicals, but also with light intensity and cloud cover. Thus, our objectives were to (1) develop and validate an improved approach for incorporating foliar photodegradation into pesticide fate models, (2) use sensitivity analysis to evaluate the effect of using chemical- and scenario-specific photodegradation rates in models, and (3) demonstrate how our pesticide dissipation model can be combined with toxicity databases to improve risk assessment for pollinators and beneficial insects. Our photodegradation approach was used in a new model, the Pesticide Dissipation from Agricultural Lands (PeDAL) model. The PeDAL model includes volatilization from plants and soil, foliar photodegradation, and leaf penetration. It was designed to predict pesticide dissipation rates (i.e. the time to reach half of the initial concentration, DT_{50}), field emission flux values, and concentration time trends in soil and plants. Our validation indicated robust model design, the sensitivity analysis showed that including chemical- and scenario-specific photodegradation rates dramatically increases accuracy in predicted DT_{50} values, and we showed that PeDAL model output can be combined with toxicity data for improved risk assessment for pollinators and beneficial insecticides.

3.10.08

A Comparison of Volatile Substance Deposition Modelling in the EU

S. Andrews, Compliance Services International

A number of formulation types (e.g. aerosol dispensers, smoke generators, cold fogging, hot fogging & vapour releasing products) can contain active substances which are volatile. In the context of both plant protection and biocidal products it may be necessary to model deposition of the active substance to soil and surface water, where environmental release cannot be excluded. This poster will look at multiple deposition models comparing: the default input assumptions and experimental refinement of these; the applicability and limitations of the models based on the underlying validation data; and variability of output where different models are applied to the same situation.

3.10.09

Fate of Pesticide Residues in Vegetative Filter Strips in Long-Term Exposure Assessments: VFSMOD Development and Analysis

R. Muñoz-Carpena, University of Florida / Agricultural & Biological Engineering; S. Reichenberger, Knoell France SAS / Environmental Fate / Modelling / GIS; R. Sur, K. Hammel, Bayer AG, Crop Science Division / Environmental Safety

Inclusion of quantitative mitigation of pesticides in regulatory environmental risk assessment (ERA) using common agricultural field conservation practices is a critical need recently identified by experts in North America and EU. Currently, mitigation by vegetative filter strips (VFS) is available by coupling the event-based model VFSMOD in continuous simulations within current long-term higher-tier surface water ERA frameworks (EU FOCUS SWAN, US EPA PWC, PRMA Canada, California CDPR PREM, etc.). In this case, the field management and pesticide-laden surface runoff at the edge of the field is calculated by the model PRZM and VFSMOD routes it from the edge of field through a VFS of desired characteristics to estimate potential load reductions before entering the aquatic environment, simulated by the receiving water body model (FOCUS TOXSWA, EPA VVWM). While under proper settings VFS could effectively reduce pesticide concentrations in surface water below thresholds of concern-*what happens to the residues trapped in the VFS*? The current ERA VFS framework uses a highly risk-conservative assumption, whereby the pesticide trapped in the VFS undergoes degradation between storm events and the surface residue (soil mixing layer and adsorbed to trapped sediment) is remobilized in full and added to the incoming pesticide load in the next event in the series. While risk conservative, this initial approach is not consistent with the nonuniform pesticide redistribution and extraction with depth used in the model PRZM within current ERA, and it has also been found too conservative for highly sorbed compounds with high specific toxicity like pyrethroids and others.Our objective is to develop

a complete VFSMOD component to quantify the fate of VFS pesticide residues between runoff events for use in long-term ERA simulations. This includes realistic assumptions of the fate of the residues, including non-linear pesticide redistribution in the soil, mass balance of the VFS soil mixing layer and sediment trapped, degradation between runoff events, and partial remobilization and carryover of the remaining residue to the next event. Initial sensitivity and limited testing with existing field data are discussed.

3.10.10

Recommendations for the Parameterization of Sediment Trapping in VFSMOD

S. Reichenberger, Knoell France SAS / Environmental Fate / Modelling / GIS; R. Sur, Bayer AG - Crop Science Division / Environmental Safety; S. Sittig, knoell Germany GmbH / E-Fate Modelling; S. Multsch, knoell Germany GmbH / Environmental Fate / Modelling / GIS; R. Muñoz-Carpena, University of Florida / Department of Hydrology Water Quality Agricultural Biological Engineering Faculty

The most widely implemented mitigation measure to reduce transfer of pesticides to surface water bodies via surface runoff are vegetative filter strips (VFS). To reliably model the reduction of surface runoff, eroded sediment and pesticide load by VFS an event-based model is needed. The most commonly used model for this purpose is VFSMOD. VFSMOD simulates reduction of total inflow (ΔQ) and reduction of incoming eroded sediment load (ΔE) mechanistically. These variables are subsequently used to calculate the reduction of pesticide load (ΔP). While ΔP can be well predicted from ΔQ, ΔE and some other variables, errors in ΔQ and ΔE will propagate to ΔP. Hence, for strongly sorbing compounds, an accurate prediction of ΔE is crucial. The most important parameter characterizing the incoming sediment in VFSMOD is the median particle diameter d50. The objective of this study was to derive a generic d50 parameterization methodology for sediment trapping in VFSMOD that can be readily used for regulatory VFS scenarios. Four studies with 16 hydrological events were selected for modelling. A first set of VFSMOD simulations, following the SWAN-VFSMOD sediment parameterization with d50 = 20 µm yielded a general overestimation of ΔE. Consequently, a maximum-likelihood-based calibration and uncertainty analysis with the DREAM-ZS algorithm was performed for the 16 events. The resulting d50 values were all in the low range (1.3-5.4 µm) and did not allow to establish a robust relationship to predict a wider range of d50 from the available explanatory variables. To increase the sample size and the range of d50 values, the comprehensive Kinston dataset for a loamy sand in North Carolina was calibrated with DREAM-ZS. Calibration was performed separately for each hydrological event. Further data points with measured particle size distributions in run-on were assimilated from the literature. The extended test data set of d50 values and explanatory variables was analysed using an extended multiple linear regression (MLR) approach and Classification and Regression Trees (CART). A good calibration of event totals and outflow hydrographs could be achieved for most events and VFS treatments of the Kinston site. The calibrated d50 values yielded a wider range (2-16 µm) than the initial 16 events. The improved d50 parameterization method derived with MLR/CART will be adopted in the next version of SWAN-VFSMOD to provide more realistic quantitative mitigation within FOCUS STEP4.

3.10.11

Implementation of Shallow Water Table Effects in Pesticide Runoff Mitigation by VFS Within Swan-Vfsmod

R. Sur, Bayer AG - Crop Science Division / Environmental Safety; R. Muñoz-Carpena, University of Florida / Department of Hydrology Water Quality Agricultural Biological Engineering Faculty; S. Reichenberger, Knoell France SAS / Environmental Fate / Modelling / GIS; K. Hammel, H. Meyer, Bayer AG - Crop Science Division / Environmental Safety; N. Kehrein, Bayer Crop Science / Data Science & Digital Transformation

Quantitative mitigation of pesticides entering surface water using vegetative filter strips (VFS) is currently available within the EU SWAN FOCUS STEP 4 suite when selecting the VFSMOD mechanistic model option. Estimates of surface runoff, sediment and pesticides simulated with PRZM are routed through the VFS where VFSMOD estimates the reductions of runoff, sediment, and pesticide. The reduced runoff is handed over to the TOXSWA aquatic model to calculate predicted environmental concentrations (PECsw). Brown et al. (2012) proposed VFSMOD parametrization rules including the selection of VFS soils and other characteristics for use in the EU FOCUS R1 to R4 (Rx) SWAN scenarios. The resulting soil parametrization rules apply for soils under common (no water table, nWT) vertical infiltration, described in VFSMOD by the Green-Ampt model extended for unsteady rainfall. However, the presence of a seasonal shallow water (sWT) condition is common in some EU regions. In these cases, the VFS pesticide mitigation efficiency can be limited, depending on the water table depth (WTD) and soil type. VFSMOD incorporates a sWT mechanistic infiltration component, which relies on the parametrization of the soil hydraulic characteristics, described by user-selected formulae like the Mualem-van Genuchten (MvG) equations. Following a procedure like in Brown et al. (2012) and making use of updated EU spatial soil databases, the main objective of this study is to identify Rx representative VFS soils to study the effects of sWT on pesticide mitigation for a combination of illustrative storm and pesticides and on PECsw. The selection and testing of the Rx VFS soils seeks to reflect a 90th-percentile worst case of pesticide load. The multicriteria adopted in the Rx soils selection ensured the representativeness of each of the selected soils within their Rx region by also evaluating the percentile of important soil parameters for noWT (K_s, S_{av}) and sWT infiltration conditions (fillable pore volume f_{pv}). The framework consisted of 4 steps: (a) spatial soil database analysis for VFS Rx mitigation scenarios; (b) selection of VFS candidate soils; (c) analysis of effects of sWT and sorption on pesticide load for individual storm events; (d) Effect of sWT on long-term STEP 4 SWAN VFS mitigation simulations. Proposed Rx VFS soils and important parameters for the SWAN implementation will be presented. The implementation of the new sWT VFS mitigation component provides a more realistic description of pesticide reduction in accordance with higher-tier STEP 4 EU FOCUS objectives.

3.10.15

Evaluation of SWAT+ Rule-Based Probabilistic Pesticide Applications in a High Intensity Agricultural Watershed in Belgium

H. Rathjens, B. Miguez, M.F. Winchell, Stone Environmental; R. Sur, Bayer AG - Crop Science Division / Environmental Safety

The Soil and Water Assessment Tool (SWAT) has been a leading international 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 two main advantages over the currently used SWAT model for landscape level watershed modeling for pesticide risks assessments: (1) flexible spatial representation of landscape features and their interactions and (2) advanced agricultural management practices, including rule-based probabilistic pesticide applications. Here, we provide an overview of the new features of SWAT+ and present the application of SWAT+ in a high agricultural intensity watershed in Belgium. We focus on assessing whether implementing the SWAT+ probabilistic pesticide application approach results in similar pesticide concentrations compared to what is achieved using detailed field-level application data available from a farmer survey. The preliminary results indicate that when using SWAT+ and a realistic rule-set of conditional applications, basic knowledge about pesticide management operations in a watershed (typical application rate, PCT, and application window by crop) is sufficient for predicting daily pesticide concentration distributions. The distributions obtained from the rule-based applications were very similar to the distributions achieved based on detailed field level farmer-survey applications, and close to concentration distributions observed in monitoring data.

3.10.16

Landscape Characterization With Google Earth Engine: Functionality Supporting High Resolution Spatiotemporal Analyses Utilizing Satellite Imagery

C.E. Holmes, Virginia Polytechnic and State University; F. Löw; T. Schad, Bayer AG Crop Science Division /Environmental Modelling; C.M. Holmes, Applied Analysis Solutions, LLC

Assessing pesticide fate in the environment requires assumptions or empirical information regarding the landscape in which pesticides are used and areas in proximity. Regulatory exposure scenarios have defined environmental factors allowing consistent and transparent use for all stakeholders. Spatially explicit exposure studies incorporate locally-relevant information on a specific environment, quantitatively selected to be placed into a larger regional context. The effort and cost of these landscape-based studies have steadily decreased as more and better data and tools become available. This presentation will introduce functionality in Google Earth Engine that increases efficiency and transparency in landscape characterization studies while reducing time and effort needed. Earth Engine is a platform developed by Google that brings together geospatial datasets, satellite imagery, and image analysis functions to create a robust platform that can be accessed through the web. Earth Engine's large pre-processed image collections, simple coding interface, and readily available analysis functions make it an inexpensive and user-friendly tool for landscape-based studies. Demonstrations include use of high temporal (5-7 day revisit), high spectral (13 bands), and moderate spatial resolution (10m) from European Space Agency's Sentinel constellation. Example results include vegetation change detection, land cover classification, image summarization by user-define areas, and charting output. Collaboration between Earth Engine users is encouraged vis-à-vis the Developer Forum, a community where users can ask and answer questions

alongside developers of the platform. While a Google Earth Engine account is required to develop code, apps can be published that allow shareable tools to be created that can demonstrate capabilities in an interactive format without requiring an account. User data uploads to Earth Engine along with any code that is written can be shared with other Earth Engine users, increasing transparency and collaboration among team members. Computations are performed on Google's servers removing processing requirements often needed by traditional analysis methods and desktop software. Expansive data and efficient functionality available in Earth Engine allow for easier access to the latest satellite data and modern image processing methods. The outcomes can provide timely and detailed information to support landscape-scale pesticide exposure assessments.

3.10.17

Spatiotemporal Explicit Surface Water Exposure Assessment Using Catchment Modelling

S. Multsch, F. Krebs, knoell Germany GmbH / Environmental Safety; S. Reichenberger, Knoell France SAS / Environmental Fate / Modelling / GIS; P. Kraft, L. Breuer, Justus Liebig University Giessen / Institute for Landscape Ecology and Resources Management; W. Beltman, L. Wipfler, Wageningen Environmental Research; S. Bub, University of Koblenz-Landau; T. Schad, Bayer AG Crop Science Division / Environmental Modelling

Tier 4 effect modelling focuses on the landscape scale assessment of pesticide use, aquatic exposure and species populations (EFSA, 2013). Such an approach requires spatiotemporal explicit input data from hydrological and exposure models. Thus, the objective of this study was the development of a hydrological catchment model which accounts for processes at the landscape scale (FOCUS, 2007): Exposure calculation for inter-connected water bodies, surface water routing through the stream network, dynamic inflow from upper streams and adjacent landscape areas and varying river cross-sectional shapes (rectangular, trapezoidal, triangular) and geometry (length, bottom with, channel depth, bank slope). The study area was the Rummen catchment in Belgium which is characterized by a dense cultivation of orchards (3120 ha). Streams extent over a length of 146 km which were discretized into 1708 single river segments with a length of ~100m. Small headwater streams account with 55% for the largest fraction of the stream network. Channel depth, bottom width and bank slope were defined on the basis of an online field observation using Google Earth to parameterize trapezoidal cross-sectional shapes. A catchment model was implemented using the Catchment Modelling Framework (Kraft et al., 2011). Water inflows into single river segments were calculated by multiplying the specific discharge measured at the outlet by the specific land area which drains into the river segment. Water flow between river segments was calculated using a diffusive wave approach. Solute degradation as well as adsorption and desorption between water and sediment were calculated according to STEPS1-2-3-4 (Klein, 2007). The simulation period covers 26 years (1992-1997 warm-up; 1998-2017 assessment period). Annual drift from orchards was calculated as an annual rate of 0.27 mg/m² wet water surface on 366 reaches on March 5th each year. The analysis of the stream network of 146 km in total length showed that the hydrological patterns were realistic with respect to the stream size. The spatiotemporal explicit simulation of hydrological fluxes and solute transport allows for the aggregation of exposure patterns according to the current standard exposure assessment (edge-of-field approach) as well as for the direct input into effect modelling. The proposed new tools and methods follow a generic approach and are transferable to other regions as well.

3.10.18

Problem Definition of a Harmonised Framework for Spatially Distributed Leaching Modelling (SDLM) of Pesticides

B. Jene, BASF SE / Environmental Fate; A. Tiktak, PBL Netherlands Environmental Assessment Agency / Water Agriculture and Food; A. Ghafoor, KEMI; A. Poot, Ctgb; E. van den Berg, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; C.G. Hoogeweg, Waterborne Environmental, Inc. / Modeling; M. Klein, Fraunhofer IME / Ecological chemistry; M. Stemmer, Austrian Agency for Health and Food Safety / Institute for Plant Protection Products; P. Sweeney, Syngenta / Environment Product Safety; R. Sur, Bayer AG - Crop Science Division / Environmental Safety

Spatially distributed leaching modelling (SDLM) of pesticides is a methodology to estimate the leaching potential of plant protection products over an extensive spatial scale such as national or European. It is described as a higher tier in the current European Guidance for groundwater risk assessment. Whereas this option is an integral part of the tiered assessment scheme only little guidance is provided on how to conduct such spatial assessments with SDLM. Guidance on how to perform such leaching assessments is therefore needed, as well as version control for high-resolution spatial databases for the EU. It was therefore decided to establish a working group under the umbrella of the SETAC Environmental Monitoring Advisory Group on Pesticides (SETAC EMAG-PEST). This document describes the aim and scope of the work to be performed by this working group. The main products of the working group will be a harmonised modelling framework including the data needed to run these models, and documents describing the use of the framework in regulatory assessments. The framework will serve two different Tiers of the groundwater risk assessment scheme, i.e. Tier-3b and Tier-4. At Tier-3b, the framework will deliver the same exposure assessment goal as currently used in FOCUS groundwater, i.e. the 80th-spatial and temporal percentiles of the leaching concentration at 1-m depth. This exposure assessment goal is considered a conservative estimate of the real groundwater concentration. To ensure consistency of the tiered approach, the modelling framework will support all parameter refinements carried out at Tier-2. At Tier-4, the measured groundwater concentration in groundwater wells is assessed. The modelling framework plays a crucial role for the selection of vulnerable regions in which to install monitoring wells. It can also be used to demonstrate whether existing groundwater monitoring studies have been carried out at locations that are sufficiently vulnerable in view of the existing FOCUS exposure assessment goal. The modelling framework will, however, not simulate the actual concentration in the groundwater wells, because additional processes occur between 1 m depth and the position of the groundwater wells. The Working Group will consist of members from academia, regulators and industry. It will consist of a Steering Committee, a subgroup on spatial data and a subgroup on modelling. The Working Group will deliver two years after the start of the project.

3.10.19

Comprehensive Characterization of Agricultural Proximity to Surface Water in France

C.M. Holmes, Applied Analysis Solutions, LLC; P. Sweeney, Syngenta / Environment Product Safety; L. Insinga, Applied Analysis Solutions, LLC; L. Loiseau, D. Johnson, Syngenta

Regulatory exposure modeling for pesticides relies on hypothetical scenarios to accommodate landscape variability across large extents. Proximity is an important aspect to potential aquatic exposure from agricultural applications. Characterizing proximity 'in total', as opposed to selected locations, allows for reduced uncertainty regarding this aspect of off-field pesticide transport. The availability of high spatial and temporal resolution cropping and hydrographic data in France provides an opportunity to characterize agricultural proximity across the entire country. The Registre Parcellaire Graphique represents individual agricultural plots used as a reference for the Common Agricultural Policy and contains 9.4 million parcels with over 325 culture codes. We analyzed 807,000 parcels encompassing 2,825,326 ha of maize along with 3.6 million water features from BD TOPO. Multiple proximity distances were assessed from water features and intersected with maize parcels. Three methods of analysis were used to determine the total area of maize parcels potentially "impacted" (i.e., area of maize potentially impacting surface water). In the Binary Method, if any portion of the maize parcel is within the proximity distance, the entire maize parcel area is impacted. The Buffer Method considers only the portion of the parcel that directly overlaps the proximity distance to be impacted. In the Threshold Method, if a specified % of the maize parcel (the threshold) falls inside a proximity distance, the entire parcel is impacted (Binary Method) otherwise only the direct parcel area with the distance is impacted (Buffer Method). Results show that, e.g., 46% of maize area in France has at least some portion of the parcel within 30m of water (Binary Method) while the Buffer Method results in 5.2% ot total maize is within 30m of water. Using the Threshold Method and a 30m proximity distance, a 10% threshold results in 20% of the maize area being impacted, while a 30% threshold results in only 8% of the maize area impacted. Regional differences are demonstrated. This study demonstrated the viability of parcel-level proximity analyses across France. The ability to subgroup results by administrative unit allows for further exploration and initial evaluation of potential drivers in proximity. Further refinements include the ability to determine proximity based on surface water characteristics (e.g., permanent v. intermittent, natural v. artificial, flowing v. static).

3.10.20

Pesticide Transfer Risk Control in Subsurface Drainage Context by the Development of Decision-Making Tool

j. maillet-mezeray, Bayer CropScience / Regulatory Affairs; j. tournebize, a. jeantet, Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE)

The changes in the aquatic risk assessment during the process of issuing Marketing Authorisations (MA) in France led to better asses the risk of the contamination of surface water through the subsurface drainage network. The initially-proposed measures to mitigate risk from regulatory assessment led to Risk Phrases aimed at prohibiting any use of drained plots. Developments have been made in order to integrate, for some substances and some specific scenario, the possibility of indexing the clay content of the soil in the plot, therefore leaving the possibility of applying the products on drained plots with a clay content lower than 45%. Moreover, herbicide solutions on subsurface drained plots are becoming scarce due to a limited number of innovations combined with the re-approvals processes, which lead to an increasing proportion of portfolio being forbidden on these plots. Weed management then becomes a major issue for

arable cropping systems on drained plots. Unlike the prevention of runoff, no risk management measures are available to prevent the risks associated with drained plots In the current enforced risk assessment guidance documents. Nevertheless, a management measure consisting in applying these herbicides before the period of water flowing in the drains seems to reduce significantly the risk of transfer by plant protection products. Our study has several objectives. The first objective is to demonstrate, using data from La Jaillière, an experimental site, representative of FOCUS scenario D5, the relevance of the management measure by analysis of historical data base for given active substances in terms of reducing the transfer of plant protection products in drained plots. The second objective is to validate, still from the La Jaillière site, the hypothesis of a management measure based on an indicator of saturation of the soil profile before drainage flow. Secondly, a drainage flow prediction model, SIDRA RU, has been tested on around twenty drained sites across France in order to assess the robustness of discharge prediction. Finally, the dissemination of this original approach will result in the design of an internet interface, developed, as a decision-making tool for treatment in drained plots, intended for farmers and their advisers. This tool is already in the testing phase at the national level with local actors involved.

3.10.21
How Can Risk Mitigation Measures Be Included in the Regulatory Environmental Risk Assessment for Pesticides in Germany?
M. Klein, Fraunhofer IME / Ecological chemistry; S. Reichenberger, Knoell France SAS / Environmental Fate / Modelling / GIS; S. Spycher, I.A. O'Connor, EBP Schweiz AG; K. Thomas, RLP AgroScience GmbH; S. Multsch, knoell Germany GmbH / Environmental Fate / Modelling / GIS; S. Sittig, knoell Germany GmbH / E-Fate Modelling; D. Großmann, J. Flade, Federal Environment Agency (Umweltbundesamt)
Surface runoff from agricultural fields is a major input pathway of pesticides into surface waters. The aim of this project was to i) analyze the effectiveness of various mitigation measures to reduce pesticide runoff and erosion inputs into surface waters, ii) assess the suitability of the measures found effective for use in the quantitative environmental exposure assessment for authorization of plant protection products (PPP), and iii) make recommendations how the potentially suitable measures could be applied in risk assessment of PPP in Germany. Following a literature analysis, 16 risk mitigation measures were presented to five experts in the field. Measures finally selected for quantitative analysis belong to 3 groups: vegetative filter strips (VFS), soil conservation measures (including no-till) and microdams in row crops. VFS effectiveness was analysed with CART (Classification and Regression Trees) using the dataset compiled by Reichenberger et al. (2019). CART was performed for three target variables: i) relative reduction of total inflow by the VFS (ΔQ), ii) relative reduction of sediment load (ΔE), and relative reduction of pesticide load (ΔP). The main data sources for soil conservation measures were a plot database with annual runoff volumes and soil losses (Maetens et al., 2012), a literature review (Fawcett et al., 1994) and a field study with event-based data (Erlach, 2005), while for microdams the main source were the data compiled by Sittig et al. (2020). The following conclusions were drawn from the analysis: i) VFS can be recommended for application in quantitative risk assessment. However, infiltration and sedimentation should be simulated with a mechanistic model such as VFSMOD. ii) Due to the high variability of results and limited availability of high-quality data, effectiveness of mulch-till could not be quantified sufficiently well. It can therefore not be recommended for now as a regulatory mitigation measure. iii) Before recommending no-till as a regulatory mitigation measure for surface runoff and erosion, the question of potentially increased pesticide loss via leaching and drainage should be clarified. iv) Microdams in row crops can also be recommended as a regulatory mitigation measure, since they have shown to be effective and their effect can be modelled as a reduction of the runoff Curve Number. However, elaborating a CN table for e.g. the FOCUS scenarios would require an in-depth analysis of the available data.

3.10.22
An Examination of the Relationship Between Groundwater Monitoring and Modelling Estimates for Regulatory Decision Making at a European Level
p.j. sweeney, F. Rama, Syngenta / Product Safety; H. Resseler, Syngenta Agro GmbH; B. Brumhard, Syngenta GmbH / Regulatory Affairs; h. willems, Syngenta
Current European regulatory groundwater assessments in Europe almost exclusively rely upon modelling estimates at Tier 1 to demonstrate compliance of regulatory triggers. Although there is the potential to use groundwater monitoring data at Tier 4 (FOCUS 2009) the lack of clearly defined protection goals and an agreed method to evaluate monitoring data at a European level mean that it is not clear how such data should be used. We propose a method closely based upon the FOCUS methodology for groundwater which could provide such a means of evaluation. The FOCUS Equivalent Concentration (FEC) method assumes that each monitoring site approximates to a FOCUS scenario and estimates a 90th percentile groundwater concentration in a similar way to FOCUS. We demonstrate how this method can be used to demonstrate an equivalent pass/fail at the FOCUS Zone level with reference to a monitoring study conducted by Syngenta at 125 monitoring locations across Europe that have been monitored quarterly for over 5 years. In addition, we show that by identifying the locations which are most vulnerable to leaching and working with growers it is possible to reduce significantly applications on such areas.

Micro(Nano)Plastics and Associated Chemicals (I): Occurrence and Analysis

3.11.02
Characterization of Nanoplastics Released During the Washing and Abrasion of Polyester Textiles
T. Yang, EMPA Technology & Society Lab / 506; B. Nowack, EMPA / Technology and Society Lab
Nanoplastics released during the daily use of plastic products and their ability to interact with our ecosystem implied the importance for us to further investigate the risk of nanoplastics. Despite the increasing number of ecotoxicological studies on nanoplastic, the exposure information remains poorly reported. Synthetic textiles have been identified as an important source of plastic fibers in the environment and previous studies have suggested that domestic washing is a major pathway for microplastic fiber release to the environment. Therefore, we designed washing and abrasion experiments to investigate the amount and other features of nanoplastics released during our washing and wearing of polyester textiles. By using different techniques such as STXM, TEM and NTA, we were able to obtain both physical and chemical information of the nanoparticles in the after-wash solution. the presence of PET nanoplastics were confirmed by comparing the near edge X-ray absorption fine spectra (NEXAFS) of PET reference nanoparticle between the NEXAFS of our samples. We also developed a way to estimate how many nanoplastics per gram textiles were released during washing and abrasion. the ratio between microplastics and nanoplastics suggested estimated nanoplastic emission to the environment which paved way for environmental risk assessment for nanoplastics.

3.11.04
Atmospheric Deposition As a Pathway for Microplastic to Marine Environment
Y. Cho, Korea Institute of Ocean Science and Technology / Oil and POPs Research Group (OPRG); S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; M. Jang, G. Han, Korea Institute of Ocean Science and Technology (KIOST); W. Shim, Korea Institute of Ocean Science and Technology / Oil and POPs research group
The microplastics are ubiquitous in terrestrial and aquatic environments. Understanding the behavior of microplastic entering the marine environment from terrestrial source has been a major challenge. Microplastics can enter the marine environment through various source including river, wastewater treatment plants (WWTPs), and atmosphere. Several studies on rivers and WWTPs as an input source of microplastic have been conducted, however studies on the atmospheric deposition are limited. In this study, we investigated annual atmospheric deposition of microplastics in heavily urbanized semi-enclosed bay, Masan Bay, South Korea. The microplastic deposition ranged from 1.7 to 80 n/m^2/day with an average atmospheric deposition of 23 ± 18 n/m^2/day. The highest deposition of microplastic was observed during winter season (December, January, and February), having highest wind speeds predominantly from North and North-East. The major polymer type was polypropylene (PP) and polyethylene (PE). Fragment was dominant shape, and less than 300 μm size range accounted for 85% of the total non-fiber microplastics. The annual load of microplastic into the bay via atmosphere deposition was estimated as 7.4×10^{11} n/y. Our observations provide field-based evidence that atmospheric transportation can be a one of the important pathway for microplastic into the marine environment.

3.11.12
Prevalence of High-Density Microplastics in Continental Shelf and Deep Sea Waters of East Asia
S. Eo, Korea Institutue of Ocean Science and Technology (KIOST); S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; Y. Song, Korea Institute of Ocean Science and Technology; G. Han, Korea Institute of Ocean Science and Technology (KIOST); S. Seo, Korea Institute of Ocean Science and Technology; W. Shim, Korea Institute of Ocean Science and Technology / Oil and POPs research group
Microplastics are widely distributed throughout aquatic environments. Information about the vertical distribution and fate of microplastics in seawater remains limited. To elucidate the vertical distribution of microplastics, three to six vertical water column layers were sampled based on the thermocline depth, from which the vertical distribution and characteristics of microplastics larger than 20 μm were investigated in continental shelf and deep-sea waters around South Korea. In addition, microplastics incorporated into marine aggregates (aggregated fraction) were investigated to determine the contribution of aggregates to vertical transport of microplastics. The abundance of microplastics was in the range of 15–

9400 particles/m³. No consistent trend was observed in the overall vertical profiles. The size, shape and polymer compositions of microplastics at each station were generally comparable throughout the water column. Unexpectedly, high-density (HD; >1.02 g/cm³) polymers accounted for an average of 73% of total microplastics. As polymer density increased, the proportion of microplastics less than 100 µm in size increased. Due to the relatively high proportion of HD polymers in offshore waters, high-density solution should be used to extract microplastics, even from surface seawaters. The aggregated fraction accounted for 0–28.6% (average, 3.4%) of total microplastics. Marine aggregates are considered an important mechanism of transport for microplastics less dense than seawater to the deep water column, but they showed lower proportions than expected in continental shelf and deep-sea waters around South Korea.

3.11.13

Small, Everywhere and Difficult to Detect - Insights Into the Detection of Microplastic Particles in Environmental Samples Using Thermal Extraction Desorption-Gas Chromatography/Mass Spectrometry

M. Kittner, Bundesanstalt für Materialforschung und -prüfung (BAM); A. Mueller, P. Eisentraut, BAM - Federal Institute for Materials Research and Testing; K. Altmann, BAM- Federal Institute Materials Research and Testing; U. Braun, UBA - Federal Environment Agency

As global demand for polymer products increases, so does the potential amount of leakage into the environment, known as macroplastic (> 1,000 µm) and microplastic particles (MP, 1 - 1,000 µm). To date, the environmental and health consequences are unclear and unpredictable, so robust MP monitoring data are needed to identify the sources, pathways and fate of these particles. Therefore, we developed the thermoanalytical method TED-GC/MS (thermal extraction desorption - gas chromatography/mass spectrometry), which uses polymer-specific thermal decomposition products for the detection of MP in environmental samples. It allows fast identification and the determination of MP mass contents, which is crucial for assessing of their potential effects and thus for legislation and monitoring. **For a better understanding of the TED-GC/MS, this presentation will provide the first methodological insights into systematic assessments of TED-GC/MS performance for MP detection in different environmental model matrices. Results of MP determination from real samples will underline the presentation and aspects of practical application will be addressed.** To harmonise the method, it is essential to assess different analytical performance parameters (e.g. recovery, signal quality or limit of detection, LOD) to ensure comparability of data. For this purpose, standard addition of different aquatic (e.g. suspended particulate matter, limnic sediment, activated sludge) and terrestrial samples (e.g. agricultural soil) were performed with non-biodegradable standard polymers (polyethylene, PE; polyethylene terephthalate, PET; polypropylene, PP; polymethyl methacrylate, PMMA; polystyrene, PS; styrene-butadiene rubber, SBR) as well as biodegradable polymers (polybutylene adipate terephthalate PBAT; polylactide, PLA) and analysed by TED-GC/MS. These results were then compared with the measurements of the neat polymers without matrix.

3.11.14

Nanoplastics: A Distinct Class of Emerging Contaminant?

B. Nguyen, McGill University / Department of Chemical Engineering; J. Gigault, CNRS UMR 3156 / Department of Chemistry; H. El Hadri, CNRS; B. Grassl, Université de Pau et des Pays de lAdour / IPREM UMR CNRS 5254; L. Rowenczyk, N. Tufenkji, McGill University / Department of Chemical Engineering; M. Wiesner, S. Feng, Duke University / Department of Civil and Environmental Engineering

Environmental nanoplastics are often thought of as an extension of microplastics with a distinction based on an arbitrary size cut-off, typically 100 nm or 1000 nm. In our view, in terms of environmental implications and analytical challenges, a size cut-off distinction provides little guidance and a consensus on the precise definition of “nanoplastic” has yet to be reached. We instead advocate for a characteristic-based distinction between nanoplastics and microplastics. Based on existing literature and analytical methods, we established a set of characteristics, distinct from microplastics and other contaminants, that define environmental nanoplastics. Specifically, nanoplastics are distinguished from microplastics in the following ways: (1) The dominance of Brownian motion over sedimentation and buoyancy characterized by random movement of the particles in a suspension medium; (2) Departure from the geometric/ray approximation between light and matter; (3) A high proportion of molecules on the surface resulting in a higher relative importance of surface interactions compared to physical interactions; (4) The thickness of the particle diffuse layer may be comparable to the size of environmental macromolecules (leading to adsorption/heteroaggregation), while it is small compared to the size of associated microorganisms (preventing biofilm formation); (5) Sizes compatible with bio-uptake, translocation and transport across biological membranes and; (6) Short length scales that may speed the diffusive release of plastic additives and non-intentionally added substances in the original bulk plastic. In this presentation, we outline the consequences of these characteristics with respect to environmental fate, biological effects, and analytical strategies. These distinctions parallel those between engineered nanomaterials (ENMs) and their bulk counterparts. Consequently, nanoplastics research can benefit from past lessons learned studying the environmental fate, health, and safety of nanomaterials. Nevertheless, like microplastics and unlike ENMs, incidentally-produced environmental nanoplastics are diverse in composition and morphologies even amongst nanoplastics from the same source.

3.11.16

NanoRelease: Adaptation of ISO22293: 2020 Weathering Protocol to Primary Microplastics Reveals Their Fragmentation and the Formation of Nanoplastics

P. Pfohl, S. Jessl, BASF SE / Dept. Material Physics & Analytics; T. Hofmann, University of Vienna / Environmental Geosciences; W. Wohlleben, BASF SE / Dept. Material Physics & Analytics and Dept. Experimental Toxicology and Ecology

Microplastics are ubiquitous in the environment, leading to an increased interest in observing possible fragmentation mechanisms under certain conditions. Degradation depends on the polymer nature and therefore changes in mechanical properties, degree of crystallinity, molecular weight and functional groups on the surface differ for various kinds of polymers. Since biodegradation has a slow reaction mechanism, polymer degradation by UV irradiation might be equally relevant in the environment to initiate autocatalytic photo-oxidation, volume loss, cracking, and ultimately fragmentation to nanoplastics. In our research we adapt the ISO22293:2020 NanoRelease protocol, which was originally developed by US-EPA, Health Canda, BASF, and others to quantify the release of fragments from macroscopic plastic parts. Here we adapted it to assess the formation of nanoplastics via UV irradiation of primary microplastics. The protocol concerns a size-selective quantification of small fragments generated by weathering with the aid of immersed sonication. We determine the amount, shape and sizes of released polyamide-6 (PA-6) and thermoplastic polyurethane (TPU) fragments before and after aging as well as the chemical properties of the polymers themselves. Our test materials are relevant for primary microplastic powders commercialised for additive manufacturing. The used methods include analytical ultracentrifugation, UVVis spectroscopy, scanning electron microscopy, Fourier-transformed infrared spectroscopy and gel permeation chromatography. The polymer degradation depends on the chemical composition of particles and therefore varies for different types of polymers. With our research we were able to demonstrate, that the morphological and structural properties of aged TPU differ from aged PA. Furthermore TPU ethers/esters as well als aromatic/aliphatic TPUs show differences in their lag time and the sizes of released fragments. The presented results enable a correlation to the fragmentation of microplastics down to nanoplastics.

3.11.19

Development of an Environmentally Relevant Luminescent Model of Micro- and Nano-Plastic for Easy Imaging and Tracking in the Tissues of Living Organisms

N. Yakovenko, University of Toulouse, IMRCP laboratory; B. Amouroux, KU Leuven / Lab for Nanobiology; C. Coudret, C. Roux, M. Albignac, F. Collin, A. Ter Halle, University of Toulouse, CNRS / IMRCP laboratory

Plastic pollution is one of the most pressing environmental issues of our time. Currently, plastic has been found everywhere on our planet in all environmental compartments including aquatic, atmospheric, and terrestrial systems. Exposure to light, moisture, heat, biological activity, mechanical stress, and other factors causes the plastics, besides chemical modifications, to be fragmented into smaller pieces, ranging in size from a few centimetres to nanoscale. Thus, the size of the plastic debris matches those of sediments and some planktonic organisms, making them able to enter the trophic chain, which is a major concern about plastic toxicity. Due to their size and to the lack of suitable detection methods, pollution by nano-plastics has been poorly studied to date. The objective of this study is to create an environmentally relevant model of micro- and nano-plastic particles (MPs and NPs), that will be easily imaged and tracked in the tissues of living organisms. Thus, we developed MPs and NPs made of a High-Density Polyethylene (HDPE) matrix as it is one of the most common plastics found in the environment. For tracking and imaging purpose, we decided to use Lanthanide-based UpConverting Nanophosphors (Ln-UCNPs). These recently developed phosphors are very stable inorganic materials, able to convert low energy Near-Infrared photons into visible light. This allows their detection even in thick samples such as in a tissue or a small animal. To mimic the mechanical stress exerted on the plastic debris by the environmental conditions, we have selected a top-down approach to synthesize MPs and NPs. In this talk will be first presented the successful blending of UCNPs and HDPE, then the fragmentation of this tagged material by cryogenic grinder followed by zirconia bead milling. This process allows the straightforward production of a wide range of NIR-luminescent MPs and NPs. The complete characterization of the obtained MPs and NPs was achieved using the following methods: Granulometry, Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy, Differential scanning calorimetry (DSC), Thermogravimetric analysis (TGA), Small-angle X-ray

scattering (SAXS), Nanoparticle tracking analysis (NTA), Dynamic light scattering (DLS), Transmission electron microscopy and energy-dispersive X-ray spectroscopy (TEM/EDX), Fluorescence measurements, etc. Ecotoxicological assays are in progress.

3.11.21

Identifying Micro- and Nano Plastics Pollutants and Associated Additives in the Environment

J. Mayser, Markes International Ltd; L. Miles, H. Martin, C. Widdowson, Markes International

Detecting and analysing microplastics in the environment is challenging. The method must be able to detect and distinguish between a large variety of polymers. Additives, such as hardeners, flame retardants and preservatives, used during the manufacturing process and other compounds, that can adhere to the surfaces of microplastics from their environment, can affect their toxicity assessment. These additives could be used to link the microplastics found in the environment to their original use or potentially even back to the manufacturer. In this study, bottled beverages were filtered through 0.3 µm filter papers. TD–GC–MS was used to identify and measure which concentration of the plastic PET had broken down and leached into the drinks. By using direct desorption and backflushing technology, a large sample size could be easily isolated from the lab environment during analysis while ensuring a large range of volatile organic compounds (VOCs) could be trapped and analysed. The VOC-profile of the microplastics not only gave information about the polymer and its concentration within the sample, but further information about the toxicity could be extracted. This enabled the determination and tentative identification of additional compounds in the samples, such as dimethyl ether, acrolein and cyclopentane. These are used in the process of manufacturing plastics, so could assist with identifying the source of the plastics. They also tentatively identified bisphenol A. Research suggests that BPA may be an endocrine disruptor in humans, making it a compound of interest when assessing toxicity. As well as identifying chemicals used in the production of the plastics, other compounds were detected such as sucrose, acetol, deltyl extra and caffeine when analysing the microplastics from a cola sample. These are known components of cola. This data is useful in identifying the source of the plastic. These compounds give further information on the origin of the microplastic particle and its environment and origin. This further information about the origin of the polymer, as well as the origin of the microplastic particles can be gathered by investigating the full VOC-profile obtained by TD-GC-MS. This allows both polymer and particle source characterization simultaneously. Acknowledgements - This application note was developed in collaboration with Eurofins IPROMA and the data reproduced here were kindly provided by Antonio Rosado Sanz and Nuria Font Cardona.

3.11.30

Environmental Exposure Enhances the Internalization of Microplastic Particles Into Cells

A.F. Ramsperger, University of Bayreuth / Animal Ecology I & Biological Physics Group; V. Bangalore Narayana, University of Bayreuth, Universitaetsstrasse30, 95447 Bayreuth / Animal Ecology I; W. Gross, University of Bayreuth, Universitaetsstrasse30, 95447 Bayreuth / Biological Physics; J. Mohanraj, M. Thelakkat, University of Bayreuth, Universitaetsstrasse30, 95447 Bayreuth / Macromolecular Chemistry I; A. Greiner, H. Schmalz, University of Bayreuth, Universitaetsstrasse30, 95447 Bayreuth / Macromolecular Chemistry II; H. Kress, University of Bayreuth, Universitaetsstrasse30, 95447 Bayreuth / Biological Physics; C. Laforsch, University of Bayreuth / Animal Ecology I and BayCEER

Research efforts and public attention on microplastic pollution was exponentially increasing during the last years due to the observed variety of effects on an organismal and environmental level. Plastic introduced to the environment undergoes processes of degradation and fragmentises down to microplastic. Furthermore, the colonisation by microbes together with biomolecules, forms an ecocorona on microplastic particles surfaces that enhances the ingestion of microplastic by organisms. Once ingested there is evidence that microplastics harm organisms and translocate in tissues causing histological changes and inflammatory responses. The reason for cellular internalisation is unknown, since this has only been shown for specifically surface-functionalised particles. The translocation of microplastics coated with an ecocorona into tissues may occur via either paracellular or transcellular pathway. The paracellular transport occurs in between cells by gap junctions for instance, whereas the transcellular transport occurs via the internalisation into cells directly. In our study, we investigated the transcellular pathway via cellular internalisation of environmentally relevant microplastic particles. After exposing microplastic particles to fresh and salt water, we identified biomolecules forming an ecocorona on the surface of microplastic particles by using scanning electron microscopy, µ-Raman spectroscopy, and X-ray photoelectron spectroscopy. We show for the first time that the coating with an ecocorona significantly enhances the internalisation of microplastic particles into macrophages compared to pristine particles without an ecocorona. Our findings highlight the importance of using environmentally exposed microplastic particles instead of pristine particles in eco- and cytotoxicity testing. The experimental outcome may change significantly when environmentally relevant scenarios are used compared to sterile laboratory conditions. These findings will help to better understand the risks deriving from plastic pollution.

Micro(Nano)Plastics and Associated Chemicals (II): Effects, Risks and Mitigation

3.12.01

Chemicals in Plastic Consumer Products and Their Role in Plastic Toxicity

L. Sorensen, SINTEF Ocean / Environment and New Resources; A. Igartua, SINTEF Ocean; I.L. Lyngstad, Norwegian University of Science & Technology (NTNU) / Department of Biology; M. Wagner, Norwegian University of Science and Technology / Department of Biology; T. Gomes, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; A. Almeida, Norwegian Institute for Water Research (NIVA); I.B. Øverjordet, SINTEF Ocean; A. Booth, SINTEF Ocean / Environmental Technology

Toxicity assessment of most plastic materials and microplastics (MP) is typically conducted using pristine reference materials that do not accurately reflect the plastic materials and particles derived from consumer products that are normally found in the environment. Importantly, few studies have considered the role of plastic chemicals as the potential cause of toxic effects from MP exposure. Most plastic consumer products contain a suite of chemicals, including additives that provide the material with specific properties, including softeners, dyes, antioxidants, UV stabilizers, antimicrobials and flame retardants. Plastics also contain residual chemicals used in polymer and/or product production processes (e.g. monomers). In the current study, >50 plastic consumer products representing different polymer types and with a broad range of additive chemical profiles were selected, characterised and their baseline and algal toxicity investigated. All samples were commercially sourced and cut into small pieces prior to use. First, dichloromethane/ethyl acetate solvent extracts of the plastic products were produced and subjected to non-target screening using chromatography coupled to mass spectrometry. Compounds were tentatively identified based on matches (>90%) to mass spectral libraries and the total number of features determined. To allow investigation of a large sample set, data processing, including spectra deconvolution, library matching, logical filtering and searches against online PBT databases, has been automated to a large extent. A broad range of chemicals were identified in the different plastic products and the chemical profiles were found to vary significantly between the products, with some containing very little and others containing either a large number of different chemicals, specific chemicals at high concentrations, or a combination of the two. All test materials were extracted using methanol and transferred to DMSO prior to toxicity screening assessment by the marine Bacteria Luminescence Toxicity (BLT) screen and the marine microalgae (*Skeletonema pseudocostatum*) growth inhibition test. Results from both studies showed a broad range of toxicities (EC_{20} values) across the different plastic products, but that responses were generally similar in the two species. Preliminary statistical analysis indicates there could be a correlation between the number of chemicals present and the EC_{20} value determined.

3.12.04

Investigating the Interactive Effects of Microplastics and the Antimicrobial Triclosan on River Biofilms

J. Castano-Ortiz, Catalan Institute for Water Research (ICRA) / Water Quality Area; L.H. Santos, Catalan Institute for Water Research (ICRA); University of Girona / Water Quality; F. Romero, Catalan Institute for Water Research (ICRA); University of Girona; L. Cojoc, University of Girona; D. Barceló, S. Rodriguez-Mozaz, Catalan Institute for Water Research (ICRA) / Water Quality

Microplastics (MPLs) have been recognized as emerging threats to freshwater ecosystems, where they may co-occur with other emerging pollutants including Pharmaceuticals and Personal Care Products (PPCPs) like the antimicrobial triclosan (TCS). Although adsorption of certain PPCPs on MPLs has already been reported, their interactive effects on epilithic communities remain unknown. The interaction between MPLs and TCS could potentially modulate the bioaccumulation and associated impacts of TCS in non-target microbial assemblages like natural river biofilms. These play important ecological roles and represent widely used environmental models to depict disturbances associated with multiple stressors. In the present work, a mesocosm experiment was carried out to address the interactive effects of polyethylene MPLs (PE-MPLs) on the bioaccumulation and toxicity of TCS in river biofilms. These were exposed to PE-MPLs (2 mg/L) and TCS (20 µg/L) under single and combined scenarios, and compared against the control. Following short-term exposure (72h), a set of interdisciplinary tools were / will be used to explore structural and functional effects in the community (e.g. photosynthetic efficiency, enzymatic activity, metabolomics, metagenomics, scanning electron microscopy). Preliminary results indicated that PE-MPLs did not induce ecotoxicological impacts in the biofilm. Additionally, functional effects linked with the bioaccumulation of TCS (i.e.

increased photosynthetic efficiency) seemed to occur regardless of the presence of PE-MPLs. The observed functional alterations are expected to link with structural changes in the community, which will be further explored via high-throughput sequencing of the biofilm.

3.12.14

Polystyrene Nanoplastics and Arsenic Contamination: Does Life History Influence the Responses of the Polychaete Hediste diversicolor?

M. Silva, Department of Biology, University of Aveiro / Biologia; M. Oliveira, University of Aveiro & CESAM / Centre for Environmental and Marine Studies (CESAM) & Department of Biology; H. Almeida, University of Aveiro / Department of Biology; E. Figueira, University of Aveiro / Biology CESAM; A. Pires, University of Aveiro / CESAM & Department of Biology

Increasing discharges of contaminants to aquatic systems have promoted higher concentrations in the sediments, which act as sink and source. Benthic organisms can thus be affected via pore water and sediments. Metals and metalloids have been found at high concentrations in sediments. Arsenic is a frequently found metalloid that has been shown to affect benthic organisms, like polychaetes. Plastics, present in the water column, also tend to sink and have been shown to affect polychaetes. Polychaetes, such as *Hediste diversicolor*, can be the most abundant group in benthic communities and, due to their intimate contact with sediments, they are maximally exposed to harmful materials present there. Thus, this study aimed to evaluate the effects of arsenic on organisms pre-exposed to waterborne 100 nm polystyrene nanoplastics (PS NPs) and organisms never exposed to contaminants on burrowing activity and biochemical endpoints associated with oxidative status, neurotransmission, and energy metabolism in *H. diversicolor* Specimens of *H. diversicolor* were born under laboratory conditions. Half of the offspring were exposed for 14 d to 0.005 mg PS NPs/L. After exposure, pre- and non-exposed organisms were allowed to grow under controlled conditions. Pre-exposed and non-exposed organisms, 18 each per condition, were randomly distributed per 3 aquaria and exposed for 28 d to different concentrations of arsenic (0; 0.05; 0.25 mg/L). Results demonstrated that organisms exposed to arsenic took longer to burrow into the sediments, and that was associated with a decrease in cholinesterase activity, which can have severe impacts on the population and ecosystem level. However, alterations at the biochemical level were found mainly on previously exposed organisms, which highlights the consequences of mixtures of contaminants. Superoxide dismutase activity, lipid peroxidation, and protein carbonylation were higher in pre-exposed organisms. Glutathione *S*-transferases activity also increased in 0.25 mg/L of arsenic in both conditions. The relevant data obtained in this study demonstrated the severe consequences of arsenic on the behavioural and biochemical level, particularly to organisms that had been previously exposed to another contaminant. Considering that in natural environments contaminants that may interact and affect organisms, it becomes essential to study the effects of interactions of contaminants on marine biota, particularly benthic organisms.

3.12.17

Fate and Transport of Microplastics in Freshwater Systems: An Open Access Modelling Framework

M. Domercq, Stockholm University, ACES / Department of Environmental Science and Analytical Chemistry; A. Praetorius, University of Amsterdam/IBED Institute / Department of Environmental Science and Analytical Chemistry ACES; M. MacLeod, Stockholm University / Department of Environmental Science and Analytical Chemistry

As plastic pollution has become a major environmental concern over recent years, attention to the presence of microplastics (MPs), defined as plastic particles < 5 mm in size, in all compartments of the environment worldwide has grown. However, exposure data is currently far from sufficient to inform risk assessment. Furthermore, questions about their distribution between different environmental compartments, their speciation in natural systems (i.e. pristine, weathered, bound to environmental colloids and biofouled), the different exposure patterns displayed by different MPs and the identification of dominant transport pathways still remain open. Mathematical models designed to integrate fate and transport mechanisms of MPs are extremely useful tools that can provide predictions of current and future exposure scenarios, as well as improve process understanding. While a few approaches for predicting microplastic exposure concentrations have been presented in recent years, no general framework has been so far introduced that coves all relevant microplastic processes and that is adaptable to different regions and temporal scales. Here we present an open source flexible fate and transport modelling framework for microplastics in freshwater systems that integrates relevant descriptors of MP fate and transport processes and that can be parameterized for a variety of spatial and temporal scales. An initial parameterization of the model describing a generic river system shows how, though the use of this framework, we can investigate on which are the most relevant parameters affecting the MPs speciation and distribution in natural systems, and therefore provide bounding information for microplastic risk assessment. The framework is been designed as an open source flexible multimedia mass-balance fate and transport model with the aim to provide a platform for constant integration of newly acquired knowledge in the fast evolving research field of microplastics.

3.12.22

Degradation Pathway of Polypropylene Based Post-Consumer Plastic in Natural Riverbank Environment

A. Delorme, ICCF - CNRS / Photochemistry; V. Verney, CNRS -ICCF / Photochimie-CVP; H. Askanian, Université Clermont Auvergne, CNRS, SIGMA Clermont, ICCF; E. Roussel, O. Voldoire, CNRS-Geolab; F. Delor Jestin, Sigma-ICCF; G. BISSAGOU KOUMBA, UCA-ICCF; J. Peiry, CNRS-Geolab

In this work, plastic debris has been introduced into the environment to be exposed to various environmental conditions such as moisture, heat, sunlight or microbial action. Prolonged exposure to these environmental factors action causes polymers to degrade, fragment into smaller pieces and cleaved into small molecules. We investigate the degradation pathways of five identical plastic butter tubs made of polypropylene (PP), positioned in five different locations near a French wild river, in which one was buried in sediments (after having being artificially pre-weathered in lab conditions), two were placed by the riverbank, and two in the vegetation. At each location moisture and temperature were monitored and samples of each plastic tub were taken frequently for almost 4.5 years (1600 days) and sampling still continues. The degradation of plastics, in particular photodegradation, leads to structural changes of the polymer backbone, such as oxidation with formation of carbonyl functional groups, chain scission and cross-linking. These transformations at molecular level impact the overall mechanical properties of the plastic and favor embrittlement and fragmentation, leading to the generation of micro- and nano-plastics. In this contribution, we show that despite being located in different places which resulted in different UV-radiation exposure and temperature variations, the plastic butter tub followed similar trends, in that after an initial decrease in the viscosity of the polymer, we observe an increase in the overall viscosity after a longer exposition to environmental conditions (after 506 days) which suggests that a chain recombination mechanism is occurring within the polymer. This is a rather unusual observation since PP is known to, generally, undergo chain scission when it is aged, as indicated by a lowered viscosity. The conclusion is that such experiments in natural conditions may be very instructive to understand the fate of abandoned plastic wastes in the environment. However, a more robust methodology must be built-up with more samples to be able to assess longer times.

3.12.27

Microplastics in the Northeast Pacific Ocean: An Exploration Across Taxa and Spatial Scales

S.M. Brander, Oregon State University / Environmental and Molecular Toxicology; A. Bolm, Oregon State University / Department of Fisheries & Wildlife; K. Lasdin, Oregon State University / Department of Fisheries and Wildlife; J. Van Brocklin, Oregon State University / Fisheries and Wildlife; E.I. Pederson, Oregon State University / Department of Fisheries and Wildlife; J. Miller, Oregon State University / Department of Fisheries & Wildlife; N. Duplaix, T. Levi, Oregon State University / Department of Fisheries and Wildlife; S. Larson, Seattle Aquarium

Microplastics have been detected in every part of the world, and their presence across matrices (water, sediment, biota) is well-documented throughout global oceans. However, although studies on microplastic occurrence are common, large swaths of coastline and offshore regions remain unexplored when it comes to microplastic internalization in resident organisms. For this reason, risk assessment and regulatory decision-making are challenging, as information about exposure across food webs, particularly in North America, is lacking. Here we report data from three projects that include representative organisms from three different trophic levels, mostly sampled from remote areas, off the Pacific Northwest (PNW) coast of the United States (Northern California, Oregon, Washington, Alaska), in the Northeast Pacific Ocean. We are investigating, a) the occurrence of microplastics in multiple species of zooplankton (primarily crustaceans) captured offshore from the highly productive Northern California Current (NCC) sampled from N. California to central Oregon, b) microplastics ingestion in Black Rockfish (Sebastes melanops) sampled from sites within or in close proximity to marine protected areas offshore of Oregon, and c) measuring microplastics occurrence in the feces (called 'spraint') of wild sea otters (Enhydra lutris) from Alaska and planned for California. Our findings support those of others in that microplastics are widely distributed across biota types and that fibers and fragments are prevalent, with plastic contamination present in samples from the majority of sites. In short, the aim of this work is to provide a clearer picture of microplastic exposure to wild organisms inhabiting coastal and offshore areas along the PNW coast. The ultimate goal is to close knowledge gaps on the magnitude of plastic internalization across taxonomic groups, as well as the prevalence of microplastics in relatively pristine and in some cases protected areas in the Northeast Pacific Ocean. This work will assist in establishing microplastic exposure levels for use in the assessment of risk and may also play a role in understanding the need for regulation to protect coastal and marine habitat from plastic pollution.

3.12.32

Economic Valuation of Benefits From the Proposed REACH Restriction of Intentionally Added Microplastics

P. King, A. Hunt, University of Bath / Economics; S. Georgiou, Health and Safety Executive; C. Rainsford, Cosmetic, Toiletry and Perfumery Association; R. Dean, Environment Agency

This study elicited willingness to pay (WTP) values for three different measures to control the loss of intentionally added microplastics to the terrestrial and marine environments. Microplastics accumulate in the marine environment and are practically unrecoverable. However, there is considerable scientific uncertainty about both the environmental and health effects of marine microplastics and the benefits of reducing their release. This study used both a Choice Experiment (CE) and Contingent Valuation (CV) to evaluate where it was more beneficial to target restrictions at source or emissions. The CE investigated source-control in evaluating how respondents accept a trade-off between the price and performance of cosmetic products when reformulated to reduce the use of microplastics. Two CV tasks then estimated the public good benefits of research to resolve the uncertainty around human health and environmental effects of microplastics, and the public good benefits of enhanced removal of microplastics from effluents discharged from Wastewater Treatment Plants (WWTP). The difference in annual WTP; £53.24 for research and £73.71 for enhanced removal, suggests that respondents are willing to pay a substantial premium for the precautionary abatement of microplastics despite uncertainty about their negative effects. An indicative Cost-Benefit Analysis is then undertaken to evaluate the economic viability of different microplastic policy options.

3.12.33

The Impact of Plastic Additive Leachates on the Physiology of Marine Mussels: A Biomarker-Based Assessment

M. Capolupo, Alma Mater Studiorum - University of Bologna / Biological, Geological and Environmental Sciences; K. Gunaalan, University of Bologna / Department of Biological, Geological and Environmental Sciences; A. Booth, SINTEF Ocean / Environmental Technology; L. Sørensen, SINTEF Ocean / Climate and Environment; P. Valbonesi, Alma Mater Studiorum University of Bologna / Departmento of Biological, Geological and Environmental Science (BiGeA); E. Fabbri, University of Bologna / Department of Biological Geological and Environmental Sciences

Ocean contamination by plastic litter is currently cited amongst the most concrete threats to the safety of marine ecosystems. Many studies have recently investigated the adverse consequences of micro- and nanoplastic ingestion in marine organisms, with evidence of a range of physiological and sublethal effects reported. However, little remains known about the possible impacts of plastic additives leaching from plastic material into natural environment. This study evaluated the sublethal effects induced by plastic leachates on the Mediterranean mussel *Mytilus galloprovincialis* through a battery of cellular, biochemical and physiological biomarkers. Mussels were exposed for 7 days to leachates obtained from five synthetic polymers, i.e. car tire rubber (CTR), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC), which were previously found to contain an array of organic additives and metals in the ng to µg/L range. Exposure to all leachates induced a significant increase in neutral lipid content in mussels; a decreased lysosomal membrane stability was observed following exposure to the PS, PP, PVC and CTR leachates, while an increased lysosome to cytoplasm volume ratio was induced by the CTR, PP and PVC leachates. Lipid peroxidation products, i.e. malondialdehyde and lipofuscin, showed a significant accumulation in mussels exposed to PET, PS and PP leachates, and PP, PVC and CTR leachates, respectively. PET and PP leachates increased the activity of the phase-II metabolism enzyme glutathione S-transferase, while a decreased acetylcholinesterase activity was observed in mussels exposed to PVC leachates, denoting the potential for neurotoxic effects. Biomarker data were integrated using the Mussel Expert System, which assigns an A-E scaled health status index (HSI) to mussels based on the biomarkers modulation and toxicological profile. The system assigned a healthy condition (HSI = A) to mussels exposed to control conditions and PET leachates. A low stress condition (HSI = B) was assigned to PS leachates, while a moderate stress condition (HSI = C) was identified in mussels exposed to PP, PVC and CTR leachates. Overall, this study shows that plastic leachates can induce lysosomal disorders, lipid peroxidation and/or neurotoxicity in mussels, with PVC, PP and CTR leachates exhibiting the greatest adverse effects on their overall fitness.

3.12.34

Impacts of Microplastic Ingestion on Wildlife Gut Microbiomes

G. Fackelmann, Institute of Evolutionary Ecology and Conservation Genomics, Ulm University; J.F. Provencher, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health Division; M. Mallory, Acadia University / Canadian Wildlife Service; C.K. Pham, Y. Rodriguez, IMAR/OKEANOS, Universidade dos Açores / Departamento de Oceanografia e Pescas; S. Sommer, Institute of Evolutionary Ecology and Conservation Genomics, Ulm University

Since the beginning of plastic production, plastics have polluted the environment. Exposure to the elements causes them to become brittle and break into smaller fragments known as microplastics (< 5 mm). Microplastics have been found all over the globe and have been shown to impact biota in a variety of different ways. However, their potential influence on (gut) microbiomes has received little attention. As a defender of good health, the gut microbiome as a collection of microorganisms and their genomes is seen as a symbiont that not only provides hosts with essential metabolic functions, but also cross-talks with host immune systems. It has been shown to play a role in disease susceptibility, as dysbiotic microbiomes are often associated with disease. Environmental microplastics are coated with a biofilm of microorganisms and have been shown to cause inflammation, damage and blockage to the gastrointestinal tract (GIT). This, combined with the fact that wildlife is known to ingest microplastic debris, led us to postulate that the gut microbiomes in species that regularly ingest these particles could be dysbiotic, leading to potentially negative downstream health impacts. The aim of our study was to investigate if wildlife gut microbiomes are impacted by microplastics. Thus, we established a collaborative project that combines microbiome and microplastics expertise from scientists around the world using four different seabird species known to regularly ingest microplastics as our study species: Cory's shearwaters (*Calonectris borealis*), northern fulmars (*Fulmarus glacialis*), black guillemots (*Cepphus grylle*) and thick-billed murres (*Uria lomvia*). Our objectives were to test 1) if microplastic ingestion has similar impacts on gut microbiomes across different seabird species and 2) if microplastics impact the various parts of the GIT (proventriculus, gizzard, cloaca) in seabirds differently or in the same patterns. We hypothesized that the proventricular, gizzard and cloacal microbiomes of seabirds with low GIT microplastic counts will differ significantly in alpha and beta diversity compared to seabirds with higher microplastic counts, regardless of the species, and we predicted that, for the latter, microbiomes will show characteristic signs of dysbiosis such as lowered alpha diversity and more dispersed beta diversity. This study is intended as a baseline for future research looking into the impacts of microplastics on wildlife (gut) microbiomes.

Per-and Polyfluoroalkl Substances (PFAS): a Persistent Challenge for the 21st Century

3.13.01

Fluorine K-Edge X-Ray Absorption Near-Edge Structure (XANES) Spectroscopy As a Novel Tool for the Characterization of Per- and Polyfluoroalkyl Substances (PFAS) in Soils and Sludges

C. Vogel, P. Roesch, P. Wittwer, Bundesanstalt für Materialforschung und -prüfung (BAM); T. Huthwelker, Paul-Scherrer-Institut; F.G. Simon, Bundesanstalt für Materialforschung und -prüfung (BAM) / Contaminant Transfer and Environmental Technologies

Per- and polyfluoroalkyl substances (PFAS) have been used extensively in the past because of their inert chemical character and resistance to degradation by environmental influences. Since the beginning of their commercial use, PFAS have been widely exposed to the environment by application of PFAS in consumer products or as a foaming agent in firefighting foams, thus several cases of contaminated soil sites have been reported. Since the number of known PFAS already exceeds 4700, their characterization and direct analysis is challenging given the currently available techniques. Here, we introduce the novel fluorine (F) K-edge X-ray absorption near-edge structure (XANES) spectroscopy as a tool to analyze PFAS and inorganic fluorine compounds in contaminated soils and sewage sludges. While F K-edge bulk-XANES spectroscopy provides us information on the overall fluorine bonding in a sample micro X-ray fluorescence (XRF) in combination with F K-edge micro-XANES spectroscopy can also detect minor fluorine compounds and PFAS hotspots in investigated soils and sludges. Additionally, we used the combustion ion chromatography (CIC) to analyze the total amount of all PFAS as a sum parameter (extractable organic fluoride: EOF) in soils and sewage sludges. During combustion in the CIC, the PFAS in the sample gets destroyed at temperatures of approx. 1000 °C and converted into inorganic fluorides that subsequently gets quantified by ion chromatography. Thus, for the first time, we successfully combined F K-edge XANES spectroscopy and CIC as analytical tools to detect and quantify PFAS contaminants in soils and sewage sludges.

3.13.03

Per- and Polyfluoroalkyl Substance Dark Matter: Role of Total Organofluorine Analysis

H.L. Lord, Bureau Veritas Laboratories / Environmental R&D; L. Khachatryan, Bureau Veritas Laboratories / Environmental Research and Development; T. Obal, Bureau Veritas Laboratories

Significant effort continues in delineating the extent of Per- and Polyfluoroalkyl Substance (PFAS) contamination and recent advances in remedial approaches hold promise. However, the extent of remedial effort required for many of these sites remains unknown due to the presence of large PFAS precursor compounds

and other unknown or uncharacterized PFAS, which are not included as part of the suite of PFASs routinely monitored but over time may transform to the monitored compounds or otherwise contribute to the risk burden of a site. The challenge in accounting for this pool of undetermined PFAS (the Dark Matter) can be significant as its nature and quantity is generally unknown. Bureau Veritas Laboratories is actively developing technologies to determine the magnitude of total PFAS including the Dark Matter in environmental matrices such as soil, water and biota. In this presentation the available technologies for providing data on total PFAS will be reviewed, and a detailed explanation of Total Organofluorine by Combustion Ion Chromatography and its practical application will be discussed. We will describe achievements to date in developing practical methods for soil and water samples as well as lessons learned and anticipated next steps. The role that this new analysis option is likely to take in the investigation of PFAS sources and remediation will be described.

3.13.04

Suspect Screening of Per- and Polyfluoroalkyl Substances in German and Chinese River Water Affected by Point Sources

H. Joerss, Helmholtz-Zentrum Geesthacht / Environmental Chemistry; F. Menger, Swedish University of Agricultural Sciences; J. Tang, Yantai Institute of Coastal Zone Research, CAS; R. Ebinghaus, Helmholtz-Zentrum Geesthacht / Department for Environmental Chemistry; L. Ahrens, Swedish University of Agricultural Sciences SLU / Dept. of Aquatic Sciences and Assessment

Only a few dozens of the several thousand per- and polyfluoroalkyl substances (PFAS) on the global market are monitored using conventional compound-specific analytical methods. This raises the question if the commonly analyzed PFAS are representative or if they make up only a small fraction of anthropogenic PFAS releases. Moreover, early identification of emerging pollutants in source regions is of importance to take action before substances of concern become widely distributed. The aim of this study was to perform a suspect screening in German and Chinese river water affected by industrial point sources, comparing various types of point sources from regions with a different history of PFAS production. Triplicate river water samples were collected at 22 stations in autumn 2018, located up- and downstream of suspected point sources in China (n = 3) and in Germany (n = 4). After solid phase extraction using mixed-bed cartridges, analysis was performed by liquid chromatography high resolution mass spectrometry (LC-HRMS). The HRMS data was screened using a PFAS suspect list, created on the basis of the United States Environmental Protection Agency "PFAS Master List" with 5060 substances. In a first evaluation focusing on homologous series, 83 PFAS from 13 homologous series were tentatively identified. 13 of these PFAS had not been reported in the environment before, while some PFAS had been widely reported previously, e.g. hexafluoropropylene oxide dimer acid (HFPO-DA, $C_6HF_{11}O_3$). In addition, the study revealed source-specific PFAS "fingerprints". Emerging and novel PFAS were particularly detected downstream of the fluoropolymer and –monomer production sites located at the German Alz River and in the Chinese Xiaoqing River Basin. Differences between the sites were observed, for example, for the homologous series of perfluoroether carboxylic acids (PFECAs, $C_nHF_{2n-1}O_3$). Based on peak area comparison, the "long-chain" C_8-PFECA was the most prevalent compound in the Xiaoqing River Basin, whereas the C_4- and C_6-PFECAs were dominant in the Alz River. The large number of tentatively identified PFAS underlines that only based on conventional target analysis environmental and human health risks are considerably underestimated. The PFAS fingerprints improve the understanding of the contamination pattern at the investigated sites, which is relevant to estimate, prioritize and abate contributions of specific sources.

3.13.05

Identification of New PFAS in Aqueous Film-Forming Foam (AFFF)

V. Larson, University of North Dakota / Environmental Engineering; F. Xiao, S. Mallula, University of North Dakota / Civil Engineering; A. Postiglione, University of North Dakota

Poly- and perfluoroalkyl substance (PFAS) identification is limited to a few known PFAS. In 2016, the EPA released the PFAS identification method 537 and, in 2018, an updated method, Method 537.1. Both methods detect a few other PFAS for mostly long-chain PFAS (> 8 carbon chain)1. In 2019, method 533 covered short-chain PFAS to compliment Method 537.1 (long-chain PFAS). All methods are targeted PFAS identification. With more PFAS research and more information about PFAS revealed, the EPA methods were adjusted and expanded. However, recent studies are also discovering other PFAS in the environment with characteristics and behaviors differing from the known targeted PFAS2,3. Over 600 new PFAS were discovered, but their impacts are largely unknown2. Aqueous Film Forming Foam (AFFF), a fire-fighting chemical and PFAS source, may contain these other PFAS chemicals. Focusing on AFFF, several studies have elucidated the structures of the unknown PFAS2,3. Each study accomplishes PFAS identification differently. The previous research efforts used different extraction methods, chromatography, mass spectrometry (MS), ionization, and MS results interpretation. All methods used non-targeted analysis (NTA). Focusing on the mass spectrometry results interpretation, some commonalities appear in the studies: Suspect identification (Exact/Accurate Mass), Fragmentation Identification, and Kendrick Mass Defect (KMD). This study analyzed six legacy AFFF commercial products provided by the manufacturer and soil samples from past AFFF release sites using these three identification methods. This knowledge will help improve PFAS identification at AFFF contamination sites for treatment and adapt to the changing AFFF formulas.

3.13.09

Latest Developments in Analysis of Legacy & Emerging PFAS

T. Anumol, Agilent Technologies, Inc.; M. Chadha, Agilent Technologies

Per /polyfluoroalkyl substances (PFAS) continue to draw global attention to their impact on, and the important issues surrounding, one of our most critical natural resources that is water. There are over 4700 PFAS compounds in commerce many of which are thought to have adverse effects to human health . PFAS compounds are ubiquitous which makes the precise detection of them an analytical challenge. Yet, the accurate and reliable analytical chareterization of PFAS is critical to addressing other issues on remediation, treatment and regularoy policies and decision making . This presentation highlights the solutions devised on the analytical methodologies to tackle the environmental analysis of these 'Forever Chemicals'. The detection & quantification of sub-ng/L concentrations of up to 50 PFAS compounds will be discussed using LC/TQ along with the use of specific consumables and parts to eliminate PFAS background and contamination .The use of HRAM MS to detect emerging PFAS compounds that are in the environment but currently not under regulatory focus or don't have analytical standards to allow for quantification and the novel use of ion-mobility MS to separated isomeric branched and linear PFAS conformers will also be described.

3.13.10

Targeted LC-MS/MS Quantitation of Legacy and Emerging Per- and Polyfluoroalkyl Substances (PFAS) in Water Matrices

L. New, Agilent Technologies Singapore (Sales) Pte Ltd; M. Giardina, F. Mavandadi, T. Anumol, C. Gan, Agilent Technologies, Inc.

Per- and polyfluoroalkyl substances (PFAS) are chemicals widely used in consumer products and industry due to their unique and desirable chemical properties. Due to widespread usage and environmental persistence, legacy PFAS are ubiquitous in the environment and new fluorochemicals are being found in the environment frequently. Currently, there are disparate standard methods such as USEPA 533 and 537 for drinking water; and USEPA 8327, ASTM 7979 and ISO methods for non-potable waters. Furthermore, the rapidly evolving and diverse regulatory initiatives across various regions and countries have made it more challenging for laboratories to keep up with these changes while trying to develop comprehensive analytical methods for analysis in different types of matrices. Hence, a MRM databases was developed for the simultaneous analysis of more than 100 native and isotopically labelled PFAS compounds on 6470B LC/TQ. A solid phase extraction (SPE) method was optimized for the extraction of these compounds in water matrices such as drinking water and surface water. The database facilitates the quick creation of targeted screening or quantitative methods for a more comprehensive targeted PFAS analysis. A reliable and robust quantification approach of legacy and emerging PFAS from multiple compound classes in water samples is presented with low ng/L method quantification limits.

3.13.15

Per- and Polyfluoroalkyl Substances (PFAS) in Surface Sediments: Occurrence, Patterns, Non-Physical Drivers and Contribution of Unidentified Precursors in French Aquatic Environments

N. Macorps, UMR CNRS EPOC Universite Bordeaux / UMR 5805 EPOC; H. Budzinski, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC; F. Lestremau, A. Assoumani, INERIS; P. Labadie, UMR CNRS EPOC Universite Bordeaux / UMR 5805 EPOC

Sediment acts as a sink for per- and polyfluoroalkyl substances (PFAS), especially for perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs). Recently, non-targeted methods have shown the presence of numerous unknown PFAS in environmental samples. The contribution of these PFAS can be estimated by the Total Oxidisable Precursor assay (TOP), an oxidative method used to convert polyfluoroalkyl precursors (pre-PFAAs) into perfluoroalkyl acids (PFAAs). While sediment properties (grain size and organic carbon fraction, f_{oc}) were reported as controlling factors for some PFAS, the influence of non-physico-chemical drivers (e.g. population density) still needs to be investigated. Thus, this work focused on sediments from France and used optimized analytical methods and geographical information system (GIS) to fill these knowledge gaps. Extraction solvent was modified from methanol (MeOH) + ammonium hydroxide to MeOH + ammonium acetate for the analysis of 16 PFAAs, 6 alternative PFAAs and 14 pre-PFAAs covering anionic, neutral and zwitterionic species (n:2 fluorotelomer sulfonamidoalkyl betaines, n:2 FTABs). The optimized method exhibited suitable performances for its application on sediments (n=50) collected in 2018. The TOP assay was adapted to this specific method extraction. Perfluorododecanic acid,perfluorooctane sulfonic acid (PFOS) as well as perfluorooctanoic acid (PFOA) were often detected (76, 74 and 68 %

respectively). 6:2 FTAB, 8:2 FTAB and perfluoropentyl propanoic acid (FPePA) were also detected (38, 26 and 36 %, respectively). Median ∑PFAS was 1.3 ng.g^{-1} dry weight (dw) (range < LOD–23 ng.g^{-1} dw). 6:2 FTAB and 8:2 FTAB levels ranged from < 0.36 to 6.4 ng.g^{-1} dw and < 0.50 to 7.4 ng.g^{-1} dw, which is in the same order of magnitude than L-PFOS (< 0.08–5.1 ng.g^{-1} dw) advocating for the necessity to include these PFAS in targeted analysis. At nationwide scale, PFOS and ∑PFCAs accounted for 37 ± 25% and 31 ± 21% of ∑PFAS, respectively, while targeted pre-PFAAs accounted for 29 ± 27%, thereby supporting the importance of the latter in sediment and the potential contribution of untargeted pre-PFAAs monitored by the TOP assay (data under acquisition). Correlations between PFAS levels normalized by f_{oc}, unidentified pre-PFAAs and non-physico-chemical drivers with GIS are being investigated. Preliminary results showed that PFAS levels were not correlated with sample site position in the hydrographic system (proxy: Strahler number).

3.13.16

Monitoring of PFAS Distribution in Different ENvironmental Matrices in an Agricultural Area Impacted by a Fluorochemical Plant

C. Ferrario, IRSA-CNR (Brugherio) / Water Research Institute; F. Comazzi, University of Milano - Bicocca / Department of Earth and Environmental Sciences; S. Valsecchi, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute; M. Bonato, University of Padua / Department of Biology and Department of Industrial Engineering; F. Corrà, University of Padua, Department of Biology; R. Lava, ARPA, Veneto; M. Prenzato, C. Cecchinato, ARPAV; S. Polesello, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute

In 2013, large-scale contamination with PFAS was discovered in Veneto region, northern Italy, as a consequence of the emissions from a fluorochemical plant in the province of Vicenza. This PFAS discharge started from '70 and impacted both surface and groundwaters because the plant is sited in the recharge area of groundwater in Veneto plain. As a consequence, PFAS were de-tected and found in drinking water, that is one of the most important routes of the human expo-sure to these compounds, together with food consumption. In this contest, Veneto local authori-ty defined three areas with different health impacts (red, yellow and green) by matching the results of the biomonitoring study to those of chemical analysis. Even though elevated serum PFAAs concentrations were detected in the residents of the contaminated areas in Veneto, con-nected with contaminated drinking water consumption, comprehensive health risk assessment and research considering the food consumption are still lacking. For that reason, an extensive monitoring program was carried out to assess the distribution of PFAS in different environmen-tal matrices (irrigation water, soil, plants) in agriculture areas in the three zones, with a specific focus on more mobile but shorter chain PFAS. Selected sampling sites are all characterized by the presence of a water body used for irrigation, agricultural soil, a ubiquitous aquatic vegetal species (Phragmites australis) and vegetable crops (Zea mais, Lactuca sativa, Cichorium inty-bus, Allium cepa). From May 2018 to October 2020, irrigation waters were monthly collected (n = 225), while soil (n = 105) and plant samples (n = 80) were collected twice a year during harvest season. Obtained results showed that, in contrast to irrigation water pollution, soil con-tamination exactly matched with the area classification based on different levels of PFAS pres-sures. Unlike soil, there are no significant differences in plants from different areas in terms of sum of PFAS, but plants grown in red area were mainly polluted by more recent compounds (C< 8). Finally, low PFAS levels recorded in edible vegetables seem to suggest that risks for population connected to food consumption are limited.

3.13.17

Global Atmospheric Concentrations of Poly- and Perfluoroalkyl Substances (PFAS) and Volatile Methyl Siloxanes (VMS)

A. Saini, Environment and Climate Change Canada / Air Quality Research Division; S. Chinnadhurai, J.K. Schuster, Environment and Climate Change Canada; A. Eng, Environment and Climate Change Canada / Atmospheric Science & Technology Directorate; T. Harner, Environment and Climate Change Canada / Air Quality Processes Research Section

Poly- and perfluoroalkyl substances (PFAS) and volatile methyl siloxanes (VMS) are high production volume chemicals and are persistent in the environment. Among PFAS, perfluorooctane sulfonate (PFOS), its salts and precursor compound (perfluorooctane sulfonyl fluoride), were added to the Stockholm Convention (SC) on persistent organic pollutants (POPs) in 2009. Very recently in 2019, perfluorooctanoic acid (PFOA), its salts and related compounds have also been listed in SC, given their potential toxic effects of human health and environment. As such, their continuous monitoring is required to assess the effectiveness of these regulations. PFAS and VMS are globally being monitored at 20 sites under Global Atmospheric Passive Sampling (GAPS) Network since the past decade. In 2017, GAPS expanded the monitoring of these chemicals to more than 40 sites across the globe including polar, background, agricultural and urban sites. Sorbent impregnated polyurethane foam disks (SIPs) were deployed at ~45 GAPS sites in double-dome sampling chambers. Sampling was conducted from April to June 2017 and samples were stored below –10 °C until analysis. Samples are being analysed for 18 perfluoroalkyl acids, 7 neutral-PFAS (nPFAS) and 7 linear and cyclic VMS. Fluorotelomer alcohols (FTOHs) dominated the air profile among nPFAS with the levels reaching a high of 230 pg/m^3. This trend is consistent with their 7 years trend observed by Rauert et al. (2018). Urban sites had up to 10 times higher average concentrations of nPFAS than other locations. Statistical analysis of perfluoroalkyl acids is currently under progress and preliminary results have shown that perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) were predominantly detected at >80% of sites. Among perfluorosulfonic acids (PFSAs), and despite restricted production and use for over a decade, PFOS was detected at ~90% of the locations including background and polar regions. VMS, in general, were at least an order of magnitude higher than PFAS. Among VMS, detections and concentrations of cyclic-VMS (cVMS) were higher than linear-VMS (lVMS), constituting >90 % of total VMS concentrations in air. In terms of spatial distribution, urban sites (345 ng/m^3) were up to 8 times higher than at polar (42 ng/m^3), rural (103 ng/m^3) and background sites (50 ng/m^3). Upcoming analysis of the results of this study will provide the first information on global levels of PFAS and VMS from a single sampling network.

3.13.18

The Paper Industry - a Largely Overlooked Environmental Source of Perfluoroalkyl Acids (PFAA)?

H.A. Langberg, Norwegian University of Science & Technology (NTNU) / Biology; G.D. Breedveld, Norwegian Geotechnical Institute (NGI) / Geotechnics and Environment; B. Jenssen, Norwegian University of Science and Technology / Biology; G. Slinde, H. Arp, Norwegian Geotechnical Institute (NGI) / Geotechnics and Environment; M. Jartun, NIVA Norwegian Institute for Water Research; T. Rundberget, Norwegian Institute for Water Research (NIVA); S.E. Hale, Norwegian Geotechnical Institute (NGI) / Geotechnics and Environment

Per- and polyfluoroalkyl substances (PFAS) have been used for paper products such as food contact materials, packaging, and disposable paper products. Precursor compounds to perfluorinated alkyl acids (PFAA) such as *N*-ethyl perfluorooctanesulfonamido ethanol (EtFOSE) and EtFOSE based phosphate diester (SAmPAP diester), have been used for paper products since the 1970s. Human exposure to such PFAS from paper products that are in contact with food has attracted some recent attention. However, emissions of PFAS from the paper industry to the environment during production and product disposal have received little attention. This work addresses this gap. Environmental fate and transport of the specific PFAS mixture(s) used, environmental PFAS profiles associated with this type of industry, as well as emission volumes were explored. Lake Tyrifjorden in Norway was used as the case study site. The lake is home to a shutdown factory which produced paper products from 1964 to 2013. Emissions of large hydrophobic precursors to PFAA resulted in high concentrations in sediments (e.g. up to 1,872 µg kg^{-1} of SAmPAP diester) and in biota (e.g. mean 149 µg kg^{-1} perfluorooctanesulfonate (PFOS) in perch (*Perca fluviatilis*) livers) in lake Tyrifjorden. In contrast, water concentrations were low (max 0.18 ng L^{-1} PFOS). The high concentrations in biota were attributed to uptake and accumulation of PFAS from the sediments into benthic organisms, biotransformation and trophic transfer. Thus, the use of hydrophobic PFAA precursors by the paper industry has a bearing on the overall fate and transport of PFAS in the aquatic environment. Different PFAS profiles were observed in fish sampled at different sources. As an example, compared to the use of aqueous film forming firefighting foams, the production of paper products was associated with relatively high percentages of carboxylic acids (PFCA). Thus, distinct differences were observed depending on PFAS source, and these might be used for source tracking in future studies. Based on an extrapolation of concentrations in a sediment core, approximately 43 tons of PFAS were estimated to reside in lake sediments. Modelling based on the core indicated that 44-205 tons of PFAS have been emitted from the factory. The work shows that production of paper products might be a major, however largely overlooked, source of PFAS to the environment. This paper source is in addition to release of PFAS from food contact materials during the use phase.

3.13.24

Perfluorooctane Sulfonate (PFOS) Concentrations in Birds Around the World

A. Bonisoli-Alquati, California State Polytechnic University, Pomona / Department of Biological Sciences; R. Flamenco, University of Connecticut / Natural Resources & the Environment

Perfluorooctane sulfonate(PFOS) is a persistent, bioaccumulative and toxic compound that was largely phased out of production in 2002, and added to Annex B of the Stockholm Convention in 2009. PFOS is also the end product of the degradation of several other perfluoroalkyl substances (PFASs), which is upending reduction in its environmental concentrations after the phase out. Several studies have reported PFOS biomagnification in terrestrial and marine food webs. Yet no comprehensive analysis exists of PFOS concentrations in any taxonomic group through time and space. We conducted the first systematic

mapping and review of the prevalence of PFOS in bird tissues throughout the world. We collected more than 500 estimates of PFOS concentrations in blood, liver, and eggs from more than 100 bird species across all continents. PFOS concentrations in liver and eggs have increased since the 1960s, while blood concentrations have decreased since the phase out. As a result, recent PFOS concentrations were over three times higher in liver and eggs compared to blood. The overall concentrations and temporal trends also depended on ecological traits. Aquatic birds had higher concentrations than terrestrial ones. Piscivores and opportunists had the highest concentrations among all feeding guilds, while herbivores had the lowest. Larger clutches and higher longevity also positively predicted PFOS concentrations across species. Our results support extensive ecotoxicological risk from PFOS in wild populations of birds, inadequately addressed by the phase out of PFOS production. Interspecific variation is largely consistent with PFOS bioaccumulation and its biomagnification through food webs. The scarcity of estimates regarding PFOS toxicity in wild populations makes inference about toxicological risk from PFOS exposure difficult. The widespread bioaccumulative potential documented here call for urgent investigations into the toxic effects of PFOS – and other PFASs – to birds and other wildlife.

3.13.25

Investigating the Transfer of Polyfluorinated Phosphate Diesters (diPAPs) Into Maize Plants

R. Lämmer, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; J. Kowalczyk, H. Just, German Federal Institute for Risk Assessment BfR / Safety in the Food Chain; J. Breuer, Agricultural Technology Centre Augustenberg; B. Göckener, K. Severin, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; M. Gaßmann, University of Kassel / Hydrology and Substance Balance; E. Weidemann, University of Kassel / Department Water Quality Management - Modelling and Simulation; T. Stahl, Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe (CVUA-MEL); M. Bücking, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology

After the EU wide restriction for the production and use of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), alternative per- and polyfluoroalkyl substances (PFAS) with similar properties are used more frequently. The environmental fate and pathways of these substitute PFAS are not yet fully understood. In this specific study, the environmental behaviour of the substitute substances 6:2 polyfluoroalkyl phosphate diester (diPAP) and 8:2 diPAP was investigated in the pathway from the soil to maize plants. For this purpose, 6:2 diPAP and 8:2 diPAP, respectively, were mixed into PFAS free soils. Maize plants (*Zea mays*) were planted on all soils and their compartments (root, stem, leaf, husk, cob and grain) were individually analyzed for PFAS concentration after harvest. After the vegetation period, the primarily applied diPAPs make up the majority of PFAS in the soil. Perfluorinated carboxylic acids (PFCAs) are found in the soil and all plant compartments, although they were not initially applied. The detection of PFCAs with chain lengths ≤ C6 (6:2 diPAP application) or chain lengths ≤ C8 (8:2 diPAP application) in the soil and in the plant compartments indicates the degradation of diPAPs into the persistent PFCAs in the environment. 6:2 diPAP and 8:2 diPAP themselves mainly remain in the soil or in the root system, whereas the plant takes up notable quantities of PFCAs. Especially leaves and husk accumulate short-chain PFCAs such as perfluorobutanoic acid (PFBA) and perfluoropentanoic acid (PFPeA). This suggests a transport of small PFAS substances via the transpiration stream within the maize plant and an accumulation caused by evaporation of water from the leaves and shows the potential of diPAPs to contribute to the total PFCA contamination. These results provide important insights into the transfer of PFAS from contaminated soil into plants. As maize plants in particular are used as animal feed, the uptake of PFAS from field soil displays an entry path into the food chain and thus may serve as a pathway for human exposure to PFAS.

3.13.26

Ranking Controlling Factors of Per- and Polyfluoroalkyl Substances (PFAS) Bioaccumulation in Fish From an Urban River: Case Study on the Seine River (Near Paris, France)

N. Macorps, UMR CNRS EPOC Universite Bordeaux / UMR 5805 EPOC; H. Budzinski, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC; S. Guerin, V. Rocher, SIAAP; P. Labadie, UMR CNRS EPOC Universite Bordeaux / UMR 5805 EPOC

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in aquatic environments. A recent shift toward emerging PFAS is calling for new data on their occurrence and fate. Understanding their bioaccumulation and its drivers is fundamental for risk assessment purposes. While factors like fish length (i.e. proxy of age) appear to control the bioaccumulation of hydrophobic contaminants such as polychlorinated biphenyls, its influence on PFAS accumulation is not yet fully understood. Similarly, few studies have addressed the influence of trophic ecology on PFAS transfer to biota. Thus, this work aimed at filling these knowledge gaps by performing a case study on the Seine River, along a river stretch heavily impacted by human activities. In 2019, four sampling sites were selected upstream (n=1) and downstream (n=3) of the Paris conurbation (France), based on the hypothesis of a longitudinal contamination gradient. Composite surface sediment samples (n=1 per site) were collected to provide indication on the biotope contamination. European Chub (*Squalius Cephalus,* n=10-12 per site) muscles, liver and composite sediments were analyzed for 36 PFAS. Carbon and nitrogen isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) were determined in muscles as a proxy for trophic ecology. 8:2 fluorotelomer sulfonamidoalkyl betaine (8:2 FTAB) and perfluoropentyl propanoic acid were detected for the first time in Seine River sediments. C_9-C_{14} perfluoroalkyl carboxylic acids (PFCAs), perfluorooctane and perfluorodecane sulfonic acid (PFOS and PFDS), perfluorooctane sulfonamide and 10:2 fluorotelomer sulfonate were detected in all fish samples and 8:2 FTAB in muscles from the furthest downstream station. Median ∑PFAS on a wet weight (ww) basis was 7.4 $ng.g^{-1}$ ww in muscle and 54 $ng.g^{-1}$ ww in liver, which is less than published results on the downstream part of the nearby Orge River (upstream of Paris). Fish muscle and liver samples from the upstream station were significantly less contaminated than downstream stations, indicating an influence of the Paris conurbation. Sediment profiles were dominated by emerging PFAS (50 ± 19%) while they accounted for 4 ± 2% in muscles and 3 ± 0.5% in liver, suggesting a lower bioaccessibility or biotransformation of emerging PFAS. Multivariate approaches are currently being used to rank controlling factors (sex, biometry, trophic ecology) of the accumulation of PFAS in fish. Preliminary results do not show a correlation between fish size/weight and PFAS levels.

3.13.28

Transcriptional and Microbiota Changes in the Manila Clam (Ruditapes philippinarum) Following Exposures to the New PFAS C6O4

I. Bernardini, University of Padua; M. Milan, University of Padua / Department of Comparative Biomedicine and Food Science; V. Matozzo, University of Padova / Department of Biology; S. Valsecchi, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute; L. Peruzza, G. Dalla Rovere, University of Padua / Department of Comparative Biomedicine and Food Science; S. Polesello, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute; S. Iori, University of Padova / Department of Comparative Biomedicine and Food Science; M. Marin, J. Fabrello, L. Masiero, University of Padova / Department of Biology; M. Ciscato, University of Padua / Department of Biology; M. Bonato, University of Padua / Department of Biology and Department of Industrial Engineering; G. Santovito, University of Padua / Department of Biology; L. Boffo, Associazione "Vongola Verace di Chioggia"; L. Bargelloni, T. Patarnello, University of Padua / Department of Comparative Biomedicine and Food Science

The great worldwide use of fluorinated substances (PFAS), has increased the concern because the toxicity for the environment and human health, requiring hence consequent limitations of its applications. C6O4, (perfluoro ([5-methoxy-1,3-dioxolan-4-yl]oxy) acetic acid), a short-chain fluorinated compound, has been recently introduced as one of the alternative to traditional PFOA, despite scientific evidences of its effects on the environment are still unclear. Evidences on the presence of this compound in aquatic ecosystems, such as the study performed by The Regional Agency for the Protection of the Environment of Veneto (Italy) that detected high levels of C6O4 in ground-water and in the Po river, underlined a growing alarm for the potential effects of this chemical into the natural environment. Overall, the present study proposes for the first time a multi-disciplinary approach for the evaluation of the effects of C6O4 on the Manila clam (*Ruditapes philippinarum*), an edible ecologically and commercially important species. Bioaccumulation, transcriptional response and microbial communities were studied in clams exposed to environmental realistic concentrations of C6O4 (0.1 µg/L and 1 µg/L) for 7 and 21 days. Furthermore, in order to better understand if C6O4 is a valid and less hazardous alternative to a long-chained PFAS, microbial and transcriptomic alterations were investigated in clams exposed to 1 µg/L of PFOA. Results indicated that C6O4 may cause significant perturbations to the digestive gland microbiota likely determining the impairment of host physiological homeostasis. Moreover, clams exposure to both C6O4 and PFOA induced similar alterations of genes involved in several molecular pathways, including immune response, apoptosis regulation, nervous system development, lipid and cell membrane metabolism. In addition, clams exposed to C6O4 showed dose-dependent responses as well as possible narcotic or neurotoxic effects. Overall, the present study suggests that the risk for environmental contamination might not be reduced by replacing PFOA with C6O4. These results prompt the urgent need to re-evaluate the use of C6O4 without any restriction given the effects of this chemical on edible marine species that imply not only possible environmental hazard but also potential risk for human health.

3.13.31

Thermal Stability and Decomposition of Perfluoroalkyl Substances on Spent Granular Activated Carbon Used for Drinking-Water Treatment

P. Challa Sasi, A. Alinezhad, F. Xiao, University of North Dakota / Civil Engineering

The aims of this study were twofold: (1) to improve our understanding of the thermal stability of per- and polyfluoroalkyl substances; and (2) to investigate

their decomposition mechanisms on spent granular activated carbon (GAC) during thermal reactivation. We studied seven perfluoroalkyl carboxylic acids (PFCAs), three perfluoroalkyl sulfonic acids (PFSAs), and one perfluoroalkyl ether carboxylic acid (PFECA) in different atmospheres (N_2, O_2, CO_2, and air). We found that the destabilization of studied compounds during thermal treatment followed first-order kinetics. The temperature needed for thermally destabilizing PFCAs increased with the number of perfluorinated carbons (n_{CF2}). Decomposition of PFCAs such as perfluorooctanoic acid (PFOA) on GAC initiated at temperatures as low as 200 °C. The PFECA was even more readily decomposed than PFCA with the same n_{CF2}. PFSAs such as perfluorooctanesulfonic acid (PFOS), on the other hand, required a much higher temperature (≥450 °C) to decompose. Volatile organofluorine species were the main thermal decomposition product of PFOA and PFOS at low to moderate temperatures (≤600 °C). Efficient mineralization to fluoride ions (>80%) of PFOA and PFOS on GAC occurred at 700 °C or higher, accompanied by near complete PFOA and PFOS decomposition (>99.9%). Thermal decomposition pathways of PFOA were proposed.

3.13.32

Thermal Decomposition of Soilborne Per- and Polyfluoroalkyl Substances

A. Alinezhad, University of North Dakota / Department of Civil Engineering; P. Challa Sasi, F. Xiao, University of North Dakota / Civil Engineering

In this study, we demonstrate that the thermal decomposition of soilborne perfluoroalkyl carboxylic acids (PFCAs), including perfluorooctanoic acid (PFOA), occurs at temperatures as low as 150 °C. Three temperature zones were discovered. The thermal decomposition began with the homolysis of a C–C bond next to the carboxyl group of PFCAs, which formed unstable perfluoroalkyl radicals. Dual decomposition pathways seem to exist.

Plastics in Terrestrial Ecosystems: Key Considerations in the Assessment of Fate and Exposure

3.14.01

Reliable Extraction and Identification of Biodegradable Polymers Fragments From Compost and Soil: Method Validation

S. Jessl, P. Pfohl, C. Roth, L. Meyer, U. Heinemeyer, BASF SE / Dept. Material Physics & Analytics; E. Biedermann, G. Battagliarin, BASF SE; W. Wohlleben, BASF SE / Dept. Material Physics & Analytics and Dept. Experimental Toxicology and Ecology

With the global amount of plastics manufactured reaching 359 million tons in 2018 and the concern for plastic pollution increasing, the worldwide public debate around microplastics has significantly increased in the last years leading to regulatory action. For example, in January 2019 the European Chemicals Agency published a restriction proposal that will introduce bans, reporting and labelling requirements for intentially added primary microplastics to avoid their release into the environment after application. A derogation is proposed in the restriction if the polymer is biodegradable, thus putting a lot of focus on the development of biodegradable alternatives. Macro- and microplastic particles occur in various environmental matrices, such as soil, sediment, compost, or aqueous media, and while the first steps are taken towards their identification, quantification and size distribution, literature still reports a wide variation in results which can be explained by the lack of standardized methods for sample preparation as well as the subsequent analysis, highlighting the need for standardization in this reasearch field. This work focusses on developing and validating non-destructive and efficient extraction polymers for biodegradable polymer in environmental matrices, i.e. soil and compost. It first investigates the impact of acidic (Fenton) and basic oxidation (Basic Piranha) protocols on protocols on the physical and chemical properties of the biodegradable polymers and low density polyethylene (LDPE) as a durable reference material during the extraction process, as this is a crucial step towards further understanding of the status quo of microplastic contamination and for the evaluation of biodegradable polymers as improved alternatives. We demonstrate that it is crucial to consider the influence of sample preparation on the analytical results and have appropriate controls in the experiments. We show that the Fenton protocol with mild oxidation conditions can be used for the tested biodegradable polymers, whereas basic piranha induces negative effects on the polymers. These findings of the fitting oxidation protocol are then incorporated into the complete extraction process and adapted to soil and compsot as two very important environmental matrices. We demonstrate that varying the order of the steps in the extraction process depending on the type of matrix enables us to get a 100% mass recovery of the polymer particles.

3.14.03

Nanoplastics in a Contaminated Soil: Innovative Method and First Understandings on Their Behaviour

A. Wahl, CNRS UMR 6118 / Laboratoire Geosciences Rennes; M. Davranche, Géosciences Rennes / University of Rennes 1; J. Gigault, TAKUVIK CNRS, Université Laval

Under environmental conditions, plastic wastes are degraded into various debris, from the macro to the nano-scale, which inexorably end up and accumulate in every environmental compartment. Despite keen interest from the scientific community, their study in soils was nevertheless late, or even non-existent, concerning nanoplastics. Indeed, the soil is a complex matrix, rich in organic matter and other natural colloids. So far, no formal proof of the presence of nanoplastics has been shown. In this context, and within the framework of the CINAPE* project funded by the ADEME, we are interested in the effective presence of nanoplastics in the soil. Our study was based on a relevant soil that received amendments from household waste, which were enriched in plastics. Thanks to an extraction protocol and the coupling of a size fractionation method (Asymmetric Flow-Field Flow Fractionation) with a molecular analysis method (Pyrolysis Gas Chromatography and Mass Spectroscopy), we demonstrated the presence of three types of plastics in the surface horizon (polyethylene, polystyrene, polyvinyl chloride), with sizes between 30 and 150 nm. We applied this method to the entire soil profile, with additional Dynamic Light Scattering measurements, and compared the results with the microplastics distributions.These results constitute the first evidence of the existence of nanoplastics in soil and suggest that degradation processes are occurring there. Based on those results, we can thus provide an innovative methodology for detecting nanoplastics in a complex organomineral matrix such as soil. Finally, we also provide the first understandings of the fate and behaviour of nanoplastics in soils. **CINAPE-ADEME: Characterisation of the nanoplastics presence and behaviour in soil.*

3.14.04

Improving Bioturbation Models for Micro- and Nanoplastic Transport in Soils

W. Heinze, Swedish University of Agricultural Science / Department of Soil and Environment; D.M. Mitrano, ETH Zurich / Environmental Systems Science; E. Lahive, UK Centre for Ecology and Hydrology; J. Koestel, Agroscope – Standort Reckenholz / Soil Quality and Soil Use; K.A. Leicht, Swedish University of Agricultural Sciences / Department of Soil and Environment; G. Cornelis, SLU Fakturamottagning / Soil and environment

Most plastic emissions originate on land, with increasing evidence of negative impacts of micro- and nanoplastics on the soil ecosystem and crop production. However, terrestrial ecosystems remain largely neglected in research on plastic pollution. Especially agricultural soils are suspected to be exposed to larger plastic inputs and a potential source for microplastic pollution of aquatic environments. The processes that determine such fate are poorly understood for microplastics, in particular transport processes that affect the distribution of small plastics in the soil profile and beyond. Current modelling largely focuses on advective transport, often with the result that under non-saturated soil conditions transport is considered limited. This neglects other processes generally known for causing substantial redistribution of soil particles, mostly bioturbation. In order to characterise micro- and nanoplastic redistribution in soil induced by bioturbation, we conducted a series of process-studies in microcosms with the deep-burrowing earthworm *Lumbricus terrestris*. For this purpose, we used metal-doped fibrous polyethylene terephthalate microplastics and spherical polystyrene nanoplastics. *L. terrestris* caused significant transfer of fibrous micro- and spherical nanoplastics to the deeper soil profile, continuously increasing over time. A targeted analysis revealed that micro- and nanoplastics were primarily transported through the burrows. X-ray computed tomography images confirmed that a semi-permanent burrow system was established after one week, indicating that mechanical mixing was limited. With casts covering the burrow walls, and residual plastic found in earthworm guts, ingestion and excretion was considered the major transport mechanism for spherical nanoplastics. Although still relevant for fibrous microplastics, we expected a higher relative contribution of mechanical mixing to their overall transport. Thus, to avoid a systematic underestimation of micro- and nanoplastic mobility in soil, bioturbation needs to be explicitly incorporated into fate modelling. While a simple bio-diffusion model may be suitable for plastics whose transport is dominated by mechanical mixing and adhesion to the surface, it systematically underestimates transfers to the lower soil profile when particles are ingested by earthworms. The relevance of plastic size and shape for biologically mediated transport needs to be expanded to develop suitable transport models.

3.14.05

The Impact of Polyester Microfibres and Keratin Fibres on the Germination of Four Agricultural Crop Species

E.G. Harrison, Staffordshire University / Biological Sciences; K. Reiling, Staffordshire University / Department of Biological Sciences; R. Halfpenny, Staffordshire University / Department of Biological Science; C. Gwinnett, Staffordshire University / Criminal Justice and Forensic Science

Microplastics (MPs) (< 5 mm in size) are ubiquitous in all environments and are currently an emerging pollutant of interest in environmental research. Although MPs have been extensively studied in the hydrosphere, there is a dearth of studies which have investigated the terrestrial system. Of specific concern are the agricultural ecosystem where there can be a high load of MPs generated from

agricultural practices, such as the application of sewage sludge or mulching films, and as such it is important to understand the impacts microplastics may have on agricultural crop species. Currently there is little known about what impact plastic fibres, a common form of MP pollution, may present to the soil-plant system, by it has been proposed that they may cause a physical change to the soil matrix. This study investigated the hypothesis that plastics are acting as a physical soil contaminant by using both polyester and natural fibres (horsetail hair) of a similar morphology to the polyester fibre. This allowed an assessment of whether physical changes to the soil structure a potential mechanism for any germination differences may be shown in this study. The effects shown in this study have potential consequences for the agro-ecosystem and could ultimately lead to a reduced crop yield.

POPs and Emerging Pollutants: Developments in Sampling, Targeted and Non-Targeted Analysis, Modeling and Reporting to Support Policy and the Protection of the Environment and Human Health

3.15.01

Spatial and Temporal Trends of Perfluoroalkyl Substances in Global Ocean and Coastal Waters

D.C. Muir, Environment and Climate Change Canada / Aquatic Contaminants Research Division; L. Miaz, Stockholm University

The global distribution of poly- and perfluoroalkyl substances (PFAS) in open ocean waters and coastal seas has been documented in a series of studies over the past 15 years. The high water solubility, low partitioning to organic matter, and extraordinary persistence of PFOS, PFOA and other perfluoroalkylsulfonates (PFSAs) and perfluorocarboxylates (PFCAs) results in their very slow removal in oceans. However there are very few temporal trend studies for PFCAs and PFSAs in water. The goal of this study was to address three major questions: (1) What are prevailing concentrations of major PFAS in coastal waters, estuaries/river mouths, their neighboring seas and oceans? (2) What are the temporal trends in concentrations of major PFAS in these water, especially of PFOA and PFOS, given national and international chemical management actions? (3) What are the riverine discharges of major PFAS to coastal waters and how to they compare globally? All the available peer reviewed literature to June 2020, which included data on PFAS in coastal marine waters and open ocean water, as well as results for the North American Great Lakes, were reviewed and organized into a Postgis database. PFSAs (C4, C6-C8, C10 PFSAs + FOSA) and 9 PFCAs (C4-C12 PFCAs) constituted the majority of analytes (24,470 reports and with 14,456 > MDL). Highest median concentrations of ΣC7-C12 PFCAs were reported in the Bohai and Yellow Seas for 2010-14 and 2015-19 (Figure 1). The elevated levels were due to PFOA, PFBA, and PFPeA. The North Sea and Baltic Sea also had high median concentrations of PFCAs and PFSAs (Figure 1) than most other coastal regions This assessment of information available for PFAS in coastal and open oceans has demonstrated that there is sufficient data to qualitatively examine temporal and spatial trends in surface waters on a large geographic scale. A major knowledge gap is measurement in surface waters of precursors of C4-C10-PFSA and C4-C12-PFCA, both neutrals (MeFOSA, EtFOSA, MeFOSE, EtFOSE, fluorotelomer alcohols), anionic and amphoteric chemicals, and fluorotelomer mono- and di-substituted phosphate esters. Information is also very limited on PFOA and PFOS replacements such as the perfluoroether carboxylates and sulfonates.

3.15.02

Lake Superior Has Lost Over 90% of Its Hexachlorocyclohexane (HCH) Load Since 1986

T.F. Bidleman, Umea University / Chemistry; S. Backus, Environment and Climate Change Canada / Great Lakes Ecosystem Management Section; A. Dove, Environment and Climate Change Canada / Water Quality Monitoring and Surveillance Division; R. Lohmann, University of Rhode Island / Graduate School of Oceanography; D.C. Muir, C. Teixeira, Environment and Climate Change Canada / Aquatic Contaminants Research Division; L. Jantunen, Environment and Climate Change Canada / Air Quality Processes Research Section

The time trend of α- and γ-hexachlorocyclohexane (HCH) isomers in Lake Superior water was followed from 1986 to 2016, the longest record for any persistent organic pollutant (POP) in Great Lakes water. Dissipation of α-HCH and γ-HCHs was first order, with halving times ($t_{1/2}$) of 6.0 y and 8.8 y, respectively. Loss rates were not significantly different starting a decade later (1996 ─ 2016). Concentrations of β-HCH were followed from 1996 ─ 2016 and dissipated more slowly ($t_{1/2}$ = 18 y). In 1986, the lake contained an estimated 114 tonnes of α-HCH and 13.6 tonnes of γ-HCH; by 2016, only 2.9% and 8.6% of 1986 quantities remained. Halving times of both HCHs in water were significantly longer than those reported in air ($p < 0.0005$), and were significantly longer in water than those reported in lake trout for γ-HCH ($p = 0.0009$), but not for α-HCH. Microbial degradation was evident by enantioselective depletion of (+)α-HCH, which increased from 1996 to 2011. Volatilization was the main removal process for both isomers, followed by degradation (hydrolytic and microbial) and outflow through the St. Mary's River. Sedimentation was negligible. Major uncertainties in quantifying removal processes were in the two-film model for predicting volatilization and in microbial degradation rates. The study highlights the value of long-term monitoring of chemicals in water to interpreting removal processes and trends in biota.

3.15.04

Chemical Pollution in Urban Fresh Water System: A Systematic Review

A.O. Desrousseaux, University of York / Department of Environment and Geography; J.B. Sallach, University of York / Environment; A. Boxall, University of York / Department of Environment and Geography

Advances in analytical capabilities have made data on chemical pollution in fresh water systems more accessible and provide the opportunity to gain a "big picture" understanding of chemical pollution in urban river systems. So far, no attempt has been made to compile these data in order to understand how urban pollution varies over space and time. Here we present a comprehensive analysis of urban chemical pollution in fresh water systems around the World and develop a global chemical detection database based on published chemical monitoring data. The aim of this work was to identify 1) geographical biases in chemical monitoring 2) the types of chemicals, detected in different regions 3) concentration distributions of the most used and most monitored compounds. Approximately 13 000 data points were collected from 71 cities in 37 countries. A total of 919 chemicals belonging to 11 categories (human and veterinary drug, industrials chemical, pesticide, personal care product, flame retardant, food chemical, earth element, metal element, inorganic anion, stanols and sterols and petroleum chemical) were identified. USA, China and Vietnam provided the richest datasets in terms of chemicals monitored with 236, 206 and 148 unique chemicals reported, respectively. In general, countries in the northern hemisphere were more heavily monitored in terms of locations and chemicals investigated compared to the southern hemisphere. Human and veterinary drugs, industrial chemicals and pesticides were the categories most researched (330, 226 and 200 chemicals respectively). The eight other categories represented 138 chemicals altogether. Looking at chemical concentration, data ranged from 0.001 to 276 243 600 ng/L. The highest concentration was the industrial perfluorinated compound PFOA detected in a tributary in China in proximity to the largest fluorine chemical plant in the country. All categories across all continents had at least one chemical measured above 70 000 ng/L. The chemicals with the most individual detections were caffeine and carbamazepine quantified in 30 and 28 cities, respectively. To tackle chemical pollution in the future, looking at physical and chemicals properties of chemical is not enough. This systematic review showed high concentrations on every continent and from every product use category. Data shows that socio-economics, climate and technological drivers play a role in chemical emissions that cannot be ignored.

3.15.05

Developing a Multimedia Dynamic Grid Fate Model

P. Falakdin, University of Insubria (Como); E. Terzaghi, University of Insubria / Department of Science and High Technology, Como; A. Di Guardo, University of Insubria / Department of Science and High Technology

A common assumption in multimedia fate modeling is the homogeneity of the environmental compartments. Even though this approach remains useful for different applications, yet it may result in an insufficient approximation of the actual heterogeneity. Here is described the development of a new grid modelling approach to survey the effect of spatial variation in environmental conditions on the distribution of chemicals at a local scale. A dynamic multiple box version of an existing multimedia box model (SoilPlusVeg) is developed to account for the effect of wind speed and wind direction on the fate and transport of chemicals towards different compartments and geographical locations. The grid-based box model consists of a user-defined number of connected cells where each cell represents a SoilPlusVeg model unit. Hourly meteorological data such as rainfall, temperature, wind velocity, and direction as well as the height of two dynamic air compartments are incorporated into the model. The connection between the cells is through advective flows of air. Advective air, including the residual chemicals remaining after the mass-balance calculations of the cell, exit the air compartments of the current cell, and enter the corresponding air compartments of the adjacent cells. Thus, the sequence of SoilPlusVeg calculations is done cell by cell one hour at a time for all the cells in the grid system until the end of the simulation. Concentration values are calculated for the upper air, lower air, and the superficial soil compartments. The model calculates the chemical transfer from soil to lower air due to volatilization, from lower air to upper air and vice versa due to diffusion, and further deposition from lower air to the soil. The environmental scenario for the simulation (e.g. soil use, organic carbon, vegetation presence, etc.) will be based on selected contaminated areas for which measured concentrations in the surrounding of the source are available. Increasing spatial resolution and introducing a spatially resolved multiple box multimedia fate model allows to include the effect of spatial variation of several environmental conditions on the fate and transport of chemicals. This new grid

model couples the atmospheric variability to the spatial heterogeneity of the terrestrial environment. Current modelling approaches do not allow to appreciate such dynamicity, which could be relevant in evaluating the spreading of a chemical from a source.

3.15.07

Combining Lab Experiments, Non-Target Analysis and Modelling to Elucidate the Global Occurrence of Atmospherically Transformed Organophosphate Flame-Retardants

J. Liggio, Q. Liu, Environment and Climate Change Canada / Air Quality Research Division; L. Li, University of Nevada, Reno / School of Community Health Sciences; T. Harner, Environment and Climate Change Canada / Air Quality Processes Research Section; X. Zhang, Ontario Ministry of the Environment, Conservation and Parks; A. Saini, Environment and Climate Change Canada / Air Quality Research Division; H. Hung, W. Li, Environment and Climate Change Canada / Air Quality Processes Research Section; C. Hao, Ontario Ministry of the Environment Conservation and Parks / Laboratory Services Branch; K. Li, P. Lee, J. Wentzell, Environment and Climate Change Canada / Air Quality Research Division; S. Li, Peking University / College of Environmental Science and Engineering

Humans and ecosystems are exposed to tens of thousands of airborne chemicals, however, only a fraction of these chemicals have been identified. This is in part due to the current approach of identifying harmful airborne chemicals, which is largely based upon knowledge of parent chemicals in production and/or in commercial products, with minimal consideration of their transformation. During their residence time in the atmosphere, emitted parent chemicals of concern are gradually transformed to thousands of products through the photooxidation involving OH radicals. These products, via wet and dry deposition will be present in various terrestrial and aquatic ecosystems but currently at unknown levels and with unknown impacts. Tracking the complex mixture of airborne chemicals, in particular the atmospheric transformation products, is an immense analytical challenge. With the developments in high-resolution mass spectrometry, several studies have attempted to search through unidentified pollutants utilizing a non-targeted analysis technique without specifically targeting individual compounds. Despite recent advances, neither a traditional targeted, nor the non-target analysis approach in isolation are able to differentiate oxidation products from precursors, or determine their parent molecules of origin. This implies that if atmospherically transformed products are excluded from the assessment of the overall persistence, bioaccumulation, and toxicity (PBT) of a chemical of emerging concern (CEC), then the true environmental impacts over the full atmospheric lifetime may be underestimated. In the current work, a series of nine organophosphate flame-retardants (OPFRs) are investigated (as a relevant CEC), in laboratory studies to identify a large number of heterogeneous oxidation products, and to demonstrate their importance to the overall fate and toxicity of OPFRs. The product identifications are then used within a non-targeted analysis framework to confirm the presence of OPFR oxidation products in ambient particles found in megacities around the globe. Finally, the full suite of product structures are used within in-silico models to estimate the persistence, bioaccumulation and toxicity of the products. The results demonstrate that atmospheric oxidation can result in a large array of species which are present urban areas around the world, and which in many cases can have a persistence and toxicity larger than the parent molecules they originated from. The world-wide distribution of atmospherically transformed OPFRs implies that the transformation products of OPFRs and potentially many other CECs and persistent organic pollutants (POPs) present in air could be an emerging global air pollution issue and suggests that the risk assessment of a variety of CECs in air should include their transformation products moving forward.

3.15.08

Time Trends of Persistent Organic Pollutants (POPs) and Chemicals of Emerging Arctic Concern (CEAC) in Arctic Air From 25 Years of Monitoring

H. Hung, F. Wong, H. Dryfhout-Clark, Environment and Climate Change Canada / Air Quality Processes Research Section; W. Aas, Norwegian Institute for Air Research; P. Bohlin-Nizzetto, NILU - Norwegian Institute for Air Research / MILK - Environmental Chemistry Department; K. Breivik, NILU - Norwegian Institute for Air Research / Atmosphere and Climate Change Department; M. Nerentorp Mastromonaco, E. Brorström Lundén, IVL Swedish Environmental Research Institute; K. Olafsdottir, University of Iceland / Pharmacology and Toxicology; . SigurÃsson, Icelandic Meteorological Office; K. Vorkamp, Aarhus University / Environmental Science; R. Bossi, Aarhus University / Department of Environmental Science; H. Skov, Aarhus University / Arctic Research Center; H. Hakola, Finnish Meteorological Institute; E. Barresi, Environment and Climate Change Canada / National Laboratory for Environmental Testing; E. Sverko, Harbin Institute of Technology; P. Fellin, H. Li, A. Vlasenko, AirZone One Ltd.; M. Zapevalov, D. Samsonov, IPEM RPA "Typhoon"; S. Wilson, Arctic Monitoring and Assessment Programme (AMAP)

Air monitoring of chemical pollutants is one of the core components of the Arctic Monitoring and Assessment Programme (AMAP) of the Arctic Council. Such work can provide insights to the long-range transport (LRT), persistence and fate of persistent organic pollutants (POPs), most of which lack comprehensive emission inventories. The monitoring work is crucial in providing information for risk assessments in chemicals management, and for evaluating the effectiveness of chemical control measures such as the Stockholm Convention (SC). We report the long-term time trends of POPs and Chemicals of Emerging Arctic Concerns (CEAC) at eight Arctic monitoring stations, including: Alert, Canada; Zeppelin, Svalbard; StΓrhЈfţhi, Iceland; Pallas, Finland; AndΓya, Norway; Villum Research Station, Greenland; Tiksi and Amderma, Russia. In this paper, we examine the time trends of 1) pesticides and industrial chemicals, several of which have been regulated by the SC for more than 10 years, 2) chemicals used in consumer products and 3) CEACs that are not currently regulated by SC, some of which may not meet POPs criteria. We found that time trends of organochlorine pesticides, e.g. endosulfan showed declining trends in Arctic air which may be associated with regulation by the SC. Other POPs that are widely used in consumer products, such as perfluorooctane sulfonic acid (PFOS), showed a lag time between the chemical being regulated and an observed decrease in air. This is likely because consumer products which contain these POPs have a long lifespan, thus remain in use or present in waste for many years after being regulated. Polybrominated diphenyl ethers (PBDEs) also showed similar declining trend as PFOS. CEACs, e.g. perfluorohexane sulfonic acid (PFHxS), showed stable trends with relatively consistent concentration over time. The monitoring of CEACs provides baseline data used when evaluating possible needs for chemical control measures as well as evaluation of their effectiveness. Under AMAP, we will continue to maintain long-term monitoring with consistent data quality as additional chemicals are added to the SC or regulated under other national and international chemical control strategies.

3.15.10

The Integrated Atmospheric Deposition Network (IADN): From Legacy to Emerging Pollutants

M. Venier, A. Salamova, Indiana University / SPEA

IADN is a long-term monitoring program run by the U.S. Environmental Protection Agency's Great Lakes National Program Office. IADN and Environment and Climate Change Canada's Air Monitoring in the Great Lakes Basin (GLB) program formed a collaborative, binational monitoring network which began in 1990. Since then, IADN and GLB have been collecting air samples once every 12 days at seven sites. The initial focus of both these programs was on legacy pollutants like PCBs and organochlorine pesticides like DDTs. With time, as new environmental challenges emerged, new compounds were steadily incorporated to expand the overview on the presence of chemicals in the Great Lakes atmosphere. Additionally, ad hoc efforts are conducted to screen samples for new and emerging pollutants. The decline in the atmospheric concentration of several legacy pollutants is a success story showing how regulations does positively impact the environment. But new challenges are continuously surfacing and new ones are awaiting. In this presentation we'll present atmospheric concentrations of both legacy pollutants as well as emerging (i.e. current use pesticides, flame retardants, PFAS) and less studied chemicals (i.e. antioxidants).

3.15.12

Using High-Resolution Land Cover Data to Improve the Definition of Local Sources and Correlations With Atmospheric POP Concentrations

J.K. Schuster, Environment and Climate Change Canada / Air Quality Research Department; T. Harner, Environment and Climate Change Canada / Air Quality Processes Research Section; C. Rauert, The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS)

The Global Atmospheric Passive Sampling (GAPS) network, initiated in 2005 across 55 global sites, supports the global monitoring plan (GMP) of the Stockholm Convention on Persistent Organic Pollutants (POPs) by providing information on POP concentrations in air on a global scale. These data inform assessments of the long-range transport potential of POPs and the effectiveness evaluation of chemical regulation efforts, by observing changes in concentrations over time. The GAPS sites are distinguished based on their apparent proximity to potential sources as: polar, background, rural, agricultural and urban, which is a streamlined version of the site categories proposed under the GMP. Previous GAPS studies analysing the spatial distribution of POP concentrations in air observed elevated concentrations at locations that are considered remote. This indicates that localized sources and conditions affected POP concentrations at some sites. Furthermore, monitored POP concentrations at some sites diverged from trends predicted by global POP models. This was usually due to the scale and resolution of the models. The challenges of analysing complex spatial data collected under the GAPS network based on 5 simple categories (polar, background, rural, agricultural, urban) are addressed by analysing the land cover composition within a 10km, 25km and 50km radius around the GAPS sites. The eight consolidated land cover categories are: Waterbodies, Wetland, Forest, Open vegetation, Bare area, Snow, Cropland, Urban. For some GAPS sites of the

category “background”, the areas within a 50 km radius showed up to 60% designated cropland. For some GAPS sites of the category “agricultural”, the areas within a 50 km radius showed up to 70% designated urban. We found significant correlations ($p < 0.05$) between POP concentrations in air and size of the specific source areas within the radius. PCBs levels in air had a statistically significant correlation to the percentage urban land cover around the sites, while Endosulfan I, Endosulfan II, Endosulfan sulfate and α-HCH had a statistically significant correlation to the percentage cropland cover, in line with known applications as industrial and agricultural chemicals respectively. The land cover composition at the individual GAPS sites is used to create vectors that describe potential local primary sources, environmental reservoirs and remoteness.

3.15.13

Guidance on the Application of PUF Disk Passive Air Samplers for Measuring Short-Chain Chlorinated Paraffins in Air: Results From a Screening Study in Urban Air

S. Niu, Utah State University; T. Harner, Environment and Climate Change Canada / Air Quality Processes Research Section; R. Chen, Utah State University / Utah Water Research Laboratory; J. Parnis, Trent University / Cemistry; A. Saini, Environment and Climate Change Canada / Air Quality Research Division; K.J. Hageman, Utah State University / Chemistry & Biochemistry

Polyurethane foam-based passive air samplers (PUF-PASs) have been widely used for atmospheric organic pollutant monitoring. However, the uptake profile of chlorinated paraffins (CPs) using PUF-PAS is still unclear. CPs are a class of synthetic polychlorinated alkanes with thousands of CP congeners, homologs, and isomers, and have been widely used in lubricants, plastics, and metal cutting fluids. Given their persistence, toxicity, and potential long-range transport, short-chain chlorinated paraffins (SCCPs) have been listed as persistent organic pollutants under the Stockholm Convention. Moreover, SCCPs have been widely detected in the environment in China, Europe, and Australia. However, studies on SCCPs in ambient air and applications of PUF-PAS for determining SCCP concentrations and sources are still limited. In this study, we estimated the equilibrium partition coefficients between PUF and air (K_{PUF_AIR}) and between octanol and air (K_{OA}) for SCCP congeners using COSMO-RS solvation theory to investigate the uptake profiles of each structural SCCP congeners by PUF disk. We then measured SCCP concentrations in PUF disk samples collected from distinct source sectors in urban air across the Greater Toronto Area (GTA) using gas chromatography coupled with mass spectrometry (GC/MS). The analytical method performed well in an inter-laboratory study. The results showed that PUF disks were suitable for SCCPs and that time-weighted linear phase sampling was possible for congeners having log K_{OA} values greater than 8.5. The highest SCCP concentrations were identified at industrial sites, and comparable SCCP concentrations were found between the residential sites and remote sites. No consistent seasonal variation in SCCP concentrations was found in the four distinct source sectors (e.g., residential area, traffic area, industrial area, and remote area). Direct measurements of K_{PUF_AIR} values and K_{OA} values are still needed for SCCPs to improve the accuracy of derived air concentrations using PUF disk passive air samplers.

3.15.15

Comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the non-targeted screening analysis of contaminants of emerging concern in wastewater

J. Devers, Univeristy of Copenhagen; D. Pattison, J.H. Christensen, University of Copenhagen / Department of Plant and Environmental Sciences

Recent toxicological analysis of wastewater has shown that only 1-5% of the measured toxicity can be attributed to currently monitored compounds, thus highlighting a significant knowledge gap in the current understanding of toxic pollutants in wastewater[1–3]. In addition, toxicological analysis of wastewater extracts fractioned in terms of polarity have shown that the polar fraction is the main carrier of toxic effects in wastewater[4,5]. This observation points the way for future non-targeted wastewater analysis. The advent of comprehensive two-dimensional gas chromatography, allowing chromatographic separation using two separate interaction mechanisms, typically non-polar in the 1st dimension and polar in the 2nd dimension, has increased both the application range in terms of polarity as well as greatly increasing the peak capacity of conventional gas chromatography[6]. Herein, we demonstrate the development of a comprehensive two-dimensional gas chromatographic method, in conjunction with other novel and traditional tools, such as 1D and 2D retention indices, derivatisation of more polar compounds and large volume solvent vent injection. The goal is to maximise the number of low abundance compounds present in wastewater that can be identified using gas chromatography; therefore striving to narrow the knowledge gap for toxic pollutants in wastewater. 1. Neale, P. A. *et al.* Development of a bioanalytical test battery for water quality monitoring: Fingerprinting identified micropollutants and their contribution to effects in surface water. *Water Res.* **123**, 734–750 (2017). 2. Escher, B. I., Van Daele, C., Dutt, M., Tang, J. Y. M. & Altenburger, R. Most oxidative stress response in water samples comes from unknown chemicals: The need for effect-based water quality trigger values. *Environ. Sci. Technol.* **47**, 7002–7011 (2013). 3. Tang, J. Y. M. *et al.* Mixture effects of organic micropollutants present in water: Towards the development of effect-based water quality trigger values for baseline toxicity. *Water Res.* **47**, 3300–3314 (2013). 4. Hrubik, J. *et al.* Toxicological and chemical investigation of untreated municipal wastewater: Fraction- and species-specific toxicity. *Ecotoxicol. Environ. Saf.* **127**, 153–162 (2016). 5. Smital, T. *et al.* Assessment of toxicological profiles of the municipal wastewater effluents using chemical analyses and bioassays. *Ecotoxicol. Environ. Saf.* **74**, 844–851 (2011). 6. Mostafa, A., Edwards, M. & Górecki, T. Optimization aspects of comprehensive two-dimensional gas chromatography. *J. Chromatogr. A* **1255**, 38–55 (2012).

3.15.17

Nation-Wide Monitoring Campaign of 53 Contaminants of Emergent Concern in Surface Waters and Sediment (EMNAT 2018): Occurrence and PNEC Exceedance Evaluation

A. Assoumani, F. Lestremau, C. Ferret, B. Lepot, M. Salomon, INERIS; M. Dévier, H. Budzinski, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC; K. Le Menach, UMR CNRS EPOC Universite Bordeaux / EPOC UMR 5805; P. Labadie, UMR CNRS EPOC Universite Bordeaux / UMR 5805 EPOC; P. Pardon, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC; L. Wiest, E. Vulliet, University of Lyon / ISA; P. Staub, French Biodiversity Agency

The overall objective of the 2018 campaign to monitor substances of emerging concern in surface water (continental and coastal) and sediment, known as EMNAT 2018, is to provide the exercise of prioritization of emerging substances, which is being carried out in 2020, with monitoring data. This exercise should make it possible to update the list of relevant substances to be monitored (SPAS) as part of future WFD monitoring programmes, which will be implemented in the next water body management cycle (2022).This campaign, led by Ineris, collected nearly 14,000 data regarding 53 substances of emerging concern (36 biocides and 17 surfactants, selected by the Prioritization Experts Committee) in surface water and sediments on a total of 98 sites in metropolitan France and in the overseas departments and regions (DROM). Frequencies of quantification and concentration levels were determined and discussed. Warning indicators, calculated from PNEC (predicted no-effect concentrations), allowed to estimate the criticality of the risk of exceeding them (frequency and degree of PNEC exceedance).Regarding impregnation levels in the environment, with respect to surfactants, Linear Alkybenzene Sulfonic acids (LAS) were the most frequently quantified substances and at the highest median concentrations in water and sediment samples, in metropolitan France and in the DROM. Regarding biocides, fipronil was the most frequently quantified substance in water samples in metropolitan France and in the DROM, but at lower median concentrations than other biocides. Methyl nonyl ketone was the most frequently quantified substance and at higher median concentrations in sediment samples from metropolitan France and in the DROM.Based on the available PNECs, the highly critical substances with respect to exceeding the PNEC are:- LAS C11, C12 and C13 in mainland and the DROM water and sediment samples, - methyl nonyl ketone in mainland and the DROM sediment samples, - fipronil and methylisothiazolinone in mainland water samples, and - fipronil, octylisothiazolinone, methyl nonyl ketone and a triethanolamine based esterquat in mainland sediment samples.

3.15.24

Root Uptake of PCBs by Herbaceous and Woody Species: Variability During Life Cycle Stages

E. Terzaghi, University of Insubria (Como) / Department of Science and High Technology; G. Raspa, Sapienza University of Rome / Department of Chemical Engineering Materials and Environment; E. Zanardini, University of Insubria / DISAT; C. Morosini, University of Insubria / Department of Science and High Technology; S. Anelli, ERSAF; S. Armiraglio, Municipality of Brescia / Museum of Natural Sciences; A. Di Guardo, University of Insubria / Department of Science and High Technology

Polychlorinated biphenyls (PCBs) are a class of man-made chemicals which was banned at the beginning of 1980s, but are still frequently found in the environment due to their wide distribution and persistence. Plants can accumulate PCBs from soil into the roots and this process represents the first step in the food web uptake, affecting both human and ecosystem exposure and effects.Therefore, the quantification and the prediction of PCB transfer from soil to roots is fundamental for assessing human and ecological risks as well as for evaluating the success of phytoremediation procedures. From the 80/90s, many studies were performed and equations were developed to estimate root uptake (e.g., root concentration factor, RCF) of organic compounds for herbaceous and woody species, as well as for vegetables. The importance of plants in the uptake of organic contaminants from air and soil was recognized to the point that even regulatory predictive approaches (e.g., CalTOX, EUSES and TRIM.FaTE model) include a vegetation compartment to predict the exposure through the food chain. However, it has recently been pointed out the need for a more ecological realistic description of vegetation uptake for refining the current regulatory approaches for human and ecological risk assessment. In the present work, we performed a long-term greenhouse experiment in field-like conditions with a weathered contaminated soil and seven

plant species. The aims of the study were: 1) to evaluate PCB uptake variation during different phenological stages of annual and perennial plant species under field like conditions; 2) to develop new predictive species-specific RCF equations obtained using the long-term results (i.e., 3 to 18 months), and 3) to compare the newly developed equations to the currently available predictive equations for root uptake. The relationships (Log RCF vs. Log K_{OW}) obtained highlighted a species-specific and time-dependent accumulation of PCB in plants roots and an important influence of DOC and POC in affecting root uptake especially for the most hydrophobic congeners. Moreover, at lower Log K_{OW} (i.e., < 5.5), the Log RCFs of the current experiment were within the range of variability of those estimated with the literature approaches; however, at increasing hydrophobicity, they were considerably higher, up to about 3 orders of magnitude. This means that care must be taken when selecting an equation to model the root uptake of a chemical by certain plant species.

3.15.32

Development and Application of a Miniaturised 3D Printed Passive Sampler Device for Monitoring of Emerging Contaminants in River Water

A.K. Richardson, Kings College London / Department of Analytical, Environmental and Forensic Sciences; R.C. Irlam, University of Newcastle / Department of Chemistry; G.A. Mills, University of Portsmouth / School of Pharmacy and Biomedical Sciences; G.R. Fones, University of Portsmouth / School of Earth and Environmental Sciences; S. Stürzenbaum, D.A. Cowan, Kings College London / Department of Analytical, Environmental and Forensic Sciences; D.J. Neep, Agilent Technologies; L. Barron, Kings College London / School of Public Health

The design, development and deployment of a novel 3D-printed passive sampling device (3D-PSDs) in the River Thames, UK for measuring the occurrence of contaminants of emerging concern is presented here for the first time. The device was printed via stereolithography technologies using a commercially available methacrylate-based resin, which has been previously demonstrated to be stable under various pH and aqueous/solvent conditions [1]. The miniaturised 3D-PSDs has the capacity to hold five 9 mm passive sampler sorbents and consisted of three core parts which connect via an interference fit. Reproducibility between prints was high (< 1% RSD) with no significant variation in dimension between the prints. The resin of the 3D-PSDs was assessed for contaminant sorption in artificial freshwater for 160 compounds over eight days. Overall, mean loss due to sorption to the 3D-PSDs was 31.4±37.0 %. A total of 43 compounds showed increased sorption of 30.8–99.6% (mean=72.5 %) and was driven by analyte logD>2. Sampling rates (R_s) for analyte uptake by a 3D-PSDs configured with 9 mm HLB (divinylbenzene-co-N-vinylpyrrolidone) sorbent disks and corresponding overlayed PES (polyethersulfone) membranes were assessed in the River Thames for 13 days. Daily water samples and sorbent extracts were analysed by rapid direct-injection liquid chromatography triple-quadrupole mass spectrometry [2]. Overall, linear uptake was observed over eight days and sampling rates for 11 compounds lay in the range of $4x10^{-5}$ to $8.2x10^{-4}$ L/day. When using the obtained R_s values to predict the water concentration, the models had an error of 5.3±4.0 ng/L over a water concentration range of 11.7 to 74.5 ng/L. Overall, these 3D-PSDs represent a cost-effective, convenient and miniaturised approach to large-scale catchment monitoring, potentially via 'citizen science'-type programmes. **References**[1] Irlam RC, Hughes C, Parkin MC, Beardah MS, O'Donnell M, Brabazon D, Barron LP. 2020. Trace multi-class organic explosives analysis in complex matrices enabled using LEGO®-inspired clickable 3D-printed solid phase extraction block arrays. J Chromatogr A. 1629:461506. [2] Ng KT, Rapp-Wright H, Egli M, Hartmann A, Steele JC, Sosa-Hernández JE, Melchor-Martínez EM, Jacobs M, White B, Regan F *et al.* 2020. High-throughput multi-residue quantification of contaminants of emerging concern in wastewaters enabled using direct injection liquid chromatography-tandem mass spectrometry. J Hazard Mater. 398:122933.

3.15.33

Are Gas Chromatography-Retention Time Measurements of Organophosphate Esters and Other Moderately Polar Compounds Biased? Evidence From the Literature for 74 Compounds

T.F. Rodgers, University of Toronto / Chemical Engineering and Applied Chemistry; J.O. Okeme, University of Toronto / Physical and Environmental Sciences; J. Parnis, Trent University / Cemistry; K. Girdhari, University of Toronto / Earth Sciences; T.F. Bidleman, Umea University / Chemistry; Y. Wan, University of Toronto / Physical and Environmental Sciences; L. Jantunen, Environment and Climate Change Canada / Air Quality Processes Research Section; M.L. Diamond, University of Toronto / Department of Earth Sciences

Accurate physical-chemical properties are essential for predicting the toxicity, behaviour and fate of organic compounds. Few laboratories measure physical-chemical properties and the accuracy of some of the resulting predictions can be difficult to ascertain. "Conventional" methods used to measure physicochemical properties can be time consuming and require extremely high precision, making them potentially prone to error. Inter-laboratory comparisons confirm these issues, with measurements of the same property by different labs differing in some cases by orders of magnitude. "Indirect" methods, such as the gas chromatography retention time (GC-RT) method for measuring vapour pressure (P_L) and the octanol-air partition coefficient (K_{OA}) provide a means to obtain fast measurements under precisely controlled conditions, and tend to be more reliable between labs. The controlled conditions of the GC-RT method enable measurements to be made at multiple temperatures which can be used to measure the enthalpy of vaporization (ΔH_{vap}) and internal energy of transfer between octanol and air (ΔU_{OA}). However, the GC-RT method works based on the assumption that retention is driven solely by van der Waals forces, and therefore, it may produce erroneous results for polar or moderately polar compounds that partake in hydrogen bonding. We gathered literature measurements performed using the GC-RT and conventional methods for P_L, K_{OA}, ΔH_{vap} and ΔU_{OA} for 74 compounds across nine compound classes exhibiting a range of polarities (synthetic musks, NBFRs, OCPs, OPEs, PAHs, PBDEs, PCBs, phthalates and UV filters). We then correlated differences between the conventional and GC-RT measurements with Abraham's solute descriptors (representing specific and non-specific intermolecular interactions) to determine whether the GC-RT measurements differed more at higher polarities. Overall, our results showed that the GC-RT and RP-HPLC indirect methods produced highly precise results that were statistically indistinguishable from the conventional methods for P_L, K_{OA} and ΔH_{vap}, but not for ΔU_{OA}. It is possible that these indirect methods may produce higher error for more polar compounds across a larger data set including more polar compounds, especially in measuring K_{OA} where the hydrogen bonding acidity (A descriptor) is an important interaction, as our dataset contained compounds with low A values of < 0.15. We found no evidence that the results from these indirect methods were biased for more polar compounds such as the OPEs studied here.

3.15.35

Comparison of ESI Vs APCI When Measuring Acrylamide by LC-MS/MS in Drinking Water

D. Mattern, I. Garaguso, PerkinElmer

Keywords: polar contaminants, LC-MS/MS, acrylamide, EU water directive

European Union water directives set forth environmental quality standards for drinking water and have also defined a list of more than 30 priortiy substances that are deemed high risk to humans (1). One of these substances, acrylamide, is part of this list because of its high toxicity and likely carcinogenicity, thus requiring it to be monitored in water laboratories (2). Here we will present the analysis of acrylamide in drinking water with two separate runs using both ESI and APCI. Because the true dual source design of PerkinElmer's QSight LC-MS/MS system allows for the ability to easily switch between ESI and APCI, this allows for different methods to be run without any manual changes to the instrument. When measuring acrylamide with ESI we observed a very strong matrix effect, but when running the same sample with APCI the effect was drastically reduced. Moreover, the sensitivity was not compromised as lower ppt levels could be achieved. The present work aims at illustrating the performance of a targeted LC-MS/MS method using a QSight triple quadrupole mass spectrometer for the quantification of this difficult polar contaminant in drinking water. Furthermore, acrylamide could be measured as low as 10 ng/L demonstrating the extreme sensitivity of the QSight LS-MS/MS. This alone is 10x lower than what is required by the EU, which set the limit at 100 ng/L. Moreover, we were able to demonstrate that by simply direct injecting drinking water we could completely eliminate the sample preparation steps. References: 1. EU COUNCIL DIRECTIVE 98/83/EC [cited 2020 Dec. 10]. Available: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31998L0083&from=EN 2. International Agency for Research on Cancer Monograph on Acrylamide [cited 2020 Dec. 10] Available: https://monographs.iarc.fr/wp-content/uploads/2018/06/mono60-16.pdf

3.15.36

Refinement and Extension of Cosmo-Rs-Trained Fragment Contribution Models for Predicting Partition Properties of SCCPs, MCCPs and LCCPs

S. Endo, National Institute for Environmental Studies (NIES) / Health and Environmental Risk Division

Chlorinated paraffins (CPs) are complex mixtures of polychlorinated *n*-alkanes and have been detected widely in the environment. Extremely high complexity of CP products hampers the use of conventional approaches to obtain physico-chemical properties including partition coefficients. Previously, we developed a fragment contribution model (FCM) trained by COSMO-RS-calculated values to predict octanol–water (K_{ow}), air–water (K_{aw}) and octanol–air (K_{oa}) partition coefficients of CP congeners. The COSMO-RS-trained FCM takes the advantages both the sophisticated quantum chemically based COSMO-RS and the simple and quick FCM to enable prediction for >10,000 congeners within a reasonable time. The purpose of this work is to extend the COSMO-RS-FCM method from SCCPs to MCCPs and LCCPs and to provide realistic distributions of log K values for various CP technical mixtures. FCMs were trained with the previous set of 815 randomly generated congeners (C_5-C_{10}) + additional 240 CP congeners (C_{11-18}). Model validation shows that the previous training set is insufficient to provide accurate predictions for low chlorinated congeners and M/LCCP congeners. The

new training set can offer predictions with 0.11–0.33 log unit RMSE even for LCCPs. The refined FCMs thus can accurately provide quantum chemically based predictions of log *K*'s for S/M/LCCPs within a short time. To simulate industrial mixtures, structures of 10,000 CP molecules were generated for a given chain length and a chlorine content using a Monte Carlo method. The FCM predictions show that log *K* of low chlorinated (30–40 wt%) mixtures is characterized by (i) crude bell-shapes with spikes originating from low chlorinated congeners, (ii) a narrow log K_{ow} range (< 2 log units), (iii) wide log K_{aw} and K_{oa} range (>6 log units) and (iv) a strong inverse correlation between log K_{aw} and log K_{oa}. In contrast, log *K* of high chlorinated (60–70 wt%) mixtures is characterized by (i) symmetric bell-shapes for all three log *K*'s (2–3 log unit range), (ii) an increase of log K_{ow} with # of Cl, (iii) log K_{aw} distributions nearly independent of # of Cl and (iv) no or only weak correlation between log K_{aw} and log K_{oa}. The congener composition is indeed important when the environmental properties of mixtures are discussed. The ranges, medians, and other quantiles of log *K* derived from the FCMs should be useful to understand the environmental behavior and perform environmental transport modeling of CPs with diverse compositions.

Extended submission 3 - Environmental chemistry and exposure assessment: analysis, monitoring, fate and modeling

3.16.05

Facilitating the Use of the Theory of Representative Sampling for Granular Waste: Number of Particles, Sample Size and Uncertainty Arising From Sampling

G. Beggio, University of Padua / Department of Civil, Architectural and Environmental Engineering; P. Hennebert, INERIS; T. Bonato, Ca' Foscari University of Venice / Department of Environmental Sciences, Informatics and Statistics (DAIS); F. Loro, WasteandChemicals Srl; L. Maggi, ChemService srl Labanalysis Group; A. Pivato, University of Padua / Department of Civil, Architectural and Environmental Engineering

Environmental forensics assessments are often based on few analytical measurements. Biased data can, therefore, led the involved actors to be improperly charged or acquitted. Data reliability, considered in terms of uncertainty and variability, is determined by the analytical methods and the performed sampling procedures. The influence of this latter increases with highly heterogeneous materials characterized by the presence of trace, "rare" elements (both considered resources or contaminants), as occurring in the field of waste management. Nevertheless, the theoretical principles behind representative sampling are still not well understood in the waste community, which often underestimates its influence on the reliability of the resulting data and consequent decisions. Applying the binomial probability distribution, the theory of representative sampling is reviewed to facilitate its use for granular solid wastes. The concept of the number of particles *n* that shall compose a representative sample is introduced. Within *n*, the same fraction *p* shall be included of "rare" particles present in the lot to be characterized. Also, n depends on the minimum achievable variability of test results, i.e., coefficient of variation *CV* computable on a set of equivalent samples. Data are presented quantifying occurring intra and inter-laboratory variability of the most used chemical-physical and ecotoxicological analyses of homogeneous material. Accordingly, a specific formula to calculate n is proposed. Three requirements are then laid down to transform *n* into mass of sample. Representative samples must include a fraction p of particles considered "rare" in terms of i) presence/concentration of substances and ii) particles size distribution, since large waste particles are seldom characterized by the same composition of fine particles. Further, iii) the size of the sampling equipment shall prevent segregation of larger particles. The mass of a representative sample is calculated by reconciling these three conditions. According to the dedicated technical standards, the layout of a replication experiment is developed to measure the uncertainty arising from a sampling plan designed for waste classified with European Waste Code 191212 "other wastes including mixtures of materials from mechanical treatment of wastes".Practical and design challenges that can affect the correct performance of the related experimental activities and the reliability of the outcomes are discussed.

3.16.06

Multi-Residue Method Development for Antivirals During Covid-19 Pandemic

S. Mohapatra, Y. Luhua, National University of Singapore / NUS Environmental Research Institute; K.Y. Gin, National University of Singapore / Civil & Environmental Engineering/NUS Environmental Research Institute

With the unprecedented event of Covid-19, antivirals' consumption has increased worldwide, leading to increased load at the wastewater treatment plants (WWTPs). Due to their inadequate removal at conventional WWTPs, such emerging contaminants could enter the natural environment and further interfere with the aquatic biota. It is paramount to develop a robust and highly-sensitive analysis method to identify and quantify such contaminants in complex environmental matrices, including wastewater samples where matrix effects for these contaminants are high. Thus, during this crucial time, a multi-residue method based on solid-phase extraction (SPE) coupled with high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) and isotope dilution was developed to simultaneously analyze a set of pharmaceuticals such as favipiravir, darunavir, ritonavir, and chloroquine which are commonly prescribed to treat viral infections. The different SPE cartridge systems were investigated to simultaneously extract the multiple classes of pharmaceuticals in different environmental water samples. HPLC-MS/MS parameters were optimized for simultaneous analysis of the target chemicals in a single injection. The multi-residue method was evaluated in terms of the detection limit (LOD), the limit of quantification (LOQ), and isotope dilution to compensate for the losses during sample preparation and matrix effect. Finally, the method was successfully applied to detect and quantify the target pharmaceuticals in the wastewater samples.

3.16.09

Abundance and Tropical-Seasonal Distributions of Marine Microplastics in Singapore's Coastal Ecosystems

M. Jong, National University Singapore / NUS Environmental Research Institute; S. Chng, National University Singapore / Civil and Environmental Engineering; K.Y. Gin, National University of Singapore / Civil & Environmental Engineering/NUS Environmental Research Institute

Microplastics (MPs) are trans-boundary pollutants with global occurrence on land and in major aquatic bodies. Marine plastics and MPs are dispersed by the ocean gyres with potentially highly varying abundance in the coastlines influenced by maritime exposure to tidal regimes and hydrodynamics, regional macroclimate, ecosystem types and environmental matrices. The rise of marine MPs pollution erodes the health of global coastal habitats, including beaches, coral reefs and mangroves. Singapore is one of the island countries located in equatorial tropics with spatially complex hydrogeology characterized by the Monsoon weather system and surrounding straits and seas. Recent field surveillance at two key coastal ecosystems in Singapore, i.e., beaches and mangroves, adopted the NOAA methodologies with modifications for optimal MP quantification in coastal samples entailing vegetal-rich clayey sediment, sand and intertidal waters. Data revealed MPs abundance differed significantly ($p< 0.05$) between the two ecosystems with higher apparent concentrations detected in mangrove water and sediments relative to beach water and sand, suggesting potential coastal hotspots that are linked to ecosystem features such as trapping mechanisms by mangrove flora. Location-specific differentials in MPs abundance were found to corroborate with the tropical Monsoon patterns and modelled hydrodynamic floating plastic tracking for Singapore waters. Higher abundance and diversity of sedimented MPs (top-soil; up to 10^3 MP/kg dry weight sediment) and suspended MPs (surface water; ~10 MP/L) occurred during the Southwest and Northeast Monsoons as compared to the inter-Monsoon seasons. This data provides baseline and updated insights of MPs pollution in Singapore's coastal habitats which are crucial for further apportionment and prediction study and mitigation planning by ecosystem managers.

3.16.15

Occurrence, Fate and Assessment of 200 Organic Compounds and Endocrine Effects During Drinking Water Treatment at Lake Taihu, China

C. Schmid, IWW Forschung gGmbH / Water Resources Management; M. Büter, E. Dopp, T. aus der Beek, IWW Water Centre

Taihu is China´s third largest freshwater lake, situated about 100 km west of Shanghai. Located in a densely populated region, the water quality has suffered from anthropogenic activities since decades. A previous study of the Sino-German Water Supply Network (SIGN) has shown that various organic chemicals occur in hazardous concentrations, which is challenging for waterworks providing drinking water from Taihu for about 10 million people. With focus on elimination rates of organic pollutants and changing endocrine activity of water during drinking water treatment, this study aims at a better understanding of these dynamics in order to optimize the treatment processes. In three different drinking water treatment plants (DWTP), water was sampled in 6-8 process steps with regard to residence time, and more than 200 organic pollutants from industry, agriculture and domestic use were analysed according to published international standards. First results show, that 39 organic pollutants were detected at least once in raw water and 36 in the final drinking water. PFOA, PFOS, PFHxS, HCBD and iohexol were the most frequently detected compounds and were barely removed during treatment. Concentrations of perfluorinated chemicals in drinking water could pose a long-term risk to human health, in particular to vulnerable population groups. Nevertheless, the detection frequencies and concentrations of many chemicals were reduced in drinking water. As chemical target analysis will not consider all pollutants, including transformation products, cell-based bioassays were applied to screen for changing endocrine disruption. Therefore, activation and inhibition of the estrogenic or androgenic receptor (ERα resp. AR) was measured using the Chemically Acitvated Luciferase gene Expression (CALUX) assay. The bioassays did not reveal any androgenic or anti-estrogenic effects. However, estrogenic and

anti-androgenic effects up to 0.06 ng 17β-estradiol equivalents/L, respectively 6725 ng flutamide equivalents/L were measured, but also reduced during treatment, especially after treatment with ozone and activated carbon.The results show, that ozone and activated carbon are useful measures to reduce anti-androgenic and estrogenic effects and concentrations of many organic contaminants in raw water from Taihu. However, more effort is needed to increase the elimination efficiency of persistent chemicals, in particular perfluorinated compounds.

Artificial Intelligence Approaches in Environmental Risk Assessment: Bayesian Networks, Machine Learning and Predictive Modelling

4.01.01

Towards a Facilitated Use of Modelling in Environmental Risk Assessment

S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology

Within EU only, more than 100000 man-made chemicals are awaiting assessment of their risk to the environment. Based on statistical analyses providing toxicity indices at different tiers, risk assessment faces today new challenges to meet all expectations in terms of regulatory requirements together with the use of advanced and sound statistical methods. In particular, EFSA today recommends a clear and unambiguously identification of uncertainty sources [1], the use of TKTD models to refine tier 2 risk assessment in particular for plant protection products acting on aquatic organisms when exposed to environmentally realistic concentration profiles [2] and that models be documented in a transparent way ensuring reproducible results [3]. If plenty ideas, methods and tools already exist in the academic world to meet these expectations, practitioners struggle in appropriate them for reasons mostly attributable to modelers themselves. These reasons mainly come from lacks of support: (1) to easily quantify uncertainties, then their propagation to model outputs and subsequent predictions; (2) to better accept changing paradigm using new modelling approaches often appearing as black boxes, together with a lack of support to fully perceive the concrete added-value of these novelties for their daily work; (3) to easily interpret goodness-of-fit criteria and therefore trust model results in their ability to support decisions from predictions; (4) to appropriate recent user-friendly turn-key facilities, while already recognized as automatically providing toxicity indices of interest in full compliance with regulatory guidelines and risk assessment decision criteria. Based concrete case studies dealt with a suite of convenient and relevant tools freely available within an all-in-one facility, this presentation will illustrate how the above-mentioned difficulties can be overcome to facilitate the use of models in the environmental risk assessment (ERA).

4.01.03

Modeling Acetylcholine Esterase Inhibition Resulting From Exposure to a Mixture of Atrazine and Chlorpyrifos Using a Physiologically Based Kinetic Model in Fish

C. Mit, C. Tebby, T. Gueganno, INERIS / Models for Ecotoxicology and Toxicology METO; A. Bado-Nilles, INERIS / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); R. Beaudouin, INERIS / Models for Ecotoxicology and Toxicology METO

Aquatic organisms are exposed to mixtures of chemicals that may interact. Mixtures of atrazine (ATR) and chlorpyrifos (CPF) may elicit synergic effects on the permanent inhibition of acetylcholinesterase (AChE) in certain aquatic organisms, causing severe damage. Mechanistic mathematical models of toxicokinetics and toxicodynamics (TD) may be used to better characterize and understand the interactions of these two chemicals. In this study, a previously published generic physiologically-based toxicokinetic (PBTK) model for fish was adapted to ATR and CPF. A sub-model of the kinetics of one of the main metabolites of CPF, chlorpyrifos-oxon (CPF-oxon), was included, as well as a TD model. Inhibition of two esterases, AChE and carboxylesterase, by ATR, CPF and CPF-oxon, was modeled using TD modeling of quantities of total and inactive esterases. Specific attention was given to the parameterization and calibration of the model to accurately predict the concentration and effects observed in the fish using Bayesian inference and published data from fathead minnow (*Pimephales promelas*), zebrafish (*Danio rerio*) and common carp (*Cyprinus carpio* L.). A PBTK-TD for mixtures was used to predict dose-response relationships for comparison with available adult fish data. Synergistic effects of a joint exposure to ATR and CPF could not be demonstrated in adult fish.

4.01.06

The Ladder of Causation for Risk Assessment and Decision Making With Wildfire Ecology

J.F. Carriger, U.S. Environmental Protection Agency / Office of Research and Development; M. Thompson, USDA Forest Service; M.G. Barron, U.S. Environmental Protection Agency / Office of Research and Development

A conceptual ladder of causation was introduced in Judea Pearl's "Book of Why" for classifying causal queries by the amount and types of causality used. The first level is seeing, the second is doing and the third level of the ladder is imagining. Causal Bayesian network or influence diagram models can be built for addressing questions at all rungs of the ladder of causation but their importance is compounded for doing and imagining. To examine the implications of these concepts for risk assessment, causal demonstrative structures that serve each rung for both assessment and decision models were developed in hypothetical risk-based case study applications for the ecological effects of wildfires. Here we explore the possibilities for using Bayesian networks for assessing wildfire impacts to ecological systems through levels of causal representation and scenario examination. Understanding causal linkages between wildfires and drivers of the cascading effects on ecological systems remains a critical need in fire management and impact assessment. The key benefit that will be highlighted in using causal models for each rung of the ladder is the capability to incorporate knowledge and concerns from multi-disciplinary experts and stakeholders. Ultimately, Bayesian networks may facilitate understanding the factors contributing to fire susceptibility and resilience, and the prediction and assessment of wildfire risks to and impacts on ecosystems. The flexibility and quantitative capabilities of Bayesian networks can accommodate most models used by frequentist statistics and machine learning but go beyond other analytic structures through easy and powerful causal representation and calculations for wildfire risk assessment and management questions at each level of the ladder. Disclaimer: The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies the U.S. Environmental Protection Agency (EPA). The findings and conclusions in this paper are those of the author(s) and should not be construed to represent any official USDA or U.S. Government determination or policy.

4.01.08

Weight of Evidence by Conditional Probabilities: A Bayesian Network Model for Predicting Fish Acute Toxicity Based on Fish Embryo Testing

J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; A.L. Madsen, Hugin Expert; S. Belanger, The Procter & Gamble Company; T. Braunbeck, University of Heidelberg / AquaTox Centre for Organismal Studies; K.A. Connors, Procter & Gamble Company / Global Product Stewardship; M.R. Embry, Health and Environmental Sciences Institute (HESI); W.G. Landis, Western Washington University / Institute of Environmental Toxicology and Chemistry; K. Schirmer, Eawag / Environmental Toxicology; S. Scholz, Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology; R. Wolf, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; A. Lillicrap, NIVA / Section of Ecotoxicology and Risk Assessment

Fish Embryo Toxicity (FET) testing has been proposed as an alternative to using juvenile fish in acute toxicity testing, to reduce the number of live animals required for hazard assessments of chemicals. However, FET data are not yet accepted as a stand-alone replacement to juvenile fish acute toxicity data for regulatory purposes such as REACH. The European Chemicals Agency has recommended the development of a Weight-of-Evidence (WoE) approach for strengthening the evidence from FET testing. To meet this challenge, we have developed a Bayesian network (BN) model for using FET data in a probabilistic WoE approach. The purpose of the proposed BN model is to integrate information from large ecotoxicological and physico-chemical datasets, and apply it in a WoE context to predict fish acute toxicity of chemicals from data on fish embryo toxicity testing in combination with other types of information. This BN model was developed from existing data on fish embryo toxicity and acute (juvenile) fish toxicity in combination with other information for more than 200 chemicals, and aims to predict the toxicity level of each chemical by combining information in four primary pathways: (i) fish embryo toxicity, (ii) physical and chemical properties, (iii) toxicity to fish of other chemicals in the same category, and (iv) toxicity to other species (algae and *Daphnia*). In a new CEFIC LRI project, SWiFT (*Strengthening Weight of evidence for FET data to replace acute Fish Toxicity*; ECO51), this BN model will be developed further and extended with additional lines of evidence (e.g. fish gill cell-line assay, neurotoxicity and biotransformation), in order to provide more accurate predictions. More details on the lines of evidence and the underlying data will be presented in the session *Alternative Approaches to Animal Testing for Ecotoxicity Assessments and Environmental Risk Assessments*. Here, we will focus on the methodological aspects: how the main steps of a WoE approach (assembly, weighting, and integration of evidence) can be quantified and implemented in a BN model. We will address the opportunities and challenges related to topics such as: (1) quantification of lines of evidence − from associations to causal relationships; (2) accounting for uncertainty and variability in existing data, both within and between studies; (3) integration of the multiple lines of evidence; and (4) approaches to model evaluation.

4.01.10

Predicting the Dissolution of Ag Nanomaterials in Simplified Physiological and Complex Environmental Media Using the Isalos Analytics Platform

A. Papadiamantis, G. Melagraki, NovaMechanics Ltd. / Nanoinformatics; P. Silva, Universidade de Aveiro / CESAM & Department of Biology; Z. Khodaparast, University of Aveiro / Biology; S. Goncalves, Department of Biology & CESAM - University of Aveiro / CESAM & Department of Biology; C. Pinheiro, University of Aveiro; A. Tsoumanis, E. Voyiatzis, NovaMechanics Ltd.; S. Loureiro, Universidade de Aveiro / Biology; E. Valsami-Jones, I. Lynch, University of Birmingham / School of Geography, Earth and Environmental Sciences; A. Afantitis, NovaMechanics Ltd.

Nanomaterials (NMs) dissolution is one of the 15 OECD physicochemical characterisation endpoints required for the risk assessment of NMs and must be reported during NMs registration under REACH. It is a complex process affected by both the NMs characteristics (e.g. surface area, size) and the exposure medium (e.g. pH, ionic strength, natural organic matter, biomolecules) and has been linked with hazardous effects. Understanding the mechanisms driving NMs dissolution and being able to predict NMs behaviour are essential for the design and development of more stable and safer NMs in safe by design strategies. To achieve this, it is necessary to consider a wider range of potential descriptors than the set of physicochemical and media-related descriptors usually considered in literature. Such parameterisation will enable development of computational approaches to facilitate prediction of the dissolution behaviour of NMs in media of varying complexities as part of a broader set of *in silico* toxicity assessment and establishment of integrated approaches to testing and assessment (IATA). We present here a robust, predictive model developed using the experimental monitoring of Ag NMs in simplified biological (ultrapure water, pH: 7.0; simplified gastric fluid, pH: 1.5; neutral lung environment. pH: 7.0) and environmental media used in ecotoxicity testing (ASTM hardwater, pH: 7.5; Artificial Pond Water, pH: 8.0; Steinberg medium, pH: 5.5; Fish System Water, pH: 7.4; Hydra medium, pH: 7.0) for 6 timepoints (0, 2, 4, 8, 24, 48 h). The predictive model was fully validated as per the OECD principles for the validation of QSAR models. Besides the physicochemical characterisation, the dataset was enriched with atomistic, molecular and structural descriptors, to a total of 92 descriptors per NM. The most significant descriptors identified were physicochemical (coating, NM core size, geometric surface area, ζ-potential), molecular (sum of metal electronegativity divided by the number of oxygens present, absolute electronegativity) and assay-related (type of exposure medium, pH). *Acknowledgement* – AGP, EVJ, IL and AA acknowledge funding from the POST-DOC/0718/0070 project, co-funded by the European Regional Development Fund and the Republic of Cyprus Research and Innovation Foundation. PS, ZK, CP, SFG, SL thank FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020). This work was supported by the H2020 project NanoFASE, grant agreement no 646002.

4.01.11

NanoPharos: A Multi-Layered Curated Nanomaterials Database for Machine Learning Modelling

A. Papadiamantis, NovaMechanics Ltd / Nanoinformatics; A. Tsoumanis, E. Voyiatzis, NovaMechanics Ltd.; M. Ha, T. Yoon, Hanyang University / Department of Chemistry; J. Jänes, J. Burk, K. Tamm, University of Tartu / Institute of Chemistry; L.A. Saarimäki, A. Federico, A. Serra, D. Greco, Tampere University; P. Doganis, P. Karatzas, H. Sarimveis, National Technical University of Athens; E. Valsami-Jones, I. Lynch, University of Birmingham / School of Geography, Earth and Environmental Sciences; G. Melagraki, NovaMechanics Ltd. / Nanoinformatics; A. Afantitis, NovaMechanics Ltd.

Alternative testing strategies (ATS) and Integrated approaches to testing and Assessment (IATA) have been proposed to support and substitute the traditional risk assessment of NMs and nano-enabled products. Robust and validated computational approaches will play a critical role in this, but sufficient amounts of nanosafety-related data are needed, which currently remain fragmented and, in most cases, inaccessible or unusable due to insufficient metadata to enable re-use with confidence. Here we present the development of a novel database (DB), NanoPharos (https://db.nanopharos.eu/) that provides harmonised, ready-for-modelling open-access data that can be directly imported into computational workflows. Users can upload and enrich all types of nanosafety and nano-biological interactions data with pre-defined structural, atomistic and molecular descriptors, substantially increasing the added value of their datasets. The DB offers a multi-layered approach employing different levels of complexity to describe the reported NMs, systems and exposure, interaction, (eco)toxicity or mechanistic experiments. The physicochemical and the study/assay properties allow the capture of the maximum information of different NMs or even different batches of the same NM. Integration of both material and system descriptors is essential to uncover relevant mechanisms of actions from the atomic level (of the NMs) through to biomolecule, organelle, cell, organism, population and ecosystem level, to support the development of Adverse outcome Pathways (AOPs) and a wide range of nanoinformatics approaches. The DB offers the opportunity to import and/or enrich their NMs-related datasets with several distinct descriptors: physicochemical, structural, molecular, image desciptors and atomistic descriptors. The exposure, interactions, hazard and risk assessment assay part of the DB handles all relevant data along with assay-related information that can play a significant role in the acquired results and for the development of predictive models. The imported data are quality checked and, if possible, data gap filling approaches are employed or the data are flagged accordingly. This work was funded by the H2020 NanoSolveIT (Grant Agreement No. 814572) and NanoCommons (Grant Agreement No. 731032) projects. A.G.P., A.A., E.V.J. and I.L. acknowledge support from the POST-DOC/0718/0070 project, co-funded by the European Regional Development Fund and the Republic of Cyprus Research and Innovation Foundation.

4.01.12

Ecotoxicological Read-Across Models for Predicting Toxicity of Freshly Dispersed Versus Medium-Aged NMs to Daphnia magna

G. Melagraki, NovaMechanics Ltd; L.A. Ellis, The University of Birmingham / GEES; D. Varsou, NovaMechanics Ltd.; I. Lynch, University of Birmingham / School of Geography, Earth and Environmental Sciences; A. Afantitis, NovaMechanics Ltd.

Nanoinformatics models to predict the toxicity / ecotoxicity of nanomaterials (NMs) are urgently needed to support commercialization of nanotechnologies and allow grouping of NMs based on their physico-chemicals and/or (eco)toxicological properties, in order to facilitate read-across of knowledge from data-rich NMs to data-poor ones. Here we present the first ecotoxicological read-across models for predicting nanomaterials (NMs) toxicity, which were developed in accordance with ECHA's recommended strategy for grouping of NMs as a means to explore *in silico* the effects of a panel of freshly dispersed versus environmentally aged (in various media) Ag and TiO_2 NMs on the freshwater zooplankton Daphnia magna, a keystone species used in regulatory testing. The dataset used to develop the models consisted of dose-response data from 11 NMs (5 TiO_2 NMs of identical cores with different coatings, and 6 Ag NMs with different capping agents / coatings) each dispersed in three different media (a high hardness medium (HH Combo) and two representative river waters containing different amounts of natural organic matter and having different ionic strengths). The experimental hypotheses being tested were (1) that the presence of natural organic matter in the medium would reduce the toxicity of the NMs by forming an ecological corona, and (2) that environmental ageing of NMs reduces their toxicity compared to the freshly dispersed NMs irrespective of the medium composition (salt only or containing biomolecules). Based on ECHA's grouping framework the NMs were grouped into two categories - freshly dispersed and 2-years aged, which have been explored *in silico* to identify the most important features driving the toxicity in each group. The final predictive models have been validated according to OECD criteria and a QSAR model report form (QMRF) report has been produced and made available in the supplementary information to support adoption of the model for regulatory purposes.

Assessment and Management of Wastewater Effluents

4.02.02

Predicting the Aquatic Toxicity of Mixtures of the Major Ions

J. Thomas, Electric Power Research Institute; P.R. Paquin, R. Bubnyte, HDR / Water Quality Modeling

The United States Environmental Protection Agency (USEPA) has released a draft of field-based methods for evaluating regional water quality benchmarks for conductivity. Such benchmarks may be technologically difficult and costly to achieve. Although conductivity provides an integrated measure of major ion concentrations (typically Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-} and HCO_3^{-}) in water, it does not quantify ionic composition, information needed to accurately assess the potential for toxicity. Pursuant to preliminary multi-ion toxicity (MIT) efforts an electrochemical approach was found to provide a viable basis for assessing MIT. The basic idea is that the transepithelial potential (TEP) between an aquatic organism's internal fluids and external environment depends upon the major ion concentrations, and a TEP perturbation may elicit adverse effects. It is assumed that an organism's response to a shift in TEP, for a given end point and exposure duration, occurs in a stressor-dependent manner regardless of mixture composition. The data and modeling analyses in this study summarize efforts to develop a way to predict the MIT of major ion mixtures. The approach has been applied to data for two invertebrates (*Ceriodaphnia dubia* and *Daphnia magna*), a fish (*Pimephales promelas*) and a mayfly (*Neocloeon triangulifer*) exposed to various salt-amended waters. During 2016 and 2018, four alternative modeling approaches, based on concentrations or ion activities and either constant or ion-related membrane permeability coefficients, were evaluated for *C. dubia*. Osmotic effects were also considered. Based on its ease of use and good predictive ability, most recent analyses have used an approach based on ion concentrations and ion-dependent permeability coefficients. During 2017, an approach was developed to utilize commonly available small MIT datasets. In spite of its limitations, this approach facilitates applications to additional species and to chronic datasets, and helps to identify new datasets that will best support development needs. In order for the approach to be amenable to derivation of a water quality criterion, chronic datasets are also being analyzed. The ability of the model to simulate ion-specific responses of test organisms to changes in composition was also assessed. This

presentation will highlight some of the important results of recent investigations as well as the relationship of these efforts to planned physiological investigations.

4.02.06

Source Apportionment for Metals in Wastewaters

A. Peters, wca; I. Wilson, G. Merrington, WCA Environment Limited; S. Comber, Plymouth University / Environmental Science; G. Deviller, DERAC / Environmental Risk Assessment of Chemicals; C.E. Schlekat, NiPERA Inc - test; E.R. Garman, E.T. Middleton, NiPERA Inc / Ecotoxicologist; S. Baken, European Copper Institute; C. Claytor, Copper Development Association Inc. / HESD; L. Assem, International Copper Association; C. Cooper, International Zinc Association / Environment & Sustainability; F. Van Assche, IZA; A.C. Ryan, International Zinc Association; S. Carey, IMOA; J. Mertens, F. Capon, EPMF; E. Nordheim, European Aluminium Association; A. Boullemant, Rio Tinto Aluminium Pechiney

There is an increasing need for the metals sector to better understand the emissions, sources, pathways and fate of metals into the environment. For example, in the EU, this need has recently become evident from a variety of influences including policy evaluations of the Water Framework Directive and Industrial Emissions Directive. However, the relative importance of different sources of metals into wastewater systems remains unclear. It is important to identify and quantify these sources so that appropriate risk management solutions can be identified. If most sources are diffuse as opposed to arising from metal uses, then attempts at management by controlling emissions from metal uses may be ineffective. The primary focus is on emissions in Europe, but also examines the extent to which the findings can be extrapolated to other regions globally. Regions with different water use patterns compared with the UK and Europe were considered as well as regions with limited or less sophisticated wastewater treatment. Domestic sources of some metals are the main source to STPs. Runoff is a consistent and significant source of all metals. Smaller trade discharges below the E-PRTR reporting threshold are probably a significant source, but not yet unaccounted for due to lack of data. The sources of metals into wastewaters are in several cases both important and uncertain. This causes difficulties in identifying areas where action to reduce the emissions of trace metals into the environment is likely to be the most effective. It will not be practically possible to reduce releases of metals from background sources such as the incoming water supply and metals present in the diet, and this means that there is a limit to the extent to which emissions of metals from wastewaters can be reduced. Uncertainties surrounding the importance of metals from road transport sources into urban runoff, and the contribution of metals in urban runoff to the total load in wastewater more generally make addressing this issue even more complex.

4.02.07

A Tiered Approach to Produced Water Risk-Based Assessment

M.G. Smit, Shell International / Risk Science team, Shell health; S. Hayes, More Energy Ltd; W. Brown, IOGP; M. Dorward, More Energy Ltd; S. Pochee, IOGP

As different operators develop in-house approaches and tools for evaluating environmental risk, there are a variety of different assumptions, inputs, levels of conservatism and risk endpoints being applied. The International Association of Oil & Gas Producers (IOGP) recognized a need for alignment to support a more consistent application and communication of risk concepts applied to offshore produced water discharges. In 2020, as a result of a 2-year project, IOGP published a new guideline for risk-based assessment of offshore produced water discharges. This guide describes industry good practice and aims to increase understanding and acceptance of RBA techniques internationally, as an effective management tool. The RBA process aligns with the general use of risk assessment, management and mitigation approaches by oil and gas operators. By incorporating different lines of evidence and using location-specific inputs to assess PW discharges, RBA goes beyond end-of-pipe standards and provides a framework whereby the overall acceptability of the discharge can be demonstrated, and/or potential improvements identified and managed. An important element of this guidance is a 'tiered approach' that will help practitioners executing RBA at different stages of project development. Usually these stages are characterized by different levels of data availability. For instance, in the design stage no produced water samples can be taken and analysed for toxicity or chemical composition and hazardous properties need to be assessed on assumptions on composition and/or reference data. The tiered approach is not only applicable to the different stages of project development. For existing facilities this approach can also be followed to assure an appropriate level of effort is applied so that low risks situations can be screened out at an early stage, while situations with a higher level of risk can be prioritized and assessed in more detail. This presentation provides a summary of the proposed tiered approach and provides example assessments conducted at each tier. Industry alignment will support more consistent global and corporate application of RBA concepts in places where this is yet to become established practice and where the regulatory framework is evolving.

4.02.08

Mineral and Geochemical Properties, Including Particle Characteristics, of an Old Coal Ash and Slag Deposit in Istria (Croatia)

M. Petrović, Ruđer Bošković Institute; M. Dolenec, University of Ljubljana Faculty of Natural Sciences and Engineering; M. Dutour Sikiric, Rudjer Boskovic Institute; V. Strasser, Ruđer Bošković Institute; G. Medunić, University of Zagreb Faculty of Science; . Fiket, Ruńer BoxkoviD Institute

Utilization of coal combustion by-products (CCB) has been a major goal in coal waste management where currently 50-60% generated by-products are utilized. However, it is estimated that 1 billion tonnes of CCB are generated yearly**,** leaving half for disposal in landfills or ash impoundments. TodayÆ_s regulations require proper lining and leachate collection which should ensure site safety. However, the disposal of coal combustion products in unlined landfills and ash ponds was a common practice in the past. Therefore, old and unmonitored landfills present a large threat to the environment. Several of those old landfills are located in historical mining and coal burning area in the Istrian peninsula (Croatia). Previous studies already reported elevated concentrations of metals in soils, waters, and biota, indicating contamination of local areas by leaching of elements from waste. However, the processes that govern the release of toxic elements have not been addressed. The composition of coal combustion by-products varies greatly, therefore elements can have different mobility depending on the sample. This presentation aims to address those processes by mineralogical and geochemical analysis of waste, with the addition of particle characterization. The mineralogical analysis indicated secondary mineral phases which could reduce the mobility of ions (As, Se, Mo). However, other contaminants may be transported by colloids, which are most stable under alkaline conditions. Besides giving insight into the geochemical processes of leaching, the findings are useful for today's landfills predictions as they represent aged waste with altered intrinsic characteristics.

4.02.09

Ecological Comparison of the French Macroinvertebrate-Based Index and the New Multimetric Index I2M2 (Case Study)

I. Elfeky, ECOMA; M. Lot, TOTAL SE; J. Cayrou, ECOMA; J. Dalens, F. Mounède, Total; L. Kermarrec, ECOMA; A. Basseres, Total / Environment

In France, the macroinvertebrate-based index IBGN has been successfully and widely used but no longer fulfils the Water Framework Directive requirements. In this context, a new field protocol has first been adopted in order to be more representative of the whole station. It is ordinarily called MPCE and allow to calculate IBG-MPCE index with the same scoring system as IBGN. Then, the new multimetric index I2M2 has been created in agreement with WFD requirements and is now officially replacing the previous indices. The present case study focuses on the comparison between IBGN, IBG-MPCE and I2M2 for two sites submitted to an industrial discharge. Results of the first site, presenting extreme hydro morphological conditions, show that the three indices lead to the same ecological conclusions (i.e. low sensitivity, low diversity, poor/bad ecological quality classes) and do not reveal a notable difference between upstream and downstream stations. The second site results show a better ecological quality (i.e. presence of sensitive taxa, good/high ecological quality classes). IBGN and IBG-MPCE scores and ecological descriptors are similar between upstream and downstream stations whereas three I2M2 metrics decrease (i.e. Average Score Per Taxon, Richness and Shannon-Weaver). This observation could be related to the difference in calculation methodology. Indeed, by giving more ecological information, I2M2 seems to be more demanding than IBGN and IBG-MPCE since it allows to better discriminate the downstream station from the upstream one. A higher discrimination efficiency of I2M2 compared to IBGN has also been described by Mondy et al. (2012) that corroborates these results. To conclude, within the regulatory transition to this new multimetric index, a different ecological state should be expected by future users. Moreover, no difference is detected between IBGN and IBG-MPCE indices. Therefore, in the case of a historical monitoring, it is recommended to calculate for first years IBG-MPCE index as a well-known reference in addition to I2M2 index.

4.02.11

Bioanalytical Characterization and Source Identification of a High Glucocorticoid Contamination in a River Under Urban and Industrial Pressures

C. Chardon, INERIS / UMR-I 02 SEBIO (Environmental Stresses and Biomonitoring of Aquatic Ecosystems; C. Gardia-Parège, Université de Bordeaux, CNRS / UMR 5805 EPOC, LPTC; E. Maillot-Maréchal, INERIS / UMR-I 02 SEBIO (Environmental Stresses and Biomonitoring of Aquatic Ecosystems; M. Debret, Normandie Université UNIROUEN / CNRS M2C; H. Budzinski, Université de Bordeaux / UMR EPOC LPTC; S. Ait-Aissa, INERIS / UMRI SEBIO ECOT

Synthetic glucocorticoids (GCs) are a group of steroids that are massively used as anti-inflammatory drugs to treat a broad spectrum of deseases such as asthma or arthritis. They may enter surface waters *via* urban, hospital and/or pharmaceutical industry effluents and were already detected in surface waters at concentrations ranging from < 1 to tens of ng/L. In organisms, GCs are designed to act through the glucocorticoid receptor (GR) and some studies have reported adverse effects

on aquatic vertebrates, such as fish. Overall, there is still a need for knowledge on the sources and driver compounds for GR activity in different contexts. In the frame of a French national survey of EDCs in surface waters using *in vitro* bioassays, we previously identified a hotspot river site with high GR activity. To further identify the source(s) and the nature of this contamination, an investigative monitoring based on bioassay (MDA-Kb2) and chemical analyses was implemented in the current study. A contamination mapping was established on the basis of bioassay data, along a 40 km streatch upstream and downstream of the contaminated site allowing identification of the discharge of the contamination in the river and of its highly probable industrial origin in the sewerage network, although the contribution of waste waters from a hospital could not be excluded. By using a suspect screening approach based on HR-MS, fluticasone propionate as well as few other GCs were identified in the samples. Mass balance analysis based on quantitave chemical analysis confirmed fluticasone propionate main role in the measured GR activity. The high mobility and persistence of this contamination points out possible high environmental concern in terms of its fate and hazard for aquatic organisms.

Bioaccumulation Assessment and BiER (Biotransformation and Elimination Rate) in Regulation of Chemicals

4.03.01

Bioaccumulation of Pollutants in Aquatic Ecosystems: Multimedia Passive Equilibrium Sampling

E. Rojo-Nieto, Helmholtz Centre for Environmental Research GmbH - UFZ / Department Cell Toxicology; M. Muz, UFZ - Helmholtz Centre for Environmental Research / Effect Directed Analysis; J. Watzke, Helmholtz Centre for Environmental Research GmbH - UFZ / Department of Ecological Chemistry; A. Jahnke, Helmholtz Centre for Environmental Research - UFZ GmbH / Ecological chemistry

Passive Sampling Devices based on polymers such as silicone, if operated in equilibrium mode, offer a superior approach to assess bioaccumulation when compared with exhaustive extraction methods, since they avoid the need for normalization of the total concentrations of chemicals to the main sorptive phase, e.g. lipids in biota or organic carbon in sediment. Passive equilibrium sampling (PES) allows to reach equilibrium in biota from lean tissue to lipid-rich tissue, and thus to compare a set of species across trophic levels. In this study, a small Swedish lake (Ången) which is a well characterized closed ecosystem has been selected, and ten different species were studied: pike, perch, pikeperch, bream, roach, eel, ruffe, crayfish, and two species of freshwater mussels. Either muscle tissue or the entire body (carcasses) were investigated, from single or pooled individuals, depending on their size and total mass. The samples were homogenized and their lipid content was measured. In order to calculate their ecosystem-specific trophic level, $\delta^{15}N$ and $\delta^{13}C$ were also determined. The homogenized tissues were sampled using silicone sheets with three different thicknesses: 125, 250 and 350 µm to confirm equilibrium partitioning between the samples and the silicone, relocating the samplers 6 times per day over 7 days. The silicone sheets were extracted and the extracts were cleaned-up prior chemical analysis. Five sediment samples from the study area were sampled using silicone-coated glass jars, an already well stablished method for PES in this matrix. Different approaches were tested for water sampling: a Dynamic Passive Sampler device, silicone-coated jars and Large Volume Solid-Phase Extraction. The analysis and quantification of a wide range of compounds, including PAHs, PCBs, PBDEs, pesticides, musk compounds, sunscreens and antioxidants, were achieved with a gas chromatograph coupled to a high-resolution mass spectrometer (GC-HRMS Orbitrap Q-Exactive, Thermo Fisher). The present work accomplishes the challenge of achieving equilibrium partitioning for a broad range of compounds using PES in homogenized biota with different lipid content, from lean tissue to lipid-rich tissue. PESs allow to compare the thermodynamic status of contaminants in biota from different trophic levels directly, and thus, assess potential biomagnification. PESs also allow to compare directly with abiotic compartments, such as sediment.

4.03.06

Facilitating the Calculation of Bioaccumulation Factors of Active Substances Within Living Organisms

A. Ratier, UMR CNRS 5558 Laboratoire de Biométrie et Biologie évolutive (LBBE) / Laboratory of Biometry and Evolutionnary Biology; C. Lopes, G. Multari, Université Lyon 1, UMR CNRS 5558 / Laboratory of Biometry and Evolutionnary Biology; V. Mazerolles, ANSES / Agency for Food, Environmental and Occupational Health & Safety; P. Carpentier, ANSES / Regulated Products; S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology

Today, ready-to-use convenient tools in ecotoxicology to diagnose and predict the accumulation and effects of chemical substances on living organisms, accounting for various exposure situations that are known to be complex (routes of exposure, metabolization processes, cocktail effects, etc.) are extremely limited. Among available methods, toxicokinetic-toxicodynamic (TKTD) models are now strongly recommended to describe the link between exposure and effects on individual life-history traits according to time from experimental data collected through standard toxicity tests. In particular, the TK part is used to relate the exposure concentration to the internal concentration within organisms, considering various processes such as accumulation, depuration, metabolism and excretion (ADME). Some regulations (*e.g.*, plant protection products in marketing authorisation applications) require a bioaccumulation test on fish according to OECD Test guideline 305, which consists in an accumulation phase measured by a depuration one. The concentration of the substance within fishes is followed over time during both phases thus allowing to model the time course of the exposure within organisms, summarized by bioaccumulation factors. On a regulation point of view, these factors are key decision criteria for further estimating the concentration of active substances present in food items of vertebrates.This presentation will illustrate a ready-to-use web tool, $MOSAIC_{bioacc}$, automatically providing estimates of bioaccumulation factors associated with their uncertainty, as well as the fits of TK models and the estimation of their corresponding kinetic parameters. This tool facilitates the daily work of regulators, but also of any user, who benefits of a freely available and user-friendly on-line interface avoiding investing into modelling technicalities. $MOSAIC_{bioacc}$ allows consideration of the growth of organisms, as well as several uptake routes, and offers the possibility to choose sub-models according to the processes considered. This tool can also be applied to terrestrial organisms exposed to contaminated soils, or birds exposed to atmospheric contamination. $MOSAIC_{bioacc}$ goes beyond the OECD Test guideline 305 in including biotransformation processes, thus allowing users to easily performed a TK analyses accounting for metabolites. $MOSAIC_{bioacc}$ is available at https://mosaic.univ-lyon1.fr/bioacc.

4.03.11

Use of One and Multi-Compartments Toxico-Kinetic Modelling to Understand Cd and Hg's Organotropism and Fate in Gammarus fossarum

O. Gestin, Université Lyon 1, UMR CNRS 5558; T. Lacoue-Labarthe, LIENSs CNRS & Université de la Rochelle UMR 7266; M. Coquery, INRAE Lyon / Unité de Recherche Milieux Aquatiques, Ecologie et Pollutions (MAEP); N. Delorme, INRAE Lyon / UR MALY Laboratoire Ecotoxicologie; L. Dherret, INRAE Lyon / Unité de Recherche Milieux Aquatiques, Ecologie et Pollutions (MAEP); T. Ciccia, O. Geffard, INRAE Lyon / UR RIVERLY Laboratoire Ecotoxicologie; C. Lopes, Université Lyon 1, UMR CNRS 5558 / Laboratory of Biometry and Evolutionnary Biology

The use of sentinel species as bioindicators and biomonitors has been increasing for several decades. To date, almost all literature about metals' bioaccumulation concerning freshwater amphipods, and more generally freshwater invertebrates, are focused on the entire organism. Indeed, little is known about relations between external exposure concentration and their biodistribution among different tissues of freshwater invertebrates. Toxicokinetic (TK) models are powerful tools to formalize and predict how a contaminant is bioaccumulated. Two types exist: i) one compartment TK models that consider an entire organism, a tissue or an organ, as a homogeneous "box" for which uptake and elimination are only occurring between this "box" and the environment; ii) multi-compartments TK models that describe the distribution of the contaminant among different tissues of an organism. Each type has advantages and limits: i) one-compartment models are easy to implement and use but cannot give a precise view of ADME processes; ii) on the contrary, multi-compartments models are much more complex to set up but also more realistic from a physiological point of view. The aim of this study is i) to investigate the biodistribution dynamic of Cd and Hg by quantifying their accumulation and depuration in *G. fossarum*'s organs, to build one and multi-compartments TK models; and ii) to improve knowledge on the fate of these compounds in this species. Gammarids were exposed to dissolved Cd (11.08 ± 1.22 µg.L^{-1}) for 7 days or dissolved Hg (0.268 ± 0.129 µg.L^{-1}) for 4 days and then placed for 14 and 21 days, respectively, in clean water (depuration phase). At each sampling days, three pools of several gammarids ($10 \leq n \leq 18$) were dissected and organs (caeca, cephalon, intestine and remaining tissues) separated before analyses by an ICP-TQ-MS, for Cd, and a Direct Mercury Analyzer 80, for Hg. Results showed that exposure of *G. fossarum* to high concentrations of dissolved metals allows us to determine that i) compared to uptake and elimination rates of other aquatic organisms, *G. fossarum* take up the Cd as efficiently as the mussel *M. galloprovincialis*, but eliminate it more rapidly, ii) organs which accumulate and depurate the most, in terms of concentrations, are caeca and intestine for both metals; iii) for Hg the one-compartment TK models is the most relevant, while the multi-compartments TK model allows a better fit to the Cd data, demonstrating dynamic transfer of Cd among organs.

4.03.13

Validation of the Hyalella azteca Bioconcentration Test (HYBIT)

C. Schlechtriem, Fraunhofer IME / Department Bioaccumulation and Animal Metabolism; V. Kosfeld, Fraunhofer Institute for Molecular Biology and Applied Ecology IME / Bioaccumulation and Animal Metabolism; P. Pandard, INERIS / Direction of Chronic Risk Assessment; C. Rauert, Umweltbundesamt /

International Chemicals Management; S. Walter-Rohde, German Federal Environment Agency (UBA)
Bioconcentration factors (BCF) for regulatory purposes are conventionally determined experimentally by aqueous exposure bioconcentration fish tests according to OECD test guideline (TG) 305. Fish bioconcentration studies are time consuming, expensive, and use vertebrate organisms in the range of 100-200 organisms per study. The availability of alternative methods may help to reduce the use of fish for BCF testing. The *Hyalella azteca* Bioconcentration Test (HYBIT) provides a non-vertebrate alternative for fish bioconcentration studies. The suitability of *H. azteca* as an alternative test organism for bioconcentration studies was recently investigated. Eighteen substances of different hydrophobicity (log K_{OW} 0.7 - 7.8) were tested under flow-through or semi-static conditions to determine steady-state and kinetic bioconcentration factors (BCF_{SS} and BCF_K). It has been shown that the BCFs obtained from bioconcentration studies with the freshwater amphipod *H. azteca* correlate significantly (r^2=0.69) with fish BCF values described in the literature. Thus, H. azteca BCF values can be assessed in accordance with the standard B criteria, e.g. BCF > 2000 (REACH), and thereby enable the prediction of B or non-B classification in the standard fish test. Generally, technical (smaller-scale test systems) and economic reasons (less time-consuming, more cost-efficient) favour the use of *H. azteca* over fish in bioconcentration studies. In addition, significantly smaller amounts of test substance are required compared to the flow-through test with fish. A protocol for carrying out bioconcentration tests with the aquatic invertebrate species *H. azteca* under standardized conditions has been developed as part of the project CEFIC-LRI ECO40. This protocol includes both, the flow-through and semi-static test design. Validation was needed to confirm the transferability of the test protocols and to prove the reproducibility of the results obtained in order to support the development of a new OECD TG. For this purpose, a multi-laboratory ring trial involving the HYBIT was carried out in 2020. The ring test has confirmed the high potential of the *Hyalella azteca* Bioconcentration Test (HYBIT) to be used as a non-vertebrate alternative for bioconcentration studies. The transferability of the test protocols (semi-static and flow-through approach) as well as the reproducibility of the results obtained was proven supporting the development of a new OECD TG.

4.03.15
Replacing the Need for In Vivo BCF Studies - the Use of In Vitro Biotransformation Data in a WoE Approach for the REACH Registration of Fragrance Chemicals
K. Jenner, Givaudan / Global Regulatory Affairs & Product Safety; H. Laue, L. Hostettler, Givaudan Schweiz AG / Fragrances S & T; G. Sanders, Givaudan International SA / Regulatory Affairs & Product Safety; G. Kreutzer, Givaudan SA / Regulatory Affairs & Product Safety; A. Natsch, Givaudan Schweiz AG / Fragrances S &T
The REACH Regulation promotes and supports the 3Rs principles (reduction, refinement, replacement) with obligations to reduce animal use via data sharing, read-across and the implementation of alternative methods. For chemicals manufactured or imported at ≥ 100 t/y information on bioaccumulation in aquatic species is required, unless the substance has a low potential for bioaccumulation (log Kow ≤ 3). If new animal data are needed, an in vivo bioaccumulation test according to OECD 305 TG is the preferred option. The number of fish used in these studies is approximately 100 to 150 per study. In vitro assays to determine biotransformation rates by primary hepatocytes (RT-HEP) or liver S9 subcellular fractions (RT-S9) from rainbow trout have been developed and validated culminating in two new OECD test guidelines (TG 319A and 319B, respectively). In this poster, we present how we have used in vitro biotransformation data in a weight-of-evidence approach for the REACH registration of fragrance chemicals to replace the need for in vivo BCF studies. The case studies demonstrate: o Read-across justification based on similar in vitro intrinsic clearance rates for target substance and source substance with in vivo BCF data. o Application of in vitro-in vivo extrapolation to predict BCFs for use in a WoE approach along with reliable QSAR predictions. o The determination of in vitro RT-S9 data for one member of a group of structurally related chemicals to support rapid biotransformation of the analogues and hence the use of predicted BCFs from QSAR models that take biotransformation into account rather than those based solely on log Kow. o Identification of metabolites in in vitro systems to support rapid excretion in vivo. o In vitro RT-S9 testing of primary biodegradation products to address the PBT assessment. These approaches provided strong evidence that the substances were not B (i.e. BCF estimates well below the REACH B-criterion of 2000 L/Kg) and that there was no risk to higher members of a food chain through secondary poisoning. Therefore, in vivo BCF tests were not considered necessary and were waived. The number of fish that were saved by applying animal-alternative methods to the REACH registered substances covered by the above case studies was approximately 1300-1950 and demonstrates that alternative, yet conservative, evaluation approaches can be adopted to fill higher-tier hazard end-points without compromising safe use.

4.03.21
Food Web on Ice: An Application of a Benchmarked Trophic Magnification Factor Approach to Emerging Substances
V. Kosfeld, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; H. Ruedel, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Environmental Specimen Bank and Elemental Analysis; C. Schlechtriem, Fraunhofer IME / Department Bioaccumulation and Animal Metabolism; C. Rauert, Umweltbundesamt / International Chemicals Management; J. Koschorreck, Umweltbundesamt / Inland Surface Waters
Among alternative bioaccumulation endpoints that are proposed for usage in weight of evidence approaches under REACH, the trophic magnification factor (TMF) represents the most complex one. It describes accumulation processes in an entire food web in the field and can thus deliver information that is more realistic. In addition to REACH, the TMF is an interesting tool for the normalization of biota concentrations to one common trophic level in the context of the Water Framework Directive (WFD), enabling the comparison of the normalized biota concentrations with Environmental Quality Standards (EQS). A standardization of TMF studies and suggestions on what to consider upon conducting or evaluating them have already been given in the literature. The food web on ice approach presented here applies these suggestions and couples them with the sample preparation and storage protocols commonly applied in the German Environmental Specimen Bank (ESB). Standardized, cryogenic homogenates of a plankton fraction, two mussel samples and 12 whole fish samples were collected from a German lake and are now available on demand for a variety of analyses. In a first step, a plausibility check of this approach was performed by determining TMFs for well-known persistent organic pollutants (POPs). The TMFs of these benchmarking compounds all exceeded the threshold of "TMF=1" that indicates trophic magnification, confirming the suitability of the sample set to detect accumulating substances. In a second step, analyses for emerging substances were performed. A screening for some pharmaceuticals revealed that these compounds could not be detected in most of the whole fish samples, which could be due to matrix effects that obstructed the detection. The analysis of the cyclic volatile methyl siloxane decamethylcyclopentasiloxane (D5) revealed trophic dilution of the substance in the sampled lake food web. The analysis of PBDE congener #99 provided a good example of how species-specific metabolism can influence the accumulation pattern in the food web, a process which has been examined in several in vitro experiments described in the literature. Based on the results obtained, we are able to demonstrate the advantage of the food web on ice approach that enables a full comparison of all analyses applied to the food web data. For substances that have a more complex mode of action, additional analyses might be needed that can aid in explaining the observed patterns.

4.03.23
Modeling Chlordecone Transfer in Ruminants
A. Fourcot, Université de Lorraine - UdL; C. Feidt, University of Lorraine - UdL / INRA URAFPA; G. Rychen, A. Fournier, Université de Lorraine - UdL / INRA URAFPA
The use of chlordecone (CLD), a chlorinated polycyclic pesticide used in the French West Indies banana fields between 1972 and 1993, resulted in a long-term pollution of agricultural areas. This environmental scandal is also a health scandal since this molecule transfers from soils to the food chain. Animal products contribute to human exposure and the issue is therefore to reduce the contamination of these products. To protect the consumer's health and to assist the food-producing sectors, a decision support tool to decontaminate animals and ensure carcass conformity (under the Maximum Residue Limit, MRL) has been developped. Currently, the priority is related to the cattle sector. This work focused on ewes, used as an animal model for ruminants. The aim of this study was to establish a physiologically based pharmacokinetic model (PBPK) in ewes. To be able to develop this tool, *in vivo* experiments were performed in adult ewes to characterize toxicokinetic of CLD and calibrate the model. First, two experiments consisting of monitoring serum and fecal elimination kinetics following a single intravenous administration of CLD or CLDOH (chlordecol) were performed. Compartmental approaches were used to estimate toxicokinetic parameters of CLD and CLDOH. Secondly, an experiment involving sequentially slaughters during contamination and decontamination phases allowed to characterize the distribution dynamics of CLD and to calculate partition coefficient of CLD in tissues. Data from the last-mentioned experiment were also used to adjust some parameters of the model. All toxicokinetic results obtained in ewes were used to develop a physiologically based pharmacokinetic (PBPK) model to predict CLD concentrations in serum, liver, muscle, adipose tissue and faeces over time. The immediate applications of this model are to estimate withdrawall times to reach the MRL and CLD amounts excreted by animals during depuration periods. Once validated, this model will be used to develop decision support tools to assist animal sectors in CLD contaminated areas and to set up decontamination. The next steps will be to extrapolate this model to adult cattle and to growing cattle.

Bioremediation and Phyto-Remediation of Contaminated

4.04.01

Long-Year Monitoring of Transformation and Migration of Polychlorinated Biphenyls (PCBs) at a Historically Contaminated Site in t. Serpukhov (Russia) and the Approach for Its Phytoremediation

G. Vasilyeva, Institute if Physicochemical and Biological problems in Soil Science; M. Lapushkin, All-Russian Research Institute of Hydraulic Engineering and Land Reclamation, Moscow; E. Strijakova, Institute of physicochemical and biological problems in soil science RAS; A. Lankin, nstitute of Physicochemical and Biological Problems in Soil Science, RUS, t. Pushchino

Use of PCB-based insulating oil for production of power electric capacitors at the plant "Condenser" until 1988 caused the soil pollution in t. Serpukhov (Russia) with PCBs, which are classified as super-toxicants. The goal of our long year experiments was to study the ecological situation resulting from the operation of this plant and to find out an effective and cheap approach to remediate this area. It was shown that the soil on a site of 4.7 hectares located nearby the plant remained highly contaminated from 1999 until 2019. The PCB concentrations in the soils slowly reduced in the central part of the site due to their microbial degradation but increased (up to 3740 mg kg^{-1}, i.e. 62000 MPL) in the south part of the site due to PCB transfer with ground and surface waters. Microfield experiments were carried out in 2020 to study the possibility of phytoremediation of this territory using basket willow (*Salix viminalis*) and black poplar (*Populus nigra*). The experiments were carried out in bottomless plastic vessels with an area of 0.1 m^2, which were filled with the three soils (A, B or C) sampled from the same contaminated site. The total initial PCB concentrations in those soils were 24; 5 and 5390 mg kg^{-1} respectively. The control samples remained unplanted, while the other samples were planted by 1-year rooters of *P. nigra* or 6-month-old rooters of *S. viminalis* (alone or together with some organic additives). The experiments started in June, and the trees were taken out in October, the tree height and weight of their roots were measured, and the content of PCB congeners and agrochemical parameters were determined in the soils. From the very beginning, all those soils demonstrated elevated phytotoxicity, especially the highly contaminated soil C - up to 65%. A strong inhibition of photosystem 2 activity and tree growth have been also demonstrated in the same soil. On the other hand, the cultivation of black poplar and basket willow in these soils, especially with those organic additives, significantly reduced the phytotoxicity of the soils, which contributed to an increase in the growth parameters of the trees, as well as an increase in the dehydrogenase activity of the soils. These treatments led to a noticeable decrease in the total concentration of PCBs in all the soils, mainly due to the accelerated biodegradation of 3CB and, to a lesser extent, of 4CB. Thus, the effectiveness of the phytoremediation has been proved for the moderately contaminated soils.

4.04.02

Effectiveness of Poplar-Assisted Bioremediation Under Different Concentration of Persistent Organic Pollutants

V. Ancona, Water Research Institute - Italian National Research Council / Water Research Institute; P. Grenni, National Research Council of Italy (CNR) / Water Research Institute; G. Aimola, Italian National Research Council / Water Research Institute; G.L. Garbini, National Research Council of Italy / Water Research Institute; I. Rascio, D. Losacco, Italian National Research Council / Water Research Institute; L. Rolando, National Research Council of Italy / Water Research Institute; V. Uricchio, Italian National Research Council / Water Research Institute / Water Research Institute (IRSA); A. Barra Caracciolo, National Research Council - Water Research Institute / Water Research Institute

Plant-assisted bioremediaton (PABR) is an effective green technology for removing contaminants from soils. It relies on the sinergistic interactions between roots and microrganisms of the rhizosphere for removing, transforming and contain toxic compounds. Poplars were proved to be very efficient in promoting degradation of chloride organic pollutants such as lindane and polychlorobyphenils (PCBs) in both laboratory and field scale experiments. Due to their high molecular stability, low solubility in water and high tendency to adsorb on particulate phase, PCBs are extremely hard to remove from soil. In this work, some crucial aspects of the PABR strategy in recovering two historically contaminated areas, located in Southern Italy, characterized by different PCB amounts (medium and high concentrations, respectively), are illustrated.Two poplar treated areas, inside the survey site, were set up at a distance of two years from each other (2013 and 2015, respectively). In the first one, characterized by a medium PCB contamination level (PCB ~250 ng/g) about 600 poplar cuttings were planted; instead, in the second one, affected by high PCB contamination (PCB >2000 ng/g), about 750 poplar cuttings were planted.Chemical investigations of soil properties and pollutants (PCB congeners) were carried out before plantation and at different times after poplar planting, in both the PABR treated areas. Moreover, microbial analyses such as the total microbial abundance (DAPI counts), cell viability (live/dead method), microbial activity (dehydrogenase activity) and the sequencing of 16S DNA (Miseq-Illumina) were performed for evaluating microbial community structure and functioning in the poplar rhizosphere. Moreover, in March 2019, a compost (CMP) or biochar (BC) treatment was also performed on some contaminated plots inside the highest polluted poplar treated area in order to increase soil quality and promote PCB degradation.The results obtained in the first poplar treated area, affected by medium PCB contamination, showed the effectiveness of PABR in restoring this area, promoting active bacteria able to degrade and contain pollutants and increase soil quality. In the second poplar treated area, the high PCB levels and the soil nutrient impoverishment favouring stress conditions for poplars limiting their growth. The addition of the fertilizers (compost or biochar) promoted an increase in soil quality and microbial activity promoting the bioremediation processes.

4.04.12

The Potential of Super Absorbent Polymer (SAP) As Supplement Substrate of Constructed Wetlands (CWs) to Retain Pesticides From Agricultural Runoff

Y. Jing, Helmholtz Center for Environmental Research (UFZ) Leipzig; A. Butkovskyi, Helmholtz Centre for Environmental Research UFZ / Dept. of Environmental Biotechnology; M. Krauss, Helmholtz centre for environmental research - UFZ / Department of Effect-Directed Analysis; K. Nowak, TU Berlin / Institute for Environmental Research (Biology V); A. Miltner, Helmholtz Centre for Environmental Research UFZ; T. Eggen, Bioforsk; M. Kästner, Helmholtz Centre for Environmental Research UFZ / Dept Environmental Biotechnology

Agricultural runoff can carry pesticides from the field into aquatic ecosystems, posing toxicological hazard to living beings. CWs are widely used to treat agricultural runoff contaminated by pesticides and nutrients. In this study, we propose SAP, a cross-linked hydrophilic polymer, as a supplement to CWs substrates aiming to promote the removal of pesticides, especially hydrophilic ones. We hypothesize that SAP can retain pesticides in aqueous solution by sorption; and when SAP is applied in CWs, it will not only support the growth of plants but also enhance the removal of pesticides. Sorption batch experiments were conducted to test the retention capacity of SAP for MCPA, bentazone, metalaxyl, propiconazole and imidacloprid. Furthermore, SAP was applied to lab-scale horizontal subsurface flow CWs for pesticides treatment. SAP showed high retention capacity both for hydrophobic and hydrophilic pesticides by sorption. The retention capacity of SAP for the pesticides followed the order: propiconazole > imidacloprid > metalaxyl > MCPA > Bentazone. Pesticides with higher $logK_{ow}$ and neutral state were more likely to be retained by SAP. Application of SAP to CWs enhanced the retention of the pesticides. The order of pesticides removal by CWs with SAP was in agreement with their retention in SAP by sorption. Therefore, SAP can be considered as supplement substrate used in CWs to improve the removal of pesticides from agricultral runoff.

4.04.13

Evaluation of the Sorption Capacity of Sustainable Materials for Nature-Based Solution in Water Reuse

C. Valhondo, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Department of Geosciences/ Hydrosciences; G. Duporté, University of Montpellier / UMR 5569 Hydrosciences Montpellier; L. Luquot, Université de Montpellier- CNRS / Department of Geosciences; E. Gomez, HydroSciences Montpellier, IRD, CNRS, University of Montpellier / UMR 5569 Hydrosciences Montpellier

Population growth and climate change have caused an increase in the water demand. Water reuse has become an urgent need to satisfy this growing demand of water especially in the most populated regions of arid and semiarid zones . The main sources of water available for reuse are impaired waters or effluents from wastewater treatment plants (WWTP). Conventional WWTPs have not been designed to efficiently remove Contaminants of emerging concern (CECs). and therefore a broad range of such contaminants are present in these waters . Nature-based solutions (NBS) provide environmental. economic and energetic benefits compared to conventional technologies. Soil Aquifer Treatment (SAT) is a NBS technique that consists in favouring the infiltration of the treated wastewater through the vadose zone and its subsequent travel through the aquifer. The quality of the recharged water improves during the infiltration and the concentration of suspended solids, CECs, microbial pathogens, and inorganic nutrients are reduced. The main processes affecting the behavior and fate of CECs during infiltration are biodegradation and sorption. The installation of reactive barriers based on sustainable materials in the base of the infiltration basins of SAT systems has been proposed as a tool to accelerate the water quality improvement by increasing the diversity and biomass of the microbial community and the sorption sites. However, the diversity of materials available for barriers construction, both by their nature and by the range on their sorbing properties, constitute a challenge to properly determine their role in the removal of and their potential release of unexpected pollutants to the aquifers. Indeed, the selection of the most appropriate materials for reliable infiltration bassins needs appropriate characterisation before any in situ application in order to favorise suitable risk/benefit balance. In the present study several materials intended for reactive barriers design were selected for an in deep characterization: i/ of their main physico-chemical properties based on their broad diversity of origin and ii/ of their sorption capacity. The results provide key parameters affecting and controlling the CECs behavior and guide the

choice of materials for the constitution of filtering barriers. Their granulometry, hydraulic resistivity, etc, will be considered for the implementation of effective reactive barriers as well.

4.04.14

Humic Substances Mitigate the Radionuclide Effects on Water Microorganisms

N. Kudryasheva, Federal Research Center Krasnoyarsk Science Center of the Siberian Branch of the Russian Academy o; T.V. Rozhko, Krasnoyarsk State Medical Academy; O. Kolesnik, Institute of Biophysics Siberian Branch of RAS

It is known that microorganisms of soils and aqueous media are sensitive to the presence of humic substance (HS), products of natural decomposition of organic matter, which play a role of natural attenuators of environmental toxicity. We study the effects of alpha- and beta- emitting radionuclides (americium-241 and tritium, respectively) in the presence of HS under the conditions of low-dose exposures (< 0.1 Gy). Luminous marine bacterium *Photobacterium phosphoreum* was applied as a model unicellular water microorganism to monitor toxicity and activation ability of the radionuclide solutions. To imitate the marine environment for bacterial cells and to balance osmotic processes, the 3% NaCl solutions were used. The bioluminescence response of the marine bacteria to americium-241 and tritium corresponded to the "hormesis" model: it included stages of bioluminescence inhibition and activation, as well as the absence of the effect. HS were shown to decrease the inhibition and activation effects of the radionuclides on the bacterial luminescence. The changes were shown to be related with the accumulation of americium-241 in the bacterial cells [1]. The correlations between the bioluminescence intensity and the content of Reactive Oxygen Species (ROS) were found in the radioactive bacterial suspensions in the presence of tritiated water [2]. The results demonstrate an important role of HS in natural processes in the regions of low radioactive contaminations: HS can mitigate radiotoxic effects and adaptive response of microorganisms to low-dose radioactive exposure of alpha- and beta- types. The involvement of ROS to these processes was demonstrated. The results can provide a basis for predicting a response of living organisms to radiation at large territories infected with low-intensity radiation after accidents, discharges of nuclear plants, or underground mining of natural resources. [1] T.V. Rozhko, L.G. Bondareva, O.A. Mogilnaya, G.A. Vydryakova, A.Ya. Bolsunovsky, D.I. Stom, N.S. Kudryasheva. Detoxification of Am-241 Solutions by Humic Substances: Bioluminescent Monitoring. Anal.&Bioanal.Chem. 2011, V.400, N2, p.329–334. [2] T. V. Rozhko, O. V. Kolesnik, G. A. Badun, D. I. Stom and N. S. Kudryasheva. Humic Substances Mitigate the Impact of Tritium on Luminous Marine Bacteria. Involvement of Reactive Oxygen Species. Int. J. Mol. Sci. 2020, 21, 6783; doi:10.3390/ijms21186783

4.04.16

Evaluation of Ecosystem Functioning and Pollutant Norms Is an Important Issue at Remediation of Oil Contaminated Soils

E. Kovaleva, M.V. Lomonosov Moscow State University; M. Guchok, ANO Ecoterra / Department of soilscience; S. Trofimov, M.V. Lomonosov Moscow State University / Department of Soil Science

Oil and gas extraction activities result in total petroleum hydrocarbons (TPH) contaminations of soils. The quality and quantity of TPH in soils is an important issue to be investigated for understanding and improving the bioremediation strategies; study of ecosystem functioning and pollutant norms in soils is challenging because TPH could be highly toxic. Soil properties, characteristics and functions determine ecosystem functioning as a whole. Selecting biological indicators that are sensitive to a particular contaminant is a crucial task. The objectives of the study were to evaluate the quality of TPH contaminated soils (West Siberia, Russia) using chemical parameters, examine the sensitivity of some biological parameters to TPH; assess the level at which the TPH contaminated soils loses ecological function and find TPH acceptable norms. We studied podzols and peat soils in West Siberia, middle taiga, under oil impact using transects. The survival and fecundity of *Enchytraeus albidus*, catalase activity, the soil respiration parameters were chosen as biological parameters in soils. Contaminants in the area investigated are TPH, Cl, Na, K, Ca, Mg, Zn, Ni, Cu, Cd, Cr, Pb, Ba, Sr, V. All of these migrate downward in the soil profile.The TPH decreased with depth and the bulk of TPH (85 – 95%) was adsorbed by the upper peat layer. We found significant negative correlation between *Enchytraeus albidus* survival rate and the TPH contents (R^2 = – 0.86 – 0.95, P < 0.01), as well as reproduction. Enzymic activity was revealed to be a sensitive indicator to TPH contaminated soils. Catalase activity appears to be sensitive to TPH (R^2 = – 0.90 – 0.94, P < 0.01). Cmic had a tendency to increase with the TPH decrease in soils; high revel of TPH in our soils nearly inhibited the substrate respiration and also Cmic. We found the decrease of qCO_2 with the rise of the TPH dose in studied soils. Mathematic analysis allows to bind chemical and biological parameters and find the TPH acceptable norms, allowing a soil system to perform its ecological functions. Quantitatively revealing the ecological impact of TPH in soils is important to help establish regulations and cleanup guidelines for acceptable TPH concentrations in a variety of land use and habitats in soils; to choose the most appropriate remediation methods and work out cleanup guidelines.

4.04.19

Green Manure As a Source of Nutrients for Petroleum-Degrading Microorganisms

O. Kulikova, National University of Oil and Gas Gubkin University / Department of Industrial Ecology; M. Elena, E. Tsygankova, National University of Oil and Gas (Gubkin University) / Industrial Ecology

The provision of soil with nutrients is an important factor that determines the intensity of decomposition of oil and oil products. Mineral fertilizers are commonly applied to the soil to enhance its quality and stimulate the biodegradation of oil-contaminants. The presence of residual acid (free acidity), physiological acidity and alkalinity resulting from the predominant use of cations or anions by plants from fertilizers and high solubility of mineral fertilizers restrict their safe use. While animal manure and some other organic fertilizers are environmentally friendly solution, their transport far from its production site is not always feasible. Especially, using animal manure as a fertilizer for bioremediation purposes in remote areas such as the Arctic region could be an unfavourable solution. Therefore, this study was aimed to establish the effectiveness of composted crop residues (green manure, GM) as a fertilizer in comparison with more common organic fertilizer – fish meal. The experiment plan includes an evaluation of the main nutrients content is widely spread plant species – common nettle (*Urtíca dióica*) and burdock (*Árctium*) and the preparation of plant fertilizer in a fermenter with a further direct application as an NPK-source to the soil samples contaminated with diesel fuel. For the purpose of the experiment, 2 tundra gley soil samples with total petroleum hydrocarbons (TPH) concentrations of 40 g/kg and 124 g/kg were chosen. The bioremediation experiment was carried out under laboratory conditions at room temperature of 18±2˚C. The experiment's design envisaged 2 treatment scenarios – with and without preliminary washing at 60±2˚C, using glyceryl sulfonate-based surfactant Addi Max PV01. The influence of the ambient parameters was evaluated for the control pots in the experiment. A biological product (BioFuture) was chosen as a biodestructor composed of several strains of hydrocarbon-oxidising microorganisms of *Pseudomonas*, *Bacillus* and *Candida* species. The study results showed that bioremediation led to the TPH-content decline by 55-56% in general, while in the experiment with the preliminary soil washing, the overall treatment efficiency was 69-76%. However, the highest treatment efficiency was established when using GM as a source of nutrients.

4.04.24

Organic Matter Degradation and Redistribution of Sediment Associated Contaminants by Benthic Invertebrate Activities, a Potential for Bioremediation?

T.v. Meer, Wageningen Environmental Research; P. Verdonschot, L. Dokter, Wageningen Environmental Research / Freshwater Ecology; M. Kraak, University of Amsterdam / IBED-FAME

Most organisms are continuously active: they breath, eat, defecate, move and create biogenic structures, breaking down organic matter (OM) and altering the environmental conditions in their surroundings. This way, invertebrate activities can influence the condition of sediment OM and therewith the distribution of sediment associated contaminants between the various environmental compartments. This ability could possibly be used to bioremediate organic contaminants and heavy metals from contaminated environments in general, and from wastewater treatment plant (WWTP) sludge in particular. Therefore, the aim of the present study was to assess the effect of the activity of larvae of the non-biting midge *Chironomus riparius* on organic matter degradation and the redistribution of heavy metals and PAHs in sludge from a WWTP. Our results showed that the chironomid larvae reduced the total sludge mass by 32%, leading to higher concentrations of heavy metals in the remaining sludge. However, the 1.16-2.04% of metals that did partition into the overlying water, resulted in an 79-758% increase in metal concentrations in the water column. The reduction and degradation of PAHs on the other hand was not enhanced by chironomid larval activity. The concentrations of heavy metals in adult chironomids were low, while concentrations of non-essential heavy metals and PAHs were highest in the exuviae, indicating that the larvae were able to expel some of the accumulated metals and PAHs in the exuviae before emerging as adults, limiting the transport of these metals between the aquatic and terrestrial environment. We concluded that the feeding and bioturbation activity of benthic invertebrates strongly alters the condition of sediment OM and therewith the distribution of contaminants in the aquatic environment, while contaminant bioaccumulation had little effect. Although the bioremediation potential by harvesting adult chironomids seems restricted, the high reduction of OM by the chironomid larvae does result in a more concentrated, yet lower total sludge mass, possibly reducing handling costs of further treatment and contaminant removal.

Bringing Together Exposure and Effects: Towards a Mechanistic Understanding of the Environmental Risk of

Chemicals in Aquatic Ecosystems

4.05.01

Development of a Mechanistic Model to Simulate the Behavior of Chemicals in Aquatic Ecosystems

A. Dorn, RWTH Aachen University / Institute of Environmental Research; P. Dalkmann, K. Hammel, E. Hellpointner, D. Faber, C. Kaiser, E. Seidel, Bayer AG, Crop Science Division / Environmental Safety; E. Bruns, Bayer AG, Division Bayer CropScience / Ecotoxicology; H. Hollert, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology

Adsorption properties of chemicals are essential for their spatial-temporal behavior respectively their mobility in water sediment systems. Adsorptive compounds spiked into overlying water quickly adsorb on the sediment surface, which lead to an accumulation in the sediment top layer. Besides compound properties, sediment characteristics such as organic matter determine the adsorptive behavior and as a result the spatial-temporal behavior of test compounds. The spatial-temporal behavior of test compounds in water sediment systems is not considered in the framework of the environmental risk assessment of chemicals in aquatic environments. In the past years, a sampling methodology enabling the depth-related analysis of chemicals in water-sediment systems was used in water sediment systems acc. to OECD 218/219 (Sediment-Water Chironomid Toxicity Using Spiked Sediment/Spiked Water). To establish a mechanistic model, the experimental results were compared with simulated values using the TOXSWA model (Toxic Substances in Surface Waters). Adsorption coefficient and organic carbon content were relevant input parameters used in the TOXSWA model, which considers diffusion retarded by adsorption as the relevant transport process. In risk assessment of pesticidal active substances, water sediment systems acc. to OECD 218/219 (assessment of effects) and OECD 308 (assessment of exposure) are used. Test systems acc. to OECD 218/219 and OECD 308 differ e.g. in terms of sediment layer thickness, use of natural or artificial sediment. Yet, the spatial-temporal behavior of an active substance within test systems acc. to OECD 308 is not investigated. Information on this is key for a better understanding of the active substance´s exposure and in the end for a more accurate risk assessment. In this work, the spatial-temporal behavior of test compounds was assessed in water sediment systems according to OECD 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems) using artificial and two contrasting natural sediments. The results showed positive correlations between the extent of adsorption and the organic content and adsorption coefficient, respectively. The sediment depth profiles obtained from the experiment were compared to simulated results by TOXSWA showing good agreements. Therefore, the TOXSWA model can contribute to a better mechanistic understanding of chemicals in water sediment systems bringing together effect and exposure.

4.05.06

"Something From Nothing" and "Nothing From Something": Metal Mixture Toxicity to Zooplankton Communities in Laboratory Microcosms and Individual-Based Models

S.E. Hansul, Ghent University (UGent) / Laboratory for Environmental Toxicology and Aquatic Ecology; A. Fettweis, KULeuven / Department of Earth and Environmental Sciences; E.E. Smolders, KULeuven / Earth & Environmental Sciences; K. De Schamphelaere, Ghent University (UGent) / Environmental Toxicology

Environmental risk assessment of metals is to date based on the assumption that species interactions, such as resource competition, are irrelevant for community- and ecosystem level effects of chemicals. This assumption is challenged by experimental data and theoretical considerations, suggesting potentially drastic alterations in community sensitivities resulting from the presence and nature of species interactions, which raises the question whether interactive effects of metal mixtures are also affected by species interactions, and whether this occurs in a predictable manner. We therefore calibrated an Individual-based Model based on Dynamic Energy Budget theory (DEB-IBM) with individual-level, single-metal toxicity data of three *Daphnia* species, then validated the model against observed Cu-Ni-Zn toxicity to the ternary community, exposed to the single metals and their mixture at an environmentally realistic concentration ratio. We found that the DEB-IBM correctly predicted some aspects of community dynamics, like the competitive dominance of *Daphnia magna* over *D. pulex* and *D. longispina* in our test system, but failed to accurately predict dynamics of the less dominant species, as well as community extinction at an exposure concentration of the mixture where none of the single metals had an effect. In contrast to this "Something from nothing" effect, the mixture effect on total yield was often lower than the most toxic single metal, especially at lower concentrations. Furthermore, no strong mixture effects on community structure relative to the most toxic single metal were observed or predicted. In conclusion, several challenges still have to be overcome to accurately predict community-level effects of metal mixtures. We suggest that a solid understanding of the relationship between individual-level parameters and community dynamics is key to overcoming these challenges. The fact that we observed unexpected effects of metal mixtures on the community-level, which allow for contrasting conclusions based on the exact choice of endpoint, highlights the importance of ecological processes for environmental risk assessment of metals.

4.05.07

Assessing Spatial Variation in the Sensitivity of Freshwater Invertebrate Assemblages to Chemicals and the Importance of River Typology

R. Liang, The University of Sheffield / Department of Animal and Plant Sciences; L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences

Current ecological risk assessments of chemicals generally adopt a single threshold, such as the Regulatory Acceptable Concentration or Environmental Quality Standard, to the protection of natural communities. This threshold criteria is usually derived from single-species toxicity tests on a limited number species. Commonly tested species may not be protective of all species that exist in natural assemblages, as the relative sensitivity of species to a chemical is toxicant specific. In addition, natural assemblages vary in species compositions over space, indicating that all assemblages may not be equally sensitive to chemicals. Adopting this single threshold approach may lead chemical risks to be underestimated or overestimated. A major challenge with assessing the spatial variation in assemblage sensitivity is that most species in natural communities have not been used in toxicity tests for any chemical. Spatial variation in species compositions has been reported to be influenced by the variation in river typology, but how river typology relates to spatial variation in assemblage sensitivity to chemicals is unknown. This study aimed to investigate: (1) whether the sensitivity of freshwater invertebrate assemblages varies over space. If it does, what spatial pattern this spatial variation presents; (2) how spatial variation in the sensitivity of freshwater invertebrate assemblages relates to Water Framework Directive (WFD) river typologies (river catchment size, mean altitude and catchment geology). To address the aims, the study investigated the sensitivity of 4013 freshwater invertebrate assemblages across England to five pesticides (malathion, fenitrothion, chlorpyriphos, parathion-methyl (PM) and diazinon) and five heavy metals (cadmium, copper, lead, nickel and zinc). The hierarchical species sensitivity distribution model (hSSD) was used to predict the toxicity of untested species and the concentration hazardous to 5% of species (i.e. HC5) was used as a measure of assemblage sensitivity. Then, spatial variation in assemblage sensitivity was related to river typologies. Results show that the magnitude of variation in assemblage sensitivity ranged from a factor of < 5 to >2000. Variation in assemblage sensitivity was spatially patterned and was influenced by WFD river typology.

4.05.10

Increasing Ecological Relevance of Chemical Risk Assessments Using Geospatial Approaches: Results From Two Case Studies

S. Marshall, Consultant; C.M. Holmes, Applied Analysis Solutions, LLC; L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences; P. Thorbek, BASF SE / Agricultural Solutions; P. Sweeney, Syngenta / Environment Product Safety; J.C. Otte, BASF SE

This platform presentation is a synthesis of the case studies described in 2 accompanying posters (surfactant and plant protection products). Review of these posters prior to viewing the platform will provide helpful background information.

A key rationale for making geo-referenced chemical risk assessment is that it provides assessments that can be tailored to local landscape/watershed abiotic characteristics and ecology to account for spatial heterogeneity within river basins. Since heterogeneity is often reflected in localised specific environmental objectives and protection goals, spatially explicit assessments can better relate to landscape/watershed scale environmental management objectives than can current generic chemical ERA frameworks. In 2017 ECETOC initiated a Task Force to investigate current capabilities in making spatially explicit chemical risk assessment (from both an exposure and effects perspective). The results of our case studies give an indication of the potential value of making geo-referenced chemical risk assessments as well as the limitations to current capability. We demonstrate that ERA can be informed by using GIS approaches to identify locations where species assemblages may be at relatively higher risk. However, there are few ecological data sets that are sufficiently comprehensive, consistent and that span large geographic areas, e.g. pan-European, for use in risk assessment. WFD biomonitoring data (diatoms, macrophytes, benthic macroinvertebrates, fish) are useful and available European data sets for freshwater ecosystems representing a broad range of structural and functional traits useful for chemical ERA. Greater realism in assessing chemical effects could be achieved if environmental typologies and their constituent biological communities were mapped and described. Developing scenarios could provide an intermediate level of resolution between current regulatory frameworks and site-specific assessments and could form the basis for achieving a pragmatic approach to framing assessments of individual chemicals. Framing of landscape-scale risk assessment is a critical step that requires clear statement of the question to be addressed and must consider data handling, e.g. aggregation, required resolution, methods for integrating data layers.

4.05.11

A Simple Integrated Exposure - Effect Criterion for Use in Chemical Risk Assessment

K. den Haan, Association of Retired Environmental Scientists ARES / Petroleum Products & Safety; J. Hermens, Utrecht University / Institute for Risk Assessment Sciences; D. van de Meent, Association of Retired Environmental Scientists ARES; J. Parsons, University of Amsterdam / Institute for Biodiversity and Ecosystem Dynamics; N. van Straalen, Vrije Universiteit Amsterdam / Ecological Science; J. Struijs, Association of Retired Environmental Scientists ARES; D. De Zwart, DdZ Ecotox

The environmental risk assessment of new chemicals conducted under the REACH framework and elsewhere requires input of two types of criteria: an estimate of the expected concentration in environmental compartments (PEC) and the expected threshold level for ecological effects (NEC). The risk is then estimated from PEC/NEC. However, both PEC and NEC come with considerable uncertainties, i.e. they are stochastic variables and follow a probability density distribution. Rather than comparing the mean PEC with the mean NEC, one would like to take the variation in both criteria into account. This can be done by considering the convolution integral of the PEC and the NEC distribution. A straightforward formula for this was derived before by Aldenberg *et al.* (2002) and Van Straalen (1990). This has now been implemented in the SIMPLEBOX framework in the module SimpleBoxTreat4SOLUTIONS (Schoorl *et al*. 2015) and is suggested for consideration in the EUSESvs2 and EUSESvs3 risk assessment packages. In the SOLUTIONS project we have made sample calculations derived from distributions of possible exposure concentrations in EU water as well as data on distributions of short-exposure EC50 values for aquatic organisms, for the same chemicals. The convolution integral, which we call “toxic pressure” (TP) is estimated for a large number of chemicals. We propose a cut-off value of 10^{-6} as a negligible risk threshold. Our new method avoids the disputable properties of the commonly used risk quotient and includes the variation and uncertainty in both exposure and effect assessment. The method is proposed for inclusion in the REACH framework.

Effect Modelling for Regulatory Environmental Risk Assessment of Chemicals: Where Are We and What Comes Next?

4.06.01

Don't Judge a Book by Its Cover: Why Read-Across Must Be Mechanistically-Grounded

P. Bicherel, P. Thomas, F. Bauer, KREATiS

Read-Across (RA) or category approaches is the most frequently used alternative to lab experimentation for REACH registration with an average frequency of ca. 25% used in endpoint summaries in order to fulfil the information requirements (European Chemicals Agency, 2020). Therefore, further attention is needed to ensure the RA is scientifically relevant. In the ECHA documentation “Chapter R6” and the “Read-Across Assessment Framework” (RAAF), the Read-Across is defined as the use of existing information from structurally-similar substance(s) or analogue(s) (*i.e.* the ‘source’ information) to predict properties for the ‘target’ substance of interest (European Chemicals Agency, 2008, 2017). However, the term “structurally-similar” is not clearly defined and can be a subjective analysis. At some point, RA can be seen as a simplified structurally-based approach where the existence of a common functional group(s) may be considered sufficient to assume identical compound properties. Recent research on mechanisms of toxic action (MechoA) indicates that: 1) important differences of toxicity may occur with presumed analogues while 2) structurally dissimilar compounds can exert the same kind of toxicity. Several case studies are presented in this presentation in order to support these two ideas. An example is given in this presentation showing that non-specific reactive compounds defined as hard electrophiles like aldehydes, epoxides, benzaldehydes, *etc*. can have the same toxicity despite the significant structural dissimilarities between each other. In contrast simple structural differences, such as the position of a substituent around a benzene ring, can dramatically change the toxic behaviour of a compound. Therefore, benzaldehydes or phenols are likely to have variable toxicity for a given species depending on the present substitutions. Concrete examples indicate compounds can be 10 to 100 times more toxic than presumed analogues based on structural similarity only. In conclusion, the structural similarity of compounds should not be considered as a sufficient or necessary criterion to perform a RA. Rather such approaches should preferentially be based on mechanistic information as is already the case when biotransformation is involved. Care should be taken to ensure the comparison between compounds is possible in the light of the mechanism of toxic action and depending on the species and the endpoint of interest.

4.06.03

Standartox - Standardizing Toxicity Data

A. Scharmüller, University Koblenz-Landau / Institute for Environmental Sciences; R.B. Schäfer, University of Koblenz and Landau / Institute for Environmental Sciences; V.C. Schreiner, University Koblenz-Landau

An increasing number of chemicals such as pharmaceuticals, pesticides and synthetic hormones are in daily use all over the world. Except for pesticides that are released into the environment deliberately, most chemicals enter the environment as a result of their use through different paths (e.g. atmospheric emission and deposition or discharge through wastewater). In the environment, chemicals can adversely affect populations and communities and in turn related ecosystem functions. To evaluate the risks from chemicals for ecosystems data on their toxicity, which is typically produced in standardized ecotoxicological laboratory tests, is required. However, for many chemicals, multiple ecotoxicity values are available for the same test organism, which can vary strongly. Current (meta-)databases, including the United States Environmental Protection Agency ECOTOXicology knowledgebase (EPA ECOTOX) only collect ecotoxicological test data, without allowing for further aggregation steps. Others, such as the Pesticide Property Data Base (PPDB) are confined to specific chemicals, here pesticides, thereby ignoring vast amounts of ecotoxicity data. We therefore developed Standartox, a tool and database that aims to overcome the limitations of other databases by continuously incorporating the ever-growing number of test results in an automated process workflow that ultimately leads to a single aggregated data point for a specific chemical-organism test combination, representing the toxicity of a chemical. Standartox comes with two front-ends, a web application (standartox.uni-landau.de) and an R package (standartox), which accesses an application programming interface providing convenience structures and thereby largely reducing processing time for users. Standartox provides the basis for reproducible science and combines information from different sources to simplify the derivation of more advanced risk indicators such as Species Sensitivity Distributions (SSD) and Toxic Units (TU), which represent two prominent concepts to assess effects on organisms in ecotoxicology. It constitutes not only a one-time compiled database but rather a scalable method that steadily incorporates new ecotoxicological test data in an automated manner, allowing for constant improvement of toxicity estimations over time. To, date Standartox provides access to more than 605,000 ecotoxicity data
points and its aggregates.

4.06.04

On the Influence of Uncertainty on SSD Analyses -Case Studies With Non-Terrestrial Target PLANTS

S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology; D. WU, Université Lyon 1 - CNRS 5558; A. Ratier, UMR CNRS 5558 Laboratoire de Biométrie et Biologie évolutive (LBBE) / Laboratory of Biometry and Evolutionnary Biology; G. Multari, University Lyon 1; V. Ducrot, Bayer Ag / Environmental Safety - Environmental Effects

Today, SSD analyses are a key tool for Tier 2 environmental risk assessment of chemicals. They provide a reliable assessment of the range of sensitivities within plant or animal communities and allow to estimate the hazardous concentration prone to affect 5% of the species (HC_5). An HC_5 estimate can be obtained from the fit of a probability distribution on a sufficient collection of toxicity values from a statistically sound analysis. Toxicity values are derived from the fit of a regression model on toxicity test data observed at several treatment levels at a target time point. This fit provides toxicity values as point estimates, associated with an uncertainty interval, either a confidence or a credible interval. Nevertheless, this uncertainty is rarely accounted for in ERA.In the case of non-terrestrial target plants (NTTP), treatment levels are called tested rates. The toxicity test data analysis leads to the estimate of 50% Effective Rates (ER_{50}), that are then used as inputs for the SSD analysis. Up to ten species are usually considered for ERA of herbicides. The used tested rates are selected prior to the experiments with little prior knowledge on the sensitivity of species to the herbicides. Consequently, unbound values (ER_{50} < lowest tested rate or ER_{50} > highest tested rate) can occur instead of uncertainty intervals, especially when the range of tested rates does not match the sensitivity of a species or when this species is not affected at the highest application rate.The Guidance Document on Terrestrial Ecotoxicology (GDTE) provides no advice on how to deal with neither uncertainty intervals nor unbound ER_{50} values within SSD analyses. As a consequence, the common practice is to ignore the uncertainty by considering point estimates, and either to discard unbound ER_{50} values or to substitute them with arbitrary values. Such practices constitute a loss of valuable information with drawbacks: (i) the range of remaining ER_{50}values may not cover the full range of sensitivities; (ii) if unbound ER_{50} values occur for many species, after discarding them, the data set might not be sufficient enough to allow the SSD analysis to be performed.In this presentation, based on several case studies, we will show how it is possible to account for both the uncertainty based on bounded intervals for ER_{50} values and unbound ER_{50} values into SSD analyses. In particular, we will explore their influence on the HC_5 estimate.

4.06.09

Capturing Reversible Events in Survival Tests: GUTS - Immobility

B. Goussen, IBACON GmbH / Ecological Modelling; T. Jager, DEBtox Research; A. Gergs, Bayer Ag / Research & Development, Crop Science Effect Modelling

Traditional environmental risk assessment of plant protection products (PPP) is usually based on controlled laboratory experiments. These experiments are often conducted under constant exposure concentrations. This approach has the benefits of simplicity. However, the tested exposure patterns are therefore far from the real world ones. The use of mechanistic modelling like toxicokinetics- toxicodynamics (TKTD) models has the potential to overcome these challenges. The assessment of the survival endpoints via a TKTD approaches is increasingly considered in Environmental Risk Assessment (ERA). One of the leading approaches in this field is the General Unified Threshold model for Survival (GUTS). This model has recently been judged as "ready to use in aquatic ERA" by the EFSA in its Scientific Opinion on TKTD modelling. In its current form, this model is limited to survival or death events (all-or-nothing) that can only occurs once in the lifetime of an organism. However, it has been shown, in some species like *Daphnia magna* for instance, that an intermediate reversible immobility state can exist under pulse exposure; organisms may recover. In the current project, we extended the GUTS model to allow for an additional state – immobility – and for the recovery of immobile organisms. This three-state model is a departure from the traditional GUTS approach which requires a rethinking of the statistical framework used. Here we present the GUTS-immobility extension with its statistical approach as well as case study showcasing the application of this model. This model has the potential to (i) help better understanding mechanisms behind immobility and mortality, (ii) reduce the need to censor data, and (iii) analyse data from tests where intermittent exposure was performed for animal-welfare reasons.

4.06.10

BeeGUTS - a TKTD Model for the Interpretation and Extrapolation of Honey Bee Survival Data

J. Baas, Wageningen Environmental Research - WUR; B. Goussen, IBACON GmbH; M. Miles, Bayer CropScience UK / Environmental Safety; T. Preuss, Bayer AG Crop Science Division; I. Roessink, Wageningen Environmental Research / Environmental Risk Assessment

Effects of pesticides on adult honey bees are currently assessed by OECD standardized tests on acute and chronic oral exposure as well as on acute contact exposure. The typical exposure duration is 48 hrs in the acute tests and 240 hrs in the chronic tests. The tests are evaluated individually and the lowest value of any of the tests is taken for further risk assessment. This approach implies that the sensitivity of a bee to a pesticide depends on the type of test that is carried out. This is of course not the case. In addition the test results expressed as a 48 hr LD_{50} (or the dose that kills 50% of the exposed bees at 48 hrs) or a 240 hr LC_{50} (the concentration of the pesticide in the food that kills 50 % of the exposed bees after 240 hrs of exposure) are not suitable to be used in further evaluations for field relevant concentrations, where exposure concentrations are not constant over time. Therefore, a mechanistic integrative assessment was developed where all tests can be interpreted and evaluated within one consistent framework with one set of parameter values. The developed approach uses a Toxicokinetic Toxicodynamic (TKTD) modelling framework based on the Generalized Unified Threshold model for Survival (GUTS) that takes into account the specifics of the test and the physiology of the bee; the BeeGUTS model. Raw data were made available for chronic, acute oral and acute contact tests for 17 individual pesticides. For all compounds for which consistent datasets were available the model was able to accurately describe the time course of the effects for the three different exposure routes (acute oral and contact, and chronic oral) with one set of parameter values and one consistent modelling framework. For the other compounds the model was able to identify any discrepancies in the data and can therefore be used as a validity check for toxicity values used in honey bee risk assessment. This new integrative approach moving from single point estimates of toxicity and exposure to a holistic link between exposure and effect will allow for a higher confidence of honey bee toxicity assessment in the future and a much-improved extrapolation potential of effects for different species and/or for field realistic exposure conditions.

4.06.13

Using DEB - TKTD for ENvironmental Risk Assessment: A Case Study With Daphnia magna

B. Goussen, M. Trijau, IBACON GmbH / Ecological Modelling; C. Boerschig, P. Emnet, IBACON GmbH / Aquatic Ecotoxicology; T. Jager, DEBtox Research; R. Ashauer, Syngenta Crop Protection AG / Environment

Traditional environmental risk assessment of chemicals is usually based on controlled laboratory experiments. These experiments are often conducted under constant exposure concentrations. These experimental conditions are often far removed from real conditions where time-variable exposure scenarios are the norm. Mechanistic modelling is recently gaining interest in regulatory environmental risk assessment as it allows considering within an integrated framework more realistic environmental scenarios. This is especially the case for toxicokinetic - toxicodynamics (TKTD) models. EFSA recently published a Scientific Opinion assessing the use of these models for survival and sublethal effects of plant protection products (PPP) on individual organisms. Models based on the Dynamic Energy Budget theory adapted for ecotoxicology (DEB-TKTD models) are the leading approach to assess sublethal effects of chemicals on individuals. DEB-TKTD models offer a comprehensive framework to analyse and extrapolate sublethal effects (growth and reproduction) of chemicals on individual organisms across their whole life cycle. However, their use still needs to be standardised and expanded in order to increase confidence for use in risk assessment. Here we illustrate via a case study how DEB-TKTD modelling can be used in the environmental risk assessment of chemicals. Our study followed the suggested steps outlined in the EFSA Scientific Opinion: (i) model calibration, (ii) model validation, (iii) forward predictions. Tailored *Daphnia magna* experiments with high resolution growth, reproduction, and survival observations were designed and conducted independently for both the calibration and validation of the DEB-TKTD model. The validated DEB-TKTD model was then used to predict the potential effects of exposure scenarios based on more realistic FOCUS profiles. This case-study showcases the advantages and challenges of using DEB-TKTD for risk assessments.

4.06.17

Reproduction Data in Deb-Based Model Analysis: On Clutch-Wise Spawning and Brood-Pouch Delays

T. Jager, DEBtox Research; M. Trijau, IBACON GmbH / Ecological Modelling; N. Sherborne, Syngenta / Environmental Safety; B. Goussen, IBACON GmbH; R. Ashauer, Syngenta Crop Protection AG / Environment

Toxicokinetic-toxicodynamic (TKTD) modelling is the only approach to make sense of the time dependence of toxic effects, and to interpret and predict consequences of time-varying exposure. These advantages are recognised in the regulatory arena, especially for environmental risk assessment (ERA) of pesticides, where time-varying exposure is the norm. A price to pay for such powerful data analysis is that modellers need to think about the link between the modelled state variables and the observations from laboratory ecotoxicity tests. For the endpoint 'reproduction', this link is not trivial, in particular when a species reproduces in clutches and even more so when eggs are deposited into the mother's brood pouch (and reproduction is scored as the release of neonates). Many of our popular aquatic test species reproduce clutch-wise and sport such a brood pouch (cladocerans, amphipods, mysids). In this contribution, we explain these and other challenges with reproduction data, and reflect on their potential impact on TKTD analysis with Dynamic-Energy Budget (DEB) models. At this moment, there are no off-the-shelf model (or data) modifications that will work for all species and all stressors. Nevertheless, both modellers and users of model results need to be aware of these considerations and their potential implications. Ignoring these challenges has the potential to cause poor performance of a calibrated DEB-TKTD in model validation, for the wrong reasons: it is not the model itself that is not applicable, but the link between the modelled properties and the observations. Model validation is required in the EFSA scientific opinion for each chemical-species combination, so an incorrect link between model and data could cause rejection of the model analysis for regulatory purposes. At this moment, modellers will need to decide how to use reproduction data on a case-by-case basis and motivate that choice in their reporting. In time, more data with pulsed exposure will be generated, and more experience gained, which will allow more structured guidance to be developed. We here discuss these challenges, and present suggestions on how to best overcome them in order to make scientifically-robust use of reproduction data in DEB-TKTD models, as applied to environmental risk assessment questions.

4.06.20

Modelling the Effects of Variability in Feeding Rate on Growth - a Vital Step for DEBtox Modelling

T. Martin, The University of York / Department of Environment and Geography; M. Hodson, University of York / Department of Environment & Geography; R. Ashauer, Syngenta Crop Protection AG / Environment

A major limitation of dietary toxicity studies on rodents is that food consumption often differs between treatments. The control treatment serves as a reference of how animals would have grown if not for the toxicant in their diet, but this comparison unavoidably conflates the effects of toxicity and feeding rate on body weight over time. A key advantage of toxicity models based on dynamic energy budget theory (DEBtox) is that chemical stress and food consumption are separate model inputs, so their effects on growth rate can be separated. To reduce data requirements, DEB uses a simplified feeding input, f (dimensionless, 0-1), based on food availability. It is assumed that with food available *ad libitum*, animals feed at the maximum rate relative to their body size, such that $f = 1$. However, this assumption is contradicted by observed food consumption in dietary toxicity studies.This mismatch has received relatively little attention, but accurately modelling the effects of food intake on growth rate is required to derive toxicodynamic parameters that reflect only a chemical's toxicity. Addressing this issue is therefore essential for the accurate extrapolation of toxic effects from laboratory data to more realistic exposure scenarios. Moreover, this allows growth models to serve as an analytical tool, providing additional insight into the results of chronic toxicity studies. Here we trial a new method for calculating the value of f, based on the observed relationships between food consumption and body size in

laboratory rats. We compare model results of the new method with those of the conventional DEB method and a previous effort to calculate *f* using observed food consumption data. Our results showed that the new method modeled total body weight over time most accurately. Additionally, modeled reserve dynamics were compared to percentage body fat over time reported in the literature. Again, our new method matched data most closely, suggesting that it provides the most realistic simulation of the biological processes underlying growth. The new method relies on the assumption that digestive efficiency increases as a function of body size, as has been observed between species. This proposed relationship could be verified through data collection. Doing so would strengthen the basis of DEB theory and support the case for its use in ecological risk assessment.

4.06.22

Modelling the Effect of Time-Variable Exposure to Herbicides in the Kielstau Watershed

N. O'Connor, The University of Sheffield / Department of Animal and Plant Sciences; U. Ulrich, N. Fohrer, Christian-Albrechts University Kiel / Institute for Natural Resource Conservation, Dep of Hydrology and Water Resources Management; A. Focks, University of Osnabruck / Institute of Environmental Systems Research

In regulatory risk assessment, usually simulated concentrations are used to characterise exposure. Together with effect thresholds, this information is used to determine risk estimates. Toxicokinetic/Toxicodynamic (TKTD) models were deemed suitable alternatives to higher tier experimental assessments in standard chemical risk assessments to analyse both effects on survival and sub-lethal effects (e.g. effects on reproduction or growth). These mechanistic models are capable of simulating the effects of complex exposure profiles while considering time-variable environmental conditions, offering a genuine method to extrapolate from lab to field conditions. We investigate the use of a *Lemna* TKTD model for the risk assessment of three herbicides. In a first step, simulated exposure dynamics were used to calculate effect risk estimates, so called EP_{50} values. From a regulatory perspective, it is interesting to see how simulated exposures may compare to time-variable concentrations measured in monitoring campaigns. Therefore, in a next step, we compared the risk estimates when measured exposure dynamics of three herbicides were considered. In a last step, we investigated the influence of environmental scenarios (considering temperature, irradiation and nutrient levels) where the optimal conditions, default within the *Lemna* model, were exchanged with measured values. It was found that increasing consideration to realistic environmental scenarios (optimal>limiting>measured) resulted in a decreased margin of safety. Measured (time-variable) environmental conditions resulted in safety margins 81%, 10% and 28% lower than those under optimal (constant) conditions, for metazachlor and flufenacet and terbuthylazine respectively. All EP_{50} values were above the Assessment Factor (AF) of 10. EP_{10} values of herbicides applied in autumn were below the AF. Ultimately, the definition of the environmental scenario had a decisive impact on the risk assessment outcome, with real environmental conditions resulting in lower safety margins at both the 50% and 10% effect level. Additional results indicate that seasonal differences in environmental conditions have a profound impact on the potential of *Lemna* populations to recover from herbicide exposure, with potential for recovery greater following spring than autumn applications. It remains to be tested how calculated endpoints of the *Lemna* TKTD model may fit into the current tiered procedure.

4.06.24

Modelling Population-Level Risks for Large-Bodied Apex Predators Emerging From the Individual-Level Effects of Organic Pollution

P. Vermeiren, J.A. Hendriks, Radboud University / Environmental Science; A.M. Ragas, Radbound University / Environmental Science

Environmental pollution is one of the main drivers of the current biodiversity crisis and particularly threatens large-bodied apex predators because of its potential to chronically affect organisms over their lifespans, and the ability of some pollutants, including organic chemicals, to accumulate throughout the food web. To improve realism in analyses of population risk from organic pollutants, we develop and test an individual-based model, the NEOPOP model, to simulate effects of organic pollution on the population dynamics of apex predators. A Dynamic Energy Budget (DEB) module forms the core of the NEOPOP model, and allows us to account for characteristics such as dietary changes, late sexual maturity, breeding periodicity, and parental care common to many large-bodied apex predators. Input parameter values are varied among individuals, to explicitly include individual trait variation, and the model is simulated with input data regarding environmental conditions within the spatial context of each individual. A bioaccumulation module determines internal pollutant concentrations given diet preferences, pollutant concentrations in prey, and bioaccumulation factors. (Sub-)lethal effects of pollutants, e.g. mortality, reduced size or lower number of offspring, emerge via interaction of pollutants with DEB bioenergetic processes such as maintenance, growth and reproduction. Declines in American alligator populations co-occurring with intensive agricultural development in Florida, USA, are used as a case study. Results indicate that current data is poorly available for reptiles; is highly scattered across species, chemicals and endpoints; lack a homogeneized format; and in many cases rely on field observations which are subject to multiple environmental conditions. Nonetheless, hypotheses regarding potentially affected mechanisms could be derived from current knowledge, and simulations compared with observed field data allowing a mechanistically based model structure to be derived. The high uncertainty regardig bioaccumulation, the magnitude of toxic effects, and dose response curves requires increased attention to analytical techniques allowing for both forward uncertainty propagation and backward parameter inference. Further development of the NEOPOP model requires approaches to improve uncertainty inherent to models for wildlife populations, and application of the model towards other species and chemicals.

4.06.25

Something From Nothing? Simulating Mixture Toxicity Effects on Daphnia magna Populations With Deb-Ibm

K. Vlaeminck, Arche consulting / Laboratory of Environmental Toxicology and Aquatic Ecology; K. Viaene, ARCHE; P. Van Sprang, Arche consulting; K. De Schamphelaere, Ghent University (UGent) / Environmental Toxicology

Ecological risk assessment (ERA) aims to predict the effects of man-made chemicals on the ecosystem. A potential complication occurs when using data assessed on individuals with single chemicals to estimate the impact on populations exposed to mixtures of these chemicals. One specific issue arising is the “something from nothing” phenomenon. “Something from nothing” could occur when each individual chemical is harmless on its own while causing a significant effect when dosed in combination. In addition, it is unclear how population-level mechanisms contribute to the “something from nothing” phenomenon. The current study aims to increase our understanding of the impact of mixtures on populations using a combination of experiments and mechanistic modelling. An individual-based model (IBM) implementation of the Dynamic Energy Budget (DEB) theory was developed to predict mixture toxicity effects on *D. magna* populations. The IBM is based on the Dynamic Energy Budget theory (DEBtox) and the General Unified Threshold model for Survival (GUTS) for lethal effects. The DEBtox and GUTS sub-models were calibrated based on individual-level data (growth, reproduction and survival) from a standard chronic *D. magna* toxicity test for four selected chemicals (i.e., pyrene, dicofol, alfa-HCH, and endosulfan). A population experiment (8 weeks) was performed to assess effects of these chemicals on *D. magna* populations. An implementation for mixture toxicity, based on the general statistical models for mixture toxicity (independent action and concentration addition), were integrated in the DEB-IBM. We validated the implementation using the data of the population experiment. In addition, we were able to explain the trends observed in the population experiment using the model. For instance, alfa-HCH on its own showed no effects on population density (compared to the control), whereas in combination with pyrene or dicofol, exerted larger-than-expected effects (e.g., pyrene on its own decreased equilibrium population density with 41%, whereas in combination with alfa-HCH there was a decrease of 71%). Using the extended DEB-IBM, we were able to explain the “something from nothing” phenomena from the DEBtox-theory. Overall, we highlight the applicability of mechanistic models, such as DEBtox and GUTS, for mixtures. DEBtox- and GUTS-based IBMs can extrapolate effects observed at the individual level to eventually predict relevant mixture toxicity effects at the population level.

4.06.28

Modelling Ecosystems in Mesocosms: A Ring Study Approach With Four Aquatic Systems Models

A. Schmolke, Waterborne Environmental, Inc. / -; N. Galic, Syngenta Crop Protection LLC / Environmental Safety; S.M. Bartell, Cardno Inc. / Science and Enviroment; I.A. O'Connor, S. Spycher, EBP Schweiz AG; N. Schuwirth, Eawag, Swiss Federal Institute of Aquatic Science and Technology; T. Strauss, gaiac Research Institute; D.V. Preziosi, R.A. Pastorok, Integral Consulting, Inc.; P. Ebke, J. Schmidt, MESOCOSM GmbH Institut für Gewässerschutz; F. Abiakar, Waterborne Environmental, Inc.; J. Collins, Waterborne Environmental, Inc. / Effects Assessment; R. Ashauer, Syngenta Crop Protection AG / Environment

Aquatic systems models (ASMs) represent food-web interactions in an aquatic community and interactions with environmental conditions. Ecological models, including ASMs, are valuable tools in pesticide risk assessments because they can be applied to a range of biotic and abiotic conditions, as well as to a variety of exposure scenarios which would be impractical to test empirically. Four ASMs (Streambugs, AQUATOX, CASM, and StoLam with STREAMcom) that have been developed, published and applied in pesticide risk assessments and other contexts are the subjects of this ring study. Model inputs and outputs are compared among the ASMs, using mesocosm data generated and provided by MESOCOSM GmbH. The ASM ring study includes: a) analysis and preparation of mesocosm control and treatment data, b) definition of the food web represented across ASMs, c) parameterization of the ASMs, d) calibration of the ASMs to mesocosm control data, e) validation of the calibrated ASMs against mesocosm control data not used in the calibration, f) calibration of the ASMs to mesocosm treatment data. All steps are documented in detail, following the recommendations

of the good modeling practice. We will present methods and results of the steps a) – d). We outline an approach for defining a mesocosm food web that can be represented by multiple ASMs, and the parameterization and calibration of the ASMs to the available mesocosm data. The approach provides important insights into the strengths and limitations of different ASMs for this particular modelling exercise through comparison of the model outputs with each other and with empirical data. In the next steps of the ring study, we will evaluate the ASMs using independent mesocosm data from the same test site, and simulate treatment effects for an example pesticide.

4.06.31

Towards Aquatic Riks Assessment at Landscape Scale

L. Wipfler, Wageningen Environmental Research; W. Beltman, Wageningen Environmental Research / Environmental Risk Assessment; H. Baveco, Wageningen University and Research; T. Schad, Bayer AG Crop Science Division / Environmental Modelling

Risk assessment at landscape scales is considered in Europe as an option to improve the realism and relevance of regulatory Environmental Risk Assessment for pesticides. Spatially distributed models can, in connection with geographical information predict emissions, exposure and environmental effects of pesticides at regional scales. This allows to quantify local risk and to assess these risks in the context of the complex, interconnected and spatially variable landscape. Examples of integrated approaches at landscape scales exist in the literature but they are scarce and of an ad-hoc nature. Still missing is a generic framework that links protection goals to landscape scale assessment endpoints and operationalizes the regulatory requirement of 'no unacceptable risk' at the landscape scale. For appropriate communication with risk managers such a framework is crucial. Currently, exposure and effects are assessed separately and at the local (field) scale. The risk associated with pesticide use is considered acceptable when the Predicted Environmental Concentration (PEC) is lower than the so-called Regulatory Acceptable Concentration (RAC), whereby the PEC is the outcome of the exposure assessment and the RAC the outcome of the effect assessment. For a spatially and temporally explicit, landscape-level quantification of risk the distinction between exposure and effect is no longer relevant: effects of pesticide use are quantified in a spatially distributed way over the entire landscape. This implies that the use of the PEC/RAC ratio should be abandoned and a new thinking framework is needed. Recently, the scientific opinion on the use of TKTD models[1] provided concrete suggestions on how to extend the risk assessment from single PEC values to time-variable concentration profiles, but no spatially distributed approach has (yet) been developed. We propose our ideas on how to build such a framework while taking the existing EU (aquatic) risk assessment as a starting point.

4.06.32

Aquatic Risk Assessment at Catchment Scale - Case Study on Insecticide Exposure Concentrations and Effects on Macroinvertebrates

W. Beltman, Wageningen Environmental Research / Environmental Risk Assessment; H. Baveco, Wageningen University and Research; L. Wipfler, Wageningen Environmental Research; M. Braakhekke, Wageningen Environmental Research / Team Environmental Risk Assessment; B. Buddendorf, Wageningen UR / ERA; S. Multsch, knoell Germany GmbH / Environmental Fate / Modelling / GIS; T. Schad, Bayer AG Crop Science Division / Environmental Modelling

Landscape modelling is important for future environmental risk assessment of aquatic ecosystems for plant protection products (PPP). In recognition of spatial and/or temporal variability, more realism can be included by assessing the risk at landscape level. The combined application of exposure, chemical fate and ecological effect models at individual and population levels allows for spatially and temporally explicit quantification of risks. A case study is presented in which PPP concentrations and effects on macroinvertebrates in surface water are simulated after application of an insecticide in fruit orchards within a meso scale river basin. The model application provides spatially and temporally distributed effects on the selected organism abundance. A landscape model was developed which integrates hydrological, PPP use, pesticide fate, and ecological effect modules. The hydrological model is being developed based on the hydrological programming library 'Catchment Modelling Framework (CMF). In the landscape model the water flows and depths are used by modules for spray drift (xDrift) e-fate (Cascade_TOXSWA) and GUTS effect (TKTD) as well as an individual-based population model based on Dynamic Energy Budget (DEB) theory. The fate of the PPP is simulated for all reaches in the catchment. The GUTS model predicts survival probabilities and margins of safety for selected species. The population model predicts population level changes for target species. In this case study a strongly sorbing substance entered the water via spray drift resulting in PECs and LP50 analysed for macroinvertebrates Asselus, Cloeon and Gammarus .The population model was only parameterized for Asellus. This general effects can be calculated on the catchment scale. The result of this case study faciltates the development of a framework for landscape risk assessment.

Environmental Risk Analysis and Regulation of Polymers

4.07.01

Phys-Chem Data of Polymers - Old Acquaintances and New Challenges

C. Lang, R. Gerhardt, BASF SE

As one common source of information, the experimental determination of physical and chemical properties is essential to every safety assessment. For products containing defined molecules, suitable guidelines are available specifying accepted methods, for example to determine water solubility. Transitioning from defined molecules to polymers, however, adds multiple dimensions of complexity as polymers inherently show a dispersity of chain lengths, chemical compositions, endgroups, or architectures. In many cases, this makes the established methods no longer applicable and even the most basic parameters, e.g. chemical identity or purity, are no longer well-defined. Additionally, the polymers to analyze will not be available in a pure form but rather in complex mixtures (IAS and NIAS), rendering the analyses even more challenging. Although the legislative requirements are still under discussion, there is a set of physical and chemical properties, which will most likely play a central role in the safety assessment of polymers. Both, the ECETOC technical reviews (1) and the latest report by Wood and PFA-Brussels (2) highlight parameters, such as molecular weight distribution, water solubility or the stability of a test item in aqueous or organic media. As already mentioned above, guidelines exist for the determination of these parameters for defined molecules. However, guidance specifically tailored for polymers is needed. In this talk, we want to illustrate some of the challenges we are facing when analyzing polymeric test items for regulatory purposes by means of exemplary case studies.References: 1 The ECETOC conceptual framework for polymer risk assessment (CF4Polymers). May 2019. Technical Report No. 133-1. http://www.ecetoc.org/wp-content/uploads/2019/06/ECETOC-TR133-1CF4Polymers.pdf The applicability of analytical tools, test methods and models for polymer risk assessment. March 2020. Technical Report No. 133-2. http://www.ecetoc.org/wpcontent/uploads/2020/03/ECETOC-TR133-2. 2 European Commission, ENV.B.2 – Sustainable Chemicals. Scientific and technical support for the development of criteria to identify and group polymers for Registration/ Evaluation under REACH and their impact assessment. Final Report. June 2020. Doc Ref. 40867-WOOD-XX-XX-RP-OP-0002_S3_PO3.5.

4.07.02

Cationic Polyquaterniums Induce Variable Toxicity to Daphnids Depending on Charge and Molecular Weight

D. Arndt, The Procter & Gamble Company / Global Product Stewardship; J. Brill, Procter & Gamble Company / Global Product Stewardship; A. Brun, Aarhus University / Department of Environmental Science; K.A. Connors, M. Lam, Procter & Gamble Company / Global Product Stewardship; H. Sanderson, Aarhus University / Environmental Science; S.E. Belanger, The Procter & Gamble Company (retired) / Global Product Stewardship

Cationic polymers, such as polyquaterniums (PQs), are often used for anti-static applications in shampoos and conditioners and in wastewater treatment processes because of their unique ability to neutralize the negative charge densities of these products. However, evidence shows that cationic polymers can induce toxicity to aquatic organisms, and these compounds have been flagged by chemical regulators as potential "polymers of concern." In addition, cationic polymers have been synthesized with a variety of structures, charges, and molecular weights (MW), and changes in these internal characteristics could change their effects in the environment and also make them more difficult to test. Therefore, this work investigates the acute toxicity of two types of low and high MW PQs, polyquaternium-6 (PQ6) and polyquaternium-10 (PQ10), to the freshwater model invertebrate, the daphnid. *Daphnia magna* and *Ceriodaphnia dubia* were exposed to PQs according to the OECD 202 standard toxicity testing guideline, and modifications to the guideline were carefully considered due to the potential for cationic polymers to sorb to anionic surfaces. PQ6 and PQ10 differed in toxicity to *Daphnia* by one (low MW) or two (high MW) orders of magnitude. A comparison of low and high MW PQ6 indicated no significant difference in toxicity between these two compounds, while a comparison of low and high MW PQ10 indicated that low MW was an order of magnitude more toxic than low MW PQ10. A comparison of PQ toxicity in *Daphnia magna* and *Ceriodaphnia dubia* was more sensitive to PQ exposure than *Daphnia magna*, and this trend was consistent for both low and high MW PQ6s and PQ10s. It is possible that the mechanism of PQ10 toxicity in daphnids could be more physical in nature , as the *Daphnia* carapaces were "sticky" and the *Daphnia* exhibited difficulty with shedding the carapace in these exposures. Although cationic polymers exhibit some characteristics of difficult to test substances, this work indicates that the toxicity of these materials can be tested using standard guidelines, as long as careful considerations of the exposure are made by the investigator. PQ toxicity differed in daphnids with regards to charge, MW, and specie. Future work will focus on exploring the variation in species sensitivity to different PQs and further investigating the physical and chemical mechanisms of PQ toxicity.

4.07.03

Cationic Polymer Environmental Toxicity: A Critical Data Review

K.A. Connors, Procter & Gamble Company / Global Product Stewardship; A. Brun, Aarhus University / Department of Environmental Science; D. Arndt, The Procter & Gamble Company / Global Product Stewardship; J.M. Rawlings, Procter and Gamble Company / Global Product Stewardship; M. Lam, Procter & Gamble Company / Global Product Stewardship; H. Sanderson, Aarhus University / Environmental Science; S.E. Belanger, The Procter & Gamble Company (retired) / Global Product Stewardship

There has been an increasing regulatory interest in the environmental hazard profile of polymers. Cationic polymers in particular have been identified as potential polymers of concern and will likely require regulatory assessment under REACH in the near future. Due to the proprietary nature of cationic polymers, there is relatively little environmental hazard data in the public domain. Here, we perform a critical review of the existing literature. A combination of specific (e.g., trade names, CAS numbers) and general terms such as "polyelectrolyte" were used to identify relevant manuscripts. A total of 674 individual experimental results were reviewed, and ~20 key publications were identified. Studies were reviewed according to current regulatory expectations for data quality, compound description, and experimental design. Key attributes were extracted from each study including information on polymer identity and characterization, experimental design, exposure attributes, and measured biological effects. Studies were also reviewed for data quality. In general, the cationic polymer data available in the public literature is of low quality and/or lacks essential details needed for a quality evaluation. Information on test substance identity was especially sparse. Very few studies reported physical/chemical details, MW distribution information, or charge density values. Given their large size, polymers are not expected to pass biological membranes and may therefore not follow the standard dose-response relationships. Experimental design, especially dose-spacing decisions, can therefore complicate toxicity interpretations. In general, very limited phys/chem details are publicly available for polymers (e.g., molecular weight, size fractions, charge density). This represents a challenge for modeling efforts. Additional insights on trophic sensitivities and toxicity mitigating factors (e.g., TOC, water quality parameters) will also be highlighted in this presentation.

4.07.04

Cationic Polymers - Aquatic Toxicity and Modelling Challenges

H. Sanderson, Aarhus University / Environmental Science; K. Roy, Jadavpur University / Department of Pharmaceutical Technology; P. Khan, Jadavpur University / Department of Pharmaceutics and Pharmaceutical Technology; A. Brun, Aarhus University / Department of Environmental Science; M. Lam, K.A. Connors, Procter & Gamble Company / Global Product Stewardship; S.E. Belanger, The Procter & Gamble Company (retired) / Global Product Stewardship

Polymers are highly diverse and understudied materials from an environmental toxicity point of view. For the past decades, polymers have largely been out of scope regarding detailed safety assessment in most regulatory programs as they are assumed to not possess relevant toxicological properties due to their size. This regulatory exclusion is currently being reconsidered. The amount of publicly available, high quality environmental toxicity data on industrial polymers such as cationic polyquaterniums is however extremely limited. We obtained data from Environment and Climate Change Canada (n = 242). The data was blinded in terms of full structural disclosure but contains information on key structural features allowing an analysis of the relative contribution of these to the overall toxicity. The area of (Q)SAR modeling for the evaluation of toxicity of polymers has remained largely unexplored. Our study revealed that a number of descriptors are relevant beyond charge density.

4.07.05

Environmental Exposure and Risk of Water-Soluble Polymers Found in UK Household Products

H.C. Brunning, University of York / Environment and Geography; J.B. Sallach, University of York / Environment; A. Boxall, University of York / Department of Environment and Geography

There are many potential emission pathways of water-soluble polymers to the environment. However, little is known about the types of water-soluble polymers which may be present in the environment, and their associated environmental risk. This study aimed to identify polymers used in common household products emitted down-the-drain in the UK, and to conduct a risk-based prioritisation of polymers for future study by estimation of worst-case predicted environmental concentrations for surface water (PEC_{SW}) and comparing these values to predicted no-effect concentrations (PNEC). An inventory of polymers found in popular brands of products including laundry detergents and personal cleansing products was built using publicly available product ingredient data. Polymers were grouped on the basis of their structure and monomer type, and their concentrations at the point of use were characterised using product patents. This data was combined with usage data from the literature, and standard values of wastewater production and dilution factors to obtain worst-case (assuming no wastewater treatment plant (WWTP) removal) PEC_{SW} estimates for the identified polymers. PNEC data were derived from species sensitivity distributions plotted using no observed effect concentrations (NOEC) obtained from the literature. A total of 186 polymers were identified across 723 products (laundry detergents, bodywash, handwash, soap bars, and bubble bath), which were subsequently divided into 23 groups. PEC_{SW} for the polymers were in the low mg L^{-1} range, with worst-case highest probable maximum PEC_{SW} values of 1.77, 1.46, and 0.88 mg L^{-1} for ethoxylated alcohol salts, alkoxylated alcohols, and polycarboxylates, respectively. Refined highest probable maximum PEC_{SW} values (accounting for WWTP removal) were derived for ethoxylated alcohol salts and alkoxylated alcohols as 0.006 and 0.035 mg L^{-1}, respectively. PNEC values were derived for the three selected polymer groups and estimated as 0.03, 0.02, and 3.07 mg L^{-1}, respectively. The study revealed that worst-case PEC_{SW} exceed PNECs for two of the three polymer groups, and refined PEC_{SW} exceeds PNEC for one polymer group. This demonstrates the potential ecotoxicological risks associated with polymers that have largely been overlooked. This data also has significant utility in providing a risk-based prioritisation of soluble polymers to help direct future research efforts.

4.07.06

Exposure to Polymethylmethacrylate NanoPlastics Induced Severe Alterations in the Morphology of Cnidarians and Amphibians

C. Venancio, University of Coimbra / Centre for Functional Ecology CFE; M. Oliveira, University of Aveiro & CESAM / Centre for Environmental and Marine Studies (CESAM) & Department of Biology; I. Lopes, University of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies

Scientific research has increased exponentially in what concerns the toxicity of nanoplastics (NPLs) towards biota. Notwithstanding, most studies focused on the most abundant NPLs (such as PS and PE) and mainly on marine species, remaining a significant knowledge gap on the potential toxicity of other polymers, equally present in environmental samples (e.g., polymethylmethacrylate - PMMA), and on the toxicity of NPLs on freshwater species. Among freshwater invertebrate species, the cnidarians have been poorly studied in ecotoxicology, despite the advantages they offer as model species, namely due to their regenerative capacity. Regarding freshwater vertebrates, amphibian species are known to be at rapid decline, thus, being chemical contamination a major cause for such decline. Therefore, identifying and assessing the extent of potential effects of contaminants (such as NPLs) on these organisms is critical to their preservation. Accordingly, this work aimed at assessing the effects of sublethal levels of PMMA-NPLs on the body morphology and regenerative capacity of *Hydra viridissima* (cnidarian) and developmental stage and body morphology of *Xenopus laevis* tadpoles (frog). Morphological changes in *H. viridissima* were detected at concentrations ≤80 mg of PMMA-NPLs/L. At 80 mg PMMA-NPLs/L, 20% of hydrants exhibited no tentacles, while the remaining presented severe changes not yet reported in the literature, specifically the presence of doubled tentacles, elbow-like tentacles and curly tentacles. Concentrations of 40 mg PMMA-NPLs significantly delayed the regeneration of the cnidarians comparatively to the hydras in the control. The tadpoles showed a higher sensitivity to PMMA-NPLs comparatively to the cnidarians. Although not inducing significant effects of on the weight of tadpoles, 1 mg of PMMA-NPLs provoked severe injuries in the abdomen zone, namely externalization of the gut more than half of the organisms presented. The results obtained in this work suggest that cnidarians and early life stages of amphibians are good indicators for assessing freshwater quality in what concerns NPLs. Other NPL polymers are known to induce injury and death on young life stages of amphibians and cnidarians; however, no data has been generated so far relatively to PMMA-NPLs. These results provide important baseline information to further understand the toxic potential of PMMA-NPLs to freshwater biota, since it provided results not reported in the scientific literature to date. This work provides important information liable to be framed into more adequate NPLs' risk assessment schemes for the freshwater compartment.

Environmental Risk Assessment of Bees and Other Arthropods Pollinators: Regulatory and Scientific Challenges for PPP and Biocides

4.08.03

Exploring the Variability of Honeybee Colony Dynamics Using the BEEHAVE Model

A. Focks, University of Osnabruck / Institute of Environmental Systems Research; A. Ippolito, EFSA - European Food Safety Authority / Pesticides Peer Review Unit

The BEEHAVE model simulates the development of a honeybee colony and its nectar and pollen foraging behavior in different landscapes. The default landscape used in BEEHAVE consists of two flower patches, which are located at different distances from the hive, and have shifted phenology, but are identical in all other aspects (size, nectar provision, pollen provision, detection probability). Understanding how colony dynamics can vary under different environmental

conditions is a fundamental step before trying to establishing benchmarks for assessing bee health. Due to current limitation of data availability, a realistic definition of landscape scenarios based on data appears very challenging. Scenarios were developed for 5 different geographical locations (D1-D5) in Europe, covering a latitudinal gradient from Sweden to southern Italy, characterised by different temporal patterns of temperature and solar irradiance, determining foraging activity and egg-laying rate. Furthermore, different landscape structures were considered by using four dimensions: 1) Flower patch fragmentation; 2) Heterogeneity in size of flowering patches; 3) Asynchrony of flowering in the different patches and 4) Heterogeneity in pollen and nectar level provided by the different patches. For each of these four dimensions three levels (low, medium, high) were assumed and translated in numeric terms. Hence, 81 different spatiotemporal landscapes have been defined for each geographical location. Overall, 405 combinations of scenarios*landscapes have simulated with BEEHAVE in 500 replicate runs per combination. Results of BEEHAVE simulations for these scenarios are analysed with respect to their influence on colony survival, variability of colony strength, forager lifespan, and honey production. A rather clear trend has been observed: In general, landscape complexity tended to increase variability between replicate runs. Patch spatial fragmentation and temporal asynchrony of flowering were always the main driver of this increase. Landscape complexity increased colony size variability mainly via food inflow variability, which in turn was determined by different food patch detection probabilities. The effect of food flow variability on colony size variability was stronger in scenarios with longer foraging periods.

4.08.04

Beehave-Ecotox - a Mechanistic Effect Model for Honeybees

T. Preuss, Bayer Ag / Environmental Safety; A. Agatz, B. Goussen, IBACON GmbH / Ecological Modelling; V. Roeben, Bayer AG / Environmental Safety; J.C. Rumkee, Syngenta / Product Safety; L. Zakharova, IBACON GmbH / Ecological Modelling; P. Thorbek, BASF SE / Agricultural Solutions

Bees are important pollinators and thus an essential part of the environmental risk assessment of pesticides in the EU. In this presentation we introduce the BEEHAVE-ecotox model, which has been developed to mechanistically link the realistic exposure of bees in the field, e.g. through foraging on nectar, pollen, and water, with subsequent effects on different levels of the colony. This model is an integral part of BEEHAVE, but can be applied to any mechanistic model of bee colony dynamics. Two alterations has been made to the original BEEHAVE model: 1) The colony setup was modified to allow the setting of specific starting conditions of the hive, which is relevant when simulating field studies. 2) An ecotox module was added to allow the simulation of effects of PPPs by following oral and contact exposure, ultimately simulating the lethal effects on adult bees and brood as a result of foraging and pollination. The ecotox module includes four submodules: an external exposure module, an in-hive fate module, a water foraging module, and an effect module. The new BEEHAVE-ecotox model was analysed and validated using laboratory studies and tunnel field studies of two PPPs with different modes of action (dimethoate and fenoxycarb) which are used as toxic standards in studies. Dose-response curves from laboratory studies were used to simulate lethal effects from oral and contact exposure. The model validation was based on two tunnel field studies. For both substances, the BEEHAVE-ecotox model was able to predict the effects on adult bee populations. The ecotox model was able to predict the relative initial effects of dimethoate on adults during the exposure phase in the tunnel and the lack of recovery in the weeks after exposure. Similar results were obtained for fenoxycarb. Model predictions after the tunnel exposure were less precise as sufficient data on the landscapes after the tunnel phase was lacking. The BEEHAVE-ecotox model is a suitable tool to predict the effects of plant protection products on bees. It is the first model to mechanistically predict PPP exposure both outside the hive to foragers and within the hive from several routes of exposure. For the regulatory risk assessment the model can potentially be used to extrapolate from laboratory to semi-field and field studies. Furthermore, it offers the possibility to study the effects in different crops and regions and to test different mitigation strategies.

4.08.05

Residues in Nectar and Pollen: Challenges in Analysis and Potential Pitfalls

A. Kiemle, Eurofins Agroscience Services EcoChem GmbH / Residue Analysis; S. Knaebe, EAS Ecotox GmbH / Ecotox Field

Pollinator species are important keystone species in our environment. This is an accepted concept and every effort is made to protect these species that are so essential for the ecology but also our economy. Already in the first risk assessment for plant protection products this fact was acknowledged with a risk assessment of honey bees as a surrogate species (EC 1991). In the newest regulation, adverse effects of plant protection products on bees are investigated even more thoroughly and not only honeybees are included but other pollinators as well (EFSA 2013). Furthermore, the risk assessment was improved by systematically including exposure assessments through the determination of residue levels in nectar and pollen (pollinator matrices). For this purpose existing analytical methods for crop residues can occasionally work as a basic concept but need to be adapted for each specific matrix that may occur in pollinator studies. The requirements for analytical methods supporting the generation of data for registrations are regulated in SANCO/3029/99 rev. 4 that also includes the method validation. But what are the various challenges and potential pitfalls that analytical laboratories face in developing analytical methods for pollen, nectar, and other bee relevant matrices? In our presentation, we will review the most important factors and most challenging tasks for the determination of PPP in pollinator samples based on our own experience. Beginning with the low sample amounts that lead to a literal one-shot analysis or the ever-increasing demand for lower LOQ (Limit of Quantification) levels, one has to take into account all aspects of the scientific process to ensure a reliable determination of pesticide residue levels in pollinator matrices. Close cooperation between biologists, ecotoxicologists and analytical chemists is therefore important for the successful evaluation of the influence of PPPs on pollinators. EC (1991): Council Directive 91/414/EEC concerning the placing of plant protection products on the market EC (2000): Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99, rev. 4, 11/07/00. EFSA (2013): Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal (2013) 11(7):3295, 266 pp.

4.08.06

Honeybee and Bumblebee Exposure to Post-Flowering Applications of an Insecticide in Apple Orchards

M. Miles, Bayer CropScience UK / Environmental Safety; A. Kiemle, Eurofins Agroscience Services EcoChem GmbH / Department of Residue Analysis; S. Knaebe, EAS Ecotox GmbH / Ecotox Field; P. Radix, R. Sur, Bayer AG - Crop Science Division / Environmental Safety

Pollinators such as Honey and bumblebees may be exposed during their foraging to a range of pesticides that are applied in agricultural practices. Applications during flowering to crops which are highly attractive for pollen and nectar represent a worst-case exposure scenario for bees. However, other exposure scenarios have been proposed such as exposure to weeds present in fields, flowering plants at field margins, adjacent flowering crops and succeeding crops (EFSA 2013). Risk assessment schemes have proposed tier I dietary exposure estimates based on worst case food consumption rates combined with default exposure levels. These exposures are expressed as dose/bee in line with the endpoints from test guideline studies. A risk assessment can then be conducted by comparing the ratio of the exposure to the study endpoint value to an agreed trigger value. One of the drawbacks of this approach is that it assumes 100% of the exposure comes from each scenario. In the case of a flowering bee-attractive crop such as oilseed rape a significant proportion of the foraged pollen and nectar may come from the treated field. In comparison the number of attractive weeds in the same crop either pre- or post-flowering offers a much lower food resource as do flowers present in the field margins. The difference in the proportion of food obtained from weeds and flowers in the field margins compared to a mass flowering crop is not accounted for at tier I. In the risk assessment exposure is based on a colony receiving 100% of its dietary needs from these sources alone. It seems unlikely that because weeds occur in fields at low densities compared to the crop that colony dietary needs could be met completely by these plants and hence the exposure to the colony at tier I is overestimated. One way to deal with this problem could be to introduce a factor to account for the proportion of diet coming from the weeds or margins at the colony or population level. However, clear guidance nor default values are available. To try to overcome some of issues surrounding exposure estimates for post-flowering applications we conducted a study to measure the concentration of an insecticide found in pollen and nectar of returning forager bees sited at the edge of five apple orchards which had received two post-flowering applications. Post-flowering apple orchards were not highly attractive to bees, however when sited at the edge returning foragers carried pollen nectar originating from the treated area. Surveys of vegetation in the orchard and surrounding areas indicated that bees forage on a wide range of plants. The test item and major metabolite were detected in pollen and nectar confirming exposure to the treated field but at low levels. From this study we shall show how exposure modelling can be used to refine the exposure of bees to post-flowering insecticide applications via in-field-weeds and compare these findings to worse-case assumptions used at tier I. Furthermore, we shall explore the relationship between honey and bumblebees to their environment to calculate factors which could be used to achieve a more realistic exposure assessment for applications made when a crop is not in flower.

Environmental Risk Assessment of Chemicals with Difficult to Test Properties: Challenges, Solutions, and Strategies

4.09.03

Challenges in an ENvironmental Risk Assessment for Fast Degrading Substances

F. Groezinger, BASF SE / Agricultural Solutions - Global Ecotoxicology; B.

Erzgraeber, S. Gebler, BASF SE / Agricultural Solutions - Global Environmental Fate Modelling; M. Habekost, BASF Corporation / Agricultural Solutions - Global Ecotoxicology

In the EU, sales of pesticides amount to 360 000 tons on average per year according to Eurostat 2020. Considering these numbers, contamination of soil and waterbodies is under critical surveillance. Hence, in the EU regulation for plant protection products, persistency is one of the properties which can trigger a cut-off if the substance shows additionally certain other properties, such as high toxicity or bioaccumulative potential. Fast degradation is a characteristic which is actually desired for a plant protection product in order to avoid lasting contamination in the environment. However, EU regulation and current guidance for study evaluation as well as for risk assessments are not ready to fully cover the benefit of very short-term environmental exposure duration. Instead they pose various challenges for the approval of fast degrading substances. This presentation aims towards highlighting common challenges in the testing and environmental risk assessment of fast degrading substances in the area of aquatic ecotoxicology. Based on different case studies novel approaches on how to overcome some of these challenges are presented. For example, proposals will be made to improve the conductance and interpretation of aquatic toxicity studies via adapted analytical sampling scheme and application of kinetic degradation models. This approach enables the adequate derivation of mean measured concentration as time-weighted average even if recovery of the test substance is not given at the end of the exposure period. Furthermore, examples will be given how lacking information in older aquatic studies could be compensated for by considering information gained in studies e.g. from environmental fate and thus may reduce the need for a repetition of aquatic (vertebrate) studies. The examples presented highlight the challenges and implicit levels of conservatism which are applied to fast degrading substances in the current European pesticide assessment scheme. For the evaluation of such substances in risk assessment, environmental behaviour should be considered and compared to realistic environmental scenarios. Ways forward are presented and a discussion on the inclusion into upcoming guidance documents is recommended in order to (i) reduce unnecessary (vertebrate) testing and (ii) come to tailored risk assessments for fast degrading substances.

4.09.06

Measurements and Predictions of Vapor Pressures of Chlorinated Paraffin Congeners From Technical Mixtures

J. Hammer, National Institute for Environmental Studies (NIES) / Center for Health and Environmental Risk Research; H. Matsukami, National Institute for Environmental Studies / Center for Material Cycles and Waste Management Research; S. Endo, National Institute for Environmental Studies (NIES) / Health and Environmental Risk Division

Chlorinated paraffins (CPs) are a complex group of human-made chemicals, some of which are considered persistent organic pollutants (POPs) and are detected in various organisms and in environmental samples even from remote regions. To properly predict their environmental fate and effects it is important to understand their physical-chemical properties including vapor pressure. In this study, the first direct measurements of the partial vapor pressure (P) of CP congener groups (carbon chain length: C10-13 and chlorine number 4-10) above CP technical mixtures are presented. P for congeners from several industrial CP mixtures with different congener distribution was measured between 20 – 50°C using a generator column method. Saturation vapor pressure P^* for each congener group as a pseudo-chemical was estimated following the Raoult's law with the activity coefficient of 1. The enthalpy of vaporization (ΔH_{vap}) was determined using the slope of the logarithmic vapor pressure versus the reciprocal temperature. The results show that P^* decreased with increasing carbon chain length and Cl content and ΔH_{vap} ranged between 40 and 103 kJ/mol. For CPs with chain length longer than C13 vapor pressure was too low to be measurable in our experimental setting. The data was compared with estimations from literature and with COSMOtherm-based predictions. Depending on congener groups, deviations between measured log P^* values and values predicted from the COSMOtherm based method ranged between 0.01 to 0.57 log units (RSME = 0.38).

4.09.07

The Effects of Dissolved Petroleum Hydrocarbons on Benthics Organisms: Chironomids and Amphipods

N. Indiketi, Institut national de la recherche scientifique (INRS); G. Triffault-Bouchet, Ministère de l'Environnement et de la Lutte contre les changements climatiques (MELCC) / Centre d'expertise en analyses environnementales du Québec (CEAEQ); P. Groleau, K. Coté, L. Levert, MELCC; P. Couture, Université du Québec, INRS / Centre Eau Terre Environnement

New projects of oil exploration and exploitation are developed across the World to meet the growing demand for petroleum. One of them is the oil sands industry in Canada, which produces unconventional oils, bitumen, diluted for transport and called dilbit (diluted bitumen). However, despite advances in our knowledge of the ecotoxicological risk that these products represent, their effects on benthic organisms following a spill are still largely unknown. In order to fill these gaps, this study aims to determine the lethal and sublethal effects of two dilbits (Bluesky and Clearwater McMurray) and one conventional oil (Lloydminster) for two freshwater benthic invertebrates: *Chironomus riparius*, a non-biting midge, and *Hyalella azteca*, an amphipod. The objective of this study is to assess the toxicity of dissolved hydrocarbons, resulting from the physical dispersion of oil, immediately after a spill on the invertebrates selected. To this end, organisms were exposed for 7 days for chironomids and 14 days for amphipods to a fraction containing soluble hydrocarbons (WAF: water accommodated fraction; 10 g/L, 18 h of agitation, followed by 6 h of sedimentation). After exposure, the effects of hydrocarbons were determined using growth inhibition, mortality and antioxidant capacities. Dissolved hydrocarbons induced no clear effects on these biomarkers for *Hyalella azteca* and *Chironomus* riparius for concentration of 25 % and below. Assays for higher concentration are ongoing. Moreover, the presence of a natural sediment seems to provide a protective role against dissolved hydrocarbons. This study will contribute to improve our knowledge of diluted bitumen toxicity to freshwater invertebrates, needed to take decisions for spill hazard management in the aquatic environment

4.09.10

Effects of Short-Chain Chlorinated Paraffins on the Expression of Key Genes of Gammarus pulex at Two Exposure Times

J.W. Jaegers, Université de Liège (ULiege) / Sciences de la Vie; C. Joaquim-Justo, University of Liege / Laboratory of Animal Ecology et Ecotoxicology; E. Gismondi, LEAE - University of Liege / Laboratory of Animal Ecology et Ecotoxicology

Short-chain chlorinated paraffins (SCCPs) are organic alkane formulations that are released in the environment by industrial processes and electronic waste. They are widely dispersed by wind and water, and therefore have been found in numerous ecosystems and organisms worldwide, where they are not readily biodegradable. This long environmental half-life coupled with continuous dispersal on a planetary scale means that organisms may repeatedly be exposed to them at various time scales. Thus, this work aimed to investigate the effects of SCCPs on the relative mRNA levels of numerous genes involved in vital biological functions (osmoregulation, oxidative stress, the endocrine system, oxygen transportation, immunity and antitoxic defences) of males of the freshwater amphipod *Gammarus pulex.* They were exposed to environmentally relevant concentrations of SCCPs (10, 100, and 1000 ng/L) at two exposure times (7 and 21 days). Results indicate that SCCPs significantly modified the expression of genes from all the studied functions, depending on exposure time. All studied functions were especially impacted in the acute 7 days exposure, while only the expression of genes involved in oxygen transportation, apoptosis regulation, and antioxidant functions was modified after 21 days. This work concludes that SCCPs impacted the expression of genes coding for antioxidant and antitoxic defences as well as endocrine, osmoregulatory, apoptotic and immune functions of *G. pulex* at ng/L concentrations, with this effect changing over time. The effects of these contaminants are under-studied in invertebrates, however these results indicate that further investigations may be warranted and needed to understand their potential effects on freshwater ecosystems.

Interactions between Global Climate Change and Contaminants: the Need for a New Environmental Assessment Paradigm

4.10.01

The ECORISK2050 Project: Predicting the Effects of Global Change on the Emission, Fate, Effects and Risks of Chemicals in Aquatic Ecosystems

P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences; S. Welch, NIVA - Norwegian Institute for Water Research / Ecotoxicology; T. Lane, University of York / Environment Department; A.O. Desrousseaux, University of York / Department of Environment and Geography; A. Mangold-Doring, Wageningen University & Research / Environmental Sciences; F. Polazzo, IMDEA Water Institute / Aquatic Ecotoxicology; J. van Dijk, Copernicus Institute of Sustainable Development, Utrecht University / PRAS unit; J.D. Hader, Stockholm University / Department of Environmental Science and Analytical Chemistry; M. Hermann, Wageningen University and Research / Environmental Sciences; P. Nagesh, Copernicus Institute of Sustainable Development Utrecht University / Environmental Sciences; R. Gajraj, University of Natural Resources and Life Sciences (BOKU); S.K. Roth, Stockholm University, ACES / Department of Environmental Science and Analytical Chemistry; S. Mentzel, NIVA - Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment; A. Sobek, Stockholm University / Department of Environmental Science; J. Eitzinger, BOKU; J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; M. MacLeod, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; M. Grung, NIVA / Ecotoxicology and Risk Assessment; A. Rico, IMDEA Water Institute; A. van Wezel, University of Amsterdam/IBED Institute; B. Chefetz, The Hebrew University of Jerusalem / Soil and Water Sciences; S.C. Dekker, Copernicus Institute of Sustainable

Development Utrecht University / Department of Environmental Sciences; A. Boxall, University of York / Department of Environment and Geography
By 2050, the world population will reach nine billion people and three quarters of the global population will live in cities. The development path to 2050 will be marked by shifts in land use and weather patterns, and by changes in the way water and food resources are obtained and managed all over the world. These global changes (GCs) will affect the emissions, environmental transport pathways and fate of chemicals, and thus affect the exposure of the natural environment to chemicals. Future changes may also alter the sensitivity of ecosystems to chemical exposure. To help address these issues, the ECORISK2050 project brings together a world leading and interdisciplinary consortium of universities, research institutes, industry and regulatory and governmental authorities to deliver a cohort of Early Stage Researchers (ESRs). The coupled training goals and research objectives of the project are: (1) to assess how the inputs of chemicals from agriculture and urban environments and their fate and transport are affected by different environmental conditions, including those of specific EU regions, and how this will change under GC scenarios in order to assess the likely increase in chemical risks to human and ecosystem health; (2) to identify potential adaptation and mitigation strategies that can be implemented in the short and medium term to abate unacceptable changes in risks, and use the GC scenarios to propose robust implementation pathways, and (3) to develop a set of tools for use by industry and policy makers that allow the impacts of a range of GC-related drivers of chemical risks to be assessed and managed. The project will deliver the next generation of scientists, consultants and industry and governmental decision-makers who have the knowledge and skill sets required to address the changing pressures that chemicals emitted by agricultural and urban activities pose to aquatic systems on the path to 2050 and beyond.

4.10.02

Looking Towards Chemical Emissions in the Future: A Framework to Extend Shared Socio-Economics Pathways (SSPs) to Single Chemical or Group of Chemicals

A.O. Desrousseaux, University of York / Department of Environment and Geography; P. Nagesh, Copernicus Institute of Sustainable Development Utrecht University / Environmental Sciences; R. Gajraj, University of Natural Resources and Life Sciences (BOKU); A. Boxall, University of York / Department of Environment and Geography; S.C. Dekker, Copernicus Institute of Sustainable Development Utrecht University / Department of Environmental Sciences; J. Eitzinger, BOKU; K. Kok, Wageningen University / Soil Geography and Landscape Group
Since the publication of the 5 Shared Socio-economics Pathways (SSPs) by the Intergovernmental Panel on Climate Change (IPCC) in 2017, many sectors had developed their own scenarios to study the future. Despite knowledges that chemicals are used massively in our daily lives and are causing harmful effects on the environment, the future outlook of chemicals going though socio-economic change, climate change and technological development is uncertain because not studied yet. Projected societal changes by the IPCC over the next 30 years on chemical emissions is unknown. Protocols and methodologies have been published to extend SSPs to specific sector. Those methods are usually developed with the identification of common key drivers that will influence the storyline and with identification of study scales (could be local, city, country, agriculture field scale etc). Because of the heterogenous characteristic of chemicals, define key driver and relevant scale for all chemicals emissions is not easy. Chemicals include pharmaceuticals, pesticides, personal care products, preservatives, flame retardants and many others, all used and emitted in the environment via different paths. Moreover, physical and chemical properties of chemicals will influence persistence of chemicals and therefore the scale at which they should be studied. No common drivers and relevant scale can be determined for all chemicals. Therefore, to study chemicals in the future with scenario and overcome the specification of chemicals, we propose here a framework to extend SSPs to context-specific chemical emissions. This framework aims to develop scenarios for a single chemical or group of chemicals sharing similar features such as in usage (ex: antidepressant emissions in 2050 in Europe) or characteristics (perfluorinated chemicals in cities in Europe). The framework, inspired of other published methodologies has 4 steps: 1) define characteristics of scenario; 2) define connection between SSP drivers and thematic focus; 3) define key drivers and 4) develop scenarios. This framework allows the study of any chemicals in the future and can be used by researcher without the involvement of shareholders and with limiting time and financial resources. Chemical emissions in the future have to develop to be studied to develop adaptations and mitigations strategies with on strong knowledges and to identify future chemical pollution challenges.

4.10.03

Effects of Climate Change on Edge-Of-Field Agrochemical Exposure to Freshwaters

T. Sinclair, University of Sheffield / Animal and Plant Sciences; A. Boxall, University of York / Department of Environment and Geography; R. Williams, Centre for Ecology & Hydrology Maclean Building; L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences
Agrochemical application is a common input to agricultural systems but going forwards climate change may alter chemical risk and use. Transport of agrochemicals through soil represents a pollution risk to non-target ecosystems, with chemical properties and weather events, particularly precipitation, influencing transport through the soil matrix. Climate change will affect localised weather patterns resulting in an increased frequency of extreme events, potentially affecting agrochemical exposure. However, how chemical properties will interact with future climate change conditions to affect exposure is largely unknown. Here we assess how the environmentally relevant chemical properties, most prominently DT50 and kOC, from a suite of diverse agrochemicals interact with climate change to affect edge-of-field exposure to freshwater ecosystems. Agrochemical exposures have been predicted in PRZM for baseline (1981-2000) and future (2061-2080) utilising high resolution UKCP18 climate data for the UK. Sites were selected along a climatic gradient and chemical applications in summer and winter were modelled capturing spatial and seasonal differences in climate change. Chemicals fell into three distinct groups based upon their DT50 and kOC values. While chemicals in one group exhibit little change in exposure, the group of rapidly degrading chemicals were expected to see marked rises of ~200-400% compared to the baseline. These increases were more pronounced for Summer applications and in dryer sites. These results indicate that climate change can have dramatic effects of freshwater exposure to agrochemicals, but this is chemical dependent. Chemicals with rapid degradation times, usually pharmaceuticals exhibit large increases, potentially due to climate change causing an increased probability extreme rainfall events before degradation occurs. Consequently how and when different agrochemicals are applied to land may need to account for future climatic conditions.

4.10.04

Combined Effects of Heatwaves and Micropollutants on Freshwater Ecosystems: Towards an Integrated Assessment of Extreme Events in Multiple Stressors Research

F. Polazzo, IMDEA Water Institute / Aquatic Ecotoxicology; A. Mangold-Doring, Wageningen University & Research / Environmental Sciences; M. Hermann, Wageningen University and Research / Environmental Sciences; S.K. Roth, Stockholm University, ACES / Department of Environmental Science and Analytical Chemistry; A. Rico, IMDEA Water Institute; A. Sobek, Stockholm University / Department of Environmental Science; P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences; M. jackson, Oxford University / Department of Zoology
Worldwide, freshwater ecosystems are deteriorating at an unprecedented rate. Multiple anthropogenic disturbances drive the so-called Global Change. Habitat transformation is leading to various and still unpredictable changes in biodiversity, species interactions, and ecosystem functioning. Among the several drivers of Global Change, Climate Change is one of the main and most concerning ones. Climate Change is composed of different processes and phenomena. In recent years, extreme weather events have emerged as one of the most important facets of Climate Change. Extreme events have been shown to drive shifts in species composition and distribution, thus facilitating changes in ecosystem functioning. Heatwaves (HWs) are weather extremes characterized by a short-term, rapid increase in temperature able to affect a wide array of living organisms, from microorganisms to humans, across all ecosystem types. HWs are of particular concern since their magnitude and frequency is predicted to increase in the future. Extreme weather events are not the only disturbances affecting aquatic ecosystems. Chemical pollution is a serious threat to aquatic ecosystems. However, among the large number of stressors affecting the freshwater realm, chemicals have been somewhat overlooked. Although the effects of chemicals on freshwater biota have been studied intensively, our understanding on the combined effects of HWs and micropollutants is too limited. To highlight the major knowledge gaps of the field and to indicate avenues for future research, we reviewed the literature dealing with the effect of HWs (alone and in combination with chemicals) on different trophic levels of freshwater ecosystems. Eventually, we provide recommendations on how using appropriate methods and endpoints to disentangle the combined effects of HWs and chemicals, which also contribute to increase the environmental relevance of chemical risk assessments.

4.10.06

Increased Daily Temperature Fluctuations Overrule the Ability of Gradual Thermal Evolution to Offset the Increased Pesticide Toxicity Under Global Warming

J. Verheyen, V. Delnat, KU Leuven / Biology; R. Stoks, KU Leuven / Biology - Evolutionary Stress Ecology and Ecotoxicology
The widespread evidence that global warming can increase species sensitivities to chemical toxicants, and vice versa, and the recent insight that thermal evolution may mitigate these effects is crucial to predict the future impact of toxicants in a warming world. Nevertheless, a major component of global warming, the predicted increase in daily temperature fluctuations (DTFs) by the IPCC scenario RCP 8.5, has been ignored at the interface of evolutionary ecotoxicology and

global change biology. We studied whether 4 °C warming and a 5 °C DTF increase (to 10 °C DTF) magnified the negative impact of the insecticide chlorpyrifos in larvae of low- and high-latitude populations of the damselfly *Ischnura elegans*. While 4 °C warming only increased chlorpyrifos-induced mortality in high-latitude larvae, the high (10 °C) DTF increased chlorpyrifos-induced larval mortality at both latitudes. Chlorpyrifos reduced the heat tolerance; however, this was buffered by latitude-specific thermal adaptation to both mean temperature and DTF. Integrating our results in a space-for-time substitution indicated that gradual thermal evolution in high-latitude larvae may offset the negative effects of chlorpyrifos on heat tolerance under warming, unless the expected DTF increase is taken into account. Our results highlight the crucial importance of jointly integrating DTFs and thermal evolution to improve risk assessment of toxicants under global warming.

4.10.07

The Influence of Temperature on the Lethal and Sublethal Effects of Two Insecticides, Imidacloprid and Flupyradifurone to Gammarus pulex

A. Huang, Wageningen Environmental Research - WUR; A. Mangold-Doring, Wageningen University & Research / Environmental Sciences; A. Focks, University of Osnabruck / Institute of Environmental Systems Research; P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences

Neonicotinoids are worldwide used insecticides, and cause high concern to aquatic system as they readily enter aquatic ecosystems. Among them, imidacloprid (IMI) is most used, but most applications are banned within Europe. The novel pesticide flupyradifurone (Flupy) is structurally close to imidacloprid and considered as the substitute of imidacloprid in the market. However, the toxicity of Flupy was largely unknown to non-targeted aquatic species. Beyond the conventional toxicity assessment, the influence of temperature on effects of Flupy and IMI appears relevant for two reasons. Firstly, previous studies have found that tropical species are more sensitive to IMI than temperate ones; secondly, under current climate warming background, a higher temperature scenario is more realistic. Hence, a toxicity assessment at different temperatures can shed some light on the importance of temperature in the risk assessment. For this, we plan to perform a 28 day chronic toxicity test with IMI and Flupy with *G. pulex* using five concentration levels plus control under three different temperatures. The temperature ranged from 7 to 24 °C to fully covered the field variation in temperature. The mortality and immobilization was determined every two to three days. Moreover, the food consumption of *G. pulex*, the internal concentration of exposed compounds, and the biotransformation metabolites will also be compared between different temperature and concentration levels. Based on preliminary results, the time to effect significantly decreased with increasing temperature, and high temperature increased the toxicity of Flupy. In detail, the organisms showed rapid effects at 24°C, and no effect under 7 °C until day 14. The time to reach 50% immobilisation at 30 µg/L was 4, 11 and 28 days under 24, 18, and 7 °C, respectively. On day 4, the EC_{10} of Flupy was 2 ± 0.8 µg/L at 24 °C, however, there was no effect observed at both 7 and 18 °C. On day 7, the control organisms at 24 °C showed high mortality, indicating that 24 degree was over the thermal tolerance of the winter generation of *G. pulex*. On day 28, the EC_{50} at 18 °C was 10 ± 10 µg/L and it was 31 ± 20 µg/L at 7 °C. Furthermore, the food consumption at 18 °C was higher than at 7 and 24 °C for *G. pulex*. In both 7 and 18 °C, the highest concentration of Flupy considerably decreased the food consumption. The experiments with IMI and further experiments with Flupy will be performed in December 2020.

4.10.09

Probabilistic Risk Assessment of Pesticides Under Present and Future Agricultural and Climate Scenarios Using a Bayesian Network

S. Mentzel, NIVA - Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment; M. Grung, NIVA / Ecotoxicology and Risk Assessment; K. Tollefsen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; M. Stenrød, Norwegian Institute of Bioeconomy Research NIBIO / Department of Pesticides and Natural Products Chemistry; R. Holten, Norwegian Institute of Bioeconomy Research (NIBIO) / Department of Pesticides and Natural Products Chemistry; J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment

In Northern Europe, future changes in land-use and weather patterns are expected to result in increased precipitation and temperature, potentially causing an increase in plant disease and insect pests. In addition, predicted population increase will change production demands, in turn altering agricultural practices, such as crop types and thus use patterns of pesticides. Against this uncertain landscape, these variabilities and magnitudes of pesticide exposure to the aquatic environment need to be accounted for better in current probabilistic risk assessment. In order to improve ecological risk assessment, this study explores an alternative approach to probabilistic risk assessment using a Bayesian Network, as these can serve as meta-models that link selected input and output variables from other models and information sources. The developed model integrates variability in both exposure and effects in the calculation of risk estimate. We focus on environmental risk of pesticides in two Norwegian case study region representatives of northern Europe. Using pesticide fate and transport models (e.g. WISPE), environmental factors such as soil and site parameters together with chemical properties and climate scenarios (current and predicted) are linked to the exposure of a pesticide in the selected study area. In the long term, the use of tools based on Bayesian Network models will allow for a more refined assessment and targeted management of ecological risks by industry and policy makers.

Marine and Freshwater Pelagic and Benthic Harmful Algal Blooms: Toxins Production, Detection, Fate, Effects, Monitoring and Management.

4.11.01

Wide-Spread Retinoids Associated With Cyanobacterial Water Blooms and Microbial Mats in Aquatic Ecosystems Elicit Teratogenic Effects

K. Hilscherova, Masaryk University, Faculty of Science, RECETOX / RECETOX; L. Sehnal, M. Pipal, L. Blahova, M. Smutna, Masaryk University, Faculty of Science / RECETOX

Some cyanobacteria have been identified as producers of compounds affecting retinoid signaling with potential adverse effects on organisms. Our field studies investigated 36 phytoplankton blooms dominated by various species in independent water bodies in central Europe, where biomass samples and surrounding water were collected. Another field study focused on microbial mats in Antarctica was undertaken on the James Ross Island, where 67 samples were collected from various aquatic ecosystems. We have analyzed content of selected retinoids and the overall capacity of samples to interact with retinoic acid receptor (retinoid-like activity). We have characterized *in vitro* and *in vivo* toxic potencies of individual detected compounds and their mixture, environmental samples and their fractions. Some of the planktonic water blooms mostly dominated by cyanobacteria showed high levels of retinoid-like activity both in biomass (up to 867 ng/g dm ATRA concentration equivalents) and their surrounding waters (up to 263 ng/L). Some retinoids were observed across all sites with the highest cumulative concentration over 2200 ng/g dm in biomass and 135 ng/L in water. The samples of microbial mats from lakes, ponds, streams, seepages, wetlands and wet rock at Antarctica were dominated by cyanobacteria, followed by algae and other microbes. Several retinoids were detected in some of the microbial mat samples with concentrations ranging from not detected to relatively high level 2570 ng/g dm (retinal). All retinoids detected in the field studies caused retinoid-like activity *in vitro* and teratogenic effects in zebrafish embryos. The relative potencies of the individual compounds compared to ATRA (REP 1) were between 0.018 (retinal) and 0.69 (5,6epoxy-ATRA). The zFET showed adverse effects at low concentrations of several retinoids with spine and craniofacial deformations being most common. Relative potencies for individual compounds and effects of fractions and reconstituted mixtures from the *in vitro* assay showed good predictability towards effects *in vivo*. This research documents common production of compounds with retinoid-like activity by cyanobacterial water blooms from temperate regions as well as by microbial mats in polar regions. In the case of temperate regions, the development of massive water blooms can lead to greater levels of retinoid compounds posing risk to exposed organisms. The work was supported by the Czech Science Foundation Grant No.18-15199S, and 20-04676X.

4.11.02

Differential Labeling of Chemically Modified Cyanotoxins and Lipids Among Cyanobacteria Planktothrix and Microcystis

R. Moron Asensio, Research department for Limnology, Mondsee / Research Department for Limnology; R. Kurmayer, University of Innsbruck / Research Department for Limnology

The cyanoHAB forming cyanobacteria genera *Microcystis* or *Planktothrix* frequently produce high intracellular amounts of cyanotoxins such as microcystin and other bioactive peptides. In this study chemically modified peptides have been localized on a subcellular level in *Microcystis* and *Planktothrix* through applying the copper-catalyzed alkyl-azide cycloaddition (CuAAC) reaction. For this purpose, unnatural amino acids carrying alkyl or azide moieties were fed to individual Planktothrix strains No371/1 and CYA126/8 as well as *M. aeruginosa* strain Hofbauer showing a promiscuous incorporation of various amino acid substrates during nonribosomal peptide synthesis (NRPS). CYA126/8 mutants for peptide synthesis and *Synechocystis* PCC6803 which does not contain NRPS were used as control and processed under identical conditions. Fluorescent labeling of modified peptides was performed using the fluorophores ALEXA405 and ALEXA488. To investigate the localization of those chemically modified compounds in relation to lipid formation the lipid dye BODIPY505/515 was applied to quantify the spatial overlap as revealed from epifluorescence and high resolution microscopical imaging. Overall, the labeling of microcystins or anabaenopeptins in peptide producers revealed consistent results. While no labeling was observed in *Synechocystis* implying that unspecific labeling is

negligible. We also detected significant lipid staining in *M.aeruginosa* while lipid staining in *Synechocystis* and *Planktothrix* was low.

4.11.03

Investigation on the Occurrence of Cyanobacterial Secondary Metabolites in Lebanese Lake Karaoun Using Mass Spectrometry and Molecular Techniques

N.A. Hammoud, CNRS (LCABIE-IPREM, Université de Pau) / Laboratoire de Chimie Analytique Bio-Inorganique et Environnement UMR 5254; S. Zervou, NCSR Demokritos / Institute of Nanoscience and Nanotechnology; T. Kaloudis, EYDAP S.A.; A. Paraskevopoulou, C. Christophoridis, T.M. Triantis, A. Hiskia, NCSR Demokritos / Institute of Nanoscience and Nanotechnology; K. Slim, Lebanese Atomic Energy Commission-CNRS / Laboratory of Microorganisms and Food Irradiation; J. Szpunar, IPREM, CNRS-UPPA / Laboratoire de Chimie Analytique BioInorganique et Environnement LCABIE; A. Fadel, Lebanese Atomic Energy Commission-CNRS; R. Lobinski, CNRSUPPA, Laboratoire de Chimie Analytique Bio-Inorganique et Environnement UMR 5254

This is the first study on the wide variety of secondary metabolites produced and secreted by cyanobacterial blooms in the largest water body in Lebanon: lake Karaoun. The reservoir was built in 1960 for several purposes as irrigation, hydropower, recreation and anticipated drinking water supply. Eutrophication, first noticed in 2004, is leading to consecutive and persistent cyanobacterial blooms since 2009, that are in their turn drastically affecting the water quality and generating serious negative impacts (ecological, economic, social and sanitary). Microscopic identifications confirmed the dominance of *Aphanizomenon ovalisporum* in spring and autumn and *Microcystis aeruginosa* in summer over all other phytoplankton species. SPE-LC-MS/MS analysis was done on water samples to investigate on the occurrence of cyanotoxins (12 microcystins [dm MC-RR, MC-RR, MC-YR, MC-HtyR, dm3MC-LR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF], anatoxin-a, cylindrospermopsin and nodularin). Complementary q-PCR analysis was carried out to confirm the presence of cyanobacteria and their toxic genes. Furthermore, and due to the distinctive smell of the water, samples were analyzed by HS-SPME-GC-MS to determine the volatile organic and/or taste and odor (T&O) compounds originating from cyanobacteria and other sources. Results showed the presence of microcystins (MC-RR, MC-YR and MC-LW) and their genes in some samples. In addition, several volatile and T&O compounds were detected, with sulfur compounds accounting mostly for the characteristic septic odor. Furthermore, the presence of anthropogenic pollutants, such as toluene, is pointing out the issue of the co-existence of hazardous cyanobacterial metabolites with anthropogenic pollutants. The present study contributes to assessment of the water quality of lake Karaoun with regards to cyanobacteria metabolites in order to raise awareness about the intended usages and propose realistic mitigations to improve the water body's condition. **Keywords:** Cyanotoxins, Volatile Organic-Taste & Odor Compounds, SPE-LC-MS/MS, HS-SPME-GC-MS **Acknowledgments:** The authors warmly thank COST Actions CYANOCOST (ES1105) www.cyanocost.net, WaterTOP (CA18225), www.watertop.net, and the National Council for Scientific Research of Lebanon (CNRS-L) for granting a doctoral fellowship to Noura Alice Hammoud. **Track 4.17 – Marine and freshwater pelagic and benthic harmful algal blooms: Toxins production, detection, fate, effects, monitoring and management Presentation type: Platform**

4.11.05

Fast Detection Strategy for monitoring cyanobacterial blooms during the COVID-19 pandemic in Campania, Italy: the case of Lake Avernus

V. COSTANTINO, University Federico II / Pharmacy; G. Della Sala, University of Naples Federico II / Pharmacy; R. Teta, G. Esposito, University of Naples Federico II / Department of Pharmacy; M. Lega, M. Casazza, University of Naples "Parthenope"; M. Stornaiuolo, S. Scarpato, University of Naples Federico II

Cyanobacteria have a long history of ecological and health impacts. This unique class of photosynthetic microorganisms can generally survive in nearly all phototrophic aquatic environments, including recreational water bodies, fisheries, and reservoirs. In the last two decades, worldwide attention has been given to the ecological effects of cyanobacteria and to their production of secondary metabolites. They represent a not yet fully explored source of novel lead compounds for drug discovery, such as the dolastatins, cryptophycins, and curacins, smenamides that have in turn inspired the development of synthetic analogues with improved bioactivity and pharmacokinetics. However, cyanobacteria growing in freshwater and marine recreational areas may have a strongly negative impact on human health. Some species can form extensive blooms, and there is evidence that these are increasing during recent decades due to nutrient enrichment, especially phosphorus. Many cyanobacteria produce toxic secondary metabolites (cyanotoxins) with differing effects on health, ranging from mild skin irritations to severe illness. We have initiated a worldwide program to study their chemistry and biochemistry. In this communication, I wish to report our multidisciplinary strategy (*Fast Detection Strategy*, FDS) suitable for an early detection and constant monitoring of cyanobacterial blooms. This strategy combines remote/proximal sensing technology with analytical/biotechnological analyses. In this study, we used *Fast Detection Strategy* (FDS) strategy to analyse Lake Avernus 2020 bloom and we report on: satellite imaging data recorded pre- and during COVID-19 lockdown (in Italy it was between 6th of March to 15th of June 2020) that pointed out to a different "bloom" situation in the Lake Avernus. These data showed the presence of cyanoHAB that guided the sampling that was done on 11 February 2020. Samples's lab analyses confirmed: a. the cyanoHAB, by identification of the *Microcistys sp* b) the identification of MC-FR) the identification of the genes Mcy.

4.11.06

Environmental Photochemical Degradation of Cyanobacterial Toxins and Emerging Cyanopeptides

R.S. Natumi, Eawag; S. Marcotullio, Ecole Polytechnique Fédérale de Lausanne (EPFL); E. Janssen, Eawag / Environmental Chemistry

Intensified cyanobacterial bloom events threaten water quality due to adverse effects associated with the release of toxins and other bioactive metabolites including cyanopeptides. Many cyanopeptides are produced simultaneously from one specie. However, their environmental behavior has not been studied for cyanopeptides other than microcystins. To assess the risks associated with cyanopeptides, we need to understand their persistence and fate processes. As cyanobacterial blooms are dominant in sunlit surface water, photodegradation can be a major fate pathway. We investigated the photochemical fate of 54 cyanopeptides, including 28 microcystins, 8 anabaenopeptins, 13 cyanopeptolins, 2 cyclamides, and 3 aeruginosins, extracted from four strains of common bloom forming cyanobacteria specie. Cyanopeptides were exposed to simulated sunlight in the presence of lake matrix and pH effects were assessed from pH 7 to pH 10. Among all compounds, 15 cyanopeptides were persistent within 3 hours of sunlight exposure, 37 cyanopeptides degraded with half-lives of 5-14 hours, and 2 cyanopeptides degraded rather fast with half-lives of > 4 hours. Photochemical half-lives of 14 tyrosine-containing cyanopeptides decreased from nearly persistent conditions at pH 7 (half-live > 27 hours) to shorter half-lives at pH 10 (< 10 hours). We determined the bimolecular reaction rate constants with photochemically produced reactive oxygen species singlet oxygen that confirmed faster reactivities of the phenolate forms. These system-independent parameters can be directly applied to other exposure scenarios where the oxidant concentrations are known. Our results are among the first to quantify the environmental fate processes of cyanopeptides beyond microcystins and bring awareness to the fact that pH dependence should be considered when evaluating their degradation in surface waters. Furthermore, persistent cyanopeptides should be prioritized for the evaluation of their ecosystem and human health risks and for their abatement during drinking water treatment.

4.11.15

Constructed Wetlands to Mitigate Cyanobacterial Harmful Algal Blooms

P. Carvalho, Aarhus University, Department of Environmental Science / Department of Environmental Science; A. Potokar, Limnos d.o.o.; C.A. Ramirez-Vargas, Aarhus University / Biology; C.A. Arias, Aarhus University / Department of Biology

Water scarcity is pressing for water reuse - often the closer eutrophic water body containing cyanobacteria and toxins. In spite of the potential risks, climate change and the increasing drought periods are pushing towards a compulsory use in agriculture of water contaminated by cyanobacterial harmful algal blooms. However, the use of nature-based solutions, namely constructed wetlands, seems an attractive approach to manage cyanotoxins contaminated waters and overcome these issues. Constructed wetlands, in their different design possibilities have proven capable of cleaning many different types of water and pollutants. Under two ongoing projects we are studying the best design and operational conditions to enable a safer use of reclaimed water for irrigation.Eighteen microcosms of unsaturated systems (mimicking vertical flow constructed wetlands) were set up in a greenhouse. Filter media (sand and gravel), as well as vegetation options (unplanted, planted with Phragmites australis or Juncus effusus) are being compared. Synthetic water mimicking a eutrophic surface water body containing 10 µg L-1 of microcystin-LR and cylindrospermopsin is being used to feed the systems.The experimental set up proved to be robust, variation in inlet concentrations between the different parts of the feeding system was not significant for both microcystin-LR and cylindrospermopsin. However, differences in the effluent from the different microcosms for both cyanotoxins was also not significant. The values for removal from the systems were very variable within a 24h period. This might be due to: 1) microcosms were still adapting, or 2) the microbial community might have been disturbed by the sudden spike of cyanotoxins. New sampling campaigns are planned and a parallel experiment to characterize the biofilm structure and potential changes is currently being carried out to assess these two issues. Moreover, ongoing analytical work high-resolution mass spectrometry (HRMS) is expected to confirm if biotransformation occurred and which biodegradation pathways might be involved.Overall, preliminary results are encouraging to explore the potential of constructed wetlands to treat water contaminated with cyanotoxins. We will be excited to share the upcoming

developments of the project during the conference.

MixTox (I): Field Evaluation of Real-World Mixtures

4.12.01

Genotoxic Effects of PAHs Mixtures in Primary Hepatocytes of the Gilt-Headed Seabream (Sparus aurata)

B. Matos, NOVA School of Science and Tecnhology / Environmental Engineering; V. Branco, Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal; M. Diniz, UCIBIO, REQUIMTE / UCIBIO, REQUIMTE; M. Martins, MARE - Marine and Environmental Sciences Centre, NOVA School of Science and Technology (FCT NOVA), Caparica, Portugal / Dep. Science and Environmental Engineering

Polycyclic Aromatic Hydrocarbons are ubiquitous contaminants existing as complex mixtures in aquatic ecosystems, causing genotoxic effects, namely DNA damage, in organisms. However, evaluation of mixture mode-of-action in vivo assays is highly complex and mechanistic interactions are difficult to discern. Fish are widely used as a sensitive model for the evaluation of environmental risk. Hence, in vitro relevant models arise as important tools for mechanistic screening before validation in vivo. The Comet assay has been extensively used for the evaluation and determination of DNA strand breaks which are an indicator of the DNA damage. In the present study, we exposed primary hepatocytes of *S. aurata,* to ecologically-relevant mixtures and concentration range of PAH and analysed DNA damage by the Comet Asssay. *S. aurata* hepatocytes were isolated and exposed to four PAHs (0.1-50µM): Phenanthrene, Benzo(A)Pyrene, Fluoranthene and Benzo(B)fluoranthene, individually and in mixtures for 24h and 48h. The results showed that, for all treatments, there was a time- and concentration dependent increase in DNA damage. Also, a great increment of DNA (% in the tail) was observed at concentrations higher than 10 µM with benzo(a)pyrene and benzo(b)fluoranthene causing the strongest effects. Nevertheless, the most significant results were obtained for the mixtures of the PAHs with a significant increase of DNA, although results varied significantly according to mixture composition.Overall, these results showed that PAH mixtures aggravate relatively to exposures to individual compounds causing a major increase of DNA damage. However, mixture composition may greatly alter the final outcome.

Acknowledgement - This work was supported by Project PAHMIX - Mixtures of Environmental Carcinogens: a molecular approach to improve environmental risk assessment strategies (PTDC/CTA-AMB/29173/2017), financed by national funds from Fundação para a Ciência e Tecnologia (FCT; www.fct.pt). Beatriz Matos is financed by FCT via PhD grant 2020.09005.BD; Vasco Branco is financed by national funds via FCT through Norma Transitória - DL57/2016/CP1376/CT002. Marta Martins is financed through FCT under the Scientific Employment Stimulus - Institutional Call (CEECINST/00102/2018).

4.12.02

Do We Systematically Underestimate the Risks of Micropollutants?

M.L. de Baat, KWR Water Research Institute / Chemical Water Quality & Health; M. Kraak, University of Amsterdam / IBED-FAME

The aquatic environment is contaminated with an increasing variety of chemicals at low and fluctuating concentrations. These complex cocktails of micropollutants exert a potential threat to human health and aquatic biodiversity. This threat represents substantial scientific and methodological challenges to chemical water quality assessment approaches, as current policies focus on the chemical analysis of only a limited number of prioritized compounds. Consequently, many of the chemicals present in water samples remain unidentified, and their contribution to the toxicity of surface waters cannot be quantified. Therefore, there is a need for future-proof monitoring methods that allow for the impact assessment of the ever-changing complex chemical burden on aquatic ecosystems. Effect-based methods (i.e. bioassays) respond to the combined action of all bioactive chemicals in a sample regardless of their identification, and may therefore overcome limitations of compound-based water quality assessment methods.This poster summarizes the results of integrated compound- and effect-based water quality assessments, combining passive sampling with a variety of bioassays and (non-)target chemical analyses. Contamination source-specific chemical profiles became apparent, with metals and PAHs predominantly present at urban locations, pharmaceuticals and personal care products at WWTP locations, and pesticides at agricultural locations. Likewise, contamination source-specific bioassay responses were observed, suggesting a causal relationship between the detected compounds and the observed toxic effects. Nevertheless, generally, only a small fraction of observed bioassay responses is explained by measured compounds. In contrast, employing non-target chemical analyses revealed that the bioassays responses were positively related to the number of unknown compounds present in complex mixtures. These results underline that compound-by-compound approaches based on limited selections of identifiable chemicals will systematically overlook and underestimate the risks of the unknown contaminants that only appear in non-target chemical analyses. Hence, it is concluded that future-proof monitoring methods should combine passive sampling, bioassays that incorporate the combined action of all bioactive chemicals present, and non-target chemical analyses that quantify the environmental contaminant burden.

4.12.03

Effects of Complex Pesticide Mixtures in Agricultural Runoff on Pimephales promelas and Daphnia magna

S. Stinson, N. Egan, University of California Davis / VMAPC; X. Deng, California Environmental Protection Agency / Pesticide Regulation; S. Lawler, University of California Davis / Department of Entomology; S. Hasenbein, University of California, Davis / VMAPC; E.B. Holland, California State University, Long Beach / Biological Sciences; R.E. Connon, University of California, Davis / School of Veterinary Medicine Anatomy, Physiology and Cell Biology

Runoff entering waterways from agricultural and urban areas can introduce influxes of highly toxic, complex chemical mixtures into nearby aquatic habitats, causing rapid changes in water quality. These dynamic mixtures often include chemicals that are known to have adverse biological effects in single chemical laboratory exposure experiments. Our goal was to assess mortality, plus molecular and behavioural responses to exposure to agricultural runoff. Runoff was collected from three sites (a reclamation ditch, a tributary and the main stem of the Salinas River) located in an intensely farmed agricultural region in the Central Coast of California, USA. Four day (96h) exposures to geometric dilutions of ambient water samples were conducted using a model fish (*Pimephales promelas*) and a sensitive invertebrate (*Daphnia magna*). Mortality, differential gene expression (qPCR) and swimming behaviour were measured. Analytical chemistry detected low concentrations of eighteen individual chemicals of concern including several pyrethroids, imidacloprid, and chlorantraniliprole. For one site, the carbamate insecticide methomyl was present at a concentration well above the EPA benchmark for aquatic life. High levels of invertebrate mortality occurred even at dilutions as low as 12% in the two sites corresponding with the highest detection levels of several chemicals of concern. Differential expression of genes involved in first line detoxification and general stress pathways (CYP1a), muscular and neuronal function (RYR2) was observed in the two sites containing higher chemical concentrations. *P. promelas* and *D. magna* were significantly hyperactive relative to controls in the same sites, as measured by total distance (mm). Fish also demonstrated reduced photomotor response to light stimuli in the same two sites with the highest levels of several chemicals of concern. Altered behaviour is an ecologically important endpoint that may have implications for survival if it affects predator avoidance and/or foraging. While routine chemical screening and acute single chemical toxicity tests are useful tools to understand the short-term impacts of complex mixtures, they may not accurately reflect impacts in natural environments. These findings suggest that the use of multiple endpoints and testing strategies will greatly improve our ability to predict biological responses in a complex environment.

4.12.04

Chemical ACTivity of Persistent Organic Pollutants Mixtures As a Measure of Chemical Pollution in Caulobacter crescentus

B.J. Sánchez, Stockholm University ACES / Department of Environmental Science; K. Jonas, Stockholm University MBW; A. Sobek, Stockholm University / Department of Environmental Science

Persistent organic pollutants (POPs) are substances that pose a risk to human health and the environment. This group of organic compounds possess a particular combination of physical and chemical properties such that, once released into the environment, they remain intact for exceptionally long periods of time, as they are resistant to photolytic, chemical and some of them on biological degradation.Bacteria are a major component of the aquatic environment and, due to their capacity to bioaccumulate hydrophobic organic chemicals, they provide a fundamental route to their transport into food chains and play a major role in the recycling and uptake of contaminated organic matter. In the present study, the freshwater alphaproteobacterium *Caulobacter crescentus* (*C. crescentus*) was used as a well-established model organism regarding cell division, morphology and stress responses, making it a good candidate to investigate how chemical mixtures affect bacteria on the cellular level and, in consequence, microbial functions in the ecosystem.Our present work builds on assessing how *C. crescentus* reacts to exposure to individual PAH and PCB congeners and to their chemical mixtures. Stable exposure concentrations will be maintained through the use of passive dosing. To assess the potential toxicity associated with mixture exposure, concentrations of the individual compounds in each mixture, below its specific toxicity threshold, will be converted to chemical activity by normalizing to its solubility in water and, thereafter, the chemical activity of individual compounds will be added up to generate the total chemical activity load of the mixture. For the passive dosing, silicone rods will be loaded by partitioning from a methanol solution containing the chemical compounds. *C. crescentus* cultures will be exposed to the silicone rods up to 24 hours and the resulting cellular effects will be analysed and quantified according to their cell viability, growth rate, cell shape and size, and induction of stress response pathways. POPs will be analysed before and after passive dosing exposure by gas chromatography/mass spectrometry (GC/MS) in order to address chemical sorption to bacterial cells and possible

chemical transformations by the microorganisms. Despite molecular insights into bacterial stress responses and adaptation, little is known about how ubiquitous chemicals in the environment and combined effects of chemical mixtures affect bacterial cells. The relevance of our work is based on the assessment of how the chemical activity of a mixture of chemicals present at environmentally relevant concentrations may drive biological activity and effects in bacteria.

4.12.05

General Unified Threshold Model of Survival (GUTS) - Extending the Toxicokinetic-Toxicodynamic Framework for Interaction Analyses (Synergism and Antagonism)

S. Bart, University of York / Department of Environment and Geography; T. Jager, DEBtox Research; A. Robinson, C. Badder, UK Centre for Ecology & Hydrology; E. Lahive, UK Centre for Ecology and Hydrology; C. Svendsen, CEH, Wallingford; D. Spurgeon, Centre for Ecology & Hydrology; R. Ashauer, Syngenta Crop Protection AG / Environment

The assessment of chemical mixture hazard is one of the major challenges in ecotoxicology and it is overlaid by the fact that chemicals can interact, leading to more or less effects than expected, commonly named synergistic and antagonistic interaction, respectively. The classic ad hoc approach for the assessment of mixture effects is based on the dose response curves. However, it is stuck at the level where it can highlight an interaction in mixture but cannot provide any mechanistic explanations, predictions afterward, and cannot account for time-varying concentrations that requires mechanistic models. We here propose a new approach using toxicokinetic-toxicodynamic (TK-TD) modelling. In earlier work, we extended the General Unified Threshold model of Survival (GUTS) framework with a module for mixture hazard assessment, and demonstrated its application with dedicated experiments and literature datasets. Here, we designed a dedicated mechanistic interaction module coupled with the GUTS mixture model to i) highlight interactions, ii) test hypotheses through the modelling to highlight which chemical is likely responsible for the interaction, and finally iii) simulate and predict the effect of synergistic and antagonistic mixtures. We tested the modelling approach with dedicated experiments expected to be synergistic with two species (*Enchytraeus crypticus* and *Brassicae mamestra*), and on literature data with two others species (*Bombus terrestris* and *Daphnia magna*) exposed to mixtures found to be synergistic and antagonistic with the classic approach. The results showed that our modelling approach can accurately simulate synergistic, antagonistic and potentiation interaction with different species and mixtures. The model also appears useful as a potential screening tool to explore interaction in mixture hazard assessment. We recognize and discuss the limitation of the approach which required trade-offs due to the use of the reduced GUTS model and may miss more complex interaction which does not follow the assumptions formulated in the interaction module.

4.12.06

Toxicokinetics of Pesticides in Gammarus Spp. Under Field Conditions: Comparing the Influence of Dietary Uptake to Aquatic Exposure Dynamics

B.B. Lauper, E. Anthamatten, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Chemistry; M.J. Arlos, University of Alberta / Department of Civil and Environmental Engineering; H. Singer, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Chemistry; J. Hollender, Eawag / Environmental Chemistry

A large number of organic micropollutants reach aquatic systems from various sources. Knowledge on their bioaccumulation and biotransformation are essential to mechanistically link exposure to effect and to designate appropriate environmental quality standards. Bioaccumulation and biotransformation of organic micropollutants in aquatic organisms have been investigated in laboratory experiments extensively. For several micropollutants (MPs), however, the measured internal concentrations in *Gammarus spp*. in field trials exceeded the predictions based on the laboratory data, sometimes by multiple orders of magnitude. These exeedences were mainly observed for pesticides, especially neonicotinoid insecticides and azole fungicides. In this work, we tested the impact of both dietary uptake and aqueous exposure dynamics on the toxicokinetics of pesticides in aquatic invertebrates with a field trial using caged *Gammarus spp.* deployed in a small Swiss stream known for receiving high pesticide loads. Using a fully automated mobile LC-ESI-HRMS/MS system, the aqueous concentrations of 77 micropollutants were measured at high temporal resolution (20 min for 1 month). The data from this caging study were then employed to model exposure pathways of pesticides into gammarids, with a hypothesis that several routes of exposure can contribute to the overall bioaccumulation. The whole body concentration of gammarids was modelled using a one-compartment toxicokinetic model with parameters taken from literature. Different exposure scenarios were then explored and compared to the body burden measured in caged gammarids. Our results suggest that the internal pesticide burden of gammarids is highly dynamic, with the aqueous exposure as the main influencing factor. Most biomonitoring studies only measure micropollutants in organisms via grab samples, and our observations indicate that this sampling technique can lead to significant underestimation of the acute risk for aquatic organisms. Furthermore, we demonstrated that dietary uptake does not contribute significantly to the total body burden during peak exposures. Nevertheless, it can play an important role in prolonged exposure, likely contributing to the organism's baseline exposure due to sorption of pesticides to detritus leaves that act as a food source for the gammarids.

4.12.07

Identifying Adverse Outcome Pathways (AOP) for Amsterdam City Fish by Integrated Field Monitoring

R. van der Oost, Waternet / Onderzoek en Advies

IDENTIFYING ADVERSE OUTCOME PATHWAYS (AOP) FOR AMSTERDAM CITY FISH BY INTEGRATED FIELD MONITORING Ron van der Oost[1*], David J. McKenzie[2], Frank Verweij[1], Carl Satumalay[1], Natascha van der Molen[1], Matthew J. Winter[3] & J. Kevin Chipman[4]. [1] Waternet Institute for the Urban Water Cycle, Amsterdam, The Netherlands; [2] UMR Marbec (CNRS–IRD-Ifremer-Université Montpellier), Montpellier, France; [3] College of Life and Environmental Sciences, University of Exeter, Exeter, UK; [4] Biosciences, University of Birmingham, B15 2TT Birmingham, UK, [*]: E-mail: ron.van.der.oost@waternet.nl The European City Fish project aimed to develop a generic methodology for ecological risk assessment for urban rivers. Since traditional methods only consider a small fraction of substances present in the water cycle, biological effect monitoring is required for a more reliable assessment of the pollution status. A major challenge for environmental risk assessment (ERA) is the application of adverse outcome pathways (AOP), i.e. the linking of pollutant exposure via early molecular and biochemical changes to physiological effects and, ultimately, effects on populations and ecosystems. We investigated the linkage between exposure to and responses of micropollutants at different levels. Many AOP aspects were investigated, from external and internal exposure to different classes of micropollutants, via molecular key events (MKE), impacts on organs and organisms (fish physiology), to changes in the population dynamics of fish. Risk assessment procedures were evaluated by comparing environmental quality standards, bioassay responses, biomarkers in caged and feral fish, and the impact on fish populations. Although no complete AOP was observed, indirect relationships linking pollutant exposure via MKE to impaired locomotion were demonstrated at the most polluted site near a landfill for chemical waste. The pathway indicated that several upstream key events requiring energy for stress responses and toxic defence are likely to converge at a single common MKE: increased metabolic demands. Both fish biomarkers and the bioanalytical SIMONI strategy (Smart Integrated Monitoring) appeared to be valuable indicators for micropollutant risks to fish communities. **Key words:** adverse outcome pathways, micropollutants, risk assessment, biochemical & physiological biomarkers, ecological studies

4.12.08

Pesticide and Transformation Product Distribution in Water, Sediment and Fish of a Small Lentic Waterbody: A Field Study

S. Slaby, Université de Lorraine - UL / Biology; F. le Cor, Universite de Lorraine / URAFPA; V. Dufour, L. Auger, University of Lorraine / URAFPA; L. Pasquini, Anses - French Agency for Food, Environment and Occupational Health & Safety / LHN; O. Cardoso, Office Français de la Biodiversité / Unité Sanitaire de la Faune; L. Curtet, Office Français de la Biodiversité; J. Baudouin, Office Français de la Biodiversité / Unité Sanitaire de la Faune; L. Wiest, E. Vulliet, University of Lyon / ISA; C. Feidt, University of Lorraine - UdL / INRA URAFPA; X. Dauchy, Anses - French Agency for Food, Environment and Occupational Health & Safety / LHN; D. Banas, University of Lorraine / URAFPA

All over the world, fishponds are used to produce edible fish. Like in France, fishponds are often located in agricultural catchments, and thus exposed to pesticide mixtures. However, few studies are interested in systems such as ponds, and furthermore in transformation products (TP) occurrence in freshwater. In this context, this work proposed to assess pesticide and TP concentrations in abiotic and biotic compartments of a small lentic waterbody located in an agricultural watershed during a complete fishpond cycle.Forty substances (2 fungicides, 15 herbicides, 3 insecticides, 19 TP) were analysed in a dam fishpond (north-eastern, France). Compound quantifications were performed by LC-ESI-MS/MS in water, sediment and 3 fish species (Cyprinus carpio, Scardinius erythrophthalmus, Tinca tinca).In abiotic matrices, main results showed the occurrence of complex mixtures which differ between water and sediment. Highest mean concentrations were quantified for metazachlor OXA in water (519.48 ± 56.52 ng.L^{-1}, FOQ = 1) and for benzamide in sediment (4.23 ± 0.17 ng.g^{-1} dry wt., FOQ = 1). Compared to former study conducted 10 years ago at the same site including 3 similar compounds, an improvement of environmental quality was highlighted. However, it appeared that TP can occurred in a greater extend than parent compounds. It is the case for OXA and ESA TP of flufenacet, metazachlor and S-metolachlor (pesticides still authorized) and for TP of imidacloprid and atrazine (prohibited). In fish, excepted for benzamide and prosulfocarb, contaminants were sporadically (desmethylisoproturon, desnitro-imidacloprid hydrochloride, tebuconazole) or never detected. Benzamide concentrations decreased between T0 and TF probably due to detoxification mechanisms. Inversely, fish accumulated prosulfocarb

during the fish farming cycle. It might have accumulated through ingestion of contaminated sediment particles or preys.In conclusion, our work brings evidences of pesticide and TP mixture occurrence in abiotic and biotic compartments of a small lentic waterbody. There is a need to integrate TP into monitoring campaigns and to conduct studies with mixtures found in environment to assess more precisely toxicological risks. In fish, few molecules were found, but such results should be considered to define specific concentration thresholds in fish products and must constitute warning signal to governmental authorities in order to regulate compounds of concern.

4.12.09

State of Water Contamination at the Head of the Watershed: Assessing the Presence of Pesticides and Their Transformation Products and Highlighting the Potential of Ponds on Water Quality Improvement

F. le Cor, Universite de Lorraine / URAFPA; S. Slaby, V. Dufour, A. Iuretig, University of Lorraine / URAFPA; C. Feidt, University of Lorraine - UdL / INRA URAFPA; X. Dauchy, Anses - French Agency for Food, Environment and Occupational Health & Safety / LHN; D. Banas, University of Lorraine / URAFPA

The contamination of natural water by plant protection products (PPP) is recognized on a global scale. In France for example, where 9 out of 10 streams attest of such pollution. At the head of agricultural watersheds, pesticide occurrence and concentrations are exacerbated. Rapid transfers from treated agricultural land to headwater streams with low dilution capacities are observed. Totally neglected by monitoring programs, the contamination of these watershed heads has been highlighted by few studies, but their transformation products (TPs) are still rarely taken into account. Their presence at high concentrations, stability and increasing numbers make them particularly worrying. Environmental data is little documented, especially because of technical limitations and rapid development of new PPP products. Our studies highlighted the capacity of small lentic water bodies, like ponds, to mitigate PPP pollution at the head of agricultural watersheds. These ponds are densely present at the head of hydrographic networks, where they are often used as extensive fish production tools. Their remarkable hydraulic characteristics allow PPP concentrations mitigation between upstream and downstream rivers. In this study, we first assess water contamination at the head of an intensively managed agricultural watershed. Using a liquid chromatography and tandem mass spectrometry analysis, we are able to show high PPP and TPs mixture in all water samples upstream and downstream of a pond, with a mean of 18.3 different molecules per sample. Detected molecules are, in half of the cases, TPs even regarding samples from upstream of the pond. At the same time, we provide evidence of the capacity of the studied pond to reduce PPP and TPs concentrations in downstream rivers. For 90 % of the studied molecules, the pond shows positive reduction rates favoring a decrease in PPP and TPs concentrations. Finally, environmental and toxicological thresholds are exceeded upstream of the pond during several events, with total PPP and TPs concentrations reaching 27 µg/L at the highest. In all downstream samples, no exceedance event could be observed, with total PPP and TPs concentrations up to 2.2 µg/L, attesting significant improvement in water quality. Thus, ponds should be considered more often when considering mitigation actions targeting both levels of PPP contamination in headwater streams.

4.12.12

Identification of Toxicity Drivers in ENvironmental Mixtures by Target and Non-Target Chemical Analyses

V. Dupraz, EPOC, University of Bordeaux; C. Gardia-Parège, Université de Bordeaux, CNRS / UMR 5805 EPOC, LPTC; J. Gaillard, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC; D. Ménard, IFREMER / Laboratory of Ecotoxicology; N. Tapie, M. Dévier, E. Geneste, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC; F. Akcha, S. Stachowski, IFREMER / Laboratory of Ecotoxicology; H. Budzinski, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC

Despite the improvement of methodologies used to predict the toxicity of chemical mixtures, knowledge of the chemicals composing the mixtures is usually required. Hence, evaluating the toxicity of mixtures of unknown composition still represents a big challenge. Phytoplankton play a key role in aquatic food webs and allow rapid toxicity testing in the laboratory. Therefore, this study aimed to i) assess the toxicity of real-life mixtures, represented by POCIS extracts, on two marine microalgae and ii) identify the main toxicity drivers in the tested mixtures by the mean of target and non-target chemical analyses.POCIS were used to sample 3 river sites from a wine-growing area over 4 one-month periods. Microalgae were exposed during 96 h to 5 dilutions of POCIS extracts in sterile 48-well microplates. The toxicity of the extracts was expressed as bioanalytical equivalent concentrations (BEQ) using concentrations of 8 pesticides of known toxicity measured by targeted chemical analyses (BEQchem) and a reference chemical (diuron, BEQbio). For non-target chemical analyses, a full-scan MS analysis was performed by high-resolution mass spectrometry (HRMS) to select a list of chemical features having a higher abundance in the most toxic extracts, using a dedicated workflow. Selective (list of selected features) and non-selective (all ions) fragmentation were then performed and features were identified by comparing their fragmentation spectra with spectral libraries and classified according to Schymanski scale.The toxicity of POCIS extracts was concomitant with the phytosanitary treatments schedule. Targeted analyses permitted to identify a major toxicity driver in 4 extracts, explaining 19.5% to 53.0% of BEQbio values: spiroxamine, a fungicide used in viticulture against powdery mildew. The HRMS workflow selected 1094 suspect candidates and confirmed correspondence between molecular signatures of POCIS extracts and phytosanitary treatments schedule. From the suspect and non-target screenings, the herbicides norflurazon and simazine and the antibiotic clarithromycin were retained as potential toxicity drivers due to their low EC50 reported for microalgae of 6.2, 7.6 and 0.15 µg L-1, respectively.POCIS extracts can be used as mixtures representative of the pressure generated by the wine-growing activity. Combining laboratory toxicity testing with target and non-target chemical analyses makes it possible to identify the main toxicity drivers in real-life mixtures.

MixTox (II): Regulatory Approaches to Real-World Mixtures

4.13.02

Regional Challenges of Real-Life Aquatic Mixtures

L. Posthuma, RIVM / Centre for Sustainability, Environment and Health (DMG); J. Postma, R. Keijzers, Ecofide; J. Slootweg, RIVM / DMG; B.v. Wal, STOWA

Amongst the challenges to protect and restore environmental quality is the evaluation of mixture exposures and effects on a real-life scale. In this presentation, we analyse real-life mixture exposures and the effects of those on aquatic ecosystems. Our study area is the Netherlands, for which we collected all monitoring data since the early 1950s. The challenges are to explore what the likely impacts of those are, and whether or not management efforts and large budgetary investments help to forward water quality. We calculated relative spatial and temporal differences across surface water bodies, based on the monitoring data, so as to describe spatial differences in the relative levels of the mixture toxic pressure, for separate compound groups and the total mixtures. We found substantial regional differences in the relative importance of various compound groups contributing to the net mixture impact metric (the mixture toxic pressure). This stresses the need to not only start analyses and management by looking from a chemo-centric viewpoint (“what chemical does what harm”?) but also from a holistic viewpoint (“where do mixtures matter most, and due to which compound groups”?). We also developed a way to evaluate whether the mixture toxic pressure of compounds, compound groups or the total mixtures improved over the last decade, by comparing distribution functions. Again, starting from a holistic point of view, we were able to show that management efforts indeed should anticipate that a solely chemo-centric approach does not help to avoid “regrettable substitution”, whereby a risky compound is forbidden, whilst a replacement-compound causes similar environmental problems.

4.13.05

The LUXPEST Project - Screening of Locally Relevant Pesticides and Their Transformation Products in Luxembourgish Waters

J. Krier, University of Luxembourg / Environmental Cheminformatics Group; E. Schymanski, R. Singh, T. Kondic, University of Luxembourg / Luxembourg Centre for Systems Biomedicine (LCSB); P. Diderich, Luxembourgish Ministry

The diversity of organic pollutants, such as pesticides, and the lack of (publicly available) information about them is a huge challenge for environmental sciences, engineering, and regulation. Suspect screening based on high-resolution liquid chromatography-mass spectrometry (LC-HRMS) has enormous potential to help characterize the presence of pesticides and their transformation products (TPs). In LUXPEST, Luxembourgish river samples were examined for the presence of pesticides and their TPs. A suspect screening workflow was established, including LC-HRMS, suspect list generation, pre-screening, and spectral annotation. The pesticide suspect list was compiled of many lists from various sources, including multilingual Luxembourgish sources, along with authorization status and use information (DOI: 10.5281/zenodo.3862688). Afterwards, data analysis was performed using *Shinyscreen*, a new, open-access application developed in house, *MetFrag*, freely available software for spectra annotation of small molecules, and custom-made scripts. This revealed the presence of 162 potential pesticide masses (with 36 compounds achieving a level 2a). Following this, a TP suspect list was generated based on the 36 tentatively identified pesticides with a spectral library match above 0.9 (via *MetFrag*), equating to an identification confidence level 2a. A total of 181 TPs were selected through literature mining with *PubChem* and *HSDB* and screened in the samples to reveal 135 potential TP masses. After the tentative identification, the relationship between pesticides and TPs, plus the spatial and temporal distribution was investigated, to obtain an overview of the Luxembourgish surface water pesticide contamination. The spatial analysis revealed that the overall highest average number of compounds was found in the river “Alzette”, potentially under the influence of multiple pesticide sources. The temporal distribution revealed the expected hotspots of compounds in July and September. After suspect screening, the results were verified via the purchase of

selected (available) reference standards, with 23 compounds confirmed and quantified in the surface water samples. The outcomes of this study are being used to improve monitoring in Luxembourg and will lead to a better understanding of the origins and interactions of pesticides and transformation products in Luxembourgish rivers.

4.13.08

Quantifying Combined Toxicity to Better Protect Fish Consumers

E. Shaw, N.R. Urban, Michigan Technological University / Civil and Environmental Engineering

National policies and international conventions have led to decreased emissions of many contaminants, but some compounds persist in the environment for decades. Variable phase partitioning facilitates selective transport, leading to latitudinal patterns for some contaminants (e.g., PCBs). Waterbody characteristics affect aquatic food webs and thus exert influence on contaminant concentrations in fish. Mercury and PCBs each have multiple modes of action, and individual thresholds have been developed to protect human health. Little attention has been focused on quantifying threshold concentrations for combined toxic effects of simultaneous exposure. To better protect human health from mercury and PCBs accumulated in fish, the objective of this work is to elucidate combined toxicity patterns associated with waterbody characteristics. Specifically, this research asks, what waterbody characteristics most significantly influence combined toxicity of contaminants in fish? Waterbody characteristics (EPA National Lake Assessment, 2007, and NHD LakeCat) and mercury and PCB fish tissue concentrations (EPA National Lake Fish Tissue Survey, 2000) have been compiled. Calculated hazard quotients (HQ) will be used to identify spatial patterns of combined toxicity based on waterbody characteristics. Additive hazard quotients will be compared; the first approach will consider a shared endpoint (e.g., neurological effects) and the second will consider the most sensitive endpoint for each compound. The reference concentration will vary based on the health effect (RfD). Multivariate statistical analysis (e.g., principal component analysis) will be used to explain patterns in HQ throughout the U.S. Analysis of HQs across different dimensions may improve understanding of how conditions interact to affect combined toxicity. Regional patterns exist but differ for a variety of environmental variables (e.g., chlorophyll-a, DOC, nutrient concentrations, pH) that may affect contaminants. Additionally, national patterns in fish tissue concentrations of mercury and PCBs are not the same. This work identifies the environmental variables statistically associated with risk of cumulative effects of the two most prevalent fish contaminants. This work can inform policy on atmospheric emissions and deposition rates or allowable environmental concentrations guidelines for safe fish consumption.

4.13.10

Aquatic Environmental Risk Assessment for Crop Protection Mixtures - Is There an Efficient and Protective Approach?

A. Gladbach, Bayer AG, Crop Science Division / Research & Development, Disease Management; A. Weyers, Bayer AG - Crop Science Division / EnSa. Ecotoxicology; M. Ebeling, Bayer AG Crop Science Division / Ecotoxicology - Terrestrial Vertebrates Expert Team; C. Dietrich, M. Wang, WSC Scientific GmbH / .

Assessments of potential effects due to mixture toxicity in plant protection products (PPP) are part of the registration requirements in the EU. The most recent regulatory approach to address aquatic mixture toxicity is the guidance on tiered risk assessment for PPP for aquatic organisms in edge-of-field surface waters (EFSA 2013). The approach, however, is rather complex and requires several dozen individual assessments for typical cases (six to eight toxicological endpoints, depending on the PPP type, for each of e.g. nine exposure scenarios for the European Central Zone, excluding mitigation measures). In the present study, the existing risk assessment approaches are assessed for potential efficiency gains while mantaining the level of protection. It could be shown that an optimized approach for the aquatic ERA of PPP mixtures can be derived based on the previously established principles and assessment schemes. A step-wise approach is proposed: Step 1: Check if all RQs from n single substances are below 1/n. Step 2: If at least one RQ exceeds 1/n, search for a risk driver. If 90% of the risk (or more) is due to one substance, a risk driver is identified and the risk assessment is based on single substances in the following. Step 3: For cases where no risk driver can be identified, the mixture toxicity risk assessment is continued with the evaluation of the worst-case RQmix corresponding to the worst-case scenario and the worst-case risk assessment, respectively. If the worst-case RQmix is below the trigger value of 1, the risk of the mixture is considered to be acceptable. Otherwise, refinement options (e.g. PEC refinements) have to be considered before repeating the proposed risk assessment scheme. The alternative 3-Step approach led in most cases to the same conclusions as EFSA (2013). The results showed that in some cases results from both approaches differ to some extent. These differences were due to the use of measured data and questionable toxicity driver identifications in the EFSA (2013) scheme. It significantly increased efficiency resulting in a great potential for workload reduction for both regulatory agencies and applicants while mantaining the same level of protection.

4.13.12

Effect-Based Trigger Values for Mixtures of Chemicals in Surface Water Detected With In Vitro Bioassays

B. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; P.A. Neale, Griffith University / Australian Rivers Institute

Effect-based trigger (EBT) values for in vitro bioassays are important for surface water quality monitoring because they define the threshold between acceptable and poor water quality. EBTs have been derived for highly specific bioassays, such as hormone-receptor activation in reporter gene bioassays, by reading across from existing chemical guideline values. This read-across method is not easily applicable to bioassays indicative of adaptive stress responses, which are triggered by many different chemicals, and activation of nuclear receptors for xenobiotic metabolism, to which many chemicals bind with rather low specificity. Mixture factors have been invoked to deal with these bioassays to accommodate the mixture effects but the resulting trigger values were not able to differentiate well between contaminated and clean water samples. We propose an alternative approach to define the EBT from the distribution of specificity ratios of all active chemicals thus accounting for mixture effects. Specificity ratios are the ratio between the predicted baseline toxicity of a chemical in a given bioassay and its measured specific endpoint. Unlike many previous read-across methods to derive EBTs, the proposed method accounts for mixture effects and includes all chemicals, not only high-potency chemicals. The EBTs were derived from a cytotoxicity EBT that was defined as equivalent to 1% of cytotoxicity in a native surface water sample. The cytotoxicity EBT was scaled by the median of the log-normal distribution of specificity ratios to derive the EBT for effects specific for each bioassay. We illustrate the new approach using the example of the AREc32 assay indicative of the oxidative stress response and two nuclear receptor assays targeting the peroxisome proliferator activated receptor PPAR□ and the arylhydrocarbon receptor AhR. The EBTs were less conservative than previously proposed but were able to differentiate untreated and insufficiently treated wastewater from wastewater treatment plant effluent with secondary or tertiary treatment and surface water. Keywords: in vitro bioassays; mixtures;

4.13.15

From Exposure to Risk: What Can We Learn From Large Scale Chemical Monitoring and Chemical Mixtures ERA?

I.M. Rodea-Palomares, Bayer CropScience NA / Environmental Effects & Risk Assessment; A. Weyers, Bayer AG - Crop Science Division / EnSa. Ecotoxicology

Vital human activities such as agriculture and industrial production result in emission of synthetic chemicals (such as pesticides, industrial chemicals, or pharmaceuticals). The potential combined effects from these emissions are perceived to threat terrestrial and aquatic ecosystems. Environmental Risk Assessment (ERA) establishes a science-based framework in which both, exposure and effects are characterized to determine the likelihood (risk) of detrimental effects to non-target organisms. ERA typically follows a tiered approach in which lower tiers screen out “no concern” versus “potential concern” situations to determine if further studies are required at higher tiers (i.e., via improved and detailed exposure and or/effects characterizations) to further evaluate potential concerns identified at lower tiers. Evaluating environmental mixtures in ERA constitutes an increased level of complexity that can be understood as a higher tier refinement over single chemical ERAs. Price et al. 2011, 2012 proposed a classification scheme that allows for classifying environmental mixtures of any degree of complexity in different categories depending on whether they represent a concern or not, and if a concern, whether it is mainly driven by a one or multiple components in the mixtures. The approach is based on two well accepted chemical mixture metrics: The Hazard Index (HI) and the Maximum Cumulative Ratio (MCR) and is readily applicable to any environmental mixture for which analytical determinations above the limit of detection for any number of chemicals are reported, and for which basic ecotoxicological data (such as NOECs or LD_{50}) is available. In this work, we explored a set of case studies that used large public chemical monitoring databases and evaluated using the Price et al. classification scheme to what extent the exposure to multiple chemicals (as revealed by levels measured in chemical monitoring) translates into a “complex mixtures” chemical risk concern. In addition, we explored the added value of chemical mixtures ERA (as a higher tier refinement) over single chemicals ERA to identify environmental samples that might pose a concern for the ecological receptors.

Pharmaceuticals in the Environment - a Global Challenge

4.14.01

Human Pharmaceuticals: Evaluation of Regulatory Fate and Effect Data to Improve Future ENvironmental Risk Assessment

D. Gildemeister, Umweltbundesamt / German Environment Agency / IV2.2 Pharmceuticals; S. Schwarz, Umweltbundesamt (UBA) / Pharmaceuticals; A. Hein, German Environment Agency (UBA) / Section Pharmaceuticals; J.

Bachmann, German Environment Agency (UBA) / Section IV2.2 Environmental Risk Assessment of Pharmaceuticals
The guideline on the environmental risk assessment (ERA) of medicinal products for human use (EMEA/CHMP/SWP/4447/00 corr 2) is currently under revision. For Germany, the German Environment Agency (UBA) is tasked with the evaluation of the ERA. Over the last decade, this regulatory work resulted in a database containing effect data on approximately 300 active pharmaceutical ingredients (APIs). The database was evaluated to draw conclusions on how the current assessment approach may be improved and what can be concluded about data completeness and environmental risks. In reference to active pharmaceutical ingredients (APIs) sold in Germany still a large data gap was identified. For the majority no regulatory data on aquatic effects are available within the dataset at UBA. Calculated risks, considering the data on ecotoxicity and consumption-based PECsw (Predicted Environmental Concentration) values, show that approx. 9 % of investigated APIs have a risk quotient higher than 1. Aquatic effect data were available for 309 different APIs. Overall, a 5 % NOEC of 450 ng/L (PNEC = 45 ng/L) was derived, which is in close range to the action limit of the current guidance of 10 ng/L for experimental investigations. A clearly most sensitive organism group was only visible for antibiotics and for endocrine active substances (EAS). Adsorption data show that many substances accumulate in relevant concentrations in sewage sludge but currently do not have to undergo a soil risk assessment. The ecotoxicity dataset for the terrestrial compartment was biased, because data were only available for highly adsorbing APIs. Fate data derived from OECD 308 studies show high persistence of many APIs: approximately 43 % of all APIs have to be classified as very persistent; 12 % show $DegT_{50}$ values in a range where abiotic or biotic degradation is not likely. In conclusion, reliable effect data are only available for a small portion of the human pharmaceuticals currently in use. For several substance groups, major data gaps prevail. The PEC action limit of 10 ng/L in the current guideline is in a relevant range. Furthermore, the currently proposed strategies for a tailored risk assessment of antibiotics and EAS are supported. For the terrestrial ERA, the proposed approach of the new draft guideline could help to identify risks of APIs that were formerly overlooked. A scientifically sound solution how to identify and treat highly persistent APIs in risk assessments is needed.

4.14.02
Use of Pharmaceutical Sales Data for Efficient Prediction of Environmental Risk
S. Welch, NIVA - Norwegian Institute for Water Research / Ecotoxicology; K. Olsen, Norwegian Institute of Public Health / WHO Collaborating Centre for Drug Statistics Methodology; J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; M. Nouri Sharikabad, Norwegian Institute of Public Health / WHO Collaborating Centre for Drug Statistics Methodology; K. Tollefsen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; M. Grung, NIVA / Ecotoxicology and Risk Assessment
The effective management and mitigation of pharmaceutical risks to the environment in the future will require realistic and efficient prediction of risk under a variety of climate and population scenarios. Here, we present an important first step in the development of such a program; the prediction of environmental concentrations (PEC) of active pharmaceuticals ingredients and the associated risk in Norwegian surface waters using data for all pharmaceutical sales in Norway, a high-income Nordic nation with an aging population. By combining the PECs derived from the national sales data with Predicted No Effect Concentrations available from Pharmaceutical Specialities in Sweden (FASS), we are able to predict Risk Quotients for substances across a wide range of drug classes. Initial results suggest that sex hormones such as levonogestrel and ethinylestradiol are key drivers of environmental risk, with RQs between 100 and 10000, while other notably high-risk substances (RQ > 1) include the antiseptic chlorhexidine, the statin simavstatin, the painkillers naproxen, paracetamol, and ibuprofen, the antineoplastic abiterone and the antibiotics ciprofloaxin and amoxicilin. Futher, given the propensity for drugs targeting similar receptors to do so with similar or identical modes of action, it is probable that mixture effects are going un-noticed, due to the restricted focus scope of current risk assessments on single chemicals. To our knowledge, our approach provides an unprecedented level of detail and accuracy for predicting environmental concentrations, in particular due to our careful inclusion of combination drugs. We intend next to compare our predictions to measured environmental concentrations, and later to develop the model here to allow for the prediction of future environmental risks under said climate and demographic scenarios.

4.14.06
Medicating the Amazon: Presence and Ecological Risks of Pharmaceuticals in the Amazon River
A. Rico, IMDEA Water Institute; R. de Oliveira, State University of Campinas; G. Silva de Souza Nunes, Federal University of Pernanbuco; C. Rizzi, S. Villa, University of Milano Bicocca / Department of Earth & Environmental Sciences; I. López, IMDEA Water Institute; M. Vighi, IMDEA Water Institute / Earth and Environmental Sciences; A.V. Waichman, Universidade Federal do Amazonas
The Amazon is the largest river basin globally and contains about 40% of the world's remaining tropical rainforest, hosting a vast diversity of terrestrial and aquatic organisms. Only 10% of the population inhabiting the major cities of the Amazon are connected to sewage treatment facilities. This implies that most wastewater produced by the inhabitants of the Amazonian region are discharged untreated into the Amazon River, constituting a major environmental pathway for pharmaceuticals and other chemicals consumed by modern societies. Here we provide the first large-scale monitoring of pharmaceuticals and other substances related to human presence (psychostimulants, personal-care products, hormones) in the Amazon river, three of its major tributaries (Negro, Tapajós and Tocantins Rivers), and smaller streams crossing the cities of Manaus, Santarém, Macapá and Belém. We confirm that urban areas constitute important hot-spots for chemical contamination, with contaminant mixtures affecting up to 50-80% of aquatic species. The compounds showing the largest chronic risks were caffeine, paracetamol, estrone, furosemide, ibuprofen, paraxanthine, benzoylecgonine and 17β-Estradiol. Moreover, we identified several ubiquitous compounds in the Amazon River which can be used as tracers of anthropogenic pressure (caffeine, paracetamol). We conclude that the chemical burden created by Amazonian cities significantly contributes to a biodiversity loss in the region. Environmental policies should be implemented to improve the sanitation system and to monitor the chemical status of the Amazon River.

4.14.11
Presence of Selected Antimicrobial Pharmaceutical Residues in Various ENvironmental Compartments in Selected Finnish, Kenyan and Zambian Sites
P.K. Kairigo, University of Jyvaskyla / Biological and Environmental Sciences; E.K. Ngumba, Jomo Kenyatta University of Agriculture and Technology (JKUAT) / Department of Chemistry; C. Muriuki, Jomo Kenyatta University of Agriculture and Technology (JKUAT) / Soil water and environmental engineering; A. Gachanja, Jomo Kenyatta University of Agriculture and Technology / Chemistry; J.R. Messo, Jomo Kenyatta University of Agriculture and Technology (JKUAT) / Soil, water and environmental engineering; T. Tuhkanen, University of Jyvaskyla / Department of Biology and Environmental Science
Presence of active pharmaceutical antimicrobials in aquatic environment is an issue of major concern due to their potential in propagation of antimicrobial resistance and possible toxicity to aquatic organisms. The focus of this study was to ascertain the environmental concentrations of selected antibiotics and antiretroviral drugs in the urban hydrological cycles of selected municipalities of Kenya, Zambia and Finland. The samples included untreated (influent) and treated (effluent) municipal wastewater, effluent suspended matter (SPM), surface water, river sediments, groundwater and source separated urine (SSU). The analyzed pharmaceuticals included three antiretroviral drugs (nevirapine, zidovudine and lamivudine) and seven antibiotics (trimethoprim, sulfamethoxazole, ciprofloxacin, norfloxacin, tetracycline, doxycycline and amoxicillin). The study revealed measured environmental concentrations (MEC) in surface water in Kenya and Zambia was several orders of magnitude higher than in Finland (Jyväskylä). MEC in Jyväskylä were only up to 0.054 µg/L, while MEC's in Nairobi and Lusaka peaked 13.8 µg/L and 49.7 µg/L, respectively. The mean concentrations in treated municipal wastewater ranged between 0.016 and 0.54 µg/L in Jyväskylä, 0.08 to 55.8 µg/L in Lusaka and 0.066 to 4 µg/L in Nairobi. The MEC in treated wastewater SPM in Kenya ranged between 11 µg/kg to 31117 µg/kg. Pharmaceuticals in groundwater and SSU were analyzed in samples from Lusaka. The compounds were only sporadically present in groundwater with concentrations below detection limit to peak at 880 ng/L. However, high concentrations up to several mg/L were measured in source separated urine with 7740 µg/L, 12800 µg/L and 10010 µg/L for sulfamethoxazole, trimethoprim and lamivudine, respectively. Preliminary risk assessment indicated Finnish samples had low to medium risk while the Kenyan and Zambian samples had medium to high risk for resistance selection. There was less environmental contamination in Finland as compared to Kenyan and Zambian sites. Moreover, risk for evolution of antimicrobial resistance was greater in the developing countries. The SPM was a major route for emission of pharmaceuticals into the environment in Kenya probably due the direct discharge of untreated sewage and wastewater treatment lagoon systems. The high concentration of pharmaceuticals in SSU is an indicator of the great potential of source separation as a critical barrier to environmental contamination.

4.14.13
Effect of Organic Fertilizers on Antibiotic and Antibiotic Resistance Gene Spread in Agroecosystems
A. Visca, National Research Council-Water Research Institute / Water Research Institute; P. Grenni, National Research Council of Italy (CNR) / Water Research Institute; L. Patrolecco, F. Spataro, J. Rauseo, Institute of Polar Science- National Research Council (ISP-CNR) / Institute of Polar Science; G. Massini, ENEA-Italian Agency for New Technologies / Energy and Sustainable Development,

Laboratory of Biomass and Biotechnology for Energy; A. Barra Caracciolo, National Research Council - Water Research Institute / Water Research Institute
Antibiotics (ABs) are compounds used against bacterial infections, inhibiting bacterial reproduction or killing them. However, the large use of these compounds has been promoting selection of antibiotic resistance bacteria (ARB). The scientific community has nowadays the awareness that antibiotic resistance spreading through antibiotic resistance genes (ARGs) is a complex phenomenon, which involves all the anthropogenic environments (urban, agricultural ecosystems, etc.) where antibiotics are used. In this context, gut bacteria of farm animals can act as reservoirs of genetic material, which can be transferred to environmental bacteria and directly or indirectly to humans through soil manure application. Manure can also be used in anaerobic digesters for producing biogas and digestate. However, the fate of antibiotic residues and antibiotic resistance genes, spread by manure or digestate application in agricultural soils, needs to be better investigated. In this work, the effects of manure and digestate application in a soil planted with *Lactuca sativa* and spiked with a mixture of 3 antibiotics (sulfamethoxazole, ciprofloxacin and enrofloxacin) have been investigated in terms of antibiotic and antibiotic resistance genes. The *sul1* and *sul2*, *qnrS*, *qepA*, *aac-(6)'-Ib-cr* genes and the mobile genetic element *intI1* were quantified by qPCR analysis. Finally, the soil microbial community was characterized with NGS (MySeq-Illumina). The antibiotic concentrations over the experimental time were also determined.The presence of *L. sativa* in the soil promoted antibiotic degradation, especially in the case of sulfamethoxazole and ciprofloxacin. The antibiotic enrofloxacin was much more persistent than the others. Adding digestate for vegetable farming would seem desirable because it promoted higher antibiotic removal from soil. Further investigations are in progress for evaluating if antibiotics were not degraded in the soil, but absorbed by the plants.

4.14.19

Assessing Drug-Induced Oxidative Stress and Its Interactions With Temperature Using a Novel Transgenic Zebrafish Model

R. Boreham, J. Ball, M.J. Hetheridge, University of Exeter / Biosciences; S. Owen, AstraZeneca / Global Sustainability; C.R. Tyler, University of Exeter / Biosciences College of Life and Environmental Sciences

Pharmaceutical compounds discharged into the environment can potentially have harmful impacts to local wildlife as many drug targets are conserved across divergent phyla. Oxidative stress (OS) is a major mechanism by which many of these environmental contaminants can induce toxicity. However, this mechanism is relatively poorly understood, particularly with regard to multiple stressor interactions. Temperature can have a major bearing on the physiology of fish, notably affecting metabolic activity and in turn influencing the toxic effect of pollutants. Temperature can also influence the chemical and physical properties of the toxicant in the aquatic environment. As our climate warms, effects on fish (and other poikilotherms) therefore may be disproportionate to that of homeotherms.Transgenic zebrafish provide powerful tools for elucidating drug/stressor interactions. Using a novel transgenic zebrafish model for detecting OS mediated via the electrophile response element (Tg-EpRE:mCherry), we screened a range of pharmaceuticals for their ability to induce OS and assess their interaction with an environmental stressor, temperature. The screen revealed differential responses between tissue types and allowed us to establish diclofenac and paracetamol (APAP) as potent pro-oxidants. Diclofenac is a non-steroidal anti-inflammatory (NSAID) which has previously been shown to induce OS in fish at environmentally relevant concentrations, and APAP is an analgesic that causes liver damage via OS at high level exposures. Both drugs are ubiquitous in surface waters, entering via sewage treatment discharges. We show dose-dependent OS responses primarily in the liver and pronephros at APAP effect concentrations as low as 1.25mM. We are now investigating the interactive effects of temperature with drug-induced OS, beginning with APAP. APAP-induced OS is exacerbated at 33°C compared to 28°C (normal ambient temperature for zebrafish). Part of this enhanced effect is due to an increased uptake of the drug under elevated temperature. Preliminary data show a more than 50% increase in the amount of paracetamol detected in the tissue of a larvae following a 3-day exposure at 33°C compared to 28°C. Unexposed larvae also showed higher OS at 33°C, confirming that thermally-induced OS can be visualised in the Tg-EpRE:mCherry model.

4.14.21

Metabolomic Profiling and Toxicokinetics Modeling to Assess the Effects of the Pharmaceutical Diclofenac in the Aquatic Invertebrate Hyalella azteca

Q. Fu, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Department of Environmental Chemistry; E. Laczko, ETH Zurich; J. Hollender, Eawag / Environmental Chemistry

The exposure of ecologically critical invertebrate species to biologically active pharmaceuticals poses a serious risk to the aquatic ecosystem. Yet, the fate and toxic effects of pharmaceuticals on these non-target aquatic invertebrates and the underlying mechanisms are poorly studied. Herein, we investigated the toxicokinetic (TK) processes (i.e., uptake, biotransformation, and elimination) of the pharmaceutical diclofenac and its biotransformation in the freshwater invertebrate *Hyalella azteca*. We further employed mass spectrometry-based metabolomics to assess the toxic effects of diclofenac on the metabolic functions of *H. azteca* exposed to environmentally relevant concentrations (10 and 100 µg/L). The TK results showed a quick uptake of diclofenac by *H. azteca* (maximum internal concentration of 1.9 µmol/kg) and rapid formation of the conjugate diclofenac taurine (maximum internal concentration of 80.6 µmol/kg), indicating over 40 times higher accumulation of diclofenac taurine than that of diclofenac in *H. azteca*. Depuration kinetics demonstrated that the elimination of diclofenac taurine was 50-130 times slower than diclofenac in *H. azteca*. Metabolomics results suggested that diclofenac inhibited prostaglandin synthesis similar to in humans and other species (e.g., zebrafish, rainbow trout, and marine musse). Furthermore, the carnitine shuttle pathway was affected at environmentally relevant concentrations. These findings shed light on the significance of the TK process of diclofenac, especially the formation of diclofenac taurine, as well as the sub-lethal effects of diclofenac on the bulk metabolome of *H. azteca*. Combining the TK processes and metabolomics provides complementary insights and, thus a better mechanistic understanding of the effects of diclofenac in aquatic invertebrates.

4.14.25

Effect of Digestate Addition on a Natural Soil Microbial Community

G. Garbini, Italian National Research Council / Water Research Institute; J. Rauseo, L. Patrolecco, Institute of Polar Science- National Research Council (ISP-CNR) / Institute of Polar Science; N. Ademollo, National Research Council / Water Research Institute; T. Pescatore, National Research Council of Italy / Institute of Polar Science; F. Spataro, Institute of Polar Science- National Research Council (ISP-CNR) / Institute of Polar Science; A. Barra Caracciolo, National Research Council - Water Research Institute / Water Research Institute; P. Grenni, National Research Council of Italy (CNR) / Water Research Institute

Antibiotics are used for the treatment or prevention of cattle infections. Antibiotic residues can be found in cattle manure because they are only partially metabolized in treated organisms and their possible decrease in anaerobic digesters is not well known so far. When cattle manure or digestate are used as organic fertilizers, residual concentrations of antibiotics together with their related resistance gens could be transferred to soil. Sulfamethoxazole (SMX) is one of the most commonly prescribed and consumed sulfonamide antibiotic for its ability to inhibit both Gram-positive and Gram-negative bacteria. However, current knowledge about its persistence and effects on natural microbial communities in soil is quite scarce. Microcosm laboratory experiments were performed for evaluating the effects of SMX (20 mg/kg) on the structure and functioning of a soil natural microbial community treated with an anaerobic digestate obtained from a biogas digester. At fixed times (0, 8 and 61 days) microbiological abundance, viability, activity and SMX residual concentrations (ASE extraction and HPLC-MS detection) were analysed. Moreover, the microbial community diversity was investigated by NGS analyses. The results showed differences in the microbial community composition at day 8 and 61 in SMX presence if compared to the same soil without antibiotic treatment. In particular, Gram-negative bacteria were in higher percentages in the SMX spiked soil samples at both 8 and 61 days. A significant increase in Alpha-Proteobacteria was also observed at 8 days. Bacilli increased 8 days after the SMX treatment and then a sharp decrease was observed at 61 days. The overall results suggest that SMX acted as a selective pressure on the microbial community. A subsequent analysis using the Picrust2 software suggested that not only the structure, but also the functioning of the soil microbial community was influenced by the digestate addition.

4.14.26

Influence of Environmental Conditions on the Bioavailability of the Ionizable Antibiotic Ciprofloxacin to Cyanobacteria

Q. Zhang, GhEnToxLab (Ghent University) / Bioscience Engineering

Attributed to human and veterinary medicine use, the occurrence of antibiotics in surface water has been detected worldwide. It is yet unclear how antibiotics might interfere with the physiological activities of non-target organisms in aquatic environments. Water chemistry conditions, oftentimes site-specific, are key players regulating the fate and state of antibiotics in ecosystems, which further reflects on the toxins' ecotoxicity to aquatic organisms. This study examined the influence of three environmental factors: pH (7.0 ~ 9.0), water hardness (5 ~ 150.0 mgCa^{2+}/L), and dissolved organic carbon (0 ~ 24.0 mg DOC/L) on the bioavailability of the ionizable fluoroquinolone antibiotic ciprofloxacin (CIP) to the cyanobacteria *Microcystis aeruginosa*. In addition, toxicity experiment results were used to parameterize a toxicity model based on concentration additive effects. The 96-hour 50% effect concentration (96h-EC50) for CIP to *M. aeruginosa* varied within a factor of 5 between 1.2 to 5.5 ug/L, except for the one at neutral pH, where it reached 12.9 ug/L. DOC was identified as the most important regulator of CIP bioavailability and it correlated strongly with the 96h-EC50 regardless of the variation in other environmental factors. We hypothesize that the increase of the 96h-EC50 at pH 7.0 may be related to *M. aeruginosa*'s acid resistance metabolism. The concentration additive effect model performed best when focusing on the toxicity of zwitterions and CIP-DOC interactions.

Within the tested pH range, binding between the CIP zwitterion and DOC appears evident, although the binding between CIP cation and DOC remains uncertain. In summary, our results show that DOC has stronger impacts on the bioavailability of CIP than pH and water hardness. The model simulating the toxicity of an ionizable antibiotic under the influence of environmental factors can be employed to help site-specific risk assessment, as water properties are localized characteristics varying across waterbodies.

4.14.30

Metabolic, Cellular and Molecular Effects of Carbamazepine and Methylmercury After Single and Combined Exposures in Dreissena polymorpha

C. Baratange, UMR-I-02 SEBIO / SEBIO; S. Paris, I. Bonnard, Université de Reims Champagne Ardenne / UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); L. Delahaut, Université de Reims Champagne Ardenne / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); D. Grandjean, Ecole Polytechnique Fédérale de Lausanne (EPFL) / Groupe Central environnemental Laboratory; S. Sayen, University of Reims Champagne-Ardenne / ICMR, UMR CNRS 7312; A. Gallorini, Institute F.-A. Forel, University of Geneva / DEFSE; D. Renault, Université de Rennes I / UMR CNRS 6553 ECOBIO; F. Breider, Ecole Polytechnique Fédérale de Lausanne (EPFL) / GR-CEL; J. Loizeau, Institut F.-A. Forel, University of Geneva; C. Cosio, University of Reims Champagne-Ardenne / UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO)

Carbamazepine (CBZ) and Hg are widespread micropollutants in aquatic environment. Both are known to trigger similar toxicity mechanisms (*e.g.* oxidative stress, energy metabolism impairment), but their mixture has not been yet investigated. Here, we assessed single and combined exposures to get insight into the interaction between CBZ (4 µg/L) and MeHg (275 ng/L) on zebra mussel *Dreissena polymorpha*, frequently used as freshwater model in ecotoxicology and biomonitoring. Both CBZ and MeHg were bioaccumulated after 7 days of exposure, with a 100 x higher enrichment factor for MeHg, and no interaction on this endpoint was observed. MeHg did not alter the levels of the 31 quantified metabolites by targeted metabolomics, while CBZ decreased amounts of α-aminobutyric acid. On the contrary, mixture reduced amounts of 25 metabolites, mainly involved in diverse amino acids and energetic pathways, and in antioxidant response, supporting synergistic interaction on this endpoint. Histopathological analyses showed no particular alterations of cells in gills and digestive glands in controls or CBZ-exposed mussels. However, MeHg-exposed mussels showed strong alterations of cells in gills respiratory surface and in digestive tubules (necrosis, fibrosis, inflammation …), suggesting a likely reduction of function in these organs. Mixture-exposed mussels showed similar alterations than MeHg-exposed mussels, suggesting no interaction on this endpoint. Antioxidant biomarkers indicated that CBZ induced catalase (CAT) activity in digestive glands, MeHg induced superoxide dismutase (*sod*) gene and SOD activity in gills, while mixture only increased SOD activity in gills. It suggested reactive oxygen species production in gills and/or digestive gland depending on the treatment, and an antagonistic interaction on this endpoint. Globally, mixture appeared as the most disturbing condition, with strong cellular alterations, likely impairing metabolic capacity as shown by decreased abundance of metabolites involved in energy, RedOx and amino acid metabolism. Such effects were not predictable based on responses after single exposures, confirming that further mixture studies are needed to better anticipate interaction, and to establish EQS using complementary endpoints.

4.14.31

Ciprofloxacin Caused Neurotoxicity and Oxidative Stress in a Neotropical Catfish (Rhamdia quelen) After Exposure to ENvironmental Concentrations

L. Ângelo, Federal University of Paraná / Postgraduate Program in Ecology and Conservation, Department of Pharmacology.; J.C. de Brito, Ezequiel Dias Foundation; H.C. Silva de Assis, Federal University of Paraná

The presence of antibiotics, such as ciprofloxacin (CIP), in aquatic ecosystems represents a concern for the environment and public health. They can cause adverse effects in the aquatic organisms and influence the development of resistance of pathogens present in surface waters. This study aimed to evaluate biochemical biomarkers in a catfish (*Rhamdia quelen*) exposed to environmental concentrations of 1 and 100 µg/L of CIP. The fish were exposed to CIP in a semi-static bioassay for 28 days. The concentrations were chosen considering a bibliographic review of the presence of CIP residues in two different scenarios: I) in surface waters, and II) in effluents from hospitals and pharmaceutical industries. The results showed a reduction in AChE activity in the group exposed to 100µg/L of CIP (19.97%) when compared to the control. In the liver, the exposure to 100µg/L of CIP caused SOD activity reduction (66.50%), which can be related to the increase in CAT activity (50.93%) and GPX (141.95%). The results suggested the production of reactive oxygen species (ROS) by CIP. Besides, a decrease in GSH levels (44.46%) was observed, which may be related to the increase in GPx activity, since GSH is necessary for GPx activity. The exposure to 1µg/L of CIP increased CAT activity (49.66%), but did not alter the activity of the other enzymes. Both concentrations tested caused a significant increase in liver lipoperoxidation (LPO), 67.72%, and 65.01%, respectively. In the kidney, exposure to 100µg/L of CIP increased GPx activity (87.90%), but decreased GST activity (24.13%). These changes were able to prevent the production of renal LPO. On the other hand, there was an increase in LPO (72.60%) in the group exposed to 1µg/L of CIP, which can be explained by possible changes in SOD or CAT activities, that are still under analysis. This study revealed that the exposure of fish to environmental concentrations of CIP caused neurotoxicity and affected the liver and kidney antioxidant defenses of *R. quelen*. A long-term exposure to CIP can alter the physiological state of these fish species, which can indirectly influence the behaviors, such as locomotion, predation, reproduction, and feeding.

4.14.32

Chronic Effects of Diclofenac on a Freshwater Gastropod, Lymnaea stagnalis

L. Bouly, BTSB / Biochemistry and Toxicology of Bioactive Substances (EA-7417); J. Malgouyres, E. BONNAFE, C. Vignet, Centre Universitaire JeanFrançois Champollion Albi France / Biochemistry and Toxicology of Bioactive Substances (EA-7417); F. Courant, HydroSciences Montpellier, IRD, CNRS, University of Montpellier / UMR 5569 Hydrosciences Montpellier; H. Fenet, Université de Montpellier, Hydrosciences Montpellier UMR 5569 / UMR 5569 Hydrosciences Montpellier; F. GERET, Centre Universitaire JeanFrançois Champollion Albi France / Biochemistry and Toxicology of Bioactive Substances (EA-7417)

Non-steroidal anti-inflammatory drugs (NSAIDs) are a particularly well-represented drug class in traces of drugs found in surface waters. Among these drugs, diclofenac is one of the most prescribed and consumed NSAIDs with healthy use against pains and symptoms induced by chronic inflammatory diseases. In Europe, concentrations noticed in freshwater range from few ng/L to 15 µg/L for the most important concentration detected. In aquatic ecotoxicology, a lot of studies have been conducted on fish showing an impact of low-ranges concentrations of diclofenac on neurotransmitter, on feeding behaviour, on reproduction and hatching. There is also studies on aquatic invertebrates and most of them were conducted on marine bivalves showing a diclofenac impact on metabolism, fertilization, immunity, embryo development, growth… Compared to study on marine invertebrate and on fish, the impact of diclofenac on freshwater gastropods has been only scarcely explored. The aim of this study is to provide information on chronic effects of environmental concentrations of diclofenac on a freshwater mollusk, *Lymnaea stagnalis*, with integration of different omics levels. On the first generation exposed, no effect on feeding behavior, locomotion or reproduction were highlighted, but metabolism pathways have been shown impacted. A transcriptomic analysis was also conducted to improve our understanding of molecular mechanism involved in diclofenac effect on non-targeted species. On the second generation exposed, we can see an impact of diclofenac on feeding behavior and reproduction.

4.14.34

The Antihistaminic Diphenhydramine Induced DNA Damage and Disrupted Locomotor Behavior of Zebrafish Larvae

.A. Barreto, University of Aveiro; J. Santos, University of Aveiro / Biology Department & CESAM; R. EusÃbio, University of Aveiro / CESAM & Biology Department; M. Amorim, Universidade de Aveiro / Biology &CESAM; V. Maria, Biology Department of Aveiro University / Biology & CESAM

Diphenhydramine (DPH) is a commonly used antihistamine, already quantified in surface waters and considered fairly stable in the environment. However, there is scarce data about its impact on aquatic organisms. Therefore, the aim of this study was to assess the effects of DPH on zebrafish embryos in terms of phenotypic, molecular and behavioral alterations. Zebrafish (*Danio rerio*) eggs were exposed to 0, 0.001, 0.01, 0.1, 1 and 10 mg/L DPH. The test run for 96 h and embryos were observed daily to evaluate mortality, hatching and malformations appearance. At 48 h, the heartbeat rate was also assessed. The test was extended until 120 h and larvae locomotor behavior was analyzed. A comet assay to evaluate the larvae DNA integrity after 96 h of exposure to 0, 0.01 and 10 mg/L DPH was also performed. DPH induced no significant effects in terms of survival, hatching and malformations presence of the embryos. However, 10 mg/L of DPH significantly decreased the heartbeat rate of the embryos. DPH, at 0.01 and 10 mg/L, significantly induced DNA damage of zebrafish larvae. DPH, at 10 mg/L, significantly increased the time swam by larvae and at concentrations between 0.1 to 10 mg/L decreased the zebrafish swimming distance, indicating a hypoactivity. Significant alterations in terms of the larvae path angles were also found. For instance, at 1 and 10 mg/L, DPH significantly decreased the straightforward movements (low amplitude angles ɢ_ class 4) and increased zigzag movements or movements with changes of direction (large amplitude angles – class 1), suggesting erratic swimming behavior. In conclusion, DPH induced DNA damage and disrupted locomotor behavior of zebrafish larvae at concentrations that caused no effect in terms of phenotype, showing the relevance of a multi-endpoint ecotoxicological evaluation at different biological levels. The detected effects may

trigger a cascade of negative consequences at a community level, supporting the importance of performing further studies to evaluate other parameters for a more complete understanding about the toxicity mechanisms of DPH.

Tire Wear and Microrubber Particles - from Problems to Solutions

4.15.01

Comparison and Improvement of Analytical Techniques for Quality Data on TWP in the Environment

P. Tromp, TNO / Circular Economy & Environment; R. Dröge, TNO / Circular Economy and Environment; I. Kooter, TNO / Environmental Health and Safety

Microplastics are of increasing concern in the environment. The mechanical abrasion of car tires by the road surface forms tire wear particles (TWP) consisting of a complex mixture of rubber, with both embedded asphalt and minerals. For predicting negative health effects and environmental impact of TWP accurate information on tyre wear generation, particle sizes and constituents is essential. In the last years, numerous research activities have started on quantifying and tracing TWP in the environment. Still, limited monitoring data are available and the majority of data lacks quality. Several analytical techniques exist, but at this date there are no harmonized methods available. To improve the quality of TWP data, in this study first we compared different analytical techniques for tracing and quantifying TWP in environmental compartments. After this, several exploratory measurement campaigns were carried out in ambient air (city center, tunnel, background), highway locations (runoff, soil, road), waterways (surface water, sediment) and sewage treatment plants (influent, effluent, sludge). Besides TWP concentrations in the environment also data on particle size, composition and relevant inorganic (nanomaterials) and organic constituents (benzothiazoles and derivates, phenyl/cyclohexyl amines and PAH) is gathered. Thermal decomposition fragments of rubber (styrene, 4-vinylcyclohexene (4-VCH) and dipentene) were measured using TED-GCMS. Additive markers benzothiazole (BT), N-cyclohexyl-1,3-benzothiazol-2-amine (NCBA) and 2-hydroxybenzothiazole (OHBT) were analyzed with LC-MSMS. Conversion factors for these markers were established by analyzing 30 most used car tires in the Netherlands. For determination of constituents of TWP additional techniques have been used, such as HR-ICPMS (metals including organic zinc), HR-SEMEDX (nano additives), thermal-optical carbon analyses (rubber, carbon black), LC-MSMS (amines) and GCMS (PAH). Size-segregated atmospheric release profiles and concentrations were established for emitted TWP (PM10: 0.1 – 1.5 µg/m^3 and PM2.5: 0.04 – 0.1 µg/m^3). Measured TWP concentrations in runoff are 1.0 and 55 mg/L for the Swedish and German site respectively, while measured TWP concentrations in surface water in the Netherlands are between 0.011 and 0.006 mg/L. Compared to additive markers pyrolysis markers are more accurate to predict environmental TWP concentrations.

4.15.03

Identification and Quantification of Microplastics in Australian Road Dust

S. Monira, RMIT University; M. Bhuiyan, R. Roychand, B. Pramanik, RMIT University / Civil Engineering

Microplastics (MPs) have been recognized as an emerging pollutant for their eco-toxicological effects in marine environments and road dust is one of the major contributors of MPs to the water environment. Because of low density and small particle size (<5mm), MPs are easily discharged into natural waterways by stormwater runoff. The aim of this study was to identify and quantify of MPs in Australian road dust. Samples were collected from residential area of Wyndham Vale city and Altona industrial area in Melbourne, Australia. Photo optical microscope was used for visual identification and quantification of MPs. The MPs observed under the microscope are classified as fragments, fibers, films and beads according to the morphology. Results found different types of road dust associated MPs such as polyethylene, polyester, polypropylene, polyethylene terephthalate, rubber and polyvinyl chloride which were validated using Fourier-transform infrared spectroscopy analysis. The main type of road dust associated MPs was microfibres which accounted for 48% in residential area. On the other hand, beads were dominant with 31% in industrial area. This was because fibers were abundant probably due to the sources, uses and the fact that fibers might tear easily of clothes and some house furniture. The results clearly show the presence of a large number of black colour tyre wear-out particles in road dust samples collected from both industrial and residential area which were accounted for 460/100gm and 90/100gm, respectively. The generation of tyre wear particles is a result of causing shear and heat through contact between tyres and road surfaces. It could be also noted that the amount and size of the particles released depend on factors such as climate (temperature), composition and structure of the tyre, the road surface, driving speed and style, pavement type and the nature of the contact.

4.15.04

Toxicity assessment of tire-related chemicals by spiked-sediment toxicity test

S. Lee, T. Tobino, The University of Tokyo / Department of Urban Engineering; F. Nakajima, Environmental Science Center, The University of Tokyo / Environmental Science Center

Tire wear particles (TWPs) are one of the important components of urban road dust which has been reported to be toxic to benthic organisms. Tire wear particles contain various groups of toxic chemicals such as polycyclic aromatic hydrocarbons (PAHs), benzothiazoles, phthalates and heavy metals, and have been observed in sediment, especially in estuarine and marine environments. However, the toxicity information of chemicals is generally limited in sediment and in salt-water environments. The objectives of this study are (1) to derive LC50 of tire-related chemicals as concentrations in sediment by spiked-sediment toxicity test with marine benthic organisms and (2) to compare the toxicity values with reported concentrations in tire and environmental sediments. Three tire-related chemicals, benzo[a]pyrene (B[a]P), fluoranthene (FLU), and 2-mercaptobenzothiazole (MBT) were separately spiked to formulated sediment and tested under static condition with continuous aeration for 10 days with a marine amphipod Grandidierella japonica. LC50 were determined as 2.0 x 102, 1.5 x 10 and 1.2 x 10 mg/kg respectively for B[a]P, FLU and MBT. The risk quotients were calculated as 1.4, 85.3 and 26.2 respectively for B[a]P, FLU, and MBT in TWPs and as 7.6 × 10-3, 6.6 × 10-2 and 2.3 × 10-4 respectively for B[a]P, FLU and MBT in environmental sediment. MBT showed the highest toxicity to the marine amphipod among the three tire-related chemicals, but FLU had the higher risk in TWPs and in the environment.

4.15.07

Differing Toxicity of Micro and Nano Tire Wear Particles and Leachate to the Development and Survival of Model Organisms Danio rerio and Daphnia magna

B. Cunningham, Oregon State University / Environmental and Molecular Toxicology; B.J. Harper, Oregon State University / Department of Environmental and Molecular Toxicology; S.M. Brander, Oregon State University / Environmental and Molecular Toxicology; S.L. Harper, Oregon State University / EMT

Tire ware particles (TWPs) originate from friction of tires on roads and are composed of a variety of materials – synthetic rubber, filling agents, oils, vulcanization agents, and other additives– each carrying their own potential toxicity. Environmental sampling has documented high levels black rubber - generally identified as tire debris - in water, sediment and organism samples. It's been estimated that on average the US generates 1,524,740 t/yr of TWPs. The consequences of the addition of tire particles to these environments are not fully understood. Past research focused on the toxicity of tire leachate, overlooking potential effects related to the particle itself. With these research gaps in mind, we designed experiments to assess potential particle and/or chemical toxicity of micro (1-20µm) and nano (< 1µm) TWPs to two model organisms, zebrafish *Danio rerio* and crustaceans *Daphnia manga*. To assess effects on development, zebrafish embryos were exposed to various concentrations of TWPs or leachate, ranging from 1.0 x 10^3 to 3.0 x 10^9 particles/ml (n=18). Exposures began 8 hours post fertilization (hpf) and lasted a total of 5 days, with assessment at two time points. In embryonic zebrafish, high concentrations of all TWP exposures significantly decreased spontaneous movement at 24hpf. Greater mortality and sublethal malformations were observed following micro TWP exposure as compared to nano and leachate exposures, and a fitted dose-response curve predicts an LC_{50} of 8.076 x 10^5 particles/ml. This indicates the toxicity may largely be attributed to the physical particles, as opposed to the chemicals they leach. Additionally, nano TWP exposures resulted in hatch delay that was not seen in leachate or micro exposures. This is some of the first research to show that exposure of zebrafish to tire microparticles themselves, and not just leachate, has detrimental effects on survival and development. The results were different when neonate *D. magna* were exposed for 48 hours to TWPs or leachate, ranging from 1.3 x 10^5 to 3.3 x 10^9 particles/ml (n=5). LC_{-50} calculated for micro and nano TWPs were 4.9 x 10^5 and 3.4 x 10^8, respectively; much of which could be explained by toxicity elicited by the leachate. In these acute exposures, no particle specific effects were noted for *Daphnia* meaning that these two aquatic species could be differentially susceptible to TWPs entering the environment.

4.15.08

Micro- and Nanoplastics: Effects of Environmentally Relevant Tire Wear Particles on Estuarine Indicator Species

J.M. Dickens, Oregon State University / CEOAS; B. Cunningham, Oregon State University / Environmental and Molecular Toxicology; S. Siddiqui, Oregon State University / Environmental & Molecular Toxicology; S. Hutton, Oregon State University / Environmental and Molecular Toxicology; E.I. Pederson, Oregon State University / Department of Fisheries and Wildlife; S.L. Harper, Oregon State University / EMT; B.J. Harper, Oregon State University / Department of Environmental and Molecular Toxicology; S.M. Brander, Oregon State University / Environmental and Molecular Toxicology

Plastic debris, comprised of a wide range of polymers and synthetic materials, is a ubiquitous source of pollution in marine and estuarine ecosystems. Micro and nanoplastics are known to have adverse effects on the habitats, diets, and

physiologies of aquatic organisms, but questions remain about the relative risk across different salinities, polymer types, concentrations, and sizes (e.g. micro vs nano). While studies confirm that high concentrations of microplastics can have deleterious effects on organisms, gaps remain in our knowledge of species response to environmentally relevant concentrations, particularly in estuaries where salinity can influence particle behaviour and potentially toxicity. Further, less is known about the effects of tire wear particles (TWP), a particle type broadly defined as plastic debris, as an organic aquatic contaminant. Generated from automobile traffic and composed of complex mixtures of oil, rubber, plastic, steel, and additives, there is an estimated 1,121,000 t/a of TWP in the United States alone, frequently detected in the coastal environment near urban areas. The estuarine indicator species inland silverside (*Menidia beryllina*) and mysid shrimp (*Americamysis bahia*) were exposed to three concentrations of TWP (60, 6000, and 60000 particles/mL) at two size fractions (1 - 20µm and < 1µm) across a salinity gradient (5, 15, and 25 ppt for *M. beryllina* and 15, 20, and 25 ppt for *A. bahia*). Additionally, individuals from both species were exposed to TWP leachate matching the highest particle concentration treatment. *M. beryllina* individuals (n=6) were exposed at 5 – 7 days post fertilization for 96 hours. *A bahia* individuals (n = 9) were exposed at 7 days old for an additional 7 days. Preliminary results in *M. beryllina* suggest that TWP size influenced swimming behaviour. Altered behaviour resultant from aquatic contaminants may lead to increased risk of predation and foraging challenges in fish and shrimp. Additionally, this could have consequences for trophic transfer as prey species may act as transport vectors, transferring TWP to predator species. While further analysis in mysid shrimp remains to be completed, the presence of adverse effects in *M. beryllina* indicates that even at current environmental levels, which are expected to continue to increase, aquatic ecosystems experience impacts dependent on the physical properties of the plastic.

4.15.09

Immune Response of Woodlice Porcellio Scaber to Microplastics Versus Natural Particulate Matter in Soil

A. Dolar, Biotechnical faculty / Biology; D. Drobne, University of Ljubljana, Biotechnical Faculty / Department of Biology; A. Jemec, University of Ljubljana, Biotechnical Fac. / Department of Biology

Key words: immune response, tire wear particles, woodlice, natural particulate matter The abrasion of tire wear is considered as one of the largest input of microplastics to the environment, in particular at the roadside. Crumb rubber from tire wear has been used in many studies with aquatic organisms. However studies on the effects of tire rubber particles on soil organisms are still rare, although the majority of emissions are released into the soil. In our study we exposed woodlice *Porcellio scaber* (Crustacea: Isopoda) to crumb rubber (< 180 µm) and two types of natural particulate matter (quartz sand and wood dust) with similar size distribution, at a concentration of 1.5% w w^{-1} in standard Lufa 2.2 soil. At different points in time during 3-weeks exposure we evaluated the changes in the *P. scaber* immune response by measuring selected immune parameters (e.g. total and differential haemocyte count, haemocyte viability, and superoxide dismutase and catalase activity) in the haemolymph. The levels of measured immune parameters in *P. scaber* haemolymph after exposure to crumb rubber in soil significantly differed from those of the control group and also from animals exposed to natural particulate matter. Crumb rubber significantly affected the total haemocyte count and differential haemocyte count, already within one week of exposure, with a remarkable peak on day four and then a gradual decline to the control levels after 3 weeks. All remarkable changes in case of microplastics exposure occurred after short-term exposure (within 1st week), while no effects on *P. scaber* were observed after long-term exposure (2nd and 3rd week) in comparison to control. On the other side, haemocyte viability did not change, neither superoxide dismutase and catalase activities in the haemplymph. For natural particulate matter no effect on *P. scaber* was observed, the levels of measured immune parameters were similar to control group, therefore they might be used as a good control in environmental studies with synthetic particulate materials.

4.15.10

In-Vitro Digestion of Tire and Road Wear Particles: Bioavailability of Metals and Polycyclic Aromatic Hydrocarbons

T. Masset, EPFL; B.J. Ferrari, Centre Ecotox; W. Dudefoi, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Department of Environmental Toxicology; K. Schirmer, Eawag / Environmental Toxicology; F. Breider, Ecole Polytechnique Fédérale de Lausanne (EPFL) / GR-CEL

The potential impact of Tire and Road Wear Particles (TRWP) on aquatic organisms has recently gained attention since the occurrence of TRWP in the aquatic environment has been observed in surface water and sediments of numerous regions. Moreover, the ingestion of TRWP by aquatic species, including fish, has recently been demonstrated. However, data regarding the bioavailability and toxicity of contaminants associated with these particles are still lacking. This study aimed (i) to characterize the solubilization potential of metals and PAHs from tire particles and TRWP in Simulated Gastric Fluids (SFG) and Simulated Intestinal Fluids (SIF) designed to mimic *Oncorhynchus mykiss* (Rainbow Trout) gut conditions and (ii) to assess the impact of influencing factors such as co-ingestion of natural food organic matter on the compounds solubilization. Our results showed that solubilization of all metals and PAHs were enhanced by up to 10-fold in simulated gut fluids compared to solubilization in the control (phosphate buffer saline solution). Zn was highly solubilized after an in-vitro digestion of 24-h with a final concentration in SIF of 3,2 $\mu g.L^{-1}$ corresponding to 23 % of the total Zn concentration in TRWP. Five PAHs (Fluoranthene, Phenanthrene, Pyrene, Benzo (e) Pyrene and Benzo (g,h,i) perylene) were quantified in SIF after an incubation time of 24-h and the concentration of individual PAHs in SIF ranged from 42 to 3092 $ng.L^{-1}$ which represented 0,4 – 1,5 % of their total concentration measured in tire particles. Metals were highly solubilized by the acidic SGF (pH = 2) but only a weak solubilization was observed in neutral SIF (pH = 7,4). Contrastingly, PAHs were not solubilized in SGF and highly solubilized in SIF which was proved to contain high quantities of proteins and surfactants forming micelles. This emphasise the importance of pH in the solubilization of metals and of bile surfactants and organic matter in the solubilization of PAHs from TRWP and brings new insights on the bioavailability of metals and organic pollutants from TRWP. Overall, our results demonstrate the importance of accounting for the ingestion of TRWP as an important route of exposure to metals and PAHs for aquatic organisms. As the solubilization of potential toxic compounds was greatly enhanced by the gut fluids and leads to bioavailability of these compounds, further studies investigating the uptake by the epithelial cells and transfer throughout the whole organism will be performed.

Using Ecosystem Services to Frame and Assess Protection Goals: Taking a Holistic Approach

4.16.01

Assessing Chemical Risk Within an Ecosystem Services Framework: Implementation and Added Value

L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences; A. Brown, Exeter University / Biosciences; J. Faber, Wageningen Environmental Research / Sustainable Land Use; N. Galic, Syngenta Crop Protection LLC / Environmental Safety; P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences; O. Warwick, Peter Fisk Associates Limited; S. Marshall, Consultant

Proof of concept studies (insecticide, metal, surfactant) were undertaken to evaluate the feasibility of adopting an ecosystem services approach to chemical environmental risk assessment for terrestrial and aquatic ecosystems. The outcomes of these studies were discussed at a multi-stakeholder workshop. Workshop participants concluded that there was added value in adopting an ecosystem services-based approach for regulatory decision making. Ecosystem services provide a common currency and a 'unifying approach' across environmental compartments, stressors and regulatory frameworks. The ecosystem services approach informs prioritisation of risk and remedial action and aids risk communication and risk management. It facilitates a more holistic assessment, enables ecosystem service trade-offs to be compared across alternative interventions, and supports comparative risk assessments and a socio-economic analysis of management options and decisions. Key to realising the added value of an ecosystem services approach to chemical risk assessment is a shift away from using a single threshold value to categorise risk, towards a consideration of the exposure-effect distribution for individual ecosystems services of interest. Also required is the development of an integrated systems-level approach across regulatory frameworks and agreement on specific protection goals and scenarios for framing environmental risk assessments and acceptability criteria for interpreting ecosystem service impact. The need to further develop tools for extrapolating toxicity data to service providers and ecosystem services delivery, including logic chains and ecological production functions, was highlighted. Also agreed was the need for methods and metrics for ecosystem service valuation to be used in assessing trade-offs between services.

4.16.02

Assessing the Feasibility and Value of Employing an Ecosystem Services Approach in Chemical ENvironmental Risk Assessment Under the Water Framework Directive

A. Brown, Exeter University / Biosciences; S. Marshall, Consultant; J. Faber, Wageningen Environmental Research / Sustainable Land Use; P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences; C. Cooper, International Zinc Association / Environment & Sustainability; P. Whitehouse, Environment Agency / Evidence; L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences

Introduction Recent multi-stakeholder workshops have identified advantages in employing an Ecosystem Services (ES) approach in chemical risk assessment, as well as challenges in implementation [1,2]. 'Chemicals: Assessment of Risks to Ecosystem Services' (CARES II) is a proof of concept project evaluating

approaches for assessing impacts on ESs and the value this may bring to regulatory decision making. Here we describe a retrospective, site-specific study assessing the risk of impacts on a range of ES before during and after a breach in the EQS for zinc in a lowland river in the UK. **Methods** This study was co-designed by representatives of the chemical industry, policy/regulatory agencies and academia during a stakeholder workshop. The study focuses on a river in the Thames catchment, in which the following ES are important: water quality regulation; habitat provisioning, recreational fishing and nature watching. We assess the feasibility of employing an ES approach in a site-specific environmental risk assessment for zinc under the Water Framework Directive (WFD). Specifically, we address the challenges of i) linking measurement endpoints for ecological receptors to ES; ii) relating measurement endpoints and assessment of ecosystem services impact to reference values; iii) assessing the risk of a specific chemical pollutant to potential ES delivery. We compare and contrast WFD- and ES- based assessments in terms of their protection of freshwater ecosystems and evaluate how an ecosystem services approach could add value to the current assessment of ecological status under the WFD. **Results and Discussion** Our results demonstrate the potential for practical application of the ES approach in retrospective chemical risk assessment, building on previous conceptual work. We show how the ES approach could add value to the WFD approach in terms of: i) evaluating the status of locally important ESs; and ii) helping to prioritise and identify where remedial measures are likely to have greatest benefit. **References** [1] Maltby et al (2018). Sci Total Environ. 621:1342-1351 [2] Faber et al (2019). Sci Total Environ. 651:1067-1077. **Key words:** Aquatic toxicity, Ecosystem services, Environmental risk assessment, Zinc

4.16.03

The Use of Ecological Models to Assess the Effects of a Plant Protection Product on Ecosystem Services Provided by an Orchard

P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences; A. Alix, Corteva Agrisciences / Risk Management; P. Thorbek, BASF SE / Agricultural Solutions; H. Baveco, Wageningen University and Research; A. Agatz, IBACON GmbH / Ecological Modelling; J. Faber, Wageningen Environmental Research / Sustainable Land Use; A. Brown, Exeter University / Biosciences; S. Marshall, Consultant; L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences

The objective of this case study was to explore the feasibility of using ecological models for applying an ecosystem services-based approach to environmental risk assessment using currently available data and methodologies. For this we used a 5 step approach: 1) selection of environmental scenario, 2) ecosystem service selection, 3) development of logic chains, 4) selection and application of ecological models and 5) detailed ecosystem service assessment. The study system is a European apple orchard managed according to integrated pest management principles. An organophosphate insecticide was used as the case study chemical. Four ecosystem services are included in this case study: soil quality regulation, pest control, pollination and recreation. Logic chains were developed for each ecosystem service and describe the link between toxicant effects on service providing units and ecosystem services delivery. For the soil quality regulation ecosystem service, springtails and earthworms were the service providing units, for the pest control ecosystem service it was ladybirds, for the pollination ecosystem service it was honey bees and for the recreation ecosystem service it was the meadow brown butterfly. All the ecological models addressed the spatio-temporal magnitude of the direct effects of the insecticide on the service providing units and ecological production functions were used to extrapolate these outcomes to the delivery of ecosystem services. For all ecosystem services a decision on the acceptability of the modelled and extrapolated effects on the service providing units could be made using the protection goals as set by the European Food Safety Authority (EFSA). Developing quantitative ecological production functions for extrapolation of ecosystem services delivery from population endpoints remains one of the major challenges. We feel that the use of ecological models can greatly add to this development, although the further development of existing ecological models, and of new models, is needed for this.

4.16.04

Towards a Systematic Method for Assessing Impacts of Chemical Pollution on Ecosystem Services of Water Systems and Filtration Services of Mussels

J. Wang, Radboud University Nijmegen / Environmental Science; R.K. Koopman, RIVM; L. Lautz, Radboud University Nijmegen; L. Posthuma, RIVM / Centre for Sustainability, Environment and Health (DMG); T. De Nijs, RIVM; F.P. Collas, Radboud University Nijmegen; T. Nolte, Radboud University Nijmegen / Department of Environmental Science; R. Leuven, Radboud University Nijmegen; J.A. Hendriks, Radboud University / Environmental Science

Chemical pollution impinges on the quality of water systems and the ecosystem services (ES) they provide. As filter-feeders, mussels provide the ES of biofiltration, while provisioning of this service is affected by chemicals. However, the economic impacts of chemical pollution on ESs are rarely quantified. Few attempts have been made to estimate the economic benefits of mussel-provided filtration services under chemical mixture exposure. Therefore, our study aimed to develop a stepwise method for quantifying chemical mixture impacts on the total ES value provided by water systems. We also developed a method for quantifying the economic benefits of mussels' filtration services for drinking water production in relation to chemical mixture exposure. The total ES value loss was estimated as a function of the multi-substance potentially affected fraction of species at the HC50 level (msPAF(HC50)), based on relationships between, subsequently: msPAF(HC50), diversity, productivity and total ES value. Regarding filtration services of mussels, economic benefits to surface water-dependent drinking water companies were estimated by linking filtration capacity to changes in river turbidity. Our studies showed that 1% msPAF(HC50) corresponded to on average 0.5% (0.05 - 1.40%) of total ES value loss. Based on the measured metal levels from 1999 to 2017 in the Rhine and Meuse Rivers (NL), dreissenid filtration services would save 110-12,000 euros/million m^3 for drinking water production when abstracting raw water at the end of respective rivers. Economic benefits increased over time due to metal emission reduction. Our study presents novel methods for assessing impacts of chemical exposure on the total value that ecosystems provide, and for quantifying the economic benefits of mussel filtration services associated with chemical exposure. With sufficient data, our methods can be applied for any chemical mixture and filter-feeder of concern. By expressing results in monetary units, we may help policymakers take a holistic view of water quality management that includes not only the costs of 'river basin management planning' under WFD, but also the value generated by our natural capital and ecosystems. We encourage the application and refinement of our systematic methods, which will contribute to thorough assessments, management and communication of chemical risks to ecosystems.

4.16.06

The Science Behind the Proposed Specific Protection Goals Options for Non-Target Terrestrial Organisms Exposed to Pesticides

S. Pieper, German Federal Environment Agency (UBA) / Plant Protection Products; A. Aldrich, Agroscope; R. Laskowski, Jagiellonian University / Terrestrial Ecology & Ecotoxicology Group; K. Machera, Benaki Phytopathological Institute / Department of Pesticides Control & Phytopharmacy, Athens, Greece; R. Smith, School of Applied Sciences, University of Huddersfield; J. Sousa, University of Coimbra / CFE - Centre for Functional Ecology, Department of Life Sciences; I. Sundh, Swedish University of Agricultural Sciences; F. Streissl, EFSA - European Food Safety Authority; C.J. Topping, Aarhus University / Department of Bioscience; M. Arena, EFSA - European Food Safety Authority / Pesticides Peer Review

Over the last five years, the Panel on Plant Protection Products and their Residues at the European Food Safety Authority (EFSA) together with its working groups have published several Scientific Opinions addressing the science behind the risk assessment of terrestrial non-target organisms exposed to pesticides (plant protection products and their active ingredients). The work focused on deriving Specific Protection Goal (SPG) options for different non-target organism groups, taking into account biodiversity and the ecosystem as required by Regulation EC 1107/2009.This contribution aims at summarising the experience gained in providing SPG options for the informed discussion between risk assessors and risk managers for very different groups of organisms (e.g. non-target arthropods, in-soil organisms and amphibians and reptiles) in agricultural systems. We present a review of the type of information needed to identify different SPG options, the scientific appraisal of the data and the chosen approach outlining the consequences of the different proposals. We highlight how the scientific basis in ecology and ecotoxicology and the legal requirements for the different organism groups can be merged to identify SPG options as proposals for risk regulation. The approach to derive SPG delivers options that must be consistent with the general protection goals of EC 1107/2009 and also with other relevant legislations in place, in order to fulfil legal requirements. Amongst lessons learned, the following data were found to be essential for the identification of SPG options: ecological vulnerability, behaviour, intrinsic recovery potential, dispersal ability and ecotoxicological sensitivity of key driver species or groups, of which only the ecotoxicological sensitivity was specifically related to the impact of pesticides. Common to all proposed SPG options is the emphasis on the correct structural and functional, spatial and temporal dimensions of the assessment and on the related environmental context. These dimensions are essential to link structural parameters to functional processes, to identify the relevance of biological effects and to appraise the suitability of available measurement endpoints for identifying effects on the protection goals. Examples of the derived SPG options related to biodiversity and ecosystem services are presented, along with identified knowledge gaps, potential pitfalls and opportunities for the risk regulation of pesticides.

Extended submission 4 - Ecological risk assessment and human health risk assessment of chemicals, mixtures and stressors and risk mitigation strategies

4.17.08

German Study on the Suitability of Bioindication With Mosses for the Detection of Atmospheric Deposition of Persistent Organic Pollutants and Microplastics: Method Development and Sample Preparation

C. Wolf, Institute of Energy and Envionmental Technology e.V. - IUTA / Air Quality & Filtration; M. Wenzel, Institut für Energie- und Umwelttechnik e. V. (IUTA); F. Itzel, Institute of Energy and Environmental Technology e.V. (IUTA); C. Kube, Institute of Energy and Environmental Technology e.V. - IUTA; A. Dreyer, ANECO; S. Nickel, W. Schröder, Planwerk; J. Tuerk, Institute of Energy and Environmental Technology e.V. (IUTA)

Mosses were and are applied as biomonitors, mainly for nitrogen and heavy metals (Schröder 2019) and although less often, for organic contaminants (Dreyer et al 2018). Whether they can also be used as biomonitors for micro-plastics is still unknown/unclear. Microplastic have been defined as plastic particles smaller than 5 mm (Arthur et al. 2009). Research on microplastic have shown, that it can be found in all environmental compartments (Möller et al. 2020), whereas the data base for aquatic systems being the largest, followed by soils and air. In this study, founded by the german environmental agency, it will be tested if mosses can be used for the detection of atmospheric deposition of micro-plastic. Therefore, a method for sample preparation, identification and quantification using TED-GC-MS and RAMAN spectroscopy is under development (Figure 1). First samples from the German Moss Survey 2020/2021 will be used for validation and to get a first impression on the suitability. Concerning POPs, the suitability of mosses as bioindicators are going to be re-evaluated after the first investigating during the pilot study in the moss monitoring 2015. A flotation device to separate the moss and plastic particles was developed and preliminary tests were conducted. For these tests moss samples were spiked with PMMA (50 µm) particles. After the separation procedure, first qualitative SEM images show a good separation between the moss sample and the spiked PMMA particles. These qualitative results must be validated by quantitative measurements and with further polymer particles and environmental samples. Figure 1: Sample preparation stepsReferences: Schröder W., Nickel S. *Environ Sci Eur* 2019 31(33): 1-15; Dreyer et al. *Environ Sci Eur* 2018 30(43): 1-14; Arthur et al. *NOOA Technical Memorandum NOS-OR&R-30*: Tacoma, WA, 2009; Möller et al., *Environ Sci Technol* 2020 54(4): 2078–2090

4.17.09

German Study on the Suitability of Bioindication With Mosses for the Detection of Atmospheric Deposition of Persistent Organic Pollutants and Microplastics: Survey Network Design Based on Decision Modelling and Statistics

S. Nickel, W. Schroeder, Planwerk

The studies funded by the Federal Environment Agency for the German Moss Survey 2020/2021 focus exclusively on persistent organic pollutants (POPs) and microplastics (MPs). These will be determined analytically in moss samples from 20 sites. Chemical and spectroscopic methods will be used for MP. In addition to this measurement program, the research participants will analyse the moss samples for heavy metals and nitrogen without financial support. For the chemical and spectroscopic analyses of moss specimens the eight sites where POPs were determined in MS2015 (Schröder et al. 2019) should be included in the MS2020/20221 monitoring network. In addition, twelve further sites should be selected from the pool of the total 400 sites of the MS2015. The criteria underlying the sample selection were as follows: Comparability with analyses from previous campaigns; Comparability with analyses from other environmental monitoring networks; Priority moss species: *Pleurozium schreberi*; Sufficient moss occurrence; Different ecosystem types; Different distances to potential emission sources; Different administrative spatial units; Spatial representation of ecoregions, land use, atmospheric deposition. Based on these criteria, a corresponding decision algorithm was developed and implemented in R. The parameters of the decision model were varied many times and the spatial distribution of the selected sampling sites was mapped in each case to support the decision. The final selected sites for the 2020/2021 MS are shown in **Figure 1**.
Figure 1. Sites for moss sampling 2020/2021 in Germany References**.** Dreyer A et al. *Environ Sci Eur* 2018 30(43): 1-14; Schröder W et al. *UBA-Texte* 91/2019; Wosniok W et al. *Environ Sci Eur* 2020 32(9):1-14

Ex-ante Life Cycle Assessment of Emerging Technologies

5.01.01

The Future of Future Scenarios in LCA

B. Steubing, CML Leiden University

Future scenarios in LCA are increasingly available, e.g. based on the combination of data from existing LCI databases such as ecoinvent and data from Integrated Assessment Models (IAMs) or other data sources. A number of innovative approaches have recently been presented to generate such future scenarios including amongst others the use of brightway and related python libraries. However, much work remains to be done as important questions still need answers: which future scenarios shall the LCA community adopt? How can future scenarios be kept up to date? Where can LCA practitioners get them? How can future scenarios be used within LCA software (by non-pythonic LCA practitioners)? This talk will attempt to provide an overview of what the next steps could be to make future scenarios practically available to the LCA community, present solutions where available, and make suggestions for some of the remaining challenges.

5.01.03

Identifying Unconstrained Suppliers Systematically for Emerging Technologies: The Case of Brown Seaweed Production

M. Ayala, P. Jouannais, M. Pizzol, Aalborg University / Planning

Identification of unconstrained suppliers is an important step for consequential LCA studies. When a new technology or product is developed, the demand increases, and consequentially the supply. the supply of the product increases. In consequential LCA, the market trend is then assessed to identify the most competitive suppliers that will be able to respond to such a change in demand from a long-term perspective. However, the current methods for unconstrained suppliers' identification are not structured and are mainly case-specific, and poorly geared to address the case of emerging technologies. In this work, a consistent, robust and replicable methodology to identify unconstrained suppliers for emerging technologies is proposed. This methodology combines quantitative and qualitative analysis. A statistical model is developed to systemically convert qualitative expert opinions into quantitative data needed to define the market mix for the product supply. The presentation starts proposing a methodological framework, which includes constraints, and continues proposing a statistical tool and determine a marginal mix for consequential LCA for the case of brown seaweed. It is particularly interesting applying the methodology to this case study due to the complexity of seaweed cascade biorefinery value chain.

5.01.04

Ex-Ante LCA for Microalgae-Based Veterinary Molecules in Finfish Aquaculture: How to Assess the ENvironmental Performance of Unknown Molecules?

P. Jouannais, M. Pizzol, Aalborg University / Planning

Diseases have been identified as a key limiting factor to the global growth of aquaculture. The sector is experiencing massive losses due to a large diversity of health issues. At the same time, a new paradigm in health management should fight antibioresistance and reduce fish farms environmental impacts. In this context, the EU-funded AquaHealth consortium intends to identify and develop new veterinary molecules produced by microalgae to treat finfish health issues. To orient the development of this early-stage technology, we perform an ex-ante and consequential LCA (with system expansion and marginal supplier identification) on a system in which microalgae production and aquaculture work in synergy. We attempt to delimit the range of possibilities for this future synergy by identifying and then modelling quantitatively the most relevant scenarios. The challenge lies in the high epistemic uncertainty in determining the microalgae candidates and the different types of bioactive molecules, while modelling the effect of the molecule on the fish farm's production and emissions is challenged by both epistemic and aleatory uncertainty. To address such complexity, we implement a stepwise approach which progressively tackles the foreseen non-linearity within the synergy. The final step is the building of a fish farm dynamic model in which different types of molecules affect variables such as Feed Conversion Ratio, Disease outbreak frequency, resistance to disease. These biologic parameters then affect the farm operational choices and associated production and emissions. The outcomes of our farm simulations are then used as input/output data for the life cycle inventory. The expected result of this approach and models is a parameterized and dynamic LCA which allows to answer questions such as: Which type of molecule provides the best environmental performance ? What are the maximal environmental impacts associated with the microalgae production for the synergy to compete with other alternatives? The conference presentation includes preliminary results answering these questions, as well as a reflection on how these methods have the potential to be generalized to other systems of emerging technologies in which a product is used to increase the efficiency of another process delivering the functional unit. Our approach particularly tackles the case of emerging techno-biological systems and attempt to address their complexity and uncertainty.

5.01.05

Proposal of a Methodological Framework for the Development of Goal, Scope and Inventory Modelling in Prospective LCA of Bio-Based Systems

N. Navarre, Faculty of Science, Leiden University / Industrial Ecology; F. Siebler, CML Leiden University / Industrial Ecology; S. Cucurachi, CML Leiden University / CML Leiden University; C. Caldeira, EC JRC; S. Sala, European Commission - Joint Research Centre / Bioeconomy unit

In the past decade, EU policies have supported the development of a strong bioeconomy. By increasing its reliance on biological resources rather than fossil resources, the EU hopes to reduce greenhouse gas emissions and limit environmental impacts. However, the environmental impacts associated with such innovations have yet to be assessed, therefore it is paramount to pre-emptively

assess their impacts to avoid costly lock-ins and unexpected environmental burdens. Prospective LCA (pLCA) has become an area of research, promoting the integration of environmental criteria in early stages of technology development. There are several methodological challenges associated with pLCA, however. The identification of alternative technologies the innovation will replace is not always clear, foreground and background systems are difficult to model due to a lack of data and current impact assessment methods may not cover novel impacts produced by emerging technologies. The current report proposes a methodological framework to address these limitations for the goal, scope, and inventory modelling in pLCA of bio-based systems. The results of the study are as follows. A valorisation study should be conducted to determine alternative functions of emerging biobased technologies. Relevant pathways should be identified and ranked according the biomass value pyramid. Should the analysis of the selection reveal undesirable impacts or hotspots, this process can be iterated to ensure a satisfactory compromise between value and environmental impacts. With a defined goal, an industrial scale project system diagram must be prepared before the processes can be upscaled using extrapolated data or proxies. To account for the background systems, integrated assessment model scenarios should be generated for all material and energetic processes used to supply the inputs to the foreground system. A decision tree framework is ultimately proposed to guide practitioners of pLCA of bio-based systems.

5.01.07

Environmental Impacts of Future Cobalt Supply Scenarios

M.v. Meide, CML Leiden University / Institute of Environmental Sciences (CML); Y. Yang, Delft University of Technology / Materials Science and Engineering (MSE); B. Steubing, CML Leiden University

Cobalt is considered an important metal in the energy transition partially because of its use in electric vehicle batteries. Demand for cobalt is expected to increase substantially up to 2050. Despite this, how the environmental impacts of cobalt production could change is currently unknown. This study provides new insight into the environmental impacts of the production of cobalt and how this could change in the future. Three variables were used to model change in the cobalt supply chain. 1) Ore grade decline, 2) primary market shares and 3) secondary market shares. Additionally, the energy transition was also taken from another study as a fourth variable. Two metal demand scenarios were used as inputs to the variables, 'business as usual' and 'sustainable development'. These four variables have been modelled between 2010 and 2050 for two policy scenarios through prospective Life Cycle Analysis. The impact increasing effect of ore grade decline and the impact decreasing effect of electricity supply work as opposing forces in the long term. It depends on the combination of variables and scenarios whether climate change impacts increase or decrease. Besides the impact results, the results from modelling and the life cycle inventories in this study can be used as a background database for other prospective life cycle analysis studies.

5.01.08

The Future Role of SCMs in Reaching Cement Climate Goals: Consequential Ex-Ante LCA on the 2020-2050 Cement Market

B. Maes, University of Antwerp / Construction; B. Craeye, A. Audenaert, M. Buyle, University of Antwerp / Faculty of Applied Engineering

The cement industry has a large environmental impact, being responsible for 7% of global CO2 emissions. The growing awareness for this environmental issue has led to Cembureau and several other stakeholders in the EU to draw out roadmaps and projections to see how the cement industry could reach the targets set out in the Paris climate agreement and EU green deal. These roadmaps show that supplementary cementitious materials (SCMs) can still play an important role in lowering cement's environmental impact. This does however require a shift in which SCMs are used, as current SCMs are typically waste materials which are already fully used. While there is no available data on how the market share will evolve, roadmaps do assert that their increased substitution demand will be met by natural pozzolans and calcined clay. This research wants to investigate how the increased use of these new SCMs in future pathways will affect an ex-ante LCA of cement. The focus is more specifically on consequential LCA, which previously did not take SCMs into account, as these where constrained materials which could not answer to a change in demand. To investigate how SCMs could impact the future environmental impact of the cement market and how uncertain this impact is, several SCMs scenarios where created with different shares for the unconstrained new SCMs. While the focus here is on SCMs and their effect on the cement market, other potential changes to the cement are also included. This is important as the value of SCMs is dependent on how much environmental impact can be avoided by substituting cement. Any change to cement therefore also influences SCMs. Not only that but, some of these changes can also be directly relevant to SCMs. Take for instance carbon capture, which is planned to be used during the calcination of clinker but could maybe also be used during the calcination of clay. Changes to the background system are included as well, more specifically electricity and fuels, as these changes influence the environmental impact of clinker and clay calcination. The assessment is performed using cornerstone scenarios to deal with the uncertainty of future events, focusing on a reference scenario and potential future pathways to reach EU climate goals. Since there is no data available on the use of carbon capture and electric kilns on calcined clay, this study will assume that similar levels of adoption take place for calcined clay, as it does for cement in the pathways.

5.01.09

Ex-Ante Life Cycle Assessment of Photovoltaic Systems Based on Large-Area Perovskite on Silicon Tandem Modules

C. Salas Redondo, Institut Photovoltaïque dIle-de-France (IPVF); C. Blanco, Institute of Environmental Sciences, Leiden University / Industrial ecology / Environmental Biology; S. Cucurachi, CML Leiden University / CML Leiden University; W. Peijnenburg, RIVM / Center for Safety of Substances and Products; L. Oberbeck, Total Gas, Renewables & Power

We have previsouly reported a comprehensive life cycle assessment (LCA) for large area (~ 2 m²) perovskite/Si tandem solar modules, with 30% power conversion efficiency (PCE), 30 years lifetime and an annual degradation of 0.5%, manufactured in a gigawatt-sized industrial scale production plant [1]. To this date and to the extent of our knowledge, that was the first one of its kind. We did an extensive compilation of the life cycle inventory (LCI) thanks to direct contact with equipment manufacturers, who provided the required data to deliver a perspective to real-life conditions. In the present study, we report the updated LCA that includes an upgraded LCI as well as the addition of the end-of-life (EoL) stage and the comparison of different energy matrix scenarios at the production stage, which are escential in a near future, where renewable energies are expected to have a larger contribution in the global energy mix. The key result found in this work is the relevance of the performance and reliability of the PV system to achieve lower environmental impacts. We tested different assumptions using a detailed yield model for the PV system which considered optimistic, realistic and pessimistic degradation rates, lifetime and PCE, among other parameters to investigate this matter. In a realistic scenario, we observed that for all the impacts assessed, the perovskite/Si tandem shows a decrease of between 16-41% in the contributions to each impact category, compared to the Si-module based reference PV system. On the contrary, in a pessimistic scenario, the perovskite/Si tandem shows an increase of between 2-41%. Moreover, contemplating different EoL scenarios permits to address strategic methodologies to implement a circular economy in the life cycle of the tandem system. Finally, the analysis anticipating future energy mix scenarios allows to define conditions to achieve further decrease of the environmental impacts. [1] Salas-Redondo C, et al. 2020. LCA of Perovskite on Si Tandem PV Modules at Industrial Scale. 37th EU PVSEC proceedings: 754 – 764.

5.01.11

Life Cycle Environmental Impacts of Hydrogen Production Scenarios Considering the Evolution of the Future French Electricity Supply Mix

M. Douziech, Mines ParisTech, PSL Research University; R. Besseau, Mines ParisTech / Centre Observation, Impacts, Energie (O.I.E.); T. Nihouarn, B. Roche, Mines ParisTech, PSL Research University; M. Gresset-Bourgeois, J. Bourmaud, RTE; P. Pérez-López, Mines ParisTech, PSL Research University / Centre Observation, Impacts, Energie (O.I.E.)

Hydrogen applications are nowadays emerging thanks to their versatility as well as the opportunity to reduce greenhouse gas emissions, contributing to (inter-)national commitments to stop global temperature rise. However, around three quarters of the world's hydrogen is currently produced from natural gas reforming, an energy intensive process producing syngas, a mix of hydrogen (H_2) and carbon monoxide. Alternatively, H_2 can be produced via electrolysis, producing H_2 and oxygen from water and electricity. Previous research highlighted that the environmental impacts of this production route are highly dependent on the electricity source. Here, we aim to compare the environmental performance of hydrogen production from natural gas reforming to the production via electrolysis considering a combination of (a) different scenarios of the shares of H_2 production pathways, (b) evolution of the French electricity mix according to projections of RTE, the French electricity transmission company, and (c) technological developments in H_2 production over the time-range from 2020 to 2050. Dynamic Life Cycle Assessment (LCA) was used to ensure a thorough and multi-criteria comparison of these alternative routes going beyond climate change impacts. Three alternative hydrogen production means were included, namely alkaline electrolyzer, proton exchange membrane (PEM), and natural gas reforming. Compared to alkaline electrolysers, PEM namely show a higher efficiency and capability to handle intermittent power. The modelling relies on the Brightway2 library in Python and the ecoinvent database adpated to account for technological improvements or supplemented using inventory data published in scientific literature. Our preliminary results show a three-times lower climate change impact of the production of H_2 via alkaline electrolyzer if the electricity used is 100% wind power compared to the French electricity mix inventoried in ecoinvent (79% nuclear, 13% hydro, 3% wind, 2% coal, 1% gas, 2% import). Depending on the evolutions of the French electricity mix's composition until 2050 switching the hydrogen production towards electrolysis could reduce the environmental impacts of this sector. To be validated, this statement requires a

thorough and complete LCA of potential hydrogen generating scenarios accounting for future market and technological evolutions in the hydrogen production sector.

5.01.14

Environmental Upscaling of a Chemical Process: The Case of a New Activator Used for the Vulcanization Process of Tires

T. Hennequin, Radboud University Nijmegen / Department of Environmental Science; L. van Vlimmeren, Radboud University / Department of Environmental Science; S. Mostoni, University of Milano-Bicocca / Dip. Scienze dei Materiali; C. Stauch, B. Fett, Fraunhofer-Institut für Silicatforschung ISC; R. Scotti, University of Milano-Bicocca / Dip. Scienze dei Materiali; R. van Zelm, Radboud University / Environmental Science

This study aims to provide recommendations in order to upscale a new chemical agent used in the vulcanization of tires. The goal is to help develop the new technology optimally from an environmental standpoint. To this end, Life cycle assessment (LCA) has been used. Life cycle inventories have been collected for the different existing technology readiness levels (TRLs). Moreover, the impacts of the future industrial production are estimated using prospective LCA. TRL 9 has been modelled using an upscaling framework tailored for chemical processes. Scenario analyses were also performed to compare different upscaling options. Notably, the use of two different solvents and the potential of solvent recovery were assessed. The new technology is an alternative activator composed of ZnO nanoparticles (NPs) anchored to silica NPs, namely, ZnO-NP@SiO_2-NP. The method of production was validated on the lab scale and is currently being upscaled within the EIT-RawMaterials project SAFE-VULCA. Modelling the impact of the future upscaled chemical agent at a high TRL was necessary to ensure a fair comparison with the incumbent activator. Besides specific recommendations for the synthesis of ZnO-NP@SiO_2-NP, this study aims to showcase findings relatable to chemical processes in general. Preliminary results showed that the new activator's impact at TRL 9 is lower than at TRL 3, by 75 and 78% for impacts on human health and ecosystems, respectively. This is the consequence of process changes and synergies as well as an increase in batch size. Moreover, across all TRLs, the main contributor to the synthesis's impact was the electricity consumed during heating, stirring, and drying. Finally, we found that solvent recovery can potentially yield an impact reduction of approx. 20%, but causes a burden shift from waste treatment to electricity consumption. The main recommendations are to optimize the electricity use and to carefully plan the treatment of waste. It is also advised to recover the solvent through distillation. Furthermore, one of the main challenges was to fairly relate the chemical agent throughout its development. Different synthesis routes were still being explored at TRL 4 and 5, making comparisons difficult. It is believed that this work is a valuable contribution to the scientific progress of both the tire and chemical engineering industries. Moreover, it intends to be an illustration of the methods developed in the prospective LCA field.

5.01.15

Using LCA to Support the Development of Novel Mobile Nutrient Recovery Technology From Anaerobic Digestion

H. Lin, M.J. Black, A. Borrion, University College London / Department of Civil Engineering & Geomatic Engineering

Digestate, as the remaining part of organic matter after Anaerobic Digestion (AD), has increasingly been realized in its rich value for nutrient recovery. Project NOMAD (Novel Organic recovery using Mobile ADvanced technology) funded by H2020 aims to establish a novel mobile unit for digestate treatment to recover rich nutrient liquid and collect fibre to produce biofertiliser and soil amenders. To support the design of the novel mobile unit from an environmental point of view, Life Cycle Assessment (LCA) methods were adopted to evaluate the environmental impacts of the NOMAD mobile unit design. A base scenario, with fossil diesel consumption, was created and assessed its environmental hotspots. Alternative scenarios were established and compared to the base scenario. 8 midpoint impact categories from ReCiPe 2016 were selected, i.e. Global warming; Ozone formation, Human health; Fine particulate matter formation; Ozone formation; Terrestrial ecosystems; Terrestrial acidification; Terrestrial ecotoxicity; Fossil resource scarcity; Water consumption. The results showed that environmental hotspots of the base scenario were due to the consumption of fossil diesel. Two scenarios with alternative biofuels, i.e. biodiesel manufactured via methyl esterification of waste cooking oil and hydrotreated vegetable oil (HVO) based on tallow, were established and assessed. The comparison between alternative biofuel scenarios and base scenario illustrated that over 60% of savings were made by alternative biofuel scenarios for 7 selected impact categories except for water consumption, which reached over 20% reduction. This suggested that alternative fuels, such as biodiesel and HVO, should be a focus for the next-stage design of the NOMAD mobile unit.

5.01.16

Ex-Ante Evaluation of Novel Biotechnology Processes for the Production of Bio-Based Succinic Acid

A. Merchan, G. Walther, RWTH Aachen University / Chair of Operations Management

The use of biomass as a renewable source of carbon instead of fossil carbon represents an opportunity for the chemical industry to attain their objectives on climate change. In this context, we are conducting research focusing on the use of second-generation biomass (therefore not competing with food production) as source of renewable carbon for the production of succinic acid. This platform chemical can be produced from both fossil-based chemicals and through the fermentation of bio-based materials. The use of succinic acid out of lignocellulose biomass is an important route for the production of biopolymers such as polybutylene succinate, 1,4-butanediol or polyurethanes. This research is concerned with a study of the environmental impacts of new biotransformation and purification technologies developed in the framework of the HyImPAct project for the production of bio-based succinic acid from maize stover produced in Germany using the Life Cycle Assessment (LCA) methodology. We have performed an ex-ante evaluation of these new biotechnological processes during early-stage development despite the missing data, limited process information and upscaling uncertainty. The outcome of this research consists of a set of potentials and limitations of novel biotechnology at an early development stage and allows to draw recommendations for process improvements. The system boundaries considered in the environmental evaluation for the production of bio-based succinic acid have been defined. Thereby, the value chain system has been divided in three sub-systems: production of second-generation biomass, distribution and material production. The material production sub-system includes the pretreatment of biomass and the biotechnological processes to produce bio-based succinic acid (i.e. biotransformation and purification). Furthermore, a comparison between the environmental impacts of the new production technologies, different industrial processes for the production of bio-based succinic acid and their fossil-based counterpart has been carried out. The conclusions drawn from this study have allowed us to analyse and select the best biotechnological processes and to identify the hotspots for their improvement from an environmental point of view. Therefore, the LCA is shown as a great tool for the design and improvement of new biotechnology.

How Life Cycle Assessment (LCA) Can Serve Effectively Environmental Foot-Printing and Policy Making: Challenges and Opportunities

5.02.01

Life Cycle Thinking and Life Cycle Assessment in EU Policies

s. sala, EC JRC; A. Amadei, European Commission Joint Research Centre / Directorate D - Sustainable resources, Land Resources Unit; E. Sanye-Mengual, European Commission - Joint Research Centre; F. Ardente, European Commission - Joint Research Centre / Directorate D - Sustainable resources, Land Resources Unit

The role of supply chains and the life cycles of products is key for ensuring sustainable consumption and production patterns (SDG12). However, the implementation of Life Cycle Thinking (LCT) and Life Cycle Assessment (LCA) in policy-making is still not harmonized. This study evaluates the historical implementation of LCT, LCA, LCC (Life Cycle Costing) and environmental footprint (Product/Organisation Environmental Footprint, PEF/OEF) in EU policies based on a systematic review (1991 - October 2020). The review includes Regulations, Directives, Decisions, Recommendations and Communications, which were categorized by year and by type, and on the basis of the covered sectors (e.g.: waste, energy, buildings, etc.). Results indicate a pivotal role of product policies in implementing LCA, particularly the Ecolabel Regulation and the Ecodesign Directive. Other relevant policies sectors include energy & fuels, waste, construction, chemicals and plastics. The PEF/OEF methods of 2013, are particularly relevant since are the first systematic attempt to have a comparable method for LCA applied in policies and green claims (organisations, products), even from a legal point of view. In the most recent years (from 2016), the number of policies and Communications related with life-cycle is growing. In particular, the 2019 Communication on the European Green Deal opens new opportunities and challenges for the implementation of LCA in policy, particularly for the PEF/OEF methods. As well, the highlighted role of a life cycle perspective in recent EU policy enhances the support of policy-making by LCA-based indicators addressing specific aspects, such as the Consumer Footprint indicator. Overall, results indicate that even if in some sectors (e.g. vehicles and waste) life cycle has been implemented with higher prescriptiveness, implementation in other sectors (e.g. food and agriculture) is only at a preliminary stage. Moreover, LCT is frequently cited only as a general and rather unspecified concept, whilst more stringent methods (LCA, PEF/OEF) are mostly cited in view of future developments. In general, despite a growing trend, the development of new stringent and mandatory life cycle requirements is still lacking. There are issues to be solved and clarified within the scientific community and at the interface between science and policy making (such as verification and market surveillance) to ensure a wider and factual implementation of LCT and LCA.

5.02.02

A Highly Resolved MRIO Database With LCIA Extensions for Transparency in Global Value Chains and Sustainable Policies

L. Cabernard, ETH Zurich / Environmental Engineering; S. Pfister, S. Hellweg, ETH Zurich / Institute of Environmental Engineering

Transparency on global value chains is crucial for sustainable policy making. Multi-regional input-output (MRIO) analysis plays a key role in providing this knowledge. However, current MRIO databases are limited in their spatial, sectoral, and temporal resolution and their LCIA coverage. Here, we present a highly resolved MRIO database with a set of environmental and socio-economic indicators and a new methodology that allows for more transparency in global value chains. We have merged the three most extensive global MRIO databases, namely EXIOBASE3, Eora26, and GTAP10, and integrated further data on commodity production and trade, as well as water and land use impacts. Our MRIO database covers 163 industrial sectors, 189 countries, time series from 1995 to 2015, and a set of environmental and socio-economic indicators, such as greenhouse gas emissions, particulate-matter related health impacts, water stress, land-use related biodiversity loss, value added, and workforce. Since the G20 are the driving force to tackle sustainable development, we use our database to assess key aspects of sustainability along G20's entire value chain of material resources (minerals, biomass, and fossil fuels). Our results show that G20's growing carbon footprint was mainly driven by the increased burning of coal and demand for minerals to build the infrastructure in emerging economies, mainly China. Together with India, China also strongly contributed to G20's increasing water stress, mainly due to the cultivation of wheat, rice, and other crops. High-income members contributed to G20's increasing environmental impacts by outsourcing their material resource production to lower-income regions with less strict environmental policies, higher water stress, and more biodiversity loss. Our results underline the importance of switching to renewable energy sources, substituting and reducing high impact minerals, and using regional comparative advantages to reduce the impacts on water and land. It further highlights the importance of extending environmental policies, such as the Paris Agreement, to a consumption perspective in order to address supply chain management in policy making. Our database and methodology allow for greater transparency in global value chains and the associated impacts to support sustainable policies.

5.02.03

Assessing Biodiversity Impacts of European Future Forest Management Strategies: Territorial and Footprint Perspective

F. Rosa, ETH Zurich / Department of Earth and Environmental Sciences; P. Lauri, International Institute for Applied Systems Analysis (IIASA) / Ecosystems Services and Management Program (ESM); A. Felton, Swedish University of Agricultural Sciences (SLU) / Southern Swedish Forest Research Centre; N. Forsell, International Institute for Applied Systems Analysis (IIASA) / Ecosystems Services and Management Program (ESM); S. Pfister, S. Hellweg, ETH Zurich / Institute of Environmental Engineering; F. di Fulvio, International Institute for Applied Systems Analysis (IIASA) / Ecosystems Services and Management Program (ESM)

The preservation of forest ecosystems is fundamental to stemming the global loss of biodiversity. Since land use is the main driver of forest loss, when planning future land use strategies it is fundamental to assess their potential impacts on biodiversity. In this context, we assessed the impacts on global species loss due to EU future alternative forest management models (AFMs) from 2020 to 2100. The analyses were conducted projecting different future land- and forest- use scenarios with the "Global Biosphere Management Model" (GLOBIOM) and estimating the potential global loss of species with the LC Impact method for land stress. A comparison of several EU forest management scenarios was performed. This was done modelling the adoption of more multifunctional management (low-intensity, close to nature practices) and set-aside on different portions of EU forest area, while considering the consequential changes in wood trade with other regions. The forest management scenarios were nested into two different climate mitigation pathways. Given the EU new strategy for biodiversity preservation, this study provides evidence-based insights in support of future EU forest management strategies. Our results showed that the more ambitious climate mitigation scenario had least impacts on species loss over time; nevertheless, biodiversity decreased in both climate mitigation pathways. When considering the impacts of EU forest management strategy, species loss in 2100 could potentially decrease with the introduction of AFMs, compared to the continuation of current practices. However, the impacts greatly depended on the type of AFM adopted, the location of imports and future demand for wood. The adoption of more multifunctional managements and set-aside on most of the EU's suitable area could decrease impacts on territorial biodiversity due to internal forest management. On the other hand, this would produce an increase of wood imports to meet EU forest biomass demand and a potential leakage of impacts within more vulnerable regions outside the EU. Therefore, in most cases, the expansion of the AFMs to smaller areas (between one quarter and half of the suitable area) turned out to be more beneficial for simultaneously maintaining territorial biodiversity and reducing the EU forest biomass global biodiversity footprint. Model improvement and proactive policies should help overcome such trade-offs between EU and global biodiversity goals.

5.02.04

LCA of Pesticide-Free Agriculture: A Swiss Case of Ex-Ante Evaluation of Political Decisions

M. Bystricky, T. Nemecek, G. Gaillard, Agroscope / Life Cycle Assessment Research Group

The use of pesticides in agriculture is increasingly questioned by the public. A Swiss Popular Initiative, which will be voted on in 2021, requests that only farmers who preserve biodiversity and have a pesticide-free production and a livestock population which can be fed with the forage produced on the farm receive direct payments. Agroscope showed that adopting this "Drinking-Water Initiative" (DWI) would lead to changes in land use and livestock numbers, as well as domestic production and income in the Swiss farming sector. We also assessed the environmental impacts of implementing the drinking-water initiative in a reference and 18 DWI scenarios. Our question was: How does the implementation of the drinking-water initiative affect the overall water pollution and biodiversity impact of the Swiss food basket? The system boundary encompassed agricultural production within Switzerland as well as imports. Thus we illustrated the impact of the sum total of agricultural raw products consumed in Switzerland. We calculated freshwater ecotoxicity of organic and inorganic pollutants, biodiversity, aquatic eutrophication with nitrogen and phosphorus, terrestrial eutrophication, and acidification as target impacts. As trade-offs, we analysed global warming potential, stratospheric ozone depletion, tropospheric ozone formation, non-renewable energy demand, abiotic resource demand, land competition, deforestation, and water scarcity. Implementing the drinking-water initiative lead to a decrease of inland freshwater ecotoxicity of organic substances by 51-75%.The other environmental impacts of domestic production decreased by 0-22%. But the environmental burden was shifted abroad: In the sum of domestic production and imports, the drinking-water initiative improved only freshwater ecotoxicity of organic substances. All other environmental impacts were similar or increased vis-à-vis the reference. The deviation was highest for water scarcity and deforestation, owing to higher imports of animal-based foods. Implementing the drinking-water initiative could reduce the pesticide and nutrient pollution of water in Switzerland as well as slightly improve domestic biodiversity. Thus, the political goal of the initiative is achievable. However, the overall impacts of the food basket would significantly increase, owing to rising food imports. For politics and society, this means that other levers have to be implemented at the same time so as to reduce these undesirable effects.

5.02.05

Employing the Consumer Footprint Indicator to Assess Circular Economy Strategies: From Product to Macro-Scale

E. Sanye-Mengual, European Commission - Joint Research Centre; T. Sinkko, EU Commission Joint Research; E. Baldereschi, European Commission - Joint Research Centre; S. Sala, European Commission - Joint Research Centre / Bioeconomy unit

Circular economy has been highlighted as a pathway towards ensuring a sustainable development with the goal of not only re-using and recycling the generated waste but also preventing its generation. At the EU level, the new Circular Economy Action Plan (CEAP) was published in 2020 towards setting the next steps for transitioning to a sustainable and competitive EU economy focusing on key value chains and intermediate products, thereby enhancing the need to consider a supply-chain approach and to focus efforts on reducing the consumption footprint. However, the environmental benefits of circular economy strategies are still under debate, e.g. closing the loop of materials might not result in a complete substitution of primary materials due to market demand leading to a circular economy rebound. Due to the relevance of value chains in circular economy, Life Cycle Assessment (LCA) can support the environmental assessment of circular economy strategies by paying attention to the entire life cycle of products. This paper aims at exploring the benefits of circular economy strategies in the EU production and consumption system while discussing the advantages and limitations of employing LCA for such assessment. 12 different circular economy statregies were evaluated covering the key value chains and intermediate products highlighted in the new CEAP. The strategies were modelled in the Consumer Footprint indicator, which was developed to evaluate the environmental impacts of EU consumption. The results of three of the scenarios are presented as examples of strategies focusing on recycling, waste prevention, and re-use. Assessing circular economy strategies is key to identify the most promising actions to be promoted and upscaled at the policy level and LCA is a comprehensive method to evaluate different typologies of circular economy strategies. However, the scenarios evalution unveiled different advantages and limitations on assessing the benefits of circular economy and the use of LCA for this purpose, including the marginal benefits of strategies at the EU macro-scale, the potential trade-offs between impact categories, the complexity of addressing combined strategies, and the limitaitons of the market context and the potential

rebound effects.

5.02.09

Environmental Modelling of Building Stocks - a Systematic Review of Potentials and Requirements for Supporting EU Policy

M. Röck, KU Leuven / Architectural Engineering; E. Baldereschi, European Commission - Joint Research Centre; E. Verellen, KU Leuven / Design and Engineering of Construction and Architecture; A. Passer, Graz University of Technology / Working Group Sustainable Construction; S. Sala, European Commission - Joint Research Centre / Bioeconomy unit; K. Allacker, KU Leuven / Design and Engineering of Construction and Architecture

The effects of multiple environmental crises are being felt increasingly across the globe. Among other environment-related challenges, the rising severity of climate change impacts is highlighting the need for a systematic shift and decarbonization of the global economy. In the European Union (EU), various policy initiatives are launched within the “European Green Deal”. Buildings and construction, being amongst the major contributors to global Greenhouse Gas (GHG) emissions and strongly related to various other environmental impacts, have a key role in decarbonization efforts. To tackle the environmental challenges at scale, policy makers require insights from environmental modelling of building stock dynamics for enabling informed decision-making. In this study, we present a systematic review of the latest scientific literature on approaches for the environmental modelling of building stocks as well as a review of the related relevant EU policy initiatives. Through synthesis of the findings, we provide insights into the strengths and limitations of existing building stock modelling approaches and identify the potentials and requirements of utilizing environmental modelling of building stock dynamics for policy support. As we characterize existing building stock modelling approaches, we show that these are mostly either life cycle, material flows or energy modelling focused, yet are aiming for extending system boundaries and comprehensive life cycle assessment. Existing studies analyse short to medium time horizons, emphasize analysis of residential buildings on urban to national scale. All studies are covering GHG related impacts, but are strongly limited on other environmental indicators. Linking existing studies to identified EU policy objectives, we find various studies investigate scenarios and strategies relevant to EU policy objectives. However, most studies emphasize energy efficiency and retrofit over investigation of potential of low carbon material uptake in reducing new construction impacts. We find clear research gaps regarding environmental hotspot analysis and monitoring of impacts across spatio-temporal scales as shortcomings in level of detail of modelling and life cycle scope of studies prevail.Future research efforts should enable comprehensive environmental assessment of building stocks across scales and emphasize the monitoring of environmental impacts from building stock development to ensure compliance with environmental targets.

5.02.10

Effect of Temporally-Resolved Electricity Mixes on the LCA of Operating a Heat Pump in Austria

D. Rixrath, Forschung Burgenland GmbH; R. Schauer, University of Applied Sciences Burgenland / Dept. Energy and Environmental Management; G. Piringer, University of Applied Sciences Burgenland / Dpt. Energy and Environmental Management

Heat pump systems are becoming increasingly popular in Austria as a low-emission residential heating system. Life cycle assessment (LCA) impacts are – for important categories – dominated by the heat pump’s electricity consumption during its use phase. Austria’s high-renewables electricity mix exhibits both seasonal and daily fluctuations, while the heat pump’s load profile is typically high in winter and can vary during the day as well. The objective of this study was to investigate the effect of a higher temporal resolution on the life cycle environmental impacts of operating a heat pump in Austria. In-house hourly load data were measured from operating a 15.1-kW heat pump, combined with hourly electricity mix for Austria from a commercial data processor. The hourly-resolved electricity mix was aggregated to daily, weekly, monthly, and annual resolutions. The functional unit for the LCA was 1 kWh of heat produced by the heat pump operation in Austria. **1. 2. 3.** Climate change impacts of the Austrian electricity consumption mix are compared between the hourly resolved, annual average and the ecoinvent consumption mix. The hourly weighted consumption mix with 0.373 kg CO2eq/kWh has the highest GWP, and the annual average mix gives 0.325 kg CO2eq/kWh. Life cycle assessment results for operating the heat pump show also that the hourly-resolved GWP100 is 14.10 % higher than the GWP annual average mix. In the other impact categories, the deviations are smaller, between -1.29 % and +12.52 %. A comparison with a natural-gas fuelled heating system shows little effect of increasing the temporal resolution of the electricity mix. Overall, a higher temporal resolution has a discernable, but moderate effect on the LCA results of operating a heat pump, even in a country such as Austria that has a high share of renewables in its electricity mix. In particular, daily and seasonal fluctuations are relevant, while there is little difference between daily, weekly, and monthly resolutions.

5.02.12

Environmental Impacts of Battery Electric Vehicles: Implications of the Cascaded Battery Life Cycle and Yearly Charging Electricity

M. Koroma, Vrije universiteit Brussel / Electrotechnical Engineering and Energy Technology,; D.M. Costa, Vrije Universiteit Brussel (VUB) / ETEC / Mobi/ Evergi; M.S. Hosen, Vrije Universiteit Brussel (VUB) / ETEC / Mobi / BIC; M. Philippot, Vrije Universiteit Brussel (VUB) - ETEC / ETEC / Mobi / Evergi; T. COOSEMANS, M. Messagie, Vrije Universiteit Brussel (VUB) / ETEC / Mobi / Evergi

The environmental performance of battery electric vehicles (BEV) is influenced by their battery size and charging electricity source. Besides, the environmental performance of BEV may consider the changes in the European Union (EU) energy sector and the interest in repurposing used BEV batteries for a second life. This study conducts a Life Cycle Assessment of the environmental impacts of a BEV, considering changes in the EU electricity mix and the repurposing of its Li-ion battery (LiB). The system boundary covers all the life cycle stages of the BEV, including its repurposed LiB cells, except the use stage of the repurposed LiB. The yearly emission profiles (2020 - 2031) of the EU electricity mix were used to estimate the BEV's well-to-tank (WTT) emissions. These were modelled based on the "Stated Policies Scenarios" for the EU reported in the World Energy Outlook 2019 report. The inventories for vehicle and battery manufacturing were based on secondary data. The ReCiPe 2016 impact assessment method was used. The used BEV battery has been repurposed for second-use in a residential application. The repurposed LiB was modelled considering battery refurbishment and assuming 50% of the used LIB cells are viable for a second lifetime of 10 years. The study also tested the sensitivity to these parameters, considering 10% to 100% cell conversion rate (CCR) and a second lifetime between 1 and 5 years. The results showed that BEV could reduce 8% in global warming potential (GWP) when future changes (increase in the share of renewable energy sources) in the charging electricity are considered. When recycling is considered, an 11% reduction in GWP was noted and a further 3% for repurposing the used LiB cells. The sensitivity analysis showed that GWP reductions occurred for a lifetime of 10 years for all CCR values. For a 5 years lifetime, GWP reductions started at 40% CCR. However, GWP increases for a lifetime of 1 year for all CCR values. The results show that available LCA studies on BEV are likely to overestimate the life cycle GWP of current BEV by at least 8% when future electricity sector changes are not considered. Thus, the environmental advantages of BEV are likely minimised. The study also found added benefits of LiB repurposing under certain conditions and confirmed the importance of recycling on BEV's net environmental performance.

How to Evaluate the Sustainability of the Transition to a Circular Economy? The Role of Life Cycle and Circularity as well as Risk Assessment Methods

5.03.01

Factor B in the Circular Footprint Formula

T. Ekvall, TERRA Ltd; M. Gottfridsson, J. Nilsson, M. Nellström, IVL Swedish Environmental Research Institute; M. Rydberg, Chalmers University of Technology / Swedish Life Cycle Center; T. Rydberg, IVL Swedish Environmental Research Institute

For material recycling to occur, waste material from a product life cycle must be made available for recycling and then used in the production of a new product. To stimulate recycling, when beneficial for the environment, life cycle assessment (LCA) should give incentives for recycling both to products that can be recycled after use and to products that can be produced from recycled material. However, most established methods for modelling recycling in LCA risk giving little or even wrong incentives. Many methods, such as the Circular Footprint Formula (CFF) in a Product Environmental Footprint (PEF), assign some of the environmental benefits of recycling to the product that uses recycled materials. This means that the incentive to send used products for recycling will be lower. If energy recovery also provides an environmental benefit, because the energy recovered substitutes energy supplied with a greater environmental impact, the LCA results may indicate that the waste should instead be sent to incineration – even when recycling is the environmentally better option. In a PEF, this risk is particularly great for textiles, because most of the environmental benefit of recycling textiles is assigned to the product that use the recycled material. The CFF includes a Factor B, which can be used for assigning part of the burdens and benefits of energy recovery to the energy instead of the product investigated. However, the default value for factor B is 0, which means that the net environmental benefit of energy recovery is fully credited to the products generating the waste. If factor B were given a higher value, the LCA results would give less incentive for energy recovery, and thus more often an incentive for material recycling. This presentation proposes an approach for calculating Factor B in CFF based on the observation that waste incineration can be described as a process with multiple jointly determining functions. Waste treatment and energy recovery both contribute to driving investments in incineration and, thereby, the volume of waste

incinerated, the quantity of energy recovered, and the quantity of energy substituted. We propose that expected revenues from gate fees and energy are an appropriate basis for calculating Factor B. Up-to-date estimates of the expected revenues in the relevant region should ideally be used for the calculations. Lacking such data, the default value B=0.6 can be used for waste incineration in Sweden.

5.03.02

Relevance of Different Allocation Procedures When Integrating Recycling Into an LCA

E. Bracquene, J. Peeters, KU Leuven / Department of Mechanical Engineering; J. Duflou, KU Leuven

In most cases, a product's composition drives its environmental profile because of the burden associated with raw material extraction and, in general, recycling is stimulated because it saves primary resources. In order to confirm the expected potential benefits, adequately assessing benefits and burdens associated with recycling activities is of increasing importance. However, integrating recycling into a lifecycle assessments (LCA) is not straightforward because it is a multi-functional process. The methodological choice between different approaches for dealing with multi-functionality in a recycling context may significantly impact the final results at individual product level and influence the decision making. Waste EEE (WEEE) is one of the fastest growing waste streams in the EU and other materials, such as metals, are today still prioritized during recycling, leaving plastics in the background. Post-consumer recycling of plastics from WEEE is a complicated task which results in a plastic recycling rate from WEEE of less than 25%. Significant increases in plastic recovery rates can be expected by clustering product categories, as clustering can avoid mixing of non-compatible plastics with overlapping material properties. A life cycle assessment (LCA) is conducted to investigate the influence of different clustering strategies on the environmental performance of waste treatment and the production of recycled plastic from LHA waste stream. In addition, the importance of using different allocation procedures when integrating recycling into an LCA is investigated in a sensitivity analysis. The findings of this study demonstrate (1) the potential benefits of implementing clusters during LHA plastic recycling, (2) the importance of using less virgin material and avoiding final waste disposal, (3) the limitation of recycling systems to reduce the overall burden associated with products, and (4) the relevance of different allocation procedures when integrating recycling into an LCA.

5.03.03

Evaluating Circular-Economy Indicators: A Case Study

A. LACHAT, Ecole des Ponts ParisTech; K. KUZMENKO, Ecole des Ponts ParisTech / Lab Navier; A. COLAS, Université Gustave Eiffel / GERS-RRO; A. FERAILLE, ENPC / Lab Navier

The Building and Public Works sector is responsible for half of the extraction of raw materials and energy consumption on a European scale. It is also responsible for the production of 227 million tonnes of waste in 2014 (43 Mt for buildings in France including 23 Mt resulting from demolition). In order to counter the aging model of linear economy (extract, use, throw away) and thus reduce the consumption of resources and the production of waste, the principle of the circular economy has emerged through the (re)democratization of recycling techniques, diversion of use and reuse. So the materials from one object are the resources for a new one. The concept is particularly concerned with resources (extractions and stocks) as well as the end of life of goods with the consideration of waste production. To measure the performance of a project from a circular economy perspective, many circularity indicators have been developed (ADEME, 2019) these last years. It includes the material recovery rate, the reincorporation rate, the Material Reutilisation Score, the Zero Waste Index and the Material Circularity Indicator. These indicators characterize material circularity over a life cycle. However, circular economy aims to take into account more cycles. The assessment is carried out over two consecutive cycles, the objective being to analyse the behaviour of the circularity indicators when taking into account several cycles. We planned to use the methodology of extending system boundaries. In this study, we apply circularity indicators named above and we evaluate impacts on a case study: a nexorade-type wooden structure (Kuzmenko, 2020). The study highlights three main observations about material circularity indicators. Indicators do not take into account the loss of intrinsic value, the loss of use value and they are not designed for several cycles. Actually, there is significant differences depending on the number of cycle under consideration. Moreover, the study gives lines of thought in order to improve the reliability of circularity indicators.

5.03.05

Resource Pressure of Carpets - a New Method for Evaluating Circular Design Improvements

H. Desing, EMPA Technology & Society Lab / Technology Society Lab; V. Lama, EMPA Technology Society Lab; R. Hischier, EMPA / Technology and Society Lab

When designing a product, many decisions are made that determine the environmental impacts the product will exert on our Planet. Indeed, prioritising a material over others, determining how much of a material will be in the finished product and how all the product-components are linked or blended together, are all choices that influence the overall sustainability of a product. Therefore, it is important to consider the environmental performance already in the design phase. In this contribution, we will showcase the application of the recently developed Resource Pressure (RP) method to assess the environmental sustainability of different carpet designs and circular strategies. This method consists of both qualitative guidelines and a quantitative indicator, evaluating the product system concerning the consumption of primary resources and generation of final waste in reference to Earth's carrying capacity. By following the design guidelines, eighteen carpet design scenarios are developed with the aim of identifying the most promising changes in design and circular strategies for improving the environmental performance of the product. In order to implement specific circular strategies, such as e.g. increasing recyclability, significant changes in the design are in some cases required. Along this line, the RP results obtained for this case study show how the RP method is able not only to assess if a specific strategy is worth to be prioritised for its influence in the reduction of the product-related environmental impacts, but also to quantify the consequences that consistently changing the design might lead to if the strategy is not eventually implemented. To evaluate the RP method and its results, the latter are compared here to a simplified LCA study for ten of the investigated carpet design scenarios. In comparison to LCA results, a close correlation was observed for most of the impact categories considered. This confirms that the RP method can, in fact, predict environmental impacts across a wide range of impact categories, without the need for detailed process data and specific LCA knowledge. It can therefore aid designers to consider the environmental dimension themselves and throughout the design process, however, does not replace but rather complement a more detailed LCA. Moreover, the simplicity of this method makes it attractive to use for a wider group of practitioners.

5.03.06

Eol-Lca in a Circular Economy: The Role of Quality Loss in Textile Recycling

A. Sudheshwar, M. Schmutz, EMPA Technology & Society Lab / Technology and Society Lab; B. Nowack, R. Hischier, EMPA / Technology and Society Lab; C. Som, Empa - Swiss Federal Laboratories for Materials Science and Technology / Technology and Society Lab

Recycling of materials is a crucial step for transitioning towards a circular economy. Increasing the share of recycled materials in production processes is feasible only if the required quality threshold is met by recycled materials; if recycled materials cannot compete with virgin materials in terms of quality, then the former would inevitably be downcycled, end-up in incineration plants, or worst, in landfills. Yet, most recycling studies focus merely on the quantity of materials collected and recycled while ignoring the quality aspect of recycled materials completely.This study focuses on the End-of-Life (EoL) of textile waste and proposes a methodology to reconcile the quality loss with quantities recovered during the recycling of textile waste. Additionally, by applying the Life Cycle Assessment (LCA) approach for various EoL scenarios for cotton and polyester (polyethylene terephthalate, PET) waste, the avoided environmental burdens from the products of EoL-treatments have been assessed and compared to provide a better understanding of the best-case waste management strategy for textile waste. The number average molar mass and the mean length of fibres have been selected as relevant quality metrics for recycled PET and cotton, respectively. Factoring-in these quality metrics in the EoL-LCA reveals a diminishing trend in the environmental benefits over multiple recycling cycles, thus underscoring the need for incorporation of recycled material quality in EoL-LCAs. As a part of the final conclusion, it is recommended to further analyse the nature of the correlation between the considered quality metrics and the actual material quality. Collaborations with the textile industry and material science would therefore be fruitful in understanding the mentioned correlation and go beyond the linear relationship established in this preliminary assessment.

5.03.07

Using Life Cycle Optimization to Analyze the Climate Change Mitigation Potential of a Circular Economy for Plastics

R. Meys, RWTH Aachen University / Chair of Technical Thermodynamics; A. Kätelhön, Carbon Minds GmbH; S. Suh, University of California Santa Barbara / Bren School of Environmental Science and Management; A. Bardow, ETH Zurich / Energy & Process Systems Engineering

The fossil-energy consumption and climate impact of plastics can be reduced by recycling plastic waste, Carbon Capture and Utilization (CCU), and biomass utilization. In this presentation, we use a global optimization model of the life-cycle of plastic production in 2050 to analyze the potential climate benefits by establishing a circular-carbon economy. Using the optimal combination of CO2, biomass, and plastic waste, a net-zero climate impact life-cycle of plastics can be achieved. To achieve these 100% emission reductions, the novel circular-carbon economy requires 61.8 % less renewable energy than the comparable linear-

carbon pathway. However, our results indicate that this reduction of overall energy demand can only be achieved by plastic recycling rates above 92%. By this means, our analysis and model highlight the capabilities of LCA to assess the benefits of the circular economy.

5.03.08

Prospective Life Cycle Assessment of Mechanical and Thermochemical Recycling of Household's Plastic Packaging Waste: Addressing Technical Quality and Potential Market Size of Recycled Materials

S. Huysveld, Ghent University / Sustainale Organic Chemistry and Technology; D. Civancik Uslu, T. Nhu Thuy, Ghent University / Sustainable Systems Engineering (STEN); S. De Meester, Ghent University / Laboratory for Circular Process Engineering (LCPE); K. Ragaert, Ghent University / Center for Polymer and Material Technologies (CPMT); J. Dewulf, Ghent University / Sustainable Systems Engineering (STEN)

A key aspect in life cycle assessment (LCA) of recycling strategies is the calculation of the avoided impacts from virgin material production. For post-consumer (PC) plastic packaging, mechanical recycling (MR) is the main recycling method today. Due to its heterogeneous composition including contamination, mechanical PC recyclates may not meet the quality of virgin alternatives leading to only a partial substitution. Moreover, closed-loop MR into food contact materials is currently not possible for the majority of plastics. In contrast, thermochemical recycling (TCR) that results in chemical building blocks is an emerging technology for closed-loop recycling of food packaging. The technical quality degradation and the different fields of application, closely related to the potential market size, should be addressed when comparing the environmental performance of MR and TCR. In this study we performed a prospective LCA of MR and TCR of 4 PC fractions in Belgium, i.e. PP, PS and mixed polyolefin (MPO) rigids, and PE films. For MR, we developed an equation for application-specific technical substitutability based on ratios of mechanical properties of the recyclate compared to the virgin material and weighting factors based on expert judgements to address their relative importance. Also, the lower potential market size for MR products was taken into account based on literature data of plastic-specific market shares of food packaging. The results for several impacts such as global warming show that both recycling options are favourable over incineration. Comparing the environmental performance of both recycling options, three key aspects can be identified. First, the lower the contamination of the waste fraction, the better the result for both. Second, MR generally has higher potential (max.) avoided impacts from virgin material production, as its products can directly be used in manufacturing, while TCR products such as naphtha should first undergo still cracking and polymerization. Third, accounting for technical quality and potential market size substantially reduces the avoided impacts from MR and brings the net impacts of both recycling options closer together. However, MR maintains the lowest net impact in most cases. This study confirms the competitiveness of TCR as emerging recycling technology, but also shows the need to further investigate the technical and legislative bottlenecks that hinder the use of mechanical recyclates as food contact materials.

5.03.11

The Second Life of an Electric Vehicle Battery Designed for Dismantling in a Stationary Storage System

M. Philippot, Vrije Universiteit Brussel (VUB) - ETEC / ETEC / Mobi / Evergi; M.S. Hosen, Vrije Universiteit Brussel (VUB) / ETEC / Mobi / BIC; M. Messagie, T. COOSEMANS, Vrije Universiteit Brussel (VUB) / ETEC / Mobi / Evergi

Electricity supply transition towards more renewable and intermittent energy sources brings a need for energy storage systems. Batteries are good candidates to store energy from renewable energy sources. In the same time, mobility is moving towards electrification with an electric car fleet that exceeds 5 million in 2018. Electric vehicle batteries at the end of their mobility life can still be used in stationary storage systems as they contain 80% of their initial capacity. Soon, these batteries will be available for 2nd life applications and circular economy concepts. This study focuses on an electric vehicle battery, designed for dismantling, that has a 2nd life application in a stationary storage system. The 2nd life application is a domestic photovoltaic installation in Belgium. The renewable electricity produced is autoconsumed by the household, the production and consumption are average for Belgium. Two scenarios are assessed: the first where the battery is reused in an electric vehicle and the second where the battery is used for stationary storage. The scope of the study contains the manufacturing of the battery, the dismantling, the 2nd use stage and to avoid allocation, the system boundaries are extended. The system boundary includes the avoidance of the production of a new battery for second use. The cells are tested to evaluate their calendar aging and their potential for 2nd life applications. The functional unit is one kWh delivered by the battery. Not only greenhouse gas emissions are evaluated but also other impacts from the ILCD list such as toxicity and acidification. These impact categories allow to analyze the compromise between climate change and other contaminations due to the energy storage in batteries. The Levelized Cost of Energy Storage (LCOES) is also assessed, to understand the costs and savings of 2nd life batteries. The data originates from a battery prototype designed for dismantling. This study is based on good quality data retrieved from prototyping of a newly designed battery and tests. It achieves to show the potential of reusing electric vehicle batteries in another or the same application.

5.03.16

Optimization the Sustainability Performance of a Biorefinery: Toward a Biorefinery Digital Twin

H. Zarafshani, KU Leuven / Material; K. Van Acker, KU Leuven / Department of Materials Engineering

While fossil fuels are responsible for 90% of the global CO_2 emissions, they also are the main source of energy delivering 87% of the consumed energy in the world. This fact brings the need to explore alternative energy resources with less impact on the environment. A potential sustainable replacement for fossil fuels is plant biomass, due to its immediate availability (44 billion ton dry mass is produced per year). The potential of biomass not only to serve as a biofuel but also as a biobased material, leads to the concept of a biorefinery. A biorefinery is a facility for conversion of entire biomass into various biofuels and chemicals. It can produce lower environmental impact compared to a conventional fossil-based refinery.The sustainability of a biorefinery can be affected by the type of input materials and process conditions. The fluctuation in the sustainability of the biorefinery performance due to fluctuating input material compositions and process parameters is difficult to capture in the current LCA methodology for emerging technologies. There is a need for a systematic method for identifying the optimal flowsheet and process parameters in order to optimize the sustainability of a biorefinery. For this optimization, an integrated platform that links all aspects of sustainability (life cycle databases and impact evaluation tools) is required. To the best of our knowledge, so far there is no single platform, which can evaluate automatically all the different sustainability aspects of a biorefinery.In this study, a digital twin of a biorefinery is introduced as a new key concept for answering this question. A digital twin is a comprehensive virtual replica of an individual product with data from the physical set-up, that will play a central role in a fully digitalized life cycle of a product. It helps us for cost- and time-effective analysis, optimization, up-scaling and virtual scenario assessment. The aim of this study is to extend the digital twin concept on biorefinery technology with the objective of auto-optimization of the process in an integrated way for environmental impact, based on life cycle sustainability assessments. Besides, a sensitivity analysis was performed to study the effect of different input and process conditions on LCA.

5.03.17

Application of Green Toxicology in Sustainable Bioeconomy Strategies - Assessment of Biosurfactants in the Project GreenToxiConomy

F.G. Weichert, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis; S. Johann, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology; C. Bertold, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis; S. Heger, RWTH Aachen University / Institute for Environmental Research; C.L. Kämpfer, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis; K. Schipper, M. Feldbrügge, Heinrich-Heine University Düsseldorf / Institute for Microbiology; T. Tiso, L.M. Blank, RWTH Aachen University / Institute of Applied Microbiology iAMB; M. Roß-Nickoll, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis; H. Hollert, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology

The exhaustion of resources, increased waste production and environmental pollution are accompanying the global population growth and indicate the need for new economic strategies. A possible future strategy is the concept of sustainable bioeconomy as emphasised by the European Commission. The Green Toxicology approach provides product-specific (eco)toxicological testing strategies to enable early decision-making and focusing on the production of less toxic entities in manufacturing, thus reducing environmental and human health impact as well as financial loss. The project GreenToxiConomy aims at implementing the Green Toxicology strategy into sustainable bioeconomy, by constant exchange of expertise between product developers and environmental scientists. To assess the product safety of selected biosurfactants (mono- and di-rhamnolipids [mono- and di-RLs], mixtures of mannosylerythritol lipids and ustilagic acids [MEL UA mix]) an effect-based bioassay battery was established and is applied within the project. To evaluate the potential of endocrine disruption and oxidative stress-related effects, mechanism-specific in vitro reporter gene assays (ERα-, AR-, Nrf2-CALUX®) were performed. The freshwater algae growth inhibition test (OECD 201), as well as the Daphnia acute immobilisation test (OECD 202), will be performed. To assess the teratogenic potential, the acute zebrafish embryo toxicity test (FET) was carried out in accordance with OECD 236. Furthermore, zebrafish eggs were exposed to selected sublethal concentrations and the larval swimming behaviour was analysed in a light/dark transition test. Results from the mechanism-specific assays indicate that none of the compounds exhibited endocrine-disrupting effects, while slight effects were observed for mono-RLs in

the Nrf2-CALUX® assay. The FET indicated acute toxicity from highest to lowest: mono-RLs>di-RLs>MEL UA mix. Behavioural effect assessment showed reduced swimming activity in MEL UA mix-exposed fish larvae. The implementation of Green Toxicology strategies within sustainable bioeconomy bear great potential to enhance early decision-making in production processes towards less harmful products for human health and the environment. This is a strong argument with respect to environmental impact and also offers economic incentives, as favourable products may be identified long before the onset of marketing and thus reducing developmental costs as well as increasing public acceptance.

LCA and Beyond - Integrating Sustainability and/or Other Dimensions in order to Improve Decision Support

5.04.01

Embracing Complexity in the Sustainability Assessment: A Computational Framework to Incorporate the Effects of Disruptions in Supply Networks

G. Larrea, LIST - Luxembourg Institute of Science a / ERIN; E. Benetto, Luxembourg Institute of Science and Technology (LIST) / Environmental Research and Innovation; A. Marvuglia, Luxembourg Institute of Science and Technology (LIST) / Environmental Research and Innovation (ERIN); T. Navarrete-Gutierrez, LIST

A supply network (SN) model can be defined as a representation of a complex system that derives from the interactions among different individuals, processes, and resources that are involved in the fulfilling of costumer's orders. Life cycle inventories (LCI) are typically simplified representations of complex and (highly) variable SN. The interactions can involve financial, material and information flows between suppliers, manufacturers, distributors, retailers, and costumers. Global SN are now more complex than ever due to the high interconnection of nodes in the network representation, the difficulty to set variables or equations to define the network evolution trajectory during time, and the network behavior that arises from nodes' interactions driven by heterogeneous motivations. Disruptions are events with low probability and high impact that force the productive system to adapt and reconfigure itself in order to survive or cope the changes. In this sense, understanding properties such as the system's restoring capacity (resilience) or its resistance to be affected by disruptive events (robustness) become significantly relevant when analyzing a SN. Different approaches to mitigate disruptive effects in SN have been proposed during the last decade, and the attempts to integrate these notions into sustainability assessment and Life Cycle Assessment (LCA) are static approaches which do not fully address the intrinsic SN complexity. The objective of this study is to propose a comprehensive framework that couples aspects of topology and system evolution into the sustainability assessment process. To fulfill this goal, we set two specific objectives: the conceptual development of the principles that nourish this approach, and the methodological framework that involved the development of a computational tool which enables the required calculations of this complexity-driven SA approach. The tool is a computational software was development and it proved to provide consisted results in terms of emissions and product flows.

5.04.02

Identification of Suitable Indicators to Assess Criticality Along the Entire Supply Chain Within Life Cycle Sustainability Assessment

M. Berr, EMPA Technology & Society Lab / Technology and Society Lab; R. Hischier, EMPA / Technology and Society Lab; P. Wäger, Empa - Swiss Federal Laboratories for Materials Science and Technology / Technology and Society Lab

Secure and sustainable supply chains are of high strategic relevance in order to realize a green and digital transformation. However, respective supply chains are often complex and exposed to a variety of risks potentially causing supply disruptions. Current criticality assessment approaches allow for evaluating supply disruption impacts at the raw materials supply only. Within a more comprehensive life cycle thinking approach, tools such as Life Cycle Assessment (LCA) and Life Cycle Sustainability Assessment (LCSA) allow to assess impacts of products along their entire life cycle. Hence, a combination and/or integration of criticality assessment into LCSA would allow for an evaluation of the latter along the entire supply chain. In the framework of an on-going Swiss National research project, a pertinent framework together with suitable indicators for such a more comprehensive criticality assessment within LCSA have been identified. This developed framework allows for identifying hotspots of supply disruption impacts along an entire product supply chain, both on the short- as well as the medium- to long-term horizons. Into this framework, supply disruption probability and vulnerability indicators, which had been collected from prominent criticality assessment methodologies and subsequently complemented as well as partly adapted, have been integrated in form of characterization factors. In the frame of this presentation, first, an overview on the setup of the developed framework is provided. Secondly, the procedure for the identification of indicators that are suitable for an integration into this framework is described. Third, challenges related to a quantification of the selected indicators with respective data from ecoinvent, EXIOBASE and the Social Hotspot Database, three LCA databases providing the currently most-comprehensive LCA-related data, are discussed. Subsequently, options to improve the indicator coverage by data acquisition from additional sources are suggested. Last but not least, a short outlook towards currently on-going case studies that aim for an operationalization and verification of the developed framework with the selected indicators is provided.

5.04.04

Comparing Different Monetization Methods for LCA

R. Arendt, TU Berlin, Department of Environmental Technology / Chair of Sustainable Engineering; T.M. Bachmann, EIFER; M. Motoshita, National Institute of Advanced Industrial Science and Technology (AIST); V.K. Bach, Technische Universitaet Berlin / Chair of Sustainable Engineering; M. Finkbeiner, Technische Universität Berlin / Chair of Sustainable Engineering

Monetizing life cycle assessment (LCA) results can be a helpful tool to support decision making, as it allows to integrate LCA into Cost-benefit-Analysis, and is one form of weighting, that enables the handling of trade-offs between impact categories. Further, it adresses the economic dimension of sustainbility, as it makes environmental and societal life cycle costing more practible. It mostly considers the damages that environmental emissions cause to ecosystems, the society and the economy, so that monetized LCA results are multidimensional and culturally diverse in nature, which is underlined by the variety of available methods. The valuation of the Area of protections (AoPs) and the geographical reference areas will be presented for the following methods: Ecovalue14, Stepwise2006, LIME3, Ecotax, EVR, EPS and the Environmental Prices Handbook. Further, we will analyze their valuation of the yearly domestic environmental damages of an average EU citizen, which ranges between 7941.13 €/capita (Ecotax) and 224.06 €/capita (LIME3). The prioritization of impact categories varies as well: Stepwise and Ecovalue assign over 50% of the per capita damages to climate change, while EPS and LIME3 assign around 50 % to mineral and fossil resource use. Choices regarding the geographical reference, and therefore income, as well as the included AoPs, strongly affect the magnitude of the monetary factors. Thus, LCA practitioners are confronted with the normative question whether every disability adjusted life year (DALY) should be weighted equally or according to the income level of the exposed individuals. While the valuation of the AoP human health was quite homogenous across methods, the valuation of the AoPs biodiversity and resources relied on different approaches that delivered a variety of monetary factors. Additionally, the inclusion of AoPs varied across methods: some methods included damages to working capacity, buildings, materials and agricultural yields, which are classically thought to be economic damages, while others did not. Further, no method assigned a priority (a value of over 20 %) to an impact category that was connected to biodiversity. The presented results support practitioners to assess the robustness of their results when using monetized results in their sustainability assessment as a basis for decision making. The inclusion of monetized LCA results in decision making advances the integration of environmental, economic and social effects.

5.04.05

Analysing the Underlying Drivers of Cities' Impact on Climate Change

P.K. Ohms, Technical University of Denmark (DTU) / Management Engineering; M. Ryberg, Technical University of Denmark (DTU) / Quantitative Sustainability Assessment, Department of Management Engineering; A. Laurent, Technical University of Denmark / Quantitative Sustainability Assessment, Department of Management Engineering; M.Z. Hauschild, Technical University of Denmark / Division for Sustainability, Department of Technology, Management and Economics

The current rapid increase in urban population and the projected growth of cities worldwide combined with growing consumption of resources, releases of emissions and production of waste related to the current state of cities, it is essential that cities adapt to reduce their impact on the environment. Studying the drivers of a city's environmental impact can greatly help identify opportunities for reducing the impacts. In this study we gathered and reviewed information from existing environmental studies of cities covering all inhabited continents and with a multiple regressional analysis we investigate the correlation between environmental impact and city characteristics, such as population density, household expenditure, temperature, and carbon footprint of energy grid mix. We only included studies that reported at least one environmental impact for more than one sector at city scale. The majority of the studies that fulfil the criteria only reported impact scores for climate change, and to achieve the largest sample size, we therefore chose to limit the quantitative analysis to climate change in this study. The retrieved pool of cities covers a wide range of city types including Asian megacities, African developing cities, and European cities with a high consumption output. In total, more than 30 cities are considered of which 40% are located in Europe, 25% in North America, 15% in Asia and 15% in the Oceania. Each of these cities face unique challenges and finding characteristics that are universally strong drivers for all cities is unlikely. To find the strongest statistical relationship we grouped cities into smaller samples using different criteria, e.g. geographical location, economy or dominating source of revenue (tourism,

industry, agriculture). To avoid a one-size-fits-all solution, we performed a targeted search for drivers of climate change impact within each type of city. The outcome of this work is an indication of which drivers explain climate change impacts for different types of cities. With the new knowledge on these drivers for different city types, we provide guidance to help form targeted recommendations and support policies on measures to reduce climate change impacts of cities.

5.04.07

Development of a Regionalized Chemicals and Plastics LCI Database for Robust Environmental Decision Support

L. Stellner, Carbon Minds GmbH; R. Meys, RWTH Aachen University / Chair of Technical Thermodynamics; A. Kätelhön, Carbon Minds GmbH

In the context of a global commitment to mitigate climate change, the chemical industry has recently been the subject of much debate in order to find strategies towards a more environmentally friendly chemical and plastics industry. To evaluate such strategies, Life Cycle Assessment (LCA) is a powerful tool to determine the environmental impacts of products and services, providing a holistic view across environmental impact categories and life cycle stages. However, to provide a reliable basis for decision-makers, LCA studies for the chemical and plastics industry require a large amount of data on chemical products and processes across global production and utilization chains. Gathering this data is especially challenging for industries with highly complex and globalized production chains, like chemicals and plastics. To support decision making, we developed a novel Life Cycle Inventory (LCI) database compliant with the ISO standards 14040 and 14044. Our chemical and plastics database currently contains approx. 200 chemicals and plastics in up to 150 regions. The database is derived from a regionalized model of the global chemical and plastics industry, built from technical data on all production technologies throughout the life cycle and market and trade data, which enables chemicals to be tracked throughout the global economy. Our bottom-up approach starts at the chemical plant level, linking information about the production site, production volume and production technology to detailed technical models for each production technology. Subsequently, these individual plants are allocated to integrated production sites and national, technology-specific or supplier-specific production mixes. Moreover, national consumption mixes are calculated by combining national production mix information to trade data. The novel LCI database aims to provide consistent and representative LCI data of chemicals and plastics, taking into account the entire supply chain and mapping differences between production technologies and locations, suppliers or regions of chemical products. Our results show significant differences between regional production or consumption mixes. In particular, due to our unprecedented modeling approach we found valuable reduction potentials to supply chain emissions using supplier-specific carbon footprints. These data support LCA practitioners in conducting more representative and reliable LCA studies leading to robust environmental decision support.

5.04.08

A Life Cycle-Based Assessment Toolbox to Assess and Improve Safety and Sustainability of Chemicals

H. Holmquist, IVL Swedish Environmental Research Institute; M. Bernander, Volvo Car Corporation / Sustainability Center; P. Fantke, Technical University of Denmark / Quantitative Sustainability Assessment, Department of Technology, Management and Economics; M. Gottfridsson, J. Gunnarsson, IVL Swedish Environmental Research Institute; A. Hellström, J. Hildenbrand, RISE; M. Johansson, AstraZeneca; A. Mullen, Astrazeneca UK Ltd; J. Olsson, Volvo cars; O. Pajalic, Perstorp; T. Rydberg, IVL Swedish Environmental Research Institute; P. Saling, BASF / Sustainability Strategy; S. Schellenberger, RISE; J. Snape, AstraZeneca UK Ltd. / Global Sustainability

The expansion of a sustainable chemical industry is key for a toxic free environment, urgently called for in EU's recent chemicals strategy and the UN Sustainable Development Goals. Substituting hazardous chemicals in industrial processes and consumer products requires not only that the selected alternatives are less hazardous, but also that the substitution does not lead to unacceptable trade-offs elsewhere in the chemical's or product's life cycle. Likewise, during process design and optimization, life cycle considerations are imperative, to prevent burden shifting from one safety, health and environmental related impact to another. To address these challenges, robust methods and tools are needed. Life cycle-based tools need to incorporate hazardous properties of molecules and materials, and risks for exposure along full life cycles. To this end a life cycle-based assessment toolbox to assess and improve safety and environmental sustainability of chemicals is under development as part of the Mistra SafeChem research programme. The starting point for building this toolbox is a tiered framework for life cycle-based alternatives assessment, well aligned to the 2014 Framework to guide selection of chemical alternatives by the US National Research Council. Building on this starting point and drawing on the available models already applied in LCA for evaluating chemical toxicity and ecotoxicity as well as the advancements in digitalization methods, the toolbox will be implemented and tested in case studies with the aim to support the development of safer and more sustainable chemicals in line with the European and global sustainable development agenda. The case studies are designed to address relevant challenges including data scarcity, circularity and decision making under uncertainty. Case study results will be used to identify further research and development needs and provide the basis for a guideline to the toolbox. The authors thank the Swedish Foundation for Strategic Environmental Research (Mistra: project Mistra SafeChem, project number 2018/11) for financial support.

5.04.14

Using Nutritional-Life Cycle Assessment to Simultaneously Improve Nutritional, Health, and ENvironmental Dimensions of Agri-Food Systems: Methodological Challenges and Case Study

A. Green, ETH Zürich & Agroscope / Institute of Food, Nutrition, and Health; T. Nemecek, Agroscope / Life Cycle Assessment; S. Smetana, German Institute of Food Technologies (DIL e.V.) / Food Data Group; A. Mathys, ETH Zurich / Institute of Food, Nutrition, and Health

Optimizing food systems from a nutritional, health, and environmental perspective is needed to ameliorate current and future sustainability challenges. However, to accomplish this, we need more and different methods in this space; one such option is nutritional-LCA. Nutritional-LCA (n-LCA) is the integration of nutrition and health into environmental LCA. Here, we explore the applications of n-LCA via a review protocol and identify methodological and data challenges; we then illustrate these issues with a case study. Specifically, we focus on nutritionally-invested environmental impacts because our food system is not only responsible for environmental impacts like climate change and biodiversity loss but also for hidden hunger and noncommunicable diseases like diabetes. Nutritionally invested footprints, or impacts, are environmental impacts measured against a nutritional functional unit (FU). However, currently, most footprints are measured using a mass-based functional unit (FU), which does not holistically reflect the key functions of food. The study is divided into two sections. For the first part, we reviewed 774 papers on the topic of methodological approaches for measuring nutritional, health, and environmental dimensions of agri-food systems. However, here, we focus specifically on LCA. Methodological challenges that we identified and addressed include the use of disqualifying nutrients in nutrient density metrics, energy standardizations of metrics, capping nutrient scores, as well as decentralized and a lack of harmonized LCA data. For the second part of the study, we implemented a case study to examine some of these methodological choices. For this, we used LCA environmental impact data and calculated nutrition scores based on food composition databases. We found that the relative rankings of environmental footprints changed when considering overall nutrient density scores or micronutrient deficiencies. Therefore, a food item that has an environmentally-friendly water footprint when measured on a kg basis may have a relatively higher footprint compared to other food items when accounting for nutrient density These changes have implications on how money should be allocated when designing improved sustainable food systems. Consequently, more research should focus on how to best operationalize n-LCA, which is still a nascent method.

5.04.15

Environmental Impacts of Foods Served in the United States National School Lunch Program

A. Stern, Tufts University / Agriculture, Food and Environment; N. Blackstone, C. Economos, T.S. Griffin, Tufts University / Friedman School of Nutrition Science and Policy

Background: High income countries must shift their dietary patterns to address the increasing environmental burdens of agriculture. Government programs and policies are an important lever in shifting these dietary patterns. In the United States, the National School Lunch Program (NSLP) has a 14 billion USD budget and maintains the power to shape the consumption habits of American children and adolescents. While the nutritional quality and cost effectiveness of NSLP are regularly assessed, the environmental impacts of producing the food for this program are not known. Baseline estimates of the environmental impacts of lunches served by NSLP are needed to make policy recommendations which encourage the design of menus with lower environmental burdens. Methods: This research used a nationally representative sample of over 2 million lunches served in NSLP during the 2014-2015 school year. Life Cycle Impact Assessment for the agricultural production phase of ingredients of lunches was performed with the following impact categories: climate change (kg CO2 eq.), land use (m2a), water consumption (m3), and freshwater (kg P eq.) and marine (kg N eq.) eutrophication. Results: The mean impact per lunch was 1.5 kg CO2 eq. climate change, 1.9 m2a crop eq. land use, 6.9E-2 m3 water consumption, and 0.4 g P eq. freshwater and 3.1 g N eq. marine eutrophication. Lunches in the top quintile of impacts contributed an outsized proportion to total impacts (~40%) suggesting that policy changes related to these lunches are a priority. Food groups varied in their contribution to each impact category; meat and dairy stood out as the greatest contributors for multiple categories, while fruits, grains, and oils were major contributors to just one impact category. Diary, fruits, and vegetables accounted for 72% of the mass of all food served but only 25% of the total GWP, whereas

meat which accounted for 8% of total lunch mass, contributed to 65% of the total GWP. Conclusions: A wide distribution of impacts was seen across lunches served in NSLP due to difference in lunch composition and calorie content. This indicates that changes in lunch composition could impact the environmental burdens from agriculture of NSLP. Low impact lunches identified in this study could act as a template for future menu planning and offer an opportunity to shift lunches in NSLP to have lower environmental impacts.

Life Cycle Impact Assessment Modeling and Application

5.05.01

Assessing Biodiversity Impacts of EU Consumption: A Comparative Analysis of Available LCIA Methods

E. Sanye-Mengual, European Commission - Joint Research Centre; F. Biganzoli, European Commission - Joint Research Center; C. Caldeira, EC JRC; C. Klöcker, S. Pfister, ETH Zurich / Institute of Environmental Engineering; F. Rosa, ETH Zurich / Department of Earth and Environmental Sciences; S. Sala, European Commission - Joint Research Centre / Bioeconomy unit

The relevance of addressing biodiversity impacts has increased in the last decades due to the outstanding effects on biodiversity loss of human activities and consequent environmental impacts. The EU Biodiversity Strategy for 2030 points out the necessity to consider the transboundary effects due to trade and supply-chains and to reduce the EU consumption footprint. For this purpose, the Life Cycle Assessment (LCA) method can support the assessment of the environmental impacts of products and organizations providing such supply-chain approach. However, multiple Life Cycle Impact Assessment (LCIA) are currentli available to assess biodiversity and there is a lack of consensus. The goal of this study is to compare the impacts on 'ecosystems quality' of currently operational LCIA methods covering biodiversity loss for unveiling convergent and divergent messages. Five LCIA methods for quantifying biodiversity impacts were compared: Recipe 2016, Impact World+, LC-Impact, Eco-scarcity, and Stepwise. The LCA-based Consumer Footprint indicator was selected as case study due to its consumption-based approach, the broad coverage of products that might contribute to different biodiversity loss drivers, and the harmonized scope of the data collection for all the products (e.g. system boundaries). The comparison of operational LCIA methods and a detailed hotspot analysis of the Consumer Footprint will enable a better understanding of the advantages and disadvantages of employing a particular LCIA method in the assessment of biodiversity impacts. Convergent and divergent messages will outline similarities and differences in the available LCIA methods, providing ley information for different stakeholders at different levels. In particular, the outputs of the study will provide information on where to focus efforts to improve quantification of biodiversity impacts. At the user level, this comparison might allow practitioners, including, businesses, to identify a preferred LCIA method to be employed depending on the scope of studies, and policy-makers to enable a clear knwoledge of the coverage and capacity of LCA for the use of biodiversity impacts data in policy-malking.

5.05.02

A Holistic Approach to Assess Value Chains' Impacts on Biodiversity

E. Martin, P. Sangines de Carcer, K. O'Brien, Quantis; A. Ernstoff, Quantis / Environmental Health Science; V. Rossi, Quantis

Biodiversity loss has now exceeded its planetary boundary. Pressure on the natural functioning of ecosystems is largely driven by production-consumption systems. Although life cycle impact assessment methodologies exist to quantify biodiversity impacts, in practice quantifying corporate biodiversity accounting is still largely lacking. Hence, there remains a gap between knowledge and practice in order to provide meaningful metrics for companies to prioritize actions to decrease their impact on biodiversity.To fill in this gap, we have developed an innovative and holistic approach to support the assessment of biodiversity impacts throughout the life cycle of products and services. The approach is based on a peer-reviewed methodology (Impact World+) which is then cross-referenced with data from life cycle inventory databases (WALDB, WFLDB, ecoinvent) and land-use change (LUC) flows modelled based on existing guidance for LUC accounting. Then we aggregated each flow's impact into an indicator of potential biodiversity impact using the regionalized IMPACT World+ method at country-level. Obtaining country-level archetypes is important to streamline multinational corporate footprinting, where corporates may only have information on the country of sourcing and not the exact location, for hundreds if not thousands of products. This methodology allows for consistent calculation of the various impact pathways on ecosystem quality as PDF.m^2.year (potentially disappeared fraction of species in a square meter in a year). This allows corporates to compare between and across value chains and identify both hot-spots (PDF.m^2.year) and levers for change (related to the impact pathways). The methods were applied to several companies. As an example, the total biodiversity impact (2 418 244 PDF.m2.year) of a volume of coconut milk was broken down per midpoint impact indicator. This showed that land transformation is the indicator that has the largest contribution to the impact on nature, followed by land occupation. To conclude, it is paramount to have a streamlined method to calculate biodiversity impacts for corporations to identify the main drivers of biodiversity throughout value chains. Our holistic approach represents a stepping-stone towards improving and up-scaling the practice of corporate accounting for biodiversity and which is a key first step to guide meaningful corporate strategies on biodiversity.

5.05.03

The Development of Environmental Impact Method for Desertification in Framework of LCIA

R. Liu, Graduate School of Environmental and Information Studies, Tokyo City University; N. Itsubo, Tokyo City University / Graduate School of Environmental and Information Studies; H. Ohashi, T. Matsui, A. Hirata, Forestry and Forest Products Research Institute

Given that the truth of desertification has occupied one-quarter of territorial land, with annually 1.2 hundred million Ha-speed spreading in the world, we call for people to face up to this global environmental problem by the middle of the century. Although the causes of desertification were varied of many, the main pressure induces it was considered as climate change and land use. Climate change dominates the macro-environmental change severely, manipulate the tendency of change in desertification, Land use play the role of speeding up the deterioration. The worsening situation for the desertification, the riskier for the related impacts act on the earth. LCIA is a kind of inclusive quantitative tool assessing the object whole life cycle gives a relative completed perspective to realize the environmental problem. However, the current LCIA framework is the lack of addressing the consequence of land use and the related issue was considered to be shortness waited to be developed. The proper circumstance in this study attempts a new try to focus on one of the land-use related impacts, desertification to be a midpoint, structuring an endpoint-based whole LCIA framework. The development of the method was composed of three components. For the first part considering the inventory of desertification as Climate change and land use, then the second part is midpoint concentrate on the desertification, the third part is desertification related impacts divided into two perspectives, addressing the ecosystem quality and human health. In this study, we execute a priority that, analysis the periodic global land use data and climatic variations data. By incorporating the data above modelling the desertification's periodic evolution, constructing the characterization factors between inventory and midpoint, for the image that the evolution of desertification per GHG emission unit. Then step to the endpoint, the first dimension is considering the biodiversity. The indicator for the biodiversity loss and the change of periodic desertification will merge to be the characterization factors connecting the midpoint and endpoint. The LIME3 model set a comprehensive model for our previous, this time we will develop a new component for the midpoints, as the same structure as LIME3.Consequently, for the future scenario, the desertification evolves in some area, for instance, the Mediterranean region, north-south America etc. the total area of desertification compares with the former report is quite close. The plant responsible for the pressure of desertification is also apparently to see, around over 60% of this kind habitat is shrinking.The study is still under processing, the phased objectives will end up with the characterization factors with the priority process.

5.05.04

Importance of Using Representative Species Datasets to Determine Effect Factors for Terrestrial Acidification

A.L. Gade, Technical University of Denmark / Quanlitative Sustainability Assessment, DTU Management; M.Z. Hauschild, Technical University of Denmark / Division for Sustainability, Department of Technology, Management and Economics; A. Laurent, Technical University of Denmark / Quantitative Sustainability Assessment, Department of Management Engineering

Terrestrial acidification has been demonstrated to be an important global issue, although major regional and local variations occur, e.g. depending on the biodiveristy landscape and the soil properties. It is commonly a default impact category in the LCIA framework and feature in many LCIA methodologies. The most up-to-date characterisation model for terrestrial acidification currently relies on effect factors (EFs) that carry important limitations: (i) they rely on a dataset of 2,409 plant species, hence far from the 383,671 accepted species of vascular plants reported in the world, (ii) they could only be recommended at biome level, given the too little data to derive accurate EFs at ecoregion level. In this study, we overcome these limitations by building on a more comprehensive dataset to derive EF values at global scale that can be differentiated at ecoregion level. We first extract georeferenced data points on observed vascular plant sepecies from a standardised, open-access, online database, clean the resulting dataset (e.g. taxonomic nomenclature), map them according to the 827 ecoregions and 14 biomes in the world. Using programming on high performance computing cluster, these datasets were matched with predicted soil pH data at 250m resolution. We then derive marginal EFs by determining species richness distributions and applying regression analysis to derive potential not-occuring fraction of species. A dataset of 113,846,141 species occurrences, corresponding to a total of 189,185 distinct species was then built, thus covering 49% of all accepted vascular plant species in the world. This can be contrasted to the 0.6% of coverage behind the EF

currently in use in LCIA for terrestrial acidification. When comparing EF values, important changes are observed between our EFs and those currently in use, suggesting that species data representativeness is important to consdier in LCIA method development. Moreover, the EF values at ecoregion level demonstrated a large spatial variability, stretching more than one order of magnitude within biomes. These results indicate that the increased dataset coverage can also enable to obtain more accurate EF values that capture local and regional specificities. We therefore recommend the implementation of our new EFs into LCIA methods for terrestrial acidification, and we advocate for further exploring the influence of dataset representativeness in other impact categories.

5.05.05
Spatially Differentiated Impact Assessment Model for Marine Eutrophication for Absolute ENvironmental Sustainability Assessments
E.B. Vea, Technical University of Denmark / Quantitative Sustainability Assessment, Department of Technology, Management and Economics; M. Ryberg, Technical University of Denmark (DTU) / Quantitative Sustainability Assessment, Department of Management Engineering; K. Richardson, University of Copenhagen / Center for Macroecology, Evolution and Climate, Globe Institute; M.Z. Hauschild, Technical University of Denmark / Division for Sustainability, Department of Technology, Management and Economics
Several methods integrating environmental boundaries (such as the planetary boundaries) with LCA have recently been developed. Such methods are referred to as Absolute Environmental Sustainability Assessment (AESA) methods and aim to assess whether a product or system actually is environmentally sustainable, not only whether it is better than a reference. A substantial knowledge gap and limitation of AESA methods is the application of globally representative average boundaries and impact assessment modelling for non-global processes such as nutrient emissions. A recent AESA study developed a spatially resolved method for quantifying impact of nitrogen emissions on the “safe operating space” (SOS) for natural soil, freshwater and coastal water. However, the spatial resolution of the model for coastal water was too coarse in the sense that exceedance of local scale SOS (in particular for areas close to shore) might be “hidden” by the inherent averaging of impacts over large marine areas. To avoid this and improve AESAs related to nutrient emissions, we aim to develop a spatially differentiated model for expressing marine eutrophication impacts of nitrogen emissions in relation to environmental boundaries for marine eutrophication at a suitable spatial resolution. To develop the AESA model, the following steps and considerations were taken; we 1) conducted a preliminary screening of limitations in current LCIA methods for marine eutrophication (other than coarse special resolution) and their applicability for AESA, 2) outlined how hydrodynamic models can be integrated with LCIA models to meet these limitations in a new AESA model for marine eutrophication, 3) screened and selected potential hydrodynamic models and tested their applicability for AESA. This study contributes to increase the spatial resolution of impact assessment of nitrogen emissions on coastal water, to improve AESAs related to nutrient emissions and greatly improve the identification of potential local exceedance of SOS. Moreover, the spatially refined model advances LCIA modelling in general by providing stronger spatial differentiation for marine eutrophication impacts, thereby, closing a known gap in existing LCIA methods.

5.05.10
Towards the Integration of Mineral Resource Dissipation in Life Cycle Inventories: Insights From Ecoinvent-Based Case Studies
F. Lai, A. Beylot, French Geological Survey, BRGM
The assessment of the impacts associated with mineral and metal resource use in Life Cycle Assessment (LCA) currently primarily relies on the concept of “resource depletion”. However, debates have recently risen within the LCA community to question this concept, therefore leading to the emergence of the “resource dissipation” concept, which considers the dissipation of a resource once it is rendered inaccessible to future users. The operationalisation of the latter concept in LCA requires further developments such as the integration of information about dissipative flows in the Life Cycle Inventories (LCI). In this context, the Joint Research Center (JRC) developed (in 2020) a new approach to account for the dissipative mineral resource flows at the level of each unit process of a system life cycle. The implementation of this approach follows two steps: 1) a resource flow analysis consisting in tracing each mineral resource input and output in each unit process system; 2) an identification of the dissipative flows, based on the resource flow analysis.This study aims at extending the application of the JRC approach to existing LCI datasets drawn from the ecoinvent v3.7 database considering, on the one hand, different cradle-to-gate metal production systems (e.g. lithium; Li), and on the other hand, the cradle-to-grave Li-ion battery system. In particular, this study aims at i) assessing the dissipative flows of mineral resources along these different systems, ii) using these results as a basis to provide insights as to the generalisation of the approach and its integration in the current and future LCI databases.As example, the cradle-to-gate production of 1 kg lithium carbonate (Li2CO3) overall generates 0.48 kg of direct dissipative mineral resource flows along its process chain. Li2CO3 production from concentrated brine accounts for 87.6% of the dissipative flows (in mass). Sodium accounts for the largest share of the dissipated resources (78%), followed by Li (13%); while final waste disposal facilities represent the main dissipation compartment (90%).The implementation of the JRC approach to the ecoinvent database appears as a promising step towards the integration of the resource dissipation concept in LCI. However, some challenges are yet to be overcome in terms of mass balance consistency in datasets and automation of the approach in order to secure the assessment of resource dissipation through the database.

Life Cycle Sustainability Assessment: Integrating the Environmental, Economic and/or Social Pillars of Sustainability

5.06.01
New Approaches of Social LCA for the Assessment of Products Along the Value Chain
P. Saling, BASF / Sustainability Strategy; T. Gruenenwald, BASF SE / CDS/S
The new SEEbalance® method is a methodology designed to evaluate all the three pillars of sustainability – environment, society and economy. This methodology has now been fundamentally revised in terms of assessing social aspects based on our own developments and the developments of the Round table for Products Social metrics where BASF is a member among other industrial partners. Within these frameworks we decided to develop qualitive factors to identify relevant social topics along value chains. For the assessment, different types of data sources were identified and applied combined with a set of decision points of the data assessment. The main goal was the development of an applicable approach, where data can be implemented from accepted third party data providers and to transfer them to easy to understand information. In addition, this information should be able to support decision-makers in the processed of further improvements of value chains. While, in theory, numerous social impacts can be assessed, it is highly ambitious to analyze, calculate, interpret, and aggregate a massive amount of impact categories in practice. For the new approach of Social Analysis under SEEbalance®, risk categories are used to allow for in-depth analysis to obtain a relatively high level of detail and completeness through manageable effort.

5.06.02
Are Equity and Fairness Quantifiable? Adapting Criteria Scoring to Sustainability and Lifecycle Assessment
S.E. Apitz, SEA Environmental Decisions Ltd
Ethical and equity considerations in decision-making encompass a broad range of issues, including social justice, equality, the polluter pays principle, distributional issues, demographic, temporal and spatial equity and business and other ethical concerns; many of these have proved difficult to quantify. All active management, including that for product development, results in (desirable and undesirable) environmental, economic and social impacts. Balancing trade-offs through sustainability, lifecycle assessment (or other) assessment poses normative questions–not just objective and science-based, but those rooted in societal values, requiring engagement and a careful consideration of diverse stakeholders’ priorities. Risks, benefits and costs of land- and water-scape management, remediation and restoration alternatives are not borne equally in terms of time, space or demographics; nor are the values and priorities of different stakeholders the same. Embedding equity considerations within assessment and decision frameworks in a quantitative and transparent manner remains challenging. Though the focus of these will be the evaluation of alternative sustainability, the objective is to trigger discussion on how these approaches might be used in social LCA. A range of issues, such as transparency, community engagement and communication of uncertainty, address the fairness of the overall decision process, rather than the relative fairness of individual alternatives. These can be evaluated and enhanced in a number of ways. On the other hand, the relative importance of various impacts to different individuals or stakeholder groups can, to some extent, be addressed with stakeholder outreach, and weighting based upon preferences. However, every impact may differ in time (e.g., duration of impacts, or delay in benefits), space (e.g., greater impacts on some neighbourhoods or regions); or demographics (e.g., unequal access or profit from benefits; impacts of gentrification or development). Such distributional effects can be addressed in a narrative manner; highlighting best management practices to address them, or they may be addressed using weighting schemes, similar to “exposure” filters, in scoring systems. A series of thought experiments and case studies (using real and hypothetical data) will explore these differing approaches, and examine their strengths, weaknesses, data needs, and feasibility.

5.06.03
Dashboard Indicators for Social and ENvironmental Screening Assessments of Novel Nutrient Recycling Agricultural Technologies
E.P. Andrade, IRTA/URV; A. Bonmatí, IRTA / ORGANIC WASTE INTEGRAL MANAGEMENT; L. Jiménez, Universitat Rovira i Virgili / Chemical

Engineering; E. Montemayor, Institute of Agrifood Research and Technology - IRTA / GIRO-Integral Organic Waste Management Program; A. Assumpcio, IRTA / GIRO-Integral Organic Waste Management Program
Dashboard information aims to communicate complex information related to current situations and historical trends and its use has been rising in many different contexts. Establishing a set of dashboard indicators (DBI) is an effective way to compare alternative situations with a baseline (i.e. current situation), as it can be easily calculated, understood and communicated. The DBI selected in the current work aim to provide novel environmental and social markers to estimate the effectiveness of new agricultural technologies in increasing nutrient recycling, developed by the Nutri2Cycle H2020 project. The selected indicators consider the main social and environmental aspects related to agriculture, as well as the goals and indicators included in the Sustainable Development Goals, the European Commission's Agri-environmental indicators and the European Green Deal. As a result, 19 environmental indicators (EI) were selected, then divided into four dimensions: 'Use of primary resources' (eight indicators), 'Emissions to environment' (six), 'Resilience to climate change' (three) and 'Productivity' (two). According to stakeholders, 17 social indicators (SI) were considered relevant for the Social Life Cycle Assessment (SLCA), and divided into: 'Workers' (six indicators), 'Value chain actors' (three), 'Local community' (six), 'Society' (seven) and 'Consumers' (two). The application of these DBI can be qualitative (yes/ no questions) or quantitative (when data is available). In addition, the assessment can be done for a current or prospective approach (when the technology is at an early Technology Readiness Level). The set of indicators were applied in a case study to evaluate the technology 'Catch crops to reduce N losses in soil and increase biogas production by anaerobic co-digestion'. The assessment provided by this technology was qualitative, and had 47% positive responses (good performance), 11% negative responses (bad performance) and 42% neutral responses (no influence or influence not known yet). Of the positive responses, 65% were from the EI and 35% from SI; the negative responses are 100% attributed to EI; and of the neutral responses, 27% was from EI and 73% from SI. Dashboard indicators do not aim to replace quantitative indicators such as those conducted through environmental or social LCA, but aim to provide an initial screening step. We have also conducted the social and environmental LCA, which have allowed us to confirm tendencies and uncertainties within them.

5.06.04

Application of Conjoint Analysis for the Life Cycle Sustainability Assessment of Electric Mobility Scenarios With a User Perspective

G.B. Bouillass, MINES ParisTECH - OIE Research Center / Centre Observation Impacts Energy O.I.E.; I. Blanc, Mines ParisTech / Centre Observation Impacts Energy O.I.E.; M. Berger, Technische Universität Berlin / Chair of Sustaiable Engineering; M. Finkbeiner, Technische Universität Berlin / Chair of Sustainable Engineering; P. Pérez-Lopez, Mines ParisTech / Centre Observation Impacts Energy O.I.E.
Mobility sector is undergoing a total shift. Public policies are pushing forward restrictive regulations in favor of energy transition and low-carbon technologies. Electric mobility has burst into market and is expected to progressively displace fossil fuel-based technologies. Users are becoming more aware not only of the environmental, but also the social and economic impacts related to their choices. Value chain actors and local authorities are, therefore, compelled to design more sustainable mobility technologies and services that fulfill user's needs and expectations. Life Cycle Sustainability Assessment (LCSA) delivers multidimensional results, including environmental impacts, economic costs, and social and socio-economic potential impacts that could perform trade-offs between the three sustainability dimensions. Using these results to support the decision-making process requires multicriteria analysis techniques. The current study proposes a combined application of Multicriteria Decision Analysis with LCSA to help choose among available mobility services from a user's perspective. Surveys and individual interviews are conducted to collect users' point of views allowing the selection of the most significant decision criteria representing the three sustainability dimensions (resource depletion, economic accessibility, adaptability, etc.). Conjoint Analysis is then applied to develop weighting factors throughout a preference analysis. In a second round of surveys, users are asked to rank different attributes combinations corresponding to alternatives sustainability performance. This approach is selected for its relevance regarding the analysis of attributes instead of a direct weighting of impacts categories. The approach is applied to a case study of daily door-to-door commuting travel scenario in France. Real-world data are used to assess sustainability indicators for selected technologies (passenger vehicles, buses, bikes) and services (shared mobility, personal vehicles, and public transportation). Further applications of this methodological framework to other case studies could support the decision-making process for end-users to better understand the effects of their choices related to mobility strategies. The global approach proposed in this work may, additionally, contribute to LCSA methodological development throughout a multicriteria analysis of the sustainability results.

5.06.05

An Lcsa-Framework to Address Finite and Variable Loop Modelling & Multifunctionality Issues Over Time

T. Schaubroeck, Luxembourg Institute of Science and Technology / Research Group EnVOC; T. Gibon, Luxembourg Institute of Science and Technology (LIST) / Environmental Research and Innovation; E. Igos, Luxembourg Institute of Science and Technology / Environmental Research and Innovation; E. Benetto, Luxembourg Institute of Science and Technology (LIST) / Environmental Research and Innovation
To evaluate the sustainability of any economic system, the impact of related products and processes should be considered, which is exactly the aim of life cycle sustainability assessment (LCSA). A first challenge for current LCSA methodology of a circular economy (CE) is the consideration of finite and variable loops (e.g. paper fibers can only be recycled a few times) in a non-steady state, since CE practices are being newly introduced and steady state may not or is not readily obtained. Second, because impact is time-dependent (e.g. financial flows are in the future commonly valued less, i.e. discounted), loops should be propagated over time. Therefore, we bring forward a general iterative modelling approach (process-by-process) in which flows are tracked. System-wide effects should also be considered, for which complex tools with associated time differentiated databases are needed, such as DYPLCA. A third challenge remains on how to potentially asign impact to products/substances and loops, which is the so-called multifunctionality issue. We present an overview of various approaches, their implications for finite variable loops, whether databases are existing and in what context they should be applied (e.g. for attributional or consequential thinking). A general six-step framework is provided to guide the practitioner in methodological choices and modelling for a certain research question. To illustrate this all, we developed a simplistic excel-tool (FLOREC BASIC) and performed two minor case studies. A fictional case on profit generation for flooring reuse for a flooring company (2 loops, with lifetime of 5 years), showcases that when considering discounting (10%), their profit drops about a third. For a case on linerboard recycling, the water scarcity impact can be about 11% higher for a realistic 5 loops of recycling instead of infinite recycling, but recycling still has a lower impact than virgin production and incineration with energy recuperation. However, when considering the consequential effect on the market, effects are the opposite; Linerboard recycling is estimated to have a higher water scarcity impact because of its higher water consumption and loss in linerboard compared to incinerating it with displacement of alternative energy sources that have a high water footprint. Our study only takes some first steps and further research is imperative. (Schauroeck et al., Resour. Conserv. Recycl. DOI: j.resconrec.2020. 105319)

5.06.06

The GeoPolEndpoint Indicator: Measuring the Economic Impact of the Use of Critical Raw Materials. Application to Li-Ion Batteries

J. Santillán-Saldivar, Université de Bordeaux / ISM CyVi; C. Helbig, T. Gaugler, A. Tuma, A. Rathgeber, University of Augsburg; A. Thorenz, University of Augsburg / Resource Strategy; G. Sonnemann, University of Bordeaux - ISM / ISM-CyVi
Energy storage has become a key concern to support efforts for reducing greenhouse gas emissions in the mobility sector, and a significant presence of Li-ion batteries (LIB) in the market resulted in a growing interest in quantifying the impacts associated to their use. Concerns about access to critical raw materials are currently not integrated in the assessment of abiotic resources use in life cycle assessment (LCA). Selected materials in LIB were assessed with the aim to understand which of these have a higher contribution to impacts analyzed from the criticality perspective at midpoint and endpoint level as a complement to LCA.The use of the Geopolitical Supply Risk Method (GeoPolRisk) has been recognized as one of the suggested practices by the UNEP Life Cycle Initiative to quantify supply risk in LCA. We build upon this methodology designed as a midpoint level indicator in life cycle sustainability assessment (LCSA) to present the GeoPolEndpoint indicator, a measure of the economic implications of the use of critical raw materials based on their price elasticity and geopolitical supply risk. **1. 2. 3.** The methodology is applied to four relevant materials in the supply chain of LIB: Aluminum, Cobalt, Copper and Nickel. We assess the mass contribution of the selected materials to the cells of LIBs with nickel-cobalt-aluminum (NCA-C) or nickel-manganese-cobalt (NMC-C) cathode active material. GeoPolRisk values from the perspective of the member countries of the OECD are calculated for the years 2015 to 2017; these midpoint level results are the base for the obtention of the endpoint level indicator. The GeoPolEndpoint method was used to assess the relative socio-economic damage attributable to the use of Al, Co, Cu and Ni in LIB in monetary units. Results show that despite Cu and Ni dominating the mass share for both types of LIB, Co stands out at the midpoint and endpoint level mainly due to the fact that it's being extracted from high risk countries outside of the OECD. Al and Cu lose relevance due to their relatively low price and low supply risk; while a predominant role of Co and Ni confirms the importance given to these materials deemed critical. Future work on this method should address how these results compare to environmental impact categories in order to complement decision making processes based on LCA, and

explore the use of the methodology for other regions or economic blocks that have a relevant role in the global economy.

5.06.08
Using Results of Attributional LCA in an Economic Sustainability Assessment
D. Schrijvers, WeLOOP / ISM CyVi; P. Loubet, Bordeaux INP / ISM-CyVi; G. Sonnemann, University of Bordeaux - ISM / ISM-CyVi
Within the Life Cycle Assessment (LCA) community, the economic sustainability of the production and consumption of products is traditionally evaluated via Life Cycle Costing. However, raw material criticality assessments are also widely used for the evaluation of the economic sustainability of a product value chain, a technology, or even a national or regional economy, e.g. by Industrial Ecologists. Environmental information often plays a role in criticality evaluations. Therefore, in this contribution, the role of environmental LCA in the evaluation of the economic sustainability of a value chain is explored. A review was conducted of more than 40 criticality assessments with different scopes. The use of environmental information within these studies is compared with the attributional (ALCA) and consequential (CLCA) perspectives applied in LCA. The review revealed that environmental impacts could play four different roles in criticality studies: 1) environmental impacts cause a high or low probability to supply disruption of a material due to potential regulations, 2) the use of a material with a high environmental or social impact affects the reputation of a company, 3) the use of a material has a high or low impact on the environment, and 4) the disrupted availability of a material has a high or low impact on the environment. The first two perspectives consider that environmental impacts can pose a risk on the material use of an economic activity, hence, they evaluate the economic sustainability of raw material use. The relevant environmental impacts are best evaluated via an ALCA, as this approach evaluates the environmental impacts that are associated with a product's value chain. The latter two perspectives consider that an increased or decreased material use can have an impact on the environment, i.e. environmental sustainability is evaluated. This is best evaluated via a CLCA, as a CLCA evaluates the impacts of a change on the environment. LCA researchers are invited to expand their economic sustainability assessments by the inclusion of criticality indicators, including the evaluation of environmentally-related value-chain risks via attributional LCAs.

5.06.09
Combined Assessment of Environmental and Economic Performance of Swiss Farms Using Life Cycle Assessment Und Data Envelopment Analysis
D. Pedolin, Agroscope; J. Six, ETH Zurich / Department of Environmental Systems Science; T. Nemecek, Agroscope / Life Cycle Assessment
Agricultural production in Switzerland and elsewhere faces challenges regarding its environmental impacts as well as its economic performance. In order to form policies and guide agricultural production into a sustainable future we need tools to assess these two dimensions simultaneously. The concept of eco-efficiency offers such a tool: Eco-efficiency expressed as environmental productivity relates production to its environmental impacts. In this study, we calculated eco-efficiency scores in two different ways: 1) output is measured in amount of produced human digestible energy [MJ] and 2) both economic output [CHF] and human digestible energy [MJ] is measured. Comparing these two approaches, we present an analysis of Swiss farms using 225 farm-year observations. The economic performance is measured in work income (revenue before wages for family work forces) and is retrieved from the Swiss farm accountancy data network (FADN). The environmental impacts are calculated using Swiss Agricultural LCA tool SALCAfarm and SimaPro using the ecoinvent3 database. The farms are characterized by 1) their farming system: Organic (N=45) and Proof of Ecological Performance PEP (N=180) (PEP demands an integrated farming practice with crop rotation, requirements regarding biodiversity, animal husbandry and usage of chemical protection products and fertilizers), 2) production region: Valley (N=125), Hills (N=58) and Mountain (N=42) as well as 3) farm size measured in utilized agricultural area UAA (6.3 ha – 63.8 ha). Eco-efficiency scores were calculated using data envelopment analysis (DEA). DEA was employed to aggregate the various environmental impacts and outputs (measured as produced amount of human digestible energy and monetary units) without having to specify normative weightings. We found mixed effects of farming-system and production region on the combined environmental-economic performance of Swiss farms, with farms in the valley region having the best performance for both organic and PEP farming systems (p-value ANOVA < 0.001).

Extended submission 5 - Life Cycle Assessment and foot-printing

5.07.02
Domestic Freshwater Use in LCA: Revisiting the Characterization of Human Health Impacts
L. Debarre, CIRAIG - École Polytechnique de Montréal / Mathematical and Industrial engineering
Insufficient water quantities in households may lead to inadequate hygiene practices. These behaviors can trigger the occurrence of water-related diseases, such as diarrheal diseases, trachoma or malnutrition. Many initiatives highlight the need for actions to be taken at the local level in order to promote universal access to clean water. In parallel, it is urgent to raise awareness among the general public (consumers, industries and decisionmakers) regarding the impacts of water use generated indirectly by products and services. In this context, life cycle assessment (LCA) appears as a relevant tool through its holistic view of the life cycle of products and services. There is still no consensus on how to model the potential impacts on human health resulting from domestic water deprivation in LCA. Two methodologies address this impact pathway in LCA, though further research needs to be carried out before any recommendation is established. Building on the work of Boulay et al. (2011), this research aims at providing revised characterization model and factors assessing the potential impact on human health induced by a domestic water deprivation, resulting from the use of 1m3 water. The fate factor is developed in line with consensus-based water assessment models, multiplying a generic scarcity index with the proportion of domestic water consumption compared to total human water consumption. Building on Boulay et al. (2011) the global effect factor is revised. The data source is updated and a novel approach is developed estimating the domestic water deficit. Differentiating between households with and without private access to water supplies, it adds the notion of inequalities in terms of the quantities of water used within a population. The country-scale exposure factor is updated, relying on the gross national income per capita as a proxy for a country capacity to adapt to water shortages. Compared to Boulay et al. (2011), the revised fate and exposure factors show lower values as a result of different methodological choices and of the overall increase of GNI, respectively. The revisited global effect factor is equal to 3.28E-3 DALY/m3, compared to 3.11E-3 in the initial model. The limitations of the current approach are discussed whilst providing novel perspectives to develop an effect factor based on comparative risk assessment to evaluate the burden of water-related diseases caused by the deprivation of domestic water.

Creating a Sustainable Future for the Marine Environment

6.01.01
JPI Oceans Knowledge Hub: Integrated Assessment of New Pollutants
I. Schulz, JPI Oceans; A. Lillicrap, NIVA Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment; A. Trujillo, Spanish State Research Agency; H. Lee Behrens, Research Council of Norway; E. Chacon, Spanish State Research Agency
Member States have indicated that better tools are required to efficiently address the monitoring and evaluation of chemical pollution especially in view of new and existing pollutants entering the marine environment. The current regulatory accepted effect-based assessment tools are limited for the marine environment. Subsequently, the amount of ecotoxicological data, related to target monitoring matrices such as sediment, is lacking.In 2019, the Joint Programming Initiative for Healthy and Productive Seas and Oceans (JPI Oceans) launched a knowledge hub on integrated assessment of new pollutants. The network consists of selected experts from JPI member countries; it aims to understand what is needed to improve the methodological basis for marine chemical status assessment. The JPI Oceans Knowledge Hub will identify the most appropriate methodology(-ies) for integrated assessments of effects of new pollutants. It will develop an overview of relevant improvements and refinements of existing methodology(-ies) will be given. This includes also research found necessary for improved effect/hazard studies, monitoring and sampling on the level of new and emerging pollutants. It is anticipated that political, economic and social dimensions will be included as the source and impact of pollutants are utterly linked with society. The following is a list of activities of the JPI Oceans Knowledge Hub which will be carried out to the extent required to reach the objectives of the Knowledge HUB:Identification of integrated assessment methodologyDefinition of input data to be applied in the integrated assessment methodologyIdentify key users of the integrated assessment methodologyIdentify test sites/regionsIdentify refinement needs of the integrated assessment methodologiesCommon definitions and terminologyRecommendations for further work

6.01.04
Creating a Sustainable Future for Offshore Infrastructure
J. Curry, Ramboll / Life Sciences; L. Alvarez, A.E. Bartram, S. Deacon, Ramboll UK Limited
In 2015 the UN established 17 Sustainable Development Goals. Sustainable Development Goal 14 (SDG 14) aims to "conserve and sustainably use the oceans, seas and marine resources for sustainable development" and consists of ten targets to reach by 2030. Sustainable decommissioning of offshore infrastructure could make a substantial contribution to SDG 14.With over 7500 oil and gas platforms spread across the continental shelves of 53 countries, the end-of-life

decommissioning of the platforms and their associated infrastructure creates a significant challenge, with current global costs estimated in the region of US$210bn. Diverse marine communities can become established during the service life of a platform, raising questions over the ecological value of decommissioning options which seek to return the ecosystem to its original, pre-installation state. A global survey of 38 environmental experts concluded partial removal options were considered to deliver better environmental outcomes than complete removal for platforms, and recommended five key ecological considerations when making individual decommissioning decisions which include provision of reef habitat, productivity of offshore ecosystems, enhancement of biodiversity, protection of the seabed from trawling, enhancement of habitat connectivity. Permitted decommissioning is also shaped by regulatory context of a given region, which may not always accept environmental justifications for partial removal. Net Environmental Benefit Analysis (NEBA) is a versatile tool which can be applied to the variety of decommissioning options, which considers both the environmental and societal externalities in relation to factors such as technical feasibility, safety and financial cost. In this study two Ramboll NEBA decommissioning case studies were compared in different marine environments; one in the North Sea and one in the Indian Ocean, to consider how both habitat, location and regulatory policy may shape decommissioning decisions.The NEBA method presented here has the flexibility to be applied to a range of different marine ecosystems and has been developed and trialled on a number of decommissioning options and platforms. By prioritising environmental benefits in decommissioning, several SDG 14 targets can be met, either directly of indirectly. This is increasingly recognised by national governments across the world who recognise the value the reef-like qualities that these infrastructure may provide.

6.01.05

Microplastics in Penguins in Antarctic Peninsula and the Scotia Sea: Geographical and Temporal Analyses 2006-2016

J. Fragão, MARE - Marine and Environmental Sciences Centre, University of Coimbra / Department of Life Sciences; F. Bessa, University of Coimbra, MARE - Marine and Environmental Sciences Centre / Department of Life Sciences, University of Coimbra; A. Barbosa, Museo Nacional de Ciencias Naturales, CSIC / Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, 28006 Madrid, Spain; P. Trathan, N. Ratcliffe, British Antarctic Survey, Natural Environment Research Council; C. Manno, British Antarctic Survey; C.M. Waluda, British Antarctic Survey, Natural Environment Research Council; P. Sobral, MARE - Faculty of Sciences and Technology, Universidade Nova de Lisboa / DCEA; V. Otero, NOVA School of Science and Technology (FCT NOVA) / Department of Conservation and Restoration; H. Guímaro, MARE - Marine and Environmental Sciences Centre, University of Coimbra / Department of Life Sciences; j. xavier, MARE - Marine and Environmental Sciences Centre, University of Coimbra / Department of Life Sciences, University of Coimbra

Marine pollution in the form of microplastics (< 5mm in size) is ubiquitous and can cause cumulative toxicity in wild biodiversity. However, they are still poorly studied in the polar regions, particularly in the Antarctic. As penguins have a widely distribution around Antarctica, and as microplastics could come via ingestion of prey, they can be used as Antarctic biological indicators. Adélie (*Pygoscelis adeliae*), Chinstrap (*Pygoscelis antarcticus*) and Gentoo (*Pygoscelis papua*) were used to assess the level of microplastics while identifying their prey (e.g. *Euphausia superba*) as a potential source of microplastics. Data collection was carried out in several breeding colonies, from the Antarctic Peninsula to South Georgia, and seasons (during December, January and February) between 2006 and 2016. The diet and the presence of microplastics was assessed by using scats, as a proxy of ingestion, in which every Antarctic krill was counted (85% , 54% and 66% of frequency of occurrence in Adélie, Chinstrap and Gentoo penguins, respectively), and measured to determine penguins diet. From a total of 317 samples, 97 anthropogenic particles were recovered from scats. Using FTIR we identified a total of 33 particles which were identified as artificial cellulose (n= 18, 55%) and microplastics (n= 10, 30%). Within microplastics, the particles found were mainly composed by polyethylene (27%) and polyester (3%). About 15% of particles were not possible to identify, but their synthetic origin was confirmed. All penguin species had microplastics, in 20% of the scats in Adélie penguins (4 microplastics in 4/20 scats) and 30% in Chinstrap (20 microplastics in 17/57 scats) and Gentoo penguins (73 microplastics in 71/240 scats). The results show that pollution by microplastics appear to not present a focal point, since the different breeding colonies distributed from north to south show similar frequency of occurrence of these particles. Additionally, no clear temporal pattern in the amount of microplastics pollution over the years was observed. This study shows that there is a permanent presence (in time and space) of microplastics in the three species of penguins, with two of these species being detected for the first time (Adélie and Chinstrap). This information will contribute with information to the improvement of policies related to plastic pollution within the Antarctic Treaty.

6.02.03

How Can Multiple Criteria Decision Analysis (MCDA) Support the Remediation of Contaminated Sites? Insights From an U.S. EPA Case Study

M. Cinelli, Poznan University of Technology; M. Gonzalez, R. Ford, J. McKernan, US EPA / ORD; S. corrente, University of Catania; M. Kadziński, R. Słowiński, Poznan University of Technology

In this research, we propose using Multiple Criteria Decision Analysis (MCDA) to aid the development of strategies for the remediation of contaminated sites. We achieve this by means of a multi-disciplinary team, including experts in decision analysis and contaminated site remediation. This team conceptualized an overview of how MCDA can support these complex and dynamic processes. Furthermore, a case study is used to illustrate the tangible contribution MCDA methods can have in providing transparent and traceable decision recommendations. Using the case study to rank remediation alternatives, this research shows the remediation process for contaminated sites is dynamic and evolves with variable features describing its decision-making challenges. A group of important decision-making characteristics that should be considered during selection of a preferred remediation strategy is presented. These features have been shown to be pivotal in steering the choice of a decision analysis method that can provide a comprehensive analysis of the considered alternatives. This analysis consists in accounting for the strengths and weaknesses that each alternative has when compared to the others, resulting in a preference-ordered ranking that condenses much information into a decision recommendation. This recommendation provides a transparent mechanism to trace the final ranking back to the individual performance of the alternatives, allowing the models to be “glass boxes” instead of “black boxes”. It was found that in order to support a quick and up-to-date application of powerful decision support techniques in the process of site remediation, decision analysts and stakeholders should interact and co-develop the process. This research also displays how such interactions can guarantee a transparent and traceable decision recommendation so that stakeholders can better understand why some alternatives perform comprehensively better than others when many inputs are used in the decision-making process.

6.02.04

Delphi Study to Identify and Assess Factors Specifying the Concern About PBT and vPvB Substances to Support Risk Management

K. Thiele, Wageningen University & Research; S. Gabbert, Wageningen University / Social Sciences; H. Tobi, Wageningen University / Biometris

PBT/vPvB substances remain in the environment over long time periods and accumulate in biota, which may lead to severe and irreversible effects in the long-run. It is not possible to reliably predict the effects of PBT/vPvB substances on the environment or on human health based on current standard practices in chemicals risk assessment. Therefore, in most European regulatory frameworks PBT/vPvB substances are regulated based on their hazards (persistence, bioaccumulation and toxicity), and not based on risk. However, it is likely that PBT/vPvB substances are not equal in terms of their potential impacts. Due to the lack of information on risks or impacts on the environment and on human health, a fundamental challenge for effective risk management of PBT/vPvB substances is to prioritise those PBT/vPvB substances for regulatory action, which are of highest concern. So far, there is no comprehensive and systematic approach identifying (i) which information on PBT/vPvB substances is relevant and (ii) how to use this information in order to support risk management decisions. Against this background, it is the purpose of this study to explore experts’ opinion on what factors of PBT/vPvB substances they consider to be important to support risk management decisions. The results of this study are intended to contribute to a better understanding of the PBT/vPvB concern. The Delphi method was successfully used in similar contexts and therefore was chosen as a suitable methodology to achieve the objectives of this study. The Delphi methods seeks to explore experts’ opinions and level of agreement by means of a series of questionnaires (termed rounds). In December 2020, the first questionnaire was sent to about 250 PBT/vPvB experts from different groups (e.g. science, authorities, industry, NGOs) and different global regions. Experts were identified on the basis of their work in relevant committees or other scientific bodies, of their publications or of volunteering in a call for experts during SETAC Europe 2019 and 2020. The Delphi study is expected to be completed in April 2021. In this contribution, we will present and discuss the results of the Delphi study.

6.02.06

Cost-Effectiveness of Persistent Chemicals With Different Indicators for 'Effect' - a Critical Evaluation

S. Gabbert, National Institute for Public Health and the Environment (RIVM) / Centre for the Safety of Substances and Products; A. de Blaeij, National Institute for Public Health and the Environment RIVM / Centre for Safety of Substances and Products; J.T. Quik, RIVM / DMG; J. Bakker, National Institute for Public Health and the Environment (RIVM) / VSP; J. de Knecht, RIVM / Centre for Safety of Substances and Products; E.M. Verbruggen, National Institute for Public Health and the Environment (RIVM) / Centre for Safety of Substances and

Products; R.J. Luit, National Institute for Public Health and the Environment RIVM
Substances with persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) properties can accumulate in environmental media and biota. However, a quantitative risk assessment and, consequently, a quantification of health and environmental impacts is considered not possible due to high uncertainty regarding safe concentration levels. As standard benefit assessment is not possible, cost-effectiveness analysis (CEA) has been used for evaluating the performance of a risk management measure (e.g. a restriction of PBT/vPvB use) in relation to a baseline scenario, assuming on-going emissions. So far, different indicators of 'effect/effectiveness' have been used in CEA for PBT/vPvB chemicals, i.e. estimates of the emission reduction, the reduction of the environmental exposure concentration at steady-state and the reduction of the cumulative environmental pollution stock. This study offers a comparative evaluation of CEA using either of the three indicators in order to (i) to examine their conceptual characteristics and methodological differences, (ii) to provide insight how they reflect persistence as a key concern associated with the use of PBT/vPvB chemicals, and (iii) to provide insight into differences regarding the interpretation of CEA results. We construct an example where CEA is applied to three hypothetical PBT/vPvB chemicals that differ regarding their intrinsic properties, in particular their reaction half-lives and mobility. Assuming an assessment period of 20 years, the cost-effectiveness ratios are calculated for two policy measures: implementation of a full stop of emissions after 10 years, and adoption of a step-wise emission reduction strategy. Costs per unit of emission reduced are assumed to be the same for both measures. Hence, total costs of the policy measures depend only on the timing of emission reduction. Our results show that the choice of the 'effect' indicator in CEA of PBT/vPvB chemicals can impact the evaluation of policy measures, and conclusions of policy measures' proportionality. In particular, using avoided emissions do not reflect the differences in environmental exposure caused by the three PBT/vPvB chemicals. Using cumulative stock estimates as effect indicator in CEA is most appropriate since it reflects the time path of pollution arising from the use of a PBT/vPvB chemical in different environmental compartments, and, thus, allows for a concern-based evaluation of risk management measures.

6.02.07
Comparing Impact Potentials of Chemical Alternatives to Prevent Regrettable Substitutions
M. Nendza, Analytisches Laboratorium; S. Hahn, Fraunhofer ITEM / Chemical Safety and Toxicology; M. Klein, Fraunhofer IME / Ecological chemistry; S. Gabbert, National Institute for Public Health and the Environment (RIVM) / Centre for the Safety of Substances and Products
Impacts from use of chemicals can be reduced by replacing substances of concern with available least harmful alternatives. To avoid regrettable substitutions, this requires reliable comparison of the relative impact potentials of the candidate for substitution with that of available alternatives. Comparative risk assessments can be used to rank many chemicals and available alternatives, but it cannot sufficiently capture so far the important aspect of potential (yet unknown) long-term impacts of (very) persistent chemicals. Comparative ranking of chemicals' relative impact potential is a key element in alternatives assessment (AA), and also used for evaluating pesticides and biocides and hazardous substances in electrical and electronic products (RoHS). Existing frameworks have largely relied on hazard criteria, in particular H-phrases, to categorise impact potential. Exposure as a key driver of a chemical's impact potential has usually not explicitly been considered. Especially for persistent substances the spatial and temporal dimensions of pollution are highly relevant for understanding long-term impacts. Dynamics of pollution stocks in water, sediment, soil and air allow to comprehensively characterise exposure over time being a prerequisite (as well as a proxy) for long-term impacts. Comparative ranking of relative impact potentials may consider hazard and exposure separately and not combined as in risk assessment, especially when exposure is not well controlled. Based on the assumption that chemicals, including persistent chemicals, may be hazardous to humans and the environment, but not all in the same way, we suggest a framework for comparative ranking of chemicals based on scores reflecting different impacts due to intrinsic properties, environmental behaviour and use. Combining these aspects into an overall impact score enables ordinal ranking of chemicals according to their relative impact potential. This allows for a comparative evaluation of impact potential across chemicals and uses. The applicability of the scoring scheme is illustrated with case study examples regarding different intrinsic properties (D4/D5), different use and emission pattern (flame retardants) and unacceptable alternatives (PFOS, PFOA).

How Can Ecotoxicology Research Be Improved to Increase Utility in Regulatory Decision Making?

6.03.02
No Risk, No Fun? - Towards a Better Protection of Soil Organisms Via Hazard-Based Criteria
B. Scholz-Starke, darwin statistics / darwin statistics; K. Ziegler, E. Hassold, German Environment Agency UBA; M. Hammers-Wirtz, Research Institute gaiac / gaiac - Research Institute for Ecosystem Analysis and Assessment; F. Stibany, RWTH Aachen University / Institute for Environmental Research
Soil organisms elude attention across chemical regulatory frameworks worldwide. Hazard-based classification systems of chemicals, as the Globally Harmonised System GHS, are mainly based on toxicity criteria derived from aquatic test systems. There are many reasons to hypothesize that soil organisms are different in many aspects of how a chemical substance could adversely affect them, compared to aquatic organisms. It has been stated in the literature that aquatic criteria protect terrestrial and soil compartments sufficiently from adverse effects of chemicals. However, there are cases reported where substances have been more toxic to terrestrial than to aquatic organisms in standardized laboratory studies. The PROSOIL project contributes to soil protection within chemical regulatory frameworks by compiling a comprehensive database of ecotoxicity indicators from international data repositories. It derives candidate lists of chemicals not identified by the existing hazard-based regulations. We used the ICS- and ETOX (German Environment Agency), the IUCLID (ECHA), and the ECOTOX databases (U.S. EPA). Building a harmonised database using historical data from various sources poses an enormous effort to aligning different conventions. Even though the approach was data-driven, the outcome of the identification of candidate substances implied subjective decisions of the persons involved as well. First, comparable groups had to be built, from organism groups (soil macro-organisms), ecotoxicological units (mg ai/kg sdw), test types (chronic laboratory), and statistical endpoints (NOEC). Thresholds of excess toxicity were defined by either point estimators of location parameters (geomean) or quantiles of the distribution of ecotoxicological values (lower 10^{th} quantile). All chemicals below the thresholds were considered very toxic in terms of CLP, while fulfilling the "T-criterion" according to PBT assessment. Depending on the "data group" considered, at least 79% of candidate substances were already classified by the CLP, but one third was in a less hazardous class than our classification. The answer, how far the existing classification based on primarily aquatic data already protects soil organisms is for the time being "partly yes, partly no". Up to one-third of substances which are deemed to be very toxic to soil organisms are currently insufficiently considered or missed by the legislation in force. Hence, a better protection of soil organisms is warranted!

6.03.04
Reliability of Fish Data for Endocrine Disruptor Hazard Assessment
R.J. Brown, wca consulting; G. Panter, WCA Environment Ltd; L.L. Lagadic, Bayer Ag / Environmental Safety; Y. Wolf, Bayer Ag; B. Holmes, Bayer Ag / Environmental Safety; D. Leverett, WCA-Environment Ltd
The European Food Safety Authority (EFSA) and European Chemicals Agency (ECHA) guidance for identifying an endocrine disruptor (ED) in non-target organisms requires that oestrogen (E), androgen (A) and steroidogenesis (S) activity is investigated in chronic fish studies (e.g. the Fish Short Term Reproduction Assay (FSTRA; OECD TG 234)), and that the ED weight of evidence assessment should also consider all available data. This means that existing published data on fish should be included and could potentially be used to conclude on the ED assessment, rather than generate new fish data. Unfortunately, there are often uncertainties over the use of published fish data to conclude on EAS activity or adversity of a substance. Generally this is not because the studies have not been conducted well or do not contain relevant endpoints but is because insufficient information has been reported to conclude on the reliability of either the study as a whole, or sometimes on individual parameters. Unlike for qualitative risk assessment, which conclude based on the most sensitive population relevant endpoints (e.g. reproduction), the ED hazard assessment is informed by both population relevant endpoints and mechanistic endpoints. The mechanistic endpoints often involve additional biological techniques (e.g. enzyme-linked immunosorbent assay (ELISA) in the case of vitellogenin analysis) or require specialist expertise for interpretation in the case of organ histopathology. If the methods or results are not fully documented it can make the data difficult to interpret, potentially leading to additional fish testing to refute or confirm a finding. The reliability of chronic fish studies for use in ED hazard assessments therefore rely on sufficient reporting of all endpoints. The reliability of more than twenty published fish reproduction studies have been assessed based on use for ED hazard assessment. Excel based tools (ToxRTool or Criteria for Reporting and Evaluating ecotoxicity Data (CRED)) were used to ensure that a reliability score (Klimisch or similar) is applied in a transparent and consistent manner. As part of the evaluation the suitability of these tools in the evaluation of ED studies are considered, including benefits, limitations, and recommendations for improvements. Common issues in the fish studies which would be expected to affect their reliability for regulatory purposes will also be discussed (supported by case studies based on recent publications).

6.03.05
Recommendations for Improving the Reliability of Published Histopathology

Data in Ecotoxicology Studies

J.C. Wolf, EPL, Inc.

Histopathology is a commonly employed endpoint in environmental toxicology research because it can provide insight into the occurrence, tissue predilection, morphologic features, spatial extent, chronologic progression, and mechanistic pathogenesis of many types of chemically-induced tissue disturbances. However, based on humane concerns for animal welfare, there has been movement in recent years to limit the extent of live animal experimentation and develop alternate strategies for assessing the potential ecotoxicologic effects of contaminant exposure. Regarding histopathology, efforts thus far have been focused primarily on the first two of the three Rs principles, i.e., replacement and reduction, while comparatively less attention has been paid to refinement. Meanwhile, current published evidence suggests that the reliability of the histopathology endpoint in ecotoxicology studies is too frequently suboptimal. Common issues in the literature include inadequate experimental designs, inappropriate and/or poorly-communicated procedures, inaccurate diagnoses, improper data presentation, incorrect data interpretation, and implausible or exaggerated conclusions. Recommendations for improving the reliability of histopathology data in ecotoxicological investigations are informed by measures pioneered in pre-clinical mammalian toxicology research, which broadly include increased availability of comparative anatomic pathology training, better understanding of toxicological principles, standardization of diagnostic terminology and criteria, mitigation of sampling and observer bias, the development of universal conventions for effectively analyzing, communicating, and publishing histopathology data, and the incorporation of quality control measures such as pathology peer review and the resolution of diagnostic differences by pathology working group. The quality of published studies may also benefit from adjustments made to the journal peer review system. Importantly, if scientific objectivity is to be viewed as a top priority, the publication of negative results needs to be incentivized to the same degree that exists for positive outcomes. Ultimately, if animals are to be sacrificed for microscopic tissue examination, it is important that every effort is made to ensure that the generated data are accurate, repeatable, and valuable for incorporation into weight-of-evidence determinations used in regulatory decision-making.

6.03.06

More Treatment Levels DO Increase Robustness of Dose-Response Curves - an Urban Legend?

H. Christl, Tier3 Solutions GmbH / Regulatroy Affairs; A. Russ, Tier3 Solutions GmbH / Regulatory Science

Regulatory testing in ecotoxicology requires an efficient use of the resources available to obtain statistically sound endpoints. Some recent recommendations are however problematic from a statistical point of view. Based either on randomised artificial data or on deterministic most extreme outcomes of a given test design, the prevailing assumptions were tested for correctness:
A) More dose levels (and fewer replicates) result in more robust dose response estimates, even with a constant total number of test systems.
B) A narrower range of closely spaced dose levels (covering only up to 20% effect) will result in distinctly more precise EC10 and EC20 estimates.
C) All or nothing tests (0% and 100% effect in adjacent dose levels) benefit from a repetition with intermediate dose levels with partial effects, so get more reliable and regulatory relevant.

Results: A) More dose levels are not relevant for the robustness of fit; only additional test systems (used for more dose levels or for more replicates) will improve robustness of curve fit. B) Testing just a narrow range of doses (causing 0 – 20% effect) may improve the certainty, but not by a large margin. This also increases the risk of invalid runs and thus should be avoided: better include dose levels up to ca 50% effect, even if the regulatory focus is on lower effect levels. C) Provided the dose spacing is not too wide (spreading factor $\leq$3.2), then tests with only 0% and 100% effect (very steep slope) do provide satisfactory robustness. Geometric means as EC50 and adjacent levels as CI cover all possible outcomes (even CI for EC10!). If a dose-response is that steep, repetition with further intermediate dose levels only proves the obvious. However, in cases with very shallow dose response, the confidence intervals will stay wide, also if further intermediate dose levels are tested.

6.03.09

Direct Measurement of Germ Cell Mutagenicity for Chironomus riparius, a Model Organism in Ecotoxicology

H.B. Doria, Senckenberg Gesellschaft für Naturforschung; A. Waldvogel, University of Cologne / Department of Ecological Genomics; M. Pfenninger, Johannes Gutenberg University Mainz / Institute for Molecular and Organismic Evolution

Mutagens are different from other toxicants because they have the power to promote heritable changes in the genome by enhancing the germline mutation rates. As a consequence, an unnatural increase on those rates can, although infrequently, lead to deterioration of population fitness and has the potential to accelerate adaptive evolution. Nonetheless, current mutagenicity tests make use of unicellular organisms or cell cultures. Thus, disregarding differences in the DNA repair mechanisms between bacteria and eukaryotes, complexity of multicellular organisms and the relevance of the exposure situation. Being, therefore, inefficient to being used in ecological risk assessments. Moreover, since mutagenicity and genotoxicity are not synonymous concepts, tests performed in multicellular complex organisms still lacks the direct observation of a higher germline mutation rate after exposure to candidate mutagens. Cadmium (Cd) is a highly toxic metal and a widespread contaminant in water bodies. It is irrefutably genotoxic, but evidence of its mutagenic potential is conflicting and relies only on *in vitro* data. To fill this knowledge gap and improve transferability of results and their employment in future regulatory decision-making process we propose a transgenerational method coupling short-term mutation accumulation lines with subsequent whole genome sequencing and a dedicated data analysis pipeline to investigate the mutagenic potential caused by chronic Cd exposure on *Chironomus riparius.* Our results show that although exposure to low, but environmentally realistic Cd concentration affected negatively the survival and developmental time in *C. riparius*, it did not affect the basal germline mutation rate nor the mutational spectrum. More importantly, because the presented experimental design makes use of a complex metazoan organism, a relevant exposure scenario and is a practical and easily implemented pipeline to detect germ cell mutagens it has the potential to be applied in ecotoxicological risk assessments.

New Standardized Methods for Nanomaterial Testing: Knowledge Transfer from Research to Regulation and Vice Versa

6.04.01

ECHA Approach to Regulating Nanomaterials From Challenges to Progresses

D. Mestre, ECHA; V. Rodriguez Unamuno, ECHA (European Chemicals Agency) / Chemical Assessment and Test Unit; A. Rodriguez Ruiz, European Chemicals Agency - ECHA; L. Deydier Stephan, European Chemicals Agency / Hazard Assessment directorate

REACH information requirements to register nanoforms, as per Annex I (on chemical safety assessment), Annex III and VI-XI (on registration information requirements) and Annex XII (on downstream user obligations), entered into force in January 2020. . ECHA is actively communicating with the registrants to develop and refine technical advices on how to comply with REACH nano-requirements. ECHA started to receive dossier updates on nanoforms and has observed that further data is being generated. While ECHA guidances are revised on a regular basis, the development of test guidelines (TGs) and guidance documents (GDs) applicable to nanomaterials is still ongoing for certain endpoints. Faced with these challenges, ECHA has increased its involvement in nano-related projects in collaboration with other agencies, academia and industry. In particular, ECHA is following NanoHarmony on nanoforms behaviour and hazard assessment; GRACIOUS on REACH Annex XI grouping and read-across adaptations; and ECETOC-Nano APP on building sets of nanoforms. ECHA is working towards providing validated methodologies and guidance as soon as possible to mitigate registrants challenges on complying with REACH regulation. Only by gathering our knowledge we will be able to achieve our common goal: safe production, commercialization and use of nanoform/nanomaterial-containing products. Communication channels between regulators, scientists and industry are opened. This collaboration is challenging but seem to be the best and most fruitful solution to gain knowledge and pursue some sound and proportionate regulatory and scientific decisions. It also presents a unique opportunity for the registrants (industry) to invest and collaborate on nano-safety issues within the European market.

6.04.03

Environmental Risk Assessment of Nanoscience and Nanotechnology in the Food and Feed Chain

E.A. Bleeker, RIVM / Center for Safety of Substances and Products; J.A. Meesters, National Institute for Public Health and the Environment (RIVM) / Center for Safety of Substances and Products; W. Peijnenburg, RIVM / Center for Safety of Substances and Products; W. Brand, National Institute for Public Health and the Environment (RIVM) / Center for Safety of Substances and Products; J.V. Tarazona, European Food Safety Authority (EFSA) / Pesticides Unit; R.A. Schoonjans, European Food Safety Authority (EFSA) / Scientific Committee and Emerging Risks Unit; J.T. Quik, RIVM / DMG

Clear guidance on conducting Environmental Risk Assessment (ERA) of nanomaterials is becoming more and more relevant. The food and feed cycle contains several application types that potentially will or already include the use of nanomaterials, i.e. food contact materials, novel foods, food additives, feed additives and plant protection products (PPPs). Due to the nature of their application (e.g. spraying and manure application to land) feed additives and PPPs are most likely to enter the environment directly and require an environmental

assessment by EFSA. We provide an overview of relevant information for ERA of nanomaterials. This overview is compiled to support EFSA in preparing guidance aimed at ERA of the application of nanoscience and nano-technology in the food and feed chain. Such guidance will complement EFSA's existing guidance on human and animal health aspects of nanomaterial risk assessment. Existing ERA guidance documents were analysed for their adequacy to cover nanomaterials. This resulted in issues that are specific for nanomaterials, e.g. related to nanospecific behaviour. To address these nanospecific issues, relevance of existing methods for different steps in ERA of nanomaterials are discussed. In exposure assessment a major issue is that nanomaterial behaviour and fate are not described by equilibrium partitioning. To predict exposure to nanomaterials, certain nanomaterial specific fate processes need to be included in existing modelling tools and in derivation of default values. In hazard and risk characterisation the main adaptations are needed in metrology and dose metrics to allow an adequate description of the fate and behaviour of nanomaterials in exposure media. These adaptations are needed to address the specific uptake mechanisms and bioavailability of nanomaterials. Such nanospecific behaviour may also impact the outcome of (chronic) toxicity studies. In particular where nanomaterials are used in encapsulation, this may result in either localised high exposure as a result of targeted release, or long term low exposure when encapsulation results in slow release of an active substance. Overall, collaboration with other EU agencies (e.g. ECHA, EMA) and organisations on method developments (e.g. OECD, ISO) is advised to avoid duplication of work and contradictory recommendations in ERA guidance for nanomaterials. This research was commissioned by the European Food Safety Authority (EFSA) (Question number: EFSA-Q-2020-00392).

6.04.04

Environmental Dispersion Stability of Organic Pigments: Relevance of Chemical Class, of Nanoforms, of Medium

W. Wohlleben, BASF / Dept. of Material Physics & Dept. of Experimental Toxicology and Ecology; H. Stratmann, BASF Schweiz AG / Dept. of Product Safety; V. Boyko, BASF SE / Dept. Material Physics & Analytics; S.R. Luederwald, B. Hidding, BASF SE / Dept. of Experimental Toxicology and Ecology; N. End, BASF Colors & Effects Switzerland AG

The TG-318 on "Dispersion stability of nanomaterials in simulated environmental media" was the first nano-specific test guideline and standardised a range of at least nine different media of varying pH, water hardness, natural organic matter.[1] The published rationale to the TG318 compared five different forms of TiO2, and GD-318 provides guidance for interpretation.[2,3] Here we implemented TG318 on more than 30 organic pigments. The resulting stability maps allow three perspectives of interpretation: a) scientific evaluation of the modulation by chemical class and nanoform descriptors: Organic pigments are hydrophobic and insoluble particles that consist of small molecules held together by strong van-der-Waals forces and/or by internal charge interactions. We tested nanoforms of perylene pigments, hydrazone pigment lakes, diketopyrrolopyrrole pigments, Cu-phthalocyanine pigments, azo-condensation pigments, at target concentrations around 1012/mL. The rationale suggested to prioritize media for grouping purposes [3]; we identify media with NOM and 1mM Ca as most suitable because they induce the most diversified results across the test set. b) regulatory conclusions on the efficacy of the TG318: If the purpose is to identify the necessity of performing aquatic and/or sediment toxicity testing, then it would have been sufficient to test only the two media of pH4; NOM; 10mM Ca and pH7; NOM; 0mM Ca, because this would have shown that all pigments have at least one medium with close to 10% dispersion stability, requiring sediment testing, and at least one medium with close to 90% dispersion stability, requiring aquatic testing. We also report on approaches to aquatic testing. The preparation of stable dispersions in standardized test media, maintaining constant exposure conditions challenges the technical feasibility, and raises concerns on environmental relevance when assessing artificial exposure scenarious. c) methodical hints on the TG implementation when detection via elemental quantification is impossible: For organics, TG-318 recommends UV/VIS detection, which we implemented and improved: Sample changing stages (e.g. Perkin Elmer, N1010567) enable quasi-continuous parallel measurement of six replicates with good reproducibility. [1]

OECD TG-318, Adopted 9 October 2017. [2]
OECD GD-318, ENV/JM/MONO(2020)9 [3]
F. Abdolahpur Monikh et al. NanoImpact, 2018, 11, 42-50.

6.04.05

Characterisation of Nanomaterials Hydrophobicity: From Research to Harmonised Testing

C. Desmet, F. Roncari, European Commission - Joint Research Centre / Directorate F Health, Consumers and Reference Materials; A. Valsesia, European Commission DG Joint Research Centre / Directorate F Health, Consumers and Reference Materials; S. Gottardo, European Commission - Joint Research Centre; J. Riego Sintes, P. Colpo, European Commission - Joint Research Centre / Directorate F Health, Consumers and Reference Materials

Several legislative frameworks, including the EU REACH Regulation 1907/2006, require reporting the octanol/water partitioning coefficient (Kow), an indicator of the fate and transport of a chemical in the aquatic compartment and a key parameter of any environmental exposure model. It has been demonstrated that nanoparticles cannot reach the thermodynamic equilibrium when partitioning between the octanol and water phases. For this reason, the use of Kow in nanomaterial risk assessment may lead to erroneous conclusions. The OECD Working Party on Manufactured Nanomaterials concluded that the existing Test Guidelines for Kow determination are not applicable to (insoluble) nanoparticles and pointed out that a surrogate such as surface hydrophobicity may be necessary. In order to fill this technological gap, a new method for the quantification of nanoparticles hydrophobicity has been developed. The method is based on the quantification of the binding affinity of nanoparticles to rationally engineered hydrophobic and hydrophilic surfaces (collectors), via the analysis of their adsorption kinetics by Dark-Field microscopy. The surface energy potential acting between each couple nanoparticle-collector is calculated using the XDLVO theory, as the sum of the electrostatic potential, the Van der Waals potential and the hydrophobic potential. The energy barrier (ΔG_{max}) induces the repulsion of the particles by the collector surface. ΔG_{max} depends on several known parameters (properties of the collectors and the particles) and other parameters to determine, including the polar component of the surface free energy of the particles (ΓP) that directly quantifies the hydrophobicity of the particles. The values for ΓP were calculated for a large set of studied particles, and were also converted in a value of equivalent contact angle with water using the Owens-Wendt equation. The validity of the method was demonstrated with a robust set of particles with different surface energy properties. We present here the method and outcome of the Inter-Laboratory Comparison exercise that was performed in the framework of an OECD Test Guideline development.

6.04.08

Influence of Graphene Oxide Nanoparticles in the Polychaete Hediste diversicolor Behaviour and Biochemical Response

M. Silva, Universidade de Aveiro; C. Sá, University of Aveiro / Department of Biology; P. Marques, University of Aveiro / Department of Mechanical Engineering & TEMA; E. Figueira, University of Aveiro / Biology CESAM; A. Pires, University of Aveiro / CESAM & Department of Biology

Engineered nanomaterials (ENMs), such as graphene oxide nanoparticles (GO NPs), have a wide array of applications. The increase of ENMs concentrations on the environment can have a negative impact on the aquatic environment. Nanoparticles suffer transformation processes when in contact with seawater, such as aggregation/agglomeration, increasing their size and density and lead to accumulation in sediments. This accumulation can easily affect benthic organisms near the bottom of the food web, such as polychaetes. The polychaete *Hediste diversicolor* mobilizes the sediments, affecting the biogeochemical cycle of nutrients and availability of contaminants. This study intends to analyze the effects of different concentrations of GO NPs on the behaviour, regeneration, antioxidant status, damage and metabolism of *H. diversicolor*. Non-regenerating *H. diversicolor* were carefully selected and amputated on the last 15 segments for the regeneration assay. Chronic (28 days) exposures were performed in the amputated organism to test conditions (0, 0.01, 0.1, 1 and 10 mg GO/L). For each condition, the corresponding aquaria (1L) were filled with seawater and sediment (2:1), 3 aquariums with 5 amputated and 1 aquarium with 5 intact organisms. Results demonstrated a decrease in the number of regenerated segments in organisms exposed to GO NPs as well as an increase in the time that organisms take to burrow into the sediments. At the lowest concentrations Protein carbonylation and Glutathione S-transferases activity decreased. The increase of the metabolic rate in exposed organisms (electron transport system) evidenced the higher energy expenditure (high use of ready energy sources –soluble sugars) to fight the toxicity generated by GO NPs. The increase in SOD activity was able to counteract the GO effects on cytosol at the lowest concentrations (lower PC levels), but not on membranes (LPO increase). At the higher concentration both cytosol and membranes were affected by GO oxidative stress. This study evidenced that exposure to GO NPs induce physiological, behavioural and biochemical alterations in the *H. diversicolor*. Considering that nanoparticles are expected to aggregate and accumulate in the sediments, this study shows that these organisms may be a target of potential harmful effects of GO NPs, which can disturb the bottom of the food web and consequently the higher trophic levels of the food web.

6.04.10

Development of an Adverse Outcome Pathway for Nanomaterial Reproductive Toxicity in Daphnia magna

K. Reilly, The University of Birmingham; Z. Guo, University of Birmingham / GEES; I. Lynch, University of Birmingham / School of Geography, Earth and Environmental Sciences

Daphnia magna are a widely used test organism in toxicity studies and have a range of chronic toxicity end points, such as growth (eye-tail length), reproduction (total offspring and time to first brood), induction of males and resting egg production are all well established end points, which can be further complimented

with sublethal markers such as lipid deposits, morphological defects, delays in moulting and changes to kairomone signalling. With increasingly complex experimental designs, such as chronic, pulsed and multigeneration toxicity studies, there is a range of data that can be used to further the understanding of Molecular Initiating Events (MIEs) and how this can lead to Adverse Outcome Pathways (AOPs) for daphnia toxicity. In addition, modelling of toxicity data can also give insights into how individual toxicity could affect the ecosystem dynamics as a whole. Toxicokinetics-toxicodynamics (TKTD) models including Dynamic Energy Budgets (DEB) utilises common end points such as growth and reproduction to access how, under various exposure conditions, daphnia can divert their energy expenditure for either maintenance, growth or reproduction. A systematic literature analysis was conducted to ascertain the potential of chemical AOPs to be applied to NMs and to identify some testable MIEs and key events (KEs) that are adaptable for testing with NMs with daphnia. In parallel, a top-down approach was applied by searching the AOP-wiki for daphnia AOs, although this was fairly limited, it provided a starting point for AOP developmental approach and identified the specific genes involved in juvenile hormone receptor induction leading to male induction (AOP 201) and excessive oxidative stress leading to oocyte apoptosis associated reproduction decline (AOP 211). This dual approach enabled a multi-pathway AOP to be developed from an MIE gut overload leading to key events including reduced calorific consumption, reduced growth including reduced carapace shedding, induction of males, and reduced mating success. Additional experimental evidence for the MIEs and KEs was generated to increase the confidence in the NM AOPs. In parallel, the data are being applied to the DEB model to provide an additional line of evidence to support the transition to reduced growth and reduced reproduction.

6.04.11

How Many Nano-Sized Titanium Dioxide Particles Are Released From Conventional Pigment Products to the Environment?

Y. Zheng, EMPA Technology & Society Lab / Technology and Society Lab; B. Nowack, EMPA / Technology and Society Lab

In recent years, concerns have been raised about the presence of nanoparticles in conventional titanium dioxide (TiO_2) pigment products. Studies have shown that 10-36% of the TiO_2 particles in food products were smaller than 100 nm. The emissions of TiO_2nanoparticles from conventional pigments have long been neglected in previous engineered materials (ENMs) exposure modeling. Quantitative knowledge of the particle size distribution released at all stages of the life cycle of nanoproducts and conventional products can help to better understand the potential risks associated with the use of TiO_2 particles. The goal of our work was to determine the emissions of TiO_2 nanoparticles from both nanoproducts and conventional pigments. To achieve this goal, we have developed a size-specific, dynamic, probabilistic material flow analysis model (ss-DPMFA), which for the first time integrates the particle size distribution into MFA modeling. In addition to providing information on mass flows, the new model utilized particle size distributions and crystal forms to describe each process, stock, and flow of TiO_2. We characterized five different forms of TiO_2 based on material functionality: (1) nano-anatase, (2) nano-rutile, (3) pigment-anatase, (4) pigment-rutile, and (5) nanocomposites used in sunscreens. The most remarkable modeling result was that before TiO_2 ENMs came onto the market in 2000, 22,400 tonnes of nano-sized particles (< 100 nm) had already been released to the environment, originating from the nano-sized fraction of conventional TiO_2 pigments. Even in 2016, 50% of the nano-sized TiO_2 particles released to surface water came from TiO_2 pigments. The obtained particle size distributions can be used as input for environmental fate risk assessment models. Our new ss-DPMFA model's additional insights about crystalline forms and surface treatment could pave the way for advanced hazard and risk assessments in ecological systems.

6.04.17

Comparative Toxicity of Cadmium and Arsenic in the Presence of Titanium Dioxide Nanoparticles and Natural Organic Matter: What Is the Role of Aging?

R. Roy, V.K. Kandrapu, R. Islam, University of Koblenz-Landau / iES Landau, Institute for Environmental Sciences; G. Kalčíková, University of Ljubljana / Faculty of Chemistry and Chemical Technology; R. Schulz, University of Koblenz-Landau / iES Landau, Institute for Environmental Sciences; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment

Titanium dioxide nanoparticles ($nTiO_2$) are frequently used in consumer products (e.g., cosmetics, cleaning agents, filters, food etc.) and thus inevitably released into freshwater systems. During their aquatic life cycle, $nTiO_2$ interact amongst others with natural organic matter (NOM) and may alter the ecotoxicity of co-occurring metals. The effect size of these interactions may be regulated by additional factors, including aging. Consequently, the present study aimed at evaluating the impact of aging on cadmium (Cd) and arsenic (As) toxicity when present in combination with $nTiO_2$ and NOM. The test design fully crossed three $nTiO_2$ levels (0.0, 0.6 and 3.0 mg/L) with two levels of NOM (0 versus 8 mg TOC/L) and seven nominal concentrations of either Cd (ranging from: 0 – 1536 µg/L) or As (ranging from: 0 – 32 mg/L) with aging durations of 0, 1, 3 and 6 days. Finally, the ecotoxicity of the aged medium was determined by involving *Daphnia magna* acute toxicity tests following the OECD 202 test guideline. The presence of both $nTiO_2$ concentrations (in combination with NOM) elevated Cd toxicity significantly by ~ 2-fold for most of the aging situations as compared to an unaged Cd solution in absence of $nTiO_2$. In contrast, As toxicity was not affected by $nTiO_2$ and NOM, an observation independent of $nTiO_2$ concentration and aging duration. While multiple other environmental factors can influence the toxicity of metal, it is evident from this study that both aging and $nTiO_2$ can influence metal toxicity. Based on this study and earlier work targeting silver and copper, it needs to be concluded that the impact of $nTiO_2$ on metal toxicity is variable and seems to depend on the charge of the metals' most toxic species.

Rethinking Science Communication: Storytelling in the Web 2.0 Era, New Tools and New Public

6.05.01

A Toxic Friendship; a Tale From Peer Reviewed to the Kids Hands

B. Gonzalez-Gaya, ESTACIÓN MARINA DE PLENTZIA. UPV/EHU / Analytical Chemistry; A. Campos-Vivanco, Vivo en Pangea; M. Vila-Costa, IDAEA CSIC / Environmental Chemistry

Among the main science outreach receivers, it is common to aim to a young audience. In general, children and youngsters are interested in nature, environment and somehow into scientific topics, no matter the level of their studies. This makes them an *a priori* appealing public. However, they are also critic, honest and get easily bored, therefore, the techniques used to transfer them research information need to be fast, fun and meaningful. Moreover, the science outreach traditionally directed to children tends to be naïve, lacks of trueness, or is full of topics. This is especially true in the out-of-date storytelling, which many times draw from a fantasy world of princesses and dragons, into which the bad is ugly and the men are the heroes. Nevertheless, we are of the opinion that also complex environmental problems (such as chemical pollution) leaded by non-traditional main protagonists (such as bacteria, asexual or even feminine characters) can be also a very appealing topic for a kids tale, and that is why we aimed to transform two of our main research publications in Nature Geosciences into an illustrated short story. The creative process to transform our science published in peer-reviewed journals to a children tale, included several steps: (1) deep understanding of the environmental problem and identification of the main concepts to communicate to kids, (2) list of actions kids can take in their lives to help to solve the problem, (3) design appealing images at equal distance between reality and kids' interest, (4) create the storyline with introduction, conflict, climax and denouement, and (5) editorial support. All steps were taken into consideration to write the many versions of the tale until the final version we are proud of. This issues including the complexities of transformation, gender issues and educational challenges will be discussed in this talk about the tale "The lonely bacterium and the toxic friends".

6.05.05

A New Level of Transparency and Stakeholder Engagement Advanced by Covid-19: A Virtual Visitor Program to Present Safety Studies for Agricultural Technologies

A. Quambusch, Bayer AG - Crop Science Division; C. Morr, Bayer AG - Crop Science Division / Regulatory Scientific Affairs

In order to increase trust in the science and safety of agricultural technologies, it is essential that the data and the procedures used in safety assessment in the regulatory environment are transparent. With the launch of the transparency website with an enormous amount of safety study data and with impressive background material on Regulatory Science, Bayer has established an ongoing dialogue with the public and stakeholders in order to reinforce the scientific rigor and safety measures involved in bringing products to the market. In 2020, Bayer has extended its commitment to transparency through a program inviting the public to observe the conduct of studies that are being done to determine if new products are safe for humans and the environment, when used according to the label instructions. Trust has to do with people. This is why Bayer's approach is to bring interested people into personal contact with Bayer scientists and to track the conduct of a GLP study live. Before the first event could take place, COVID-19 stopped all travelling and visiting activities and OpenLabs is on hold for much longer than expected. Bayer changed plans and instead of inviting people to Monheim, we'll now bring our testing facilities and scientists virtually to our visitors. Our site can be visited in a virtual environment. Technicians are filmed conducting a GLP study and scientists explain the guidelines, the process and the scientific background. In order to make this an interactive and personal experience, guided tours are organized in the virtual environment, where our scientists discuss the details of the GLP study and related topics with small groups of visitors. First being disappointed about the restrictions by the pandemic, we now see the advantages of virtual OpenLabs: We can easily host people from around the world, being inclusive for people who would not be able to travel due

to various reasons and we are not dependent on growing seasons and schedules of the lab. OpenLabs is a unique opportunity for those who wish to experience the details of Good Laboratory Practice (GLP) and how we conduct GLP studies to ensure the integrity of our study results.

6.05.07
Multiple Ways to Convey Microplastic-Related Science to Stakeholders and the Interested Public
A. Jahnke, Helmholtz Centre for Environmental Research - UFZ GmbH / Ecological chemistry; C. Rummel, Helmholtz Centre for Environmental Research (UFZ) / Department of Bioanalytical Ecotoxicology; D. Kuehnel, UFZ - Helmholtz Centre for Environmental Research
Outreach should be in the focus of every scientist, to make scientific outcomes more relevant and meaningful, to educate the public, to inform policy-makers and regulators, and after all even to make the use of tax money for funding research transparent. However, not all scientific topics are easy to communicate and raise interest for. Very different is the situation when we think about plastics in the environment. Information on this topic has increased knowledge tremendously during the last decade; you can ask almost everyone on the street, and this person will with high likelihood have heard about this issue being a serious problem. So in our case, doing research about the fate and effects of plastics in the marine environment, we do not face difficulties in raising interest or setting the scene. Our challenges are more towards conveying the right aspects and dimensions of the problem, and give laypersons knowledge as well as tools to contribute to achievable solutions. During the last 5 years, we have explored multiple ways to convey science to our (1) fellow scientists, (2) stakeholders, including policy-makers and regulators, and (3) the general public. We designed a cover art graphic for a scientific journal where our article was printed. We held an interdisciplinary stakeholder workshop and conveyed the major outcomes in a science comic designed for primary school children. We collaborated with a university-associated artist who sent us an interdisciplinary tandem of students to get inspiration for their project to create a piece of art based on impressions from our research. We used a blog and did multiple interviews for students, newspapers, radio and TV about microplastic pollution in general as well as around a scientific expedition crossing the North Pacific Garbage Patch. Additionally, we supported design and implementation of workshops for high school students who visit the UFZ Student Lab. This presentation will give an overview and personal assessment in terms of the outreach potential of these different means of communication.

Extended submission 6 - Environmental policy, risk management, and science communication

6.06.02
Testing for the Absence of Effects in Ecotoxicology - an Overview of Current Methods and Alternatives
M.M. Mair, University of Bayreuth / Junior Research Group Statistical Ecotoxicology; F. Hartig, University of Regensburg / Theroretical Ecology
Most scientific projects are designed to show the presence of effects. Standard null hypothesis significance tests with a point null hypothesis at zero are designed exactly for this goal. In other situations, however, the goal is the opposite: to demonstrate that negative side effects are absent. This is the case for instance in the registration of chemicals (e.g., under REACH) or during the approval of plant protection products. The issue is that standard hypothesis tests are ill-suited for this purpose, because they are asymmetric: a non-significant result is inconclusive and does not indicate that the null hypothesis is correct. This is because these tests do not control for type II errors, that is, false negative results. Effect size estimates and confidence intervals could be interpreted instead of p-values, but if the goal is to make a regulatory decision about the true absence of an effect, they have to be converted into a binary value. To address this challenge, several methods have been suggested in the literature and in regulatory guidelines to distinguish true negatives (true zero-effects) from a lack of statistical power in the experimental setup (e.g., due to small sample size or high variance). These include post-hoc power analyses such as the minimum detectable effect (MDE) and minimum detectable difference (MDD), and the use of upper bounds of confidence intervals to decide whether true effects are negligible. As an alternative to standard tests, interval hypothesis testing can be used to test, whether true effects are significantly smaller than a predefined effect size that is considered acceptable. A further option would be to switch to Bayesian statistics and use Bayes factors to decide whether a zero-effect (or predefined acceptable effect size) is more likely than a non-zero effect (or effect larger than acceptable). We review and compare these different methods using theory and simulation, discuss their advantages and weaknesses and argue that the inclusion of alternative methods into the toolbox of ecotoxicity research and environmental risk assessment could benefit the reliability of regulatory decisions.

COVID-19 Pandemic Consequences, Detection and Surveillance Through the Lenses of Environmental Toxicology and Chemistry Scientists

7.01.01
What Are the Relevant ENvironmental Factors for Designing Sustainable Community Masks?
M. Schmutz, EMPA Technology & Society Lab / Technology and Society Lab; R. Hischier, EMPA / Technology and Society Lab; C. Som, Empa - Swiss Federal Laboratories for Materials Science and Technology / Technology and Society Lab
Since the emergence of the COVID 19 pandemic, it became common to wear facemasks in our public daily life. The sudden worldwide increase in demand for facemasks that started in spring 2020 lead to the development of new type of facemasks, such as textile community masks. In the present crisis, it was estimated, for example in Switzerland, that 4 million masks are required per day by the civil society within the country. The high demand of facemasks also brings new concerns regarding their ecological sustainability. For instance, the use of surgical masks by the population involves high amounts of wastes that could be potentially decreased by using reusable textile masks. However, very little is known regarding their environmental sustainability and what factors are influencing it. Therefore, the goal of this study is to provide support for decision-making in a quick changing market on how textile masks could be designed with lower environmental impact by identifying the hotspots for their sustainability. To do so, a simplified Life Cycle Assessment was conducted comparing surgical masks and 2-layered cotton masks. The results show that depending on the environmental impact considered, the cotton masks was in some cases performing better than the surgical masks, and in other cases had a poorer performance. The most important factor that was identified for designing more sustainable cotton masks was their lifespan (the number of times the cotton masks can be worn before thrown away) and was followed by their weight.

7.01.02
COVID-19 Face Masks: A New Source of Human and Environmental Exposure to Plasticizers
J. Fernández-Arribas, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Department of Environmental Chemistry; T. Moreno, R. Bartroli, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Research Council (CSIC) / Geosciences; E. Eljarrat, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Research Council (CSIC) / Department of Environmental Chemistry
The COVID-19 pandemic caused by the virus SARS-COV-2 emerged in December 2019 and poses a huge global health threat. The preventive measures implemented to control and mitigate its high transmissibility involved a sudden increase in demand and consumption of plastic products by the general public, healthcare workers and service workers. Based on WHO estimation, 89 million medical masks are required for the COVID-19 response each month worldwide, as well as 129 billion face masks for general public. There are two aspects that must be considered: the environmental impact derived from the massive consumption of disposable masks and the effect on human health due to the prolonged and daily use of such masks. Organophosphate esters (OPEs) are high-production-volume chemicals widely used as plasticizers and flame retardants. This group of emerging pollutants has been raising increasing concern due to their reported toxic effects. Tri-n-butyl phosphate (TNBP) has been observed to disrupt endocrine and reproductive functions, nervous system development and is suspected carcinogen. OPEs are also associated with asthma and allergies. Moreover, some OPEs have established oral reference doses (RfD) and oral cancer slope factors (SFO). Based on these values, the non-carcinogenic and carcinogenic risks of human exposure to OPEs can be evaluated. The main objective of the present study is to evaluate the OPE occurrence in different types of masks, and to determine their human impact through prolonged and continuous use due to the current pandemic situation. Likewise, an assessment of environmental impact due to the large amount of mask waste globally generated will be carried out. OPE concentration ranges were similar in surgical and reusable masks, but higher levels were found in self-filtering masks. In both FFP2 and FFP3 masks, tris(2-ethylhexyl) phosphate (TEHP) was the major contributor to OPE levels. This compound has established RfD and SFO values by the USEPA, as well as TNBP. Hence, there is not only an environmental risk but a human exposure risk that needs to be assessed with the use of these masks, despite their greater security against the virus. Each of these different types of mask have different usable lifetimes, so that a balance needs to be found between the environmental impact of waste disposal and any potential health risk associated with OPEs.

7.01.03
Effects of Microplastic From Masks to Earthworm: Another Side Effects of COVID-19 Pandemic
J. Kwak, Konkuk University; Y. An, Konkuk University / Department of Environmental Heath Science
This study focused on the another side effects of COVID-19 in the environmental

point of view. In 30th December 2020, World Health Organization(WHO) reported 62,363,527 confirmed cases of Coronavirus Disease (COVID-19) in a global world . Unfortunately, the number of confirmed cases and deaths have been increasing. To prevent transmissions between people, some countries require wearing masks in the public spaces currently. Owing to the increasing number of use of masks, it is easier to find disused and disposed masks on the beaches, streets, or mountains compared to before COVID-19 pandemic. Therefore, the purpose of this study was set to predict the effects of microplastics which derived from medical masks. Melt blown filters were discarded from medical masks, subsequently they were cut, sieved (< 300 μm), and mixed into soil at 1000 mg/kg dry soil. Adult earthworm Eisenia andrei was exposed for 21 days in the 20°C incubator. Advers effects on earthworm individual, tissuey, and cellular levels were assessed. Fourier transform infrared (FT-IR) spectrometer confirmed that fragments and fibers which derived from mask melt blown filters were polypropylene (PP). Since exposure Day 1, excreted fragments through earthworm casts were observed. After 21 days exposure, inhibitions in coelomocytes esterase activity and spermatogenesis were observed. On the other hands, neglible effects on survival, oxidative stress in coelomocytes, and oogenesis in ovaries were caused. This study provided evidences of potential adverse impacts of microplastics which derived from medical masks to earthworm coelomocytes and male reproductive tissues. *This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2020R1A2B5B02001734).*

7.01.06

Can the Massive Release of anti-COVID-19 Pharmaceuticals Lead to a Risk for Aquatic Ecosystem?

F. Cappelli, IRSA-CNR / University of Insubria, Department of Science and High Technology; I. Fochi, Thermo Fisher Scientific SpA; O. Longoni, BrianzAcque s.r.l; M. Palumbo, Water Research Institute, CNR; S. Polesello, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute; J. Rigato, BrianzAcque s.r.l; C. Roscioli, Water Research Institute - Italian National Research Council, IRSA-CNR; M. Rusconi, A. Sala, BrianzAcque s.r.l; F. Salerno, F. Stefani, Water Research Institute - Italian National Research Council, IRSA-CNR; S. Valsecchi, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute

On last March 11th, the World Health Organization declared the novel coronavirus SARS-CoV-2 a global pandemic. Since this event, the scientific community has constantly worked in order to find the best therapy to treat the infection and the symptoms of COVID-19 disease. However, the absence of specific medicines and vaccination too, resulted in a range of therapies that have been tested during the disease's evolution leading to a massive use of pharmaceuticals, mostly of them never used so much before in such a short time. The potential inefficiency of urban wastewater treatment plants in removing these complex compounds can lead to a new environmental risk for the receiving water bodies and, consequently, for the aquatic ecosystem. The aim of the present study was to survey the presence of anti-COVID-19 drugs in wastewater and downstream river samples collected in the Lombardy region that was the first European area touched by pandemic outbreaks and the most affected region in Italy. More than sixty compounds, including drugs that belong to different therapeutic classes like antiretroviral medicines, analgesics, antibiotics and, additionally, some metabolites, were collected in an anti-COVID-19 drugs list. Suspect screening strategy by Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) was used to search for the anti-COVID-19 drugs in pre-filtered samples that were directly injected in a Q Exactive Orbitrap Mass Spectrometer. Acquisition was carried out in Full Scan-Data Dependent MS/MS (FS-ddms2) mode. All the identification process was carried out with Compound Discoverer 3.1 software (Thermo Fisher Scientific). After a filtering procedure using the software tools, candidates with an acceptable level of confidence were semi-quantified in all the water samples, obtaining the area ratio values of 21 compounds (including some metabolites). Reference materials of the tentatively identified pharmaceuticals were purchased in order to achieve the quantification. Finally, the environmental risk on aquatic ecosystem due to the massive use of anti-COVID-19 drugs was assessed.

7.01.07

Development and Application of a High-Throughput Bioanalytical Method for Sewage Surveillance for SARS-CoV-2

T. Boogaerts, University of Antwerp, Toxicological Center / Pharmaceutical Sciences; N. De Roeck, L. Jacobs, P. Delputte, Laboratory for Microbiology, Parasitology and Hygiene, University of Antwerp; A. Van Nuijs, University of Antwerp / Toxicological Center

Surveillance of influent wastewater (IWW) could be applied to track down SARS-CoV-2 outbreaks at high spatio-temporal resolution and could potentially be used as an early warning for emergence of SARS-CoV-2 circulation in the general population. A SARS-CoV-2 RNA concentration and detection method was developed and applied to IWW samples from different Belgian communities. In order to obtain high and reproducible recoveries for SARS-CoV-2 RNA, different sample concentration methods (Macrosep, Centricon, Vivaspin and Amicon centrifugal filter devices and PEG concentration) were compared in combination with different RNA extraction kits (Qiagen Viral RNA, RNeasy and Powermicrobiome and Promega Maxwell PureFood GMO and Authentication kit) with both fresh and frozen IWW. Macrosep (100 kDa) and Centricon Plus-70 (100 kDa) centrifugal filter devices resulted in the highest recoveries for SARS-CoV-2 RNA. While increasing loading volumes yielded in lower Ct-values for for all genes of interest, lowering pore sizes did not improve the sensitivity. For RNA extraction, the Qiagen RNeasy and Viral RNA manual extraction kits did not significantly differ from the automated Maxwell PureFood GMO and Authentication kit, but the use of the Qiagen Powermicrobiome extraction kit resulted in poor detection of SARS-CoV-2 RNA. Real-time qPCR was performed with four different SARS-CoV-2 primer sets (N1, N2, N3 & E). In-sample stability of SARS-CoV-2 RNA in IWW was determined at different time points and storage temperatures to assess optimal sample handling during transport and storage. We found that SARS-CoV-2 RNA is relatively stable in IWW samples for up to 6 days of storage at 4 °C. However, freeze-thawing the samples drastically influenced the in-sample stability of SARS-CoV-2 RNA with an observed loss of 55-100% already after one week of at -20°C and freezing the samples should therefore be avoided. The finalized bioanalytical assay relies on sample concentration of fresh IWW samples stored at 4 °C with Macrosep Centrifugal filter devices with a pore size of 100 kDa and a loading volume of 20 mL together with the automated Maxwell PureFood GMO and Authentication kit to ensure high-throughput. The developed method proved to be suitable to monitor temporal changes in SARS-CoV-2 RNA levels in IWW, which were similar to the patterns in the number of new COVID-19 cases within the catchments.

7.01.08

Drugs, Mental Health, and Disinfectants: Changes in Sewage Sludge Chemical Signatures During the COVID-19 Pandemic

S.L. Nason, Connecticut Agricultural Experiment Station / Environmental Science; E.Z. Lin, Yale University / Environmental Health Sciences; B.D. Eitzer, Connecticut Agricultural Experiment Station / Analytical Chemistry Department; j.p. koelmel, Yale University / School of Public Health Environmental Health Sciences YSPH Global Health Concentration; J. Peccia, Yale University / Chemical and Environmental Engineering

The COVID-19 pandemic and related shutdowns have caused massive changes in everyday activities for many people. Signs of those changes are present in the chemical signatures of wastewater and sludge that is produced during the pandemic. We began collecting daily primary sewage sludge samples from the wastewater treatment plant in New Haven, CT USA on March 19, 2020, three days before the local lockdown began, for the primary purpose of monitoring levels of the SARS-CoV-2 virus. In addition to the viral analysis, we analyzed the chemical composition of the samples using liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS). We used targeted and suspect screening strategies to identify contaminants in the sludge and found evidence of increasing opioid and antidepressant use, as well as upwards trends in chemicals used in disinfectants and sunscreens during the first 15 weeks of sampling. Additionally, we are in the process of using non-targeted methods to screen for molecular features in the data that correlate with virus levels. Though wastewater monitoring during the pandemic has largely focused on measurement of the virus itself, we believe that chemical analysis can reveal trends that are important for public and environmental health.

7.01.09

Monitoring Proteomic Biomarkers in Wastewater for Community-Wide Public Health Assessment

K. Jagadeesan, A. Kannan, B. Kasprzyk-Hordern, University of Bath / Department of Chemistry

Recently, wastewater-based epidemiology (WBE), has been established as a useful tool to verify public health status. In brief, a wastewater sample is collected at the influent of a wastewater treatment plant and analysed for wide range chemical substances including endogeneously formed human products that can be related to aspects of public health. This approach has been successfully validated and applied to monitor substances of abuse in large scale studies across the globe and to measure the concentrations of several pharmaceuticals, metabolites, and personal care products[1], and most recently to SARS-CoV-2. However, there also exists the possibility to adapt this approach for the analysis of a spectrum of endogenous biomarkers for stress and disease (e.g., proteins, lipids, and nucleosides), specifically, proteins, to evaluate the state of public health. The aim of this work is to detect and quanitify C-reactive protein (CRP), one of the positive acute-phase response proteins[2], in influent wastewater to investigate public health within populations. The challenges of analysing endogenous biomarkers such as CRP in wastewater include the dilute nature of the material and the diverse complexity of the organic matter in the influent that can interfere with the methods[3]. Hyphenated mass spectrometry (MS) demonstrated accurate and reproducible quantification of some of these endogenous biomarkers in plasma samples, by the analysis of peptides produced via enzymatic digestion of

protein targets, which is known as bottom-up (shotgun) proteomics[4]. In our initial study, one of the signature peptide of CRP showed better recovery after digestion as well as quantification in influent samples. This demonstrates the development of an LC-MS/MS method to identify and quantify CRP in wastewater at low concentration levels. This method has a potential for WBE to investigate community-wide public health status. References: 1. Kasprzyk-Hordern, B., Kondakal, V. V. R. & Baker, D. R. *Journal of Chromatography A Enantiomeric analysis of drugs of abuse in wastewater by chiral liquid chromatography coupled with tandem mass spectrometry.* 2010, *1217,* 4575-4586. 2. Ironson, G., Banerjee, N., Fitch, C. & Krause, N. *Social Science & Medicine Positive emotional well-being, health Behaviors, and inflammation measured by C-Reactive protein.* 2018, *197,* 235-243. 3. Avella, A. C., Görner, T. & de Donato, P. *Science of The Total Environment The pitfalls of protein quantification in wastewater treatment studies.* 2010, *408,* 4906-4909. 4. Kuhn, E. *et al. PROTEOMICS Quantification of C-reactive protein in the serum of patients with rheumatoid arthritis using multiple reaction monitoring mass spectrometry and 13C-labeled peptide standards.* 2004, *4,* 1175-1186. **Acknowledgement:** This work is a part of the Wastewater Fingerprinting for Public Health Assessment (ENTRUST) project funded by Wessex Water and EPSRC IAA (grant no. EP/R51164X/1).

7.01.10

Temporal Changes in Substance Use During the COVID-19 Pandemic in Belgium Through the Analysis of Influent Wastewater

T. Boogaerts, University of Antwerp, Toxicological Center / Pharmaceutical Sciences; M. Quireyns, M. De Prins, H. De Loof, University of Antwerp / Toxicological Centre; A. Covaci, University of Antwerp, Toxicological Center / Toxicological Center; A. van Nuijs, University of Antwerp, Toxicological Center / Toxicological Centre

The SARS-CoV-2 pandemic triggered the need for various measures to contain the spreading of the virus, such as social distancing, home confinement and closing of all non-essential activities. These interventions could also potentially result in altered demand and supply of substances and in limited treatment, prevention and harm-reduction campaigns in terms of substance use. A complementary approach which could deliver additional information on the consumption patterns of these substances under these complex circumstances is wastewater-based epidemiology (WBE). In WBE, day-to-day concentrations of substances in influent wastewater are measured and back-calculated to per capita mass load estimates (mg/day/1000 inhabitants). WBE was applied to evaluate temporal changes in illicit drug (i.e. cocaine, amphetamine, methamphetamine and MDMA), alcohol and psychoactive pharmaceutical use (i.e. morphine, codeine and methadone) in three Belgian communities (i.e. Antwerp, Boom and Brussels) during the the COVID-19 pandemic. Daily 24-h composite wastewater samples were collected from March 2020 until the end of June 2020. In most of the investigated locations, illicit drug and alcohol use remained stable or even increased compared to previous years. Weekly consumption patterns remained the same in 2020 compared to previous years with the highest consumption rates observed during the weekend. Interestingly, an increase in substance use in Antwerp in 2020 was also observed on the day the bars reopened and the number of contacts per household was expanded. The present study clearly shows the potential of WBE to quickly assess the impact the pandemic had on substance use in Belgium with high temporal resolution.

7.01.11

Wastewater-Based Epidemiology for Monitoring Community Derived Antibiotic and Resistant Genes

N. Sims, University of Bath / Chemistry; A. Kannan, University of Bath / Department of Chemistry; E. Holton, University of Bath / Chemistry; K. Jagadeesan, University of Bath / Department of Chemistry; R. Barden, R. Standerwick, Wessex Water; B. Kasprzyk-Hordern, University of Bath / Department of Chemistry

Antimicrobial resistance (AMR) has been hailed as one of the critical public health threats facing 21st century. The discovery and wide-spread availability of antibiotics since the 1970s revolutionised medicine, however overprescribing has resulted in the increased development of multi-drug resistance in pathogens. Current AMR surveillance within populations relies heavily on clinical data. This however covers only a small proportion of the community as samples are from those who have seeked medical aid, hence might not be representative of the wider population. Wastewater-based epidemiology (WBE) is a promising approach of sampling influent wastewater for biomarkers in order to give public health information on the population that has contributed. The analysis of AMR related biomarkers in WBE could therefore provide key spatiotemporal and comprehensive information on resistance circulating in communities giving complimentary information to current AMR clinical data. This study presents results from a year-long study in two catchment areas in the South-West if England combining the analysis of antibiotics, metabolites and resistance genes in influent wastewater to investigate AMR within populations. Using advanced analytical tools, chemistry and biological techniques have been combined together to give insight to the antimicrobial patterns and resistance of two communities Over 200 influent wastewater samples have been collected from two different catchment areas, with up to ten 24-hr composite samples from each site per month. Over 60 different antibiotics and metabolites covering a range of different classes have been investigated. Next generation DNA sequencing along with dPCR on seasonally relevant samples have given insight to the diversity of resistant genes present in wastewater. Catchment prescription data of antibiotics demonstrates seasonal prescribing patterns which is reflected from wastewater analysis.

7.01.13

Wastewater Surveillance on SARS-CoV-2 in Bangladesh: The Ongoing Dhaka City Project

R. Haque, icddr,b / Human Ecology; M. Rahman, N. Amin, icddrb / Environmental Interventions Unit, Infectious Disease Division; M. Rahman, icddrb / Virology Laboratory; Z. Mahmud, icddrb / Laboratory of Environmental Health; Z. Rahman, icddrb / Environmental Interventions Unit; R. Raqib, P. Sarker, icddrb / Nutritional Biochemistry; A. Hossain, Dhaka Water Supply & Sewerage Authority (DWASA); D. Johnston, N. Akter, UNICEF / WASH Section; M. Islam, P. Bhattacharya, KTH royal Institute of Technology / Sustainable Development, Environmental Science and Engineering

Wastewater based epidemiology (WBE) surveillance has a long history of use in public health, particularly for poliovirus and more recently antimicrobial resistance (AMR), drug and pharmaceuticals. During the ongoing COVID-19 pandemic, it is being used for the detection of SARS-CoV-2 viral RNA shed into wastewater from the upper gastrointestinal and upper respiratory system via faeces. The fact of viral shedding and persistent up to few weeks in faeces provides an advantage to the researchers around the world in utilizing wastewater system to monitor the viral prevalence and estimate the relative proportion of the asymptomatic carriers. During the eight-week of the wet season through September and October, we collected 170 grab samples from multiple untreated wastewater sources and wastewater treatment plant (WWTP) in Dhaka, Bangladesh. We identified 40-45% SARS-CoV-2 viral RNA from septic tanks, drain, surface water bodies, pumping station except WWTP based on the assay of RdRp and N-gene. SARS-CoV-2 concentrations ranged from 1200 to 334000 genomic copies/100 mL in samples where viral RNA was detected. Also, we have collected wastewater influent samples from COVID-19 dedicated hospital and non-COVID hospital to tract the difference of viral load between symptomatic and asymptomatic carriers at each hospital. We found evidence of asymptomatic carriers in non-COVID hospital and the result shows the detection rate of SARS-CoV-2 similar to COVID-19 dedicated hospital. We propose to examine the large-scale feasibility of wastewater surveillance among Dhaka City covering 21 million populations and also scale-up the wastewater surveillance in the divisional cities in the country. The sanitation system in Bangladesh is a heterogeneous and largely sewage and grey wastewater are open to the environment. Because of shared sanitation, poor faecal sludge management (FSM), inaccessibility of on-site sanitation (OSS) and different typology of sanitation facilities contributes to the sanitation crisis. Therefore, wastewater surveillance can be an effective monitoring tool and supplement to clinical testing for SARS-CoV-2 in limited-resource countries like Bangladesh with distinct sanitation system. This research will summarize the outcome of the WBE surveillance data with clinical cases. Additionally, we will transfer this knowledge to health and sanitation authority of Bangladesh Government to incorporate with the public health surveillance system for the preparedness of current and future pandemic.

Environmental Quality Affecting Agro-Food Systems and Water Resources across the Mediterranean Area

7.02.02

Risk Reduction of Chemical Residues in Soils and Crops - Impact Due to Wastewater Used for Irrigation (RESIDUE)

D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiologia / Agroquimica y Conservacion del Suelo; D. Drabkin, R. Schatten, FU Berlin / WG Geoecology; K. Terytze, Freie Universität Berlin / Earth Science; Y. Maor, Phytor Lab, Jerusalem; B. Chefetz, The Hebrew University of Jerusalem / Soil and Water Sciences; I. Re, M. Balzarotti, Consorzio Italbiotec, Milan; K. Weinfurtner, IME Fraunhofer

The current project is a cooperation of 5 partner from 4 European countries under the PRIMA research programme. It started in August 2020 and we are going to present the project concept and first project results. The goal of the project is to enhance food quality/security by an evaluation of the risks associated with agricultural techniques, using sewage sludge for soil fertilization and treated wastewater for irrigation. Associated contaminants may reach the soils and plants, representing a risk to the consumer. In the project, we develop an improved treatment of waste materials prior to spreading based on biochar techniques. We study the fate and distribution of selected relevant contaminants from treatment of the waste materials until crop harvest. ^{14}C-radiolabelled contaminants allow an

advanced evaluation to extend the risk assessment for transformation products and bound residues. As an integral part of the project, soil qualities and sustainable use of waste materials in agriculture will be an important result of the study. To ensure practical relevance of the project, advancement will be pursued by the involvement of stakeholders (farmers' cooperatives, trade associations) to evaluate the applicability of the proposed technologies. Further, a user manual will be prepared that can serve as guidance on how to implement the research results in agricultural practise. As countries like Israel have no choice to use waste materials in agriculture, the project will support e.g. the Israel export agricultural business. In Spain and Italy basically the same applies: the high rate of treated sewage sludge and wastewater used in agriculture raises the same concerns. This demonstrates the project relevance, because it will provide new insights into the risk associated to this agricultural practice, as related to chemical pollution and food-safety issues. For countries like Germany, where water limitations might get more relevance in the future as a result of climatic change, the project will be of value for the safety and quality assessment of sewage sludge application in agriculture. Sewage sludge may find a broader application again if the risk for contamination can be reduced, because sewage sludge brings valuable organic matter and plant nutrients to soil.

7.02.03

Impact of the presence of organic contaminants of emerging concern (CECs) in reclaimed wastewater used to greenhouse crop irrigation

A.R. Fernández-Alba, University of Almería; M. García-Valverde, M. Gómez-Ramos, M. Martinez Bueno, University of Almería / Department of Physics and Chemistry

The reuse of reclaimed wastewater for agricultural irrigation is increasing around the world and is becoming an alternative to combat water scarcity, specially in those areas where there are large commercial interests and which suffer water deficit. In recent years, some research works have studied the accumulation of organic contaminants of emerging concern in crops irrigated with treated wastewater [1]. To date, most of these works have been carried out in the laboratory (unrealistic agricultural conditions), under uncontrolled environmental conditions or in field trials at concentration levels higher than those expected in reclaimed water. Thus, the objective of this work was to measure the uptake of several organic contaminants with different physical-chemical properties in soil, plant tissues and tomatoes grown using reclaimed water, under realistic agricultural conditions. For that, a pilot study was carried out in a greenhouse. A total number of 31 contaminants were analyzed belonging to different compound categories: herbicides, fungicides, insecticides, analgesics, anti-inflammatories, lipid regulators, b-blockers, antiepileptic and diuretics. A modified QuEChERS extraction method followed by liquid chromatography coupled to tandem mass spectrometry was the analytical procedure used. The work allowed to determine which compounds have the capacity to reach the plant and, to develop a plant uptake model that allows estimating the levels of organic emerging contaminants in soil and plants. [1] Picó Y, Alvarez-Ruiz R, Alfarhan AH, El-Sheikh MA, Alobaid SM, Barceló D. 2019. Uptake and accumulation of emerging contaminants in soil and plant treated with wastewater under real-world environmental conditions in the Al Hayer area (Saudi Arabia). *Sci. Total Environ.*, 652, 562-572

7.02.04

Exposure to Pesticides Assessed by Wastewater- Based Epidemiology and Its Relation With the Surrounding Agro-Food Systems in a Typical Mediterranean Coastal Wetland

D. Sadutto, Environmental and Food Safety Research Group of the University of Valencia (SAMA-UV) / Desertification Research Centre (CIDE); V. Andreu, CIDE CSIC UV GV; L.V. Herrera, Environmental and Food Safety Research Group of the University of Valencia (SAMA-UV) / Desertification Research Centre (CIDE); Y. Pico, University of Valencia / Environmental Quality and Soil

The use of pesticides is between two and three million tons per year, 70% of all pesticides being used in Europe and the United States following by China. Due to their relevant toxicity that could affect the human health and environment. Wastewater Based Epidemiology (WBE) approach has been recently applied to this field with the purpose to asses human exposure to pesticide through the determination of the metabolites excreted by urine. The aim of this study was to establish concentrations and occurrence of different classes of pesticides, including parent, degradation products and human metabolites excreted by urine, such as 3-phenoxybenzoic acid a biomarker of 20 synthetic pyrethroids in (i) wastewaters of different Wastewater Treatments Plants (WWTPs) of Valencia (Spain) to determine the "facto" population exposure and (ii) in surface waters from a typical Mediterranean wetland, the Albufera Natural Park, localized near the city, to value the impact of these contaminants into environment and their relation with the exposure of the human being to these contaminants.The sample were collected from three different wastewater treatment plants (WWTPs) that treating wastewater from metropolitan area Valencia (Spain) with about 1.2 million of inhabitants. The sampling was carried out for 1 week (from February to March 2020). Instead, the surface water samples (52) were taken in an area consists of a highly eutrophic coastal lagoon surrounded mainly by rice fields. The pesticide detection was performed with a 1260 Infinity ultrahigh-performance LC system coupled to a 6410 triple-quadrupole mass spectrometer from Agilent Technologies using two precursors → product ion transitions for almost all compounds in the Multiple selected Reaction Monitoring mode (MRM). Different pesticides and metabolites are detected in influent wastewater. The most frequent biomarkers were 3-PBA and trans DCCA (biomarker of cypermethrin, cyfluthrin and permethrin) with an occurrence of 71% and 63% and with a relevant intake estimated from 125.7 to 200.3 mg/ day/ inhabitants. Moreover, the results evidenced the presence of many selected Emerging Contaminant (EC) in surface water. The most abundant ones are herbicides and fungicides, as the prochloraz. In this study was applied for the first time the WBE approach to value the pesticide exposure of Valencia population. Furthermore, the detection of pesticides in surface waters of Natural Park pointed out that is important to optimize removal treatments and to create new barriers to avoid the discharges of contaminants to these sensitive environments.

7.02.05

Benefits and Risks Related to Plant Microbiome in Depuration Systems and Water Reuse for Crop Irrigation

F. Mapelli, University of Milan- DeFENS / Department of Food, Environmental and Nutritional Sciences; V. Riva, F. Riva, University of Milan / Department of Food, Environmental and Nutritional Sciences; R. Choukr-Allah, Institut Agronomique et Vétérinaire / Hassan II, Salinity and Plant Nutrition Laboratory; A. Rashed, National Water Research Center; N. Kalogerakis, Technical University of Crete / Environmental Engineering; S. Borin, E. Crotti, University of Milan / Department of Food, Environmental and Nutritional Sciences

Water scarcity is an issue of global concern, recently exacerbated by climate changes on both sides of the Mediterranean basin. Treated wastewaters (TWWs) represent a huge source of water that become newly available after depuration and could be used for irrigation purposes to boost crop production in arid and semi-arid regions. A safe reuse of TWWs has to cope with the presence of specific contaminants that are not efficiently removed by the currently used WW treatment technologies. Examples of contaminants of emerging concern (CECs) are synthetic dyes, widely used in textile industries, pharmaceuticals, including antibiotics, and also antibiotic resistance determinants. While testing and scaling-up of new tertiary treatments for CEC removal is ongoing, phytodepuration is an available, low-cost and easy to maintain technology with the potential to decrease CEC concentrations by exploiting the activity and the positive interaction established by plant root system and the associated microbiome. We studied the cultivable bacteria recruited by *Phragmites australis* plants in different Constructed Wetlands (CWs), establishing isolate collections and screening more than 150 strains for the ability to cope with CECs typical of WWs and to promote plant growth. The most promising strains were further tested in CW microcoms, identifying candidate inoculants for microbial assisted phytodepuration of azo-dye contaminated WWs. At the same time, the identification of the bacteria isolates indicated that cultivable root-associated microbiomes in CWs include taxonomic groups that establish strong relationship with animals and humans, like *Enterobacteriaceae* and lactic acid bacteria. Antibiograms and minimum inhibitory concentration test were used to investigate the antibiotic resistance in the bacteria collections, revealing its occurence to a variable extent according to the considered molecules. Plant root system has been reported as a hot-spot for the enrichment of antibiotic resistant bacteria through Horizontal Gene Transfer (HGT) events, a key aspect for phytodepuration and in the context of water reuse for crop irrigation. We are currently studying HGT in selected strains, relevant for their environmental origin, water reuse and the interaction with crops, attempting to assess antibiotic resistance spread in the agro-food system. Ideally, this knowledge will help to maximize the quality and safety of TTW reuse in compliance with the 'One Health' concept.

Global Plastic Contamination: a Journey towards Scientifically Informed Policies and Solutions

7.03.02

Public Risk Perception of Microplastics

C. Völker, ISOE - Institute for Social-Ecological Research / Water infrastructure and risk analyses; J. Kramm, S. Werschmöller, ISOE - Institute for Social-Ecological Research

While the scientific knowledge about the environmental risk of microplastics is still incomplete, the topic receives great public attention. Among environmental scientists, critics claim that the risks of microplastics are exaggerated and lead to sensationalist media reports which in turn misinform the public. However, the public's awareness and risk perception regarding microplastics have so far been little researched and an in-depth study on the public risk perception of microplastics is still lacking. Against this background, we conducted a representative survey and investigated 1) the public's knowledge about microplastics, 2) the risk perception regarding microplastics and 3) the factors

affecting risk perception of microplastics. We conducted a representative online survey with the resident population in Germany from the age of 18 in April 2020 (N=1.027). Participants were asked about their knowledge, concerns about the effects of microplastics on the environment, sources of microplastics, actions on microplastics and science communication. The results show that microplastics are widely known in the German population. Overall, about 80 % of the respondents have heard about microplastics. About 60 % of the respondents state that they have good to very good knowledge about the emergence, distribution and effects of microplastics. However, people feel less informed about how to behave regarding microplastics (only 45 %). Questions on the participant's concerns revealed that the high knowledge about microplastics is simultaneously linked to a high risk perception (90.5 %). Only 9.5 % of the respondents show a low risk perception. In addition, the (self-assessed) knowledge about microplastics affects the respondent's risk perception. People with higher knowledge show a statistically significant higher risk perception (index: 3.29) than people with lower knowledge (index: 2.94). The results further show that respondents who heard about negative media narratives have a significant higher risk perception. To conclude, the high risk perception of the German public is mainly based on media stories and mostly not consistent with scientific evidence. Therefore, science communications should inform the public about scientific uncertainty and avoid exaggerated images rather than triggering emotions in order to tell a story. However, the oversensitive public perception should not be a reason to delay precautionary measures to combat microplastic pollution.

7.03.03

First Global Map of Risks of Microplastic in the Ocean Surface

G. Everaert, Flanders Marine Institute / Ocean and Human Health; M. De Rijcke, Flanders Marine Institute VLIZ; B. Lonneville, Flanders Marine Institute; C. Janssen, Ghent University / Blue Growth Research Lab; T. Backhaus, University of Gothenburg / Department of Biological and Environmental Sciences; J. Mees, Flanders Marine Institute VLIZ; E. Van Sebille, Utrecht University; A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group; A.I. Catarino, Flanders Marine Institute / Ocean and Human Health; M.B. Vandegehuchte, Flanders Marine Institute / Research Department

Current studies on the risk of microplastic (MP) in the aquatic environment suggest that the in situ concentrations are on average several orders of magnitude lower than the concentrations where effects are expected to occur. There is a need to identify hotspots of risk to prioritise mitigation measures, as MP concentrations are expected to increase in the future. Here, we examine the risk of floating MP in the ocean surface by integrating environmental MP concentrations with ecotoxicity data. We first quantified unacceptable levels of MP concentrations based on ecotoxicity data available in scientific literature. In parallel, we quantified past (1970), current (2010) and future (2050 and 2100) environmental concentrations of MP based on microplastic distribution. To draw conclusions about the past, current and future risk of MP at the ocean surface [< 5 m depth], we compared in situ MP concentrations with the corresponding unacceptable levels using a probabilistic approach. Effect data for 23 different species from eight phyla were included in our assessment: Arthropoda, Chordata, Cnidaria, Echinodermata, Haptophyta, Mollusca, Ochrophyta, and Rotifera. The resulting median unacceptable level was $1.21 * 10^5$ MP m^{-3} (95% CI: $7.99 * 10^3$ MP m^{-3} - $1.49 * 10^6$ MP m^{-3}). We found strong indications that organisms in parts of the Mediterranean Sea and the Yellow Sea are currently at risk. By 2100, we expect that 68.7% and 53.9% of the Mediterranean Sea and the Yellow Sea will have unfavourable conditions for marine life due to MP pollution, under a worst case and in a business as usual scenarios, respectively. Our results showed substantial spatial differences in the risk of MP in the ocean surface layer (0 - 5 m depth). Global mapping of the MP risks is instrumental to identify marine regions that need increased attention for mitigation measures.

7.03.05

A Comprehensive Overview of Chemicals Used As Plastic Monomers, Additives and Processing Aids

H. Wiesinger, ETH Zurich / Civil, Environmental and Geomatic Engineering - Ecological Systems Design; Z. Wang, S. Hellweg, ETH Zurich / Institute of Environmental Engineering

Plastics pervade every aspect of our modern life. Production of plastics requires a variety of chemical substances, including monomers, additives and processing aids; they may enable the production and processing processes of plastics, as well as impart, enhance or maintain of specific plastic properties. However, many of these substances are not chemically bound to the polymer matrix of the plastics, and may be released during production, use, disposal and recycling of plastics, leading to human and environmental exposure and possible adverse effects. In contrast to the broad range of substances being currently used, research and risk assessment have focused on a limited number of substances, particularly heavy metals, phthalates and brominated flame retardants. One major cause is due to a lack of clear oversight of substances being currently used in plastics. To combat this ongoing shortcoming and provide guidance regarding which substances to focus on, a comprehensive database of plastic monomers, additives and processing aids is compiled in this study. The database builds on publicly accessible sources including existing regulatory databases, industry databases, lists and handbooks, and peer-reviewed scientific literature. Information on their hazards, production volumes, use information (including functions, polymer type compatibilities, industrial sectors of use, and geographical distributions), and legal status are included; and, scientific and regulatory hot- and blind-spots were investigated. More than 10'000 substances are identified for potential presence in plastic products, more than 2'400 of which are substances of (potential) concern. Many of these are currently not sufficiently regulated or researched. Overall, major information and transparency gaps remain, mostly on compatible polymer types, industrial sectors of use, applied concentrations, and (harmonized) hazard data. Tackling the remaining knowledge gaps and risks will require combined efforts from the industry, civil society organizations, and scientific and regulatory institutions, including policies that incentivize information sharing along supply chains.

7.03.06

What Are the Chemicals That Migrate or Are Extractable From Food Contact Plastics?

B. Geueke, J. Boucher, Food Packaging Forum Foundation; K.J. Groh, Eawag - Swiss federal Institute of Aquatic Science and Technology / UTOX Environmental Toxicology; F. Gwosdz, entrepre.nerds; P. Jieh, Food Packaging Forum Foundation; C.D. Kassotis, Wayne State University; M. Maffini, Independent Consultant; O. Martin, Brunel University London / Institute for the Environment, health and societies; J. Muncke, Food Packaging Forum Foundation

Food packaging is a type of food contact article (FCA) that protects food, enables storage, transport, transfer of information, marketing, and facilitates food availability while reducing food waste. Around 57% of consumed foodstuffs are packaged, and most food packaging is made of plastic (69%) or has a plastic layer in direct contact with food (14%). Plastic is not an inert material and chemicals can transfer from the packaging into foodstuffs, or into the environment when packaging is landfilled or littered. Over 4200 different chemicals are possibly associated with plastic packaging but there is no systematic overview of which chemicals have been shown to migrate or are extractable from plastic FCAs. As part of the Food Contact Chemicals and human Health (FCCH) project, we compiled a systematic evidence map on chemicals that migrated or were extracted from plastic and other FCAs by analyzing around 1100 publicly available studies. To support this task, we developed a cloud-based software tool, SciExtract, which helped standardize the data extraction process and allowed multiple researchers to work in parallel. As a result of the systematic evidence map, we compiled a chemical database, the FCCmigex, which lists all food contact chemicals that have been measured and detected to migrate from FCAs, or found to be extractable from FCAs. This freely available resource will provide necessary data to better understand chemical exposures. It will also greatly reduce the current knowledge bias towards studying a few chemicals known to be used in plastic and other food packaging materials. Furthermore, the FCCmigex will facilitate recognition of trends in chemical uses in FCAs, enable further systematic reviews, and address key knowledge gaps. In a next step of the FCCH project, chemicals from the FCCmigex will be compared to human biomonitoring studies to characterize evidence of human exposure to food contact chemicals. This information will be useful in the ongoing EU policy process for revising the food contact materials regulation.

7.03.07

Microplastic Regulation Should Be More Precise to Incentivize Both Innovation and Environmental Safety

D.M. Mitrano, ETH Zurich / Environmental Systems Science; W. Wohlleben, BASF SE / Dept. Material Physics & Analytics and Dept. Experimental Toxicology and Ecology

Numerous studies have made the ubiquitous presence of plastic in the environment undeniable, and thus it no longer comes as a surprise when scientists measure the accumulation of macroplastic litter and microplastic fragments in both urban and remote sites. The presence of plastic in the environment has sparked considerable discussion amongst scientists, regulators and the general public as to how industrialization and consumerism is shaping our world. Restrictions on the intentional use of primary microplastics, small solid polymer particles in applications ranging from agriculture to cosmetics, are under discussion globally, despite uncertain microplastic hazards and prioritization amongst options for action. In some instances, replacements are technically simple and easily justified, but in others substitutions may come with more uncertainty such as significant performance questions and monetary costs. Scientific impact assessment of primary microplastics compared to their alternatives relies on a number of factors including, but not limited to, microplastic harm, existence of replacement materials, and the quality, cost and hazards of alternate materials. Here we assess the scope, effectiveness and utility of microplastic regulations with specific emphasis on the new definitions proposed by ECHA for restriction of primary microplastics under REACH. To this end, we aim to 1) provide a systematic orientation of the polymer universe, to appreciate which (micro)plastic

characteristics are relevant, measurable and enforceable, 2) cluster specific uses of solid plastic to highlight how primary microplastic can add to issues of environmental pollution and human health, 3) evaluate drivers leading to regulations and their potential for enforceability and impact and 4) suggest priority cases where regulations should be focused and precision increased to incentivize innovation of sustainable materials and promote environmental health and safety. Regulations need a precise focus and must be enforceable by measurements. Policy must carefully evaluate under which contexts microplastic use may be warranted and where incentives to replace certain microplastics can stimulate innovation of new, more competitive and environmentally conscious materials.

Cross Species Extrapolation: Opportunities in a 21st Century Regulatory Non-Animal Testing World

8.01.01
The changing landscape of chemical risk assessment: Maximising data sources to inform data gaps in a NAM world
J.L. Dorne, EFSA
.

8.01.02
Striking a Balance: Facilitating international harmonization and accommodating regional needs
K. Sullivan, Physicians Committee for Responsible Medicine / Research and Regulatory Affairs
.

8.01.03
Exploring Ways to Reduce Animal Testing in A Regulatory Context
M. Lowit, U.S. Environmental Protection Agency
.

8.01.04
Making Better Use of Toxicity Studies for Human Health by Extrapolating across Endpoints
F. Madia, European Commission - Joint Research Centre
.

From Ecological Concepts to Ecological Scenarios for Mechanistic Effect Model Applications in Risk Assessment and Management

8.02.01
Environmental scenarios: the weather and the whether
E. McVey, Ctgb / Ecotoxicology
.

8.02.02
Mechanistic modelling framework capturing effects of environmental conditions and stressors on ontogeny: the DEB (Dynamic Energy Budget) theory perspective
L. Pecquerie, IRD
.

8.02.03
Population feedback of non-lethal exposure makes few individuals suffer, but survivors mostly thrive
A.d. Roos, Universiteit van Amsterdam / Institute for Biodiversity and Ecosystem Dynamics
.

8.02.04
Ecological concepts of complex trophic interactions - bottom-up and top-down regulation of food web and community structures
R. Sahm, Umweltbundesamt / IV
.

8.02.05
From simplified to increasingly complex scenarios: how the considered number and type of biotic interactions affect risk assessment of antibiotic resistance persistence
M. Zwanzig, TU Dresden
.

8.02.06
Which species should we model? Examples of how to define focal species for the risk assessment of plant protection products
U. Hommen, Fraunhofer IME / Ecotoxicology
.

8.02.07
What to model? Selecting vulnerable species and landscape parameters for the development of ecological scenarios
A. Rico, IMDEA Water Institute
.

8.02.08
Possibilities and limitations to learn from species distribution modelling for water quality management
N. Schuwirth, Eawag, Swiss Federal Institute of Aquatic Science and Technology
.

8.02.09
xAquaticRisk - A Modular Landscape Model for Pesticide Use, Exposure, Effect and Risk Assessment of Aquatic Organisms
S. Bub, University of Koblenz-Landau
.

8.02.10
Development of terrestrial landscape scenarios for population-level risk assessment
M. Wang, WSC Scientific GmbH / .
.

8.02.11
Temperature influence on the toxicokinetics of insecticides
A. Mangold-Doring, Wageningen University & Research / Environmental Sciences
Mechanistic effect models such as toxicokinetic-toxicodynamic (TK-TD) models have gained increasing acceptance to inform lower-tier effect assessments of chemicals. These models are usually informed by laboratory toxicity experiments conducted under optimal temperatures. As increased temperature has been observed to increase the toxicity of insecticides, there is a need for tools enabling to predict the effects of chemicals under different temperatures. In this research, we investigate the influence of temperature on the toxicokinetics of insecticides using both, in vivo laboratory experiments and in silico modelling approaches. The freshwater gastropod Gammarus pulex was exposed to the insecticides imidacloprid and flupyradifurone at 7, 18, and 24 °C. The internal concentrations of the insecticides were measured during the two days uptake and three days elimination phase at 6, 24, 48, 72, 96, and 120 h after exposure. In a first step, these results were fitted to a one-compartment TK model, uptake and elimination rate constants could be estimated, and the kinetic bioconcentration factor (BCFkin) was calculated. In a second step, the Arrhenius equation will be used to modify the one-compartment TK model, to correct the rate constants for the respective temperature. The modified model will then be used to predict the internal concentrations of the compounds at the different temperatures and subsequently compare to the measured values. For imidacloprid, both uptake and elimination rate constants increased with increasing temperature. The resulting BCFkin was similar in all temperatures. Contrastingly, the BCFkin for flupyradifurone decreased with increasing temperatures, as elimination rate constants increased considerably with increasing temperatures, while uptake rate constants stayed in the same order of magnitude. These results highlight the influence of temperature on the uptake and elimination of insecticides in freshwater organisms and indicate differences of this phenomenon among compounds. The modified model potentially allows to extrapolate insecticides' toxicokinetics across different temperature. As such, this model could inform mechanistic effects models to enable a more realistic risk assessment for insecticides in the future.

8.02.12
Optimizing spatio-temporal use of non-persistent insecticides for mosquito population control
B.K. Hackenberger, Department f Biology, University of Osijek / Department of Biology
.

8.02.13
Adding realism to ecological risk assessment: an agent-based model of trout to explore the effects of multiple stressors
C. Accolla, University of Minnesota / Ecology, Evolution, and Behavior
.

How Can We as Scientists Communicate Different Types of Uncertainties Effectively When Advising Policy

8.03.01
Room to manoevre in a highly standardised and regulated context with aims for change
A. van Wezel, University of Amsterdam/IBED Institute
.

8.03.02
Improving the characterisation of uncertainty in scientific assessments of health and environmental risks
A. Hart, A & A Hart Ltd
.

8.03.03
The multiple dimensions of uncertainty in science for policy
J.P. van der Sluijs, University of Bergen
.

8.03.04
Why values are central to trust in science
H. Douglas, Michigan State University / Philosophy
When many scientists here about social and ethical (i.e. non-epistemic) values influencing science, they presume those values are biasing science, i.e. steering the science away from accurate conclusions. However, the influence of social and ethical values is not the same as bias, and indeed in some cases, can help scientists produce more accurate work. Further, because of the many important roles for values in science, including in addressing the acceptability of some uncertainties, such values are a crucial basis for trust in scientific work. This talk will explain both how to differentiate value-ladenness from bias, and why values are crucial to trust, in contrast to bias, which undermines trust. Because of the importance of values for trust, values need to be communicated in science communication.

8.03.05
'Big data' and regulators: How to reduce uncertainties and increase confidence?
J. Slobodnik, Environmental Institute
.

Stop Biodiversity Loss-Global Challenges for Business, Regulators and Society

8.04.02
The Knowledge Centre for Biodiversity of the European Commission
G. Dubois, M. Robuchon, European Commission - Joint Research Centre
.

8.04.03
Approaches to sustainable agriculture
A. Arroyo Schnell, IUCN
.

8.04.04
Biodiversity and business: Challenges, opportunities and ways forward
S. Bekker, UNEP-WCMC
.

8.04.05
Sustaining biodiversity, sustaining society: Cross-sectoral goals and sectoral regulation
S.E. Apitz, SEA Environmental Decisions Ltd
.

8.04.06
An industry strategy and scientific methodologies applied to preserve biodiversity
J. Lharidon, LOréal Research & Innovation / Life Sciences Direction
.

8.04.07
Biodiversity challenges from an ocean perspective
R. Troublé, The Tara Ocean Foundation
.

The European Green Deal (Chemicals Strategy)

8.05.01
Results of the 2020 Green Deal Discussion Forum and associated questionnaire
M. Bloor, Scotlands Rural College; A. Leopold, Calidris Environment BV / Calidris Environment BV; J. van Dijk, Copernicus Institute of Sustainable Development, Utrecht University / PRAS unit
.

8.05.02
Life Cycle Assessment approach being taken to ensure that chemicals are designed for sustainability
s. sala, EC JRC
.

8.05.03
National regulatory prospectives on the European Green Deal (Chemicals Strategy)
L. Posthuma, RIVM / Centre for Sustainability, Environment and Health (DMG); M. Wimmer, Austrian Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology
.

8.05.04
Design of chemicals taking criteria for sustainability into account at very early development stages and right through the life cycle
D. Debecker, Solvay S.A.
.

8.05.05
A case study involving chemicals contamination as an example of how the policies proposed in the Chemicals Strategy for Sustainability would help to address a situation in real life
J. Wietor, European Environmental Bureau
.

8.05.06
An industry view on working with the Chemical Strategy for Sustainability
I. Malcomber, Unilever R&D Colworth
.

Water Scarcity and Pollution in the Mediterranean European Rivers: Challenges for their Management

8.06.01
The Guadalquivir river Basin: Characteristics and management
V. Cifuentes, Oficina de Planificación Hidrológica. Confederación Hidrográfica del Guadalquivir
.

8.06.02
The management of the Catalonian River Basins in global change scenarios
A. Munne, Catalan Water Agency
.

8.06.03
Foster the innovation to address the water scarcity: where Science meets Policy for economic growth
T. Lettieri, European. Commission - Joint Research Centre / Directorate D Sustainable Resources Water and Marine Resources Unit
.

Poster Abstracts

Alternative Approaches to Animal Testing for Ecotoxicity and Environmental Risk Assessments

1.01.01
Replacing the Need for Acute Toxicity Studies in Fish: The RTgill-W1 Assay
K. Jenner, Givaudan / Global Regulatory Affairs & Product Safety; L. Hostettler, Givaudan Schweiz AG / Fragrances S & T; G. Kreutzer, Givaudan SA / Regulatory Affairs & Product Safety; H. Laue, A. Natsch, Givaudan Schweiz AG / Fragrances S &T; G. Sanders, Givaudan International SA / Regulatory Affairs & Product Safety
Testing for acute fish toxicity is an integral part of the environmental safety assessment of chemi-cals. Under REACh regulations, prior consent should be obtained from the competent authority before performing in vivo fish testing. Within certain European directives, e.g. for cosmetics, the use of vertebrate species in chemical testing is prohibited. The Fish Gill Cell Line (RTgill-W1) as-say provides a non-animal alternative that has been validated and published as an ISO standard (ISO 21115) and is currently at the advanced stage of evaluation in the OECD Test Guideline Programme. To demonstrate the applicability of this test, we have previously performed and pub-lished a benchmarking study on 38 fragrance chemicals for which we had high quality historical in vivo data on fish toxicity covering a broad range of physicochemical properties and diverse chem-istries. This study showed a very strong correlation (R2 = 0.90–0.94) between in vitro gill toxicity and in vivo fish acute toxicity. To promote regulatory acceptance and further demonstrate the high in vitro–in vivo correlation for fragrance chemicals, we present here our latest results for new chemical products or expanding product registrations where we were required to perform an in vivo acute fish study for European or global notification purposes. The challenge of assessing the toxicity of poorly soluble chemicals that are expected to have no toxic effect up to their solubility limit is also explored. Further we provide case studies, for example products used predominantly or exclusively in cosmetic applica-tions, where we have submitted registrations without in vivo testing. We demonstrate how the RTgill-W1 assay can be used as a bridging tool between in silico predictions and full in vivo testing and how it can be used to support hazard and risk assessment.

1.01.03
Performance of Three-Dimensional Rainbow Trout (Oncorhynchus mykiss) Hepatocyte Spheroids for Evaluating Biotransformation of Three Different Fragrance Chemicals
M.T. Hultman, Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; L. Hostettler, Givaudan Schweiz AG / Fragrances S & T; K. Petersen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; H. Laue, Givaudan Schweiz AG / Fragrances S & T; A. Lillicrap, NIVA / Section of Ecotoxicology and Risk Assessment
Hydrophobic chemicals which are not biotransformed have the potential for being bioaccumulative in tissue and lipid reservoirs of aquatic organisms such as fish, potentially causing both short and long-term biological effects. The assessment of a chemical's potential to bioaccumulate in fish involves as a screening method its physicochemical properties (e.g. log Kow) or conventional animal (*in vivo*) test methods (OECD TG 305). Due to ethical and economical concerns, the development of alternative methods to measure in vitro biotransformation rates in primary hepatocytes (RT-HEP) and S9 fractions (RT-S9) from rainbow trout were established (OECD TG 319A/B) to improve existing in silico predictions. Due to the limited life time of RT-S9 and RT-HEP, these assays are not suitable to measure very slowly biotransformed chemicals. One recently developed model with longer assay life time is based on three dimensional (3D) hepatic spheroids (3D-HEP) of rainbow trout. The aim of this study was to determine the biotransformation rates of three fragrance chemicals, one slowly (Cashmeran, CASH; Log Kow: 4.5), one moderately (Ambrofix, AMB; log Kow: 5.1) and one rapidly (Cyclohexyl salicylate, CS; Log Kow: 4.7) biotransformed (in the TG 139 A/B assays) using 3D-HEP. The 3D-HEP viability was measured with Trypan blue and AlamarBlue during the full period of incubation (0-72h) and decrease of the parent chemicals in active and heat-inactivated spheroids as negative control analysed by GC-MS. The in vitro intrinsic clearance rates ($CL_{IN\ VITRO,INT}$) were compared with $CL_{IN\ VITRO,INT}$ determined in RT- S9 and RT-HEP assays. The 3D-HEP were viable during the whole period of incubation (up to 72 h) for all chemicals tested. Log-linear depletion was obtained throughout the study for all chemicals in the 3D-HEP. Around 50% of the initial amount of CS, AMB and CASH was biotransformed within 4, 48 and 48h, respectively. Decrease in the heat-inactivated control was < 20% over the whole incubation time for all three chemicals. The $CL_{spheroid}$ rate of AMB and CS was 5- and 100-fold lower respectively when compared to the RT-HEP model. CASH, which is slowly biotransformed in both RT-S9 and RT-HEP, displayed a similar CL rate in the 3D-HEP. The 3D-HEP demonstrated reproducibility and a log-linear biotransformation of all three chemicals tested, indicating that 3D-HEP are highly metabolically competent for at least 72 h and are therefore suitable to measure compounds with slow CL rates.

1.01.04
Functional Characterization of a 3D Cell Culture Model (Spheroids) From the Rainbow Trout Liver Cell Line RTL-W1
S. Joyce, V. Holgersson, M. Brookman-Amissah, University of Gothenburg / Department of Biological & Environmental Sciences; T. Lammel, University of Gothenburg / Department of Biological and Environmental Sciences
In vitro models based on continuous piscine cell lines have proven to be a powerful tool in examining the toxicity of chemical compounds. However, many continuous cell lines have partially or completely lost some tissue-specific properties. The lack of specific functionalities such as lower expression levels of detoxification enzymes and transporters can lead to false-quantitative estimates of toxicity. It has been demonstrated that various continuous cell lines can regain some tissue-specific functionalities when cultured in a three-dimensional (3D) configuration, and previous results suggest that this may also hold true in the case of the rainbow trout liver cell line RTL-W1 as spheroidal aggregate cultures (spheroids) of this cell line showed higher cytochrome P450 1a (Cyp1a) mRNA expression levels compared to monolayer cultures. The objective of the sph3roiD project is to optimize 3D culture and assay protocols with respect to reproducibility and throughput, conduct a detailed characterization of the biosynthetic and detoxification capabilities of the RTL-W1 spheroids and explore their potential as an *in vitro* model for predicting fate and effects of environmental pollutants in fish (hepatic metabolization and toxicity). RTL-W1 spheroids with different sizes were produced and cultured as one spheroid per well in 96-well ultra-low attachment (ULA) plates and characterized with regard to uniformity (shape), size, and metabolic activity over a 4-week period. Metabolic activity (cell viability) was determined by measuring the spheroids' ATP content using the CellTiter-Glo®3D assay. Based on the obtained results, a seeding cell density between ~6000-8000 cells/100 µL was established as optimal to obtain spheroids with a size suitable for use in further testing (200-250µm in diameter). At present, studies are being carried out to characterize levels of selected target genes and corresponding proteins/enzymes involved in xenobiotic detoxification, such as Cyp1a, and anabolic processes, such as vitellogenin, without and upon exposure to model compounds (β-naphthoflavone and estradiol) and known environmental pollutants using RT-qPCR and plate reader-based biochemical assays. Comparative analyses of RTL-W1 cells cultured as 3D spheroids and conventional 2D monolayers planned for the near future will show whether there exist significant differences in liver-typic properties and responses of the two *in vitro* systems as hypothesized.

1.01.05
Fish Gill Cell System for Marine Toxicological Studies; Peculiarities and Applicable Cytotoxicity Assays
K.A. Bawa-Allah, University of Lagos, Nigeria / Zoology; A.A. Otitoloju, UNIVERSITY OF LAGOS / Department of Zoology; C. Hogstrand, Kings College London / Department of Nutritional Sciences
There is a continued urge in the scientific community to reduce the number of organisms used in toxicity screening of chemicals and in response to this, several *in vitro* processes are being developed to replace toxicity testing *in vivo* especially, using fish. In this study, we used an optimised rainbow trout fish gill cell system, which can withstand artificial seawater, to evaluate the acute toxic potentials of organic and inorganic pollutants to fish gill epithelial cells. Cells were cultured using the double seeded insert (DSI) technique and grown in modified L-15 culture medium. Cells with an intact gill epithelium, were exposed to arsenic (As), cadmium (Cd) and BTEX mix respectively, resuspended in artificial seawater (30‰). The effective concentration of the pollutants adversely affecting 50% of exposed cells (EC/IC_{50}) was determined using an array of cytotoxicity assays including the Lactate dehydrogenase (LDH) assay, MTT (3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenylytetrazolium bromide, a tetrazole)

assay, Alamar Blue (AB) assay as well as determination of Protein, Glutathione (GSH), and Adenosine triphosphate (ATP) content in cells. Speciation analysis of the trace elements showed that only 4% of Cd added as cadmium sulphate ($CdSO_4.8H_2O$) was present in solution as Cd^{2+} and ≤ 47% of As added as arsenic oxide (As_2O_3) was present in solution as arsenous acid (H_3AsO_3), and bioavailable to cells. Results of the cytotoxicity assays showed that the MTT and Calcein Live Cell assays gave precise prediction of the IC_{50} values of the pollutants (As, Cd and BTEX mix) against the cells, according to recommended guidelines for accurate EC/IC_{50} determination. The EC/IC_{50} values derived from the other cytotoxicity assays did not conform with the recommended guidelines. The IC_{50} calculated based on bioavailable trace element concentration was 45.8 µM and 112.1 µM As, 19.2 µM and 23.5 µM Cd, derived from MTT and Calcein Live Cell assays, respectively. This study has shown that the optimised rainbow trout gill cell can be adopted as an *in vitro* method for the toxicological profiling of waterborne chemicals in marine ecosystems and has highlighted the advantages of testing these chemicals resuspended in seawater mimicking their form in marine ecosystems. There is need for further studies to identify additional molecular and cytotoxicity assays that can accurately predict toxicity using this fish gill cell system.

1.01.06
Predicting Exposure Concentrations of Chemicals With a Wide Range of Volatility and Hydrophobicity in Different Multi-Well Plate Set-Ups
J. Stadnicka-Michalak, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; N. Bramaz, Swiss Centre for Applied Ecotoxicology Eawag-EPFL; R. Schönenberger, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Department of Environmental Toxicology; K. Schirmer, Eawag / Environmental Toxicology
Quantification of chemical toxicity in small-scale bioassays is challenging owing to little volumes used and extensive analytical resource needs. Yet, relying on nominal concentrations for effect determination maybe erroneous because loss processes like sorption or volatilization can significantly reduce the actual exposure. Mechanistic models, developed for prediction of exposure concentrations based on distribution coefficients, exist but require further validation with experimental data. Here we have performed validations, identifying strengths and weaknesses of two such models as they currently stand. We then developed empirical models for chemicals with a wide range of properties, different well-plate formats, exposure volumes, plate covers and biological entities. These models were able to predict losses for both non-volatile and volatile chemicals in different well-plate systems, confirmed by model cross-validation and extrapolation. Case study model application showed that predicted and measured chemical concentrations resulted in the same in-vitro-to-in-vivo extrapolation of fish acute toxicity data, i.e. almost 80% of data points being within the 3-fold range of line of unity compared to less than 40% for nominal concentrations. Thus, our approach can significantly improve the applicability of small-scale systems by providing accurate chemical concentrations in exposure media without resource- and time-intensive analytical measurements.

1.01.11
A Miniaturized Alga Growth-Inhibition Assay for Ecological Risk Assessment of Chemicals
A. Puertas, I. Iturria, J. Solís, BBD BioPhenix-Biobide / R&D Department; A. Muriana, BioBide / R&D Department; M. Mazón, BBD BioPhenix - Biobide / R&D
The environmental risk of new chemical substances is of increasing concern, and global regulatory authorities now require that companies conduct an appropriate testing program before approval. In this process, aquatic toxicity studies are essential to understand the environmental hazard and risk assessment. Several guidelines describe the mandatory test required for registration submissions which include freshwater microalgae (OECD 201), invertebrates (OECD 202), and fish (OECD 203, 210 and 236). During early developmental phases, chemical companies could benefit from screening assays to predict the aquatic toxicity of new substances. To this end, a miniaturized alga growth-inhibition assay has been implemented to simplify the OECD 201 test. The use of microplate format makes it possible to evaluate the toxicity of a high number of substances, cost-effectively, with a reduced amount of test substance required. Exponentially growing *Pseudokirchneriella subcapitata* was exposed to 10 concentrations of the test substances on a single 96 well-plate for 72 hours. Fluorescent intensity was measured after this incubation period on a plate reader and was used as a surrogate of alga biomass variation. The results obtained were statistically analysed to determine the toxicity parameters: EC50, EC10, EC20, NOEC, and LOEC. Several environmentally relevant compounds have been tested, including bisphenol A, copper sulphate, pesticides (penconazole and difenoconazole) and several flame retardants. The sensitivity and reproducibility of the assay are comparable to the standard assay described on the OECD 201 Guideline, providing relevant information for rapid decision-making during the environmental risk assessment of new chemicals. This miniaturized assay proved to be an efficient and cost-effective solution for high-throughput screening of aquatic toxicity during early phases of chemical substances development process.

1.01.12
Applying the 3Rs to Reduce Animal Testing in Support of the Registration of Pesticides by the U.S. Environmental Protection Agency
M. Lowit, U.S. Environmental Protection Agency; P. Ceger, D. Allen, N. Choksi, A. Daniel, Integrated Laboratory Systems, Inc.; W.P. Eckel, U.S. Environmental Protection Agency; J. Hamm, Integrated Laboratory Systems, Inc.; T. Johnson, U.S. Environmental Protection Agency; N. Kleinstreuer, NICEATM; C. Sprankle, J. Truax, Integrated Laboratory Systems, Inc.; E. Odenkirchen, U.S. Environmental Protection Agency
As part of the U.S. Environmental Protection Agency's (EPA) commitment to reducing animal testing, the Office of Pesticide Programs (OPP) is critically evaluating which studies form the basis of OPP decisions. This is part of a greater effort by EPA focused on a variety of approaches to apply the 3Rs (reduction, replacement, refinement) to the in vivo toxicity data used to assess and manage potential risk to a variety of taxonomic groups from use of pesticides. Two recently completed EPA 3Rs efforts have led to policies supporting the reduction of animals used in avian acute toxicity and fish bioconcentration testing. Current efforts focus on data retrospectives to assess data needs for fish acute toxicity and avian chronic toxicity studies as well as potential applications of in silico methods. The retrospective analysis involving fish has focused on data needs for establishing acute toxicity. OPP typically requires in vivo testing of three different fish species, a cold and a warm freshwater species and an estuarine/marine species, which in total can require 200 or more fish for a single pesticide registration. The purpose of the retrospective study was to determine whether all three species are necessary to evaluate potential risk or whether fewer species can provide OPP with sufficient information to support pesticide registration decisions protective of public health and the environment. We extracted and curated median lethal concentration (LC_{50}) values from approximately 700 of the fish acute toxicity studies submitted to the Agency between 1998 and 2016. We determined that a cold-water species (Rainbow trout; *Oncorhynchus mykiss*) was the most sensitive among the three categories considered (freshwater [cold or warm] and estuarine/marine) for more than 50% of the chemicals where a determination could be made about the most sensitive species. For the chemicals that the warm-water or estuarine-marine species was the most sensitive, the difference in toxicity between the cold-water species and the most sensitive species was determined to be relatively small and no more than 5X. These study results will inform whether fewer than three species will suffice for assessing acute lethal risk to fish and provide the basis for exploring a potential reduction in species testing that may reduce animal use and resources for this regulatory endpoint. This project was funded in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

1.01.13
Statistical Analysis of Avian Reproduction Studies: Statistical Protocol
J.W. Green, JohnWGreen-ecostats.com / Data Science and Informatics; M. Foudoulakis, Corteva agriscience / CPRS; T.B. Fredricks, Bayer CropScience / Global Regulatory Sciences; T. Carro, FMC Agricultural Solutions / Environmental Safety Assessment; J.D. Maul, Syngenta Crop Protection Inc. / Environmental Safety; S. Plautz, BASF / Agricultural Solutions, Ecotoxicology; P. Valverde, Dow AgroSciences LLC; A. Schapaugh, Bayer CropScience / Global Regulatory Sciences; X. Sopko, Corteva Agriscience™ The Agriculture Division of DowDuPont™
Detailed statistical protocols are given for avian reproduction studies used for regulatory risk assessment for continuous, quantal, and count data that addresses monotonicity (and its absence), variance heterogeneity/overdispersion, use of historical control data, model selection and model averaging, shallow and steep dose-response, effects of biologically importance, outlier identification and handling. Analyzing replicate proportions or mean counts as continuous, possibly after a transform, is usually less appropriate than analyzing quantal data as conditionally binomial and count data as Poisson and applies both to tests for NOEC and

to regression when data are adequate. Outliers include distinguishing 1 cracked eggs out of 2 laid from 1 cracked out of 40 laid. Analysis with and without outliers can help interpretation. Guidance is provided on when to use trend tests that smooth non-monotonicity (e.g., Williams) and when to use trend tests that do not smooth (e.g., Jonckheere-Terpstra) or pairwise tests. Careful model selection, including distribution assumed, is important for both selecting the 'best" fit and for model averaging that takes model uncertainty into account. Models to consider include hockey-stick and MAXSD, as well as hormetic models. Guidance is given on the role of historical control data and biologically important effect size in study interpretation.

1.01.14
Statistical Analysis of Avian Reproduction Studies: Case Studies
J.W. Green, JohnWGreen-ecostats.com / Data Science and Informatics; M. Foudoulakis, Corteva agriscience / CPRS; T.B. Fredricks, Bayer CropScience / Global Regulatory Sciences; T. Carro, FMC Agricultural Solutions / Environmental Safety Assessment; J.D. Maul, Syngenta Crop Protection Inc. / Environmental Safety; S. Plautz, BASF / Agricultural Solutions, Ecotoxicology; P. Valverde, Dow AgroSciences LLC; A. Schapaugh, Bayer CropScience / Global Regulatory Sciences; X. Sopko, Corteva Agriscience™ The Agriculture Division of DowDuPont™
Avian reproduction studies for regulatory risk assessment pose numerous challenges, including (a) limited number of doses, (b) non-monotone dose-response, (c) small effects that are statistically significant, (d) larger effects that are not statistically significant, and (e) limited knowledge of what size effects are biologically important. Case studies are presented to address these concerns. With only 3 positive doses following a monotonic dose-response: (1) the MAXSD approach can establish the lowest dose with mean response exceeding X% (e.g., X=10); (2) A trend-based test can smooth mild non-monotonicity to determine a NOEC, (3) Comparison of concurrent and historical control data can distinguish real effects from statistical aberrations. If a response curve is flat after an initial decrease, a hockey-stick model may capture the maximum effect better than a decreasing model. Where available, a level of biological concern can distinguish a result that is statistically significant but biologically irrelevant or be used to test against a relevant level of effect. In the unusual cases where 5+ treatment groups are available, careful model selection or model averaging are effective. Generalized linear & non-linear models can be more effective and biologically more appropriate than transformations to approximate normality and better able to take variance heterogeneity/overdispersion into account.

1.01.16
Design of Bioluminescent Enzymatic Sensor for the Analysis of Complex Food and Environmental Matrices
V. Kalyabina, E. Esimbekova, K. Kopylova, Siberian Federal University / Department of Biophysics; V. Kratasyuk, Siberian Federal University / Biophysical
The presence of potentially toxic compounds in complex environmental matrices, such as soil, or complex food matrices, stimulates the development of simple and effective techniques and devices. The bioluminescent enzyme inhibition-based assay has a good potency to be used as the integral analysis of complex matrices contaminated with a mixture of toxic substances. The purpose of the study was to develop a scheme for design of a new bioluminescent enzymatic sensor based on a coupled enzyme system of luminous bacteria NAD(P)H:FMN-oxidoreductase+luciferase (R + L), suitable for assessing the safety of food and environmental matrices with complex composition. The bioluminescent analysis is based on the detection of the inhibitory effect of a sample on enzymatic reactions by registering changes in the intensity of emitted light. At the first stage, the sensitivity of enzymes to a number of heavy metals and pesticides at the level of their maximum allowable content in food was established. At the next stage, the effect of the food samples as complex matrices on the coupled enzyme system R + L activity in the absence of pollutants was assessed. If necessary, we modified the procedure of the sample preparation to decrease the effect of control (unpolluted) matrices on enzymes. At the third stage model experiments were conducted to assess the sensitivity of the coupled enzyme system R + L to a complex mixture of the food sample and toxic substances. To design the bioluminescent sensor at the fourth stage we developed a biorecognition element consisting of enzymes R + L and their substrates NADH and an aliphatic aldehyde co-immobilized in a starch gel. To ensure a high activity and sensitivity of the biorecognition element we varied the ratio of its components and conditions of their immobilization in microfluidic chips. As an additional step it is possible to choose a suitable transducer providing the detection of a biosensor signal. The presented steps can be applied for the development of any enzymatic biosensor for assessing the safety of complex matrices. The use of enzymes in ecotoxicological analysis in the future will reduce the number of experiments on animals.
The reported study was funded by the RFBR, the Government of Krasnoyarsk region and the Krasnoyarsk Regional Fund of Science according to the research project no. 20-44-242001.

Alternative Methods for Safety Assessment of Nanomaterials in the Aquatic Environment

1.02.04
Ecotoxicology Assessment of PMMA Nanoplastics Using Haemocytes From Mytilus edulis
M. Michelangeli, T. Gomes, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment
Increasing amounts of nano-sized plastic particles (nanoplastics) are manufactured for a variety of applications and constitute a new area in environmental research. Nanoplastics can pose a serious risk to the aquatic environment due to their small size, high surface/volume and possible release of chemical contaminants. Thus, it is necessary to develop accurate and reliable methodologies to outline the probable ecotoxicological hazards of these particles towards aquatic organisms. Mussel haemocytes play a key role in cell immunity and the modulation and disruption of their morpho-functional activities, physiological responses and sub-cellular and molecular mechanisms have become useful models to assess the environmental impact of different contaminants. Due to their high developed processes, haemocytes are considered a target group for nanoparticles toxicity and have been implemented in both *in vivo* and *in vitro* ecotoxicological studies. The aim of this study was to employ *Mytilus edulis* haemocytes as model targets in the ecotoxicological assessment of polymethyl-methacrylate (PMMA) nanoplastics. To achieve this, a high throughput method using flow cytometry has been developed, where different haemocytes subpopulations have been detected according to their FSC and SSC properties and a wide battery of fluorescent probes have been implemented to characterize their basal biochemical and physiological status. Exposure experiments are currently ongoing in which mussel haemocytes will be exposed to nano-sized particles of PMMA, ranging from 0.025 to 5 µm, to understand their size-dependency toxicity. Different biological endpoints such as haemocytes mortality, cell viability, apoptosis, reactive oxygen species formation, cellular and mitochondrial membrane potential and lipid peroxidation will then be determined using flow cytometry. The integrated effects methodology developed has proven to be a fast, accurate and reproducible cost-effective alternative tool to quickly predict toxic effects at conditions close to an *in vivo* state. As this methodology allows the simultaneous measurement of multiple cellular parameters that can be utilized to evaluate the mechanisms of action at cellular and subcellular level, it can thus be used as an alternative tool in the ecotoxicological assessment of nanoplastics.Acknowledgement – This work was supported by NIVA's strategic research programme and the projects MicroLEACH and REVEAL funded by the Norwegian Research Council.

1.02.09
Toxicity and Antioxidant Activity of Water-Soluble Fullerene C60 Derivatives
A. Kicheeva, E.S. Kovel, FRC KSC SB RAS; N. Kudryasheva, Institute of Biophysics SB RAS; Siberian Federal University
Nanoscale structures are extremely perspective for modern technology.However, application of nanomaterials creates potential risks for the environment and living organisms. As carbon is the main constituent of living organisms,fullerene C_{60},the most abundant fullerene, has raised much interest in the field of biomedical applications. This interest was mainly due to the unique properties of this carbon allotrope including its three-dimensional structure, solubility, and chemical reactivity. Hydrated fullerene(HyFn) is a hydrophilic supramolecular complex consisting of a fullerene C_{60} macromolecule enclosed to a hydrate shell. A great deal of information has accumulated concerning the beneficial effects of HyFn,its neuroprotective,anticancer,anti-inflammatory action, mainly determined by the antioxidative capacity of HyFn,which is revealed unexpectedly at extremely low concentrations.Another extensively studied C_{60} derivative is fullerenol, where multiple hydroxyl groups are chemically bound to the surface of fullerene C_{60} case, providing the enhanced water solubility. Fullerenols are powerful direct-action antioxidants(free-radical traps) both in vitro and in vivo. The aim of the work was to compare toxic and antioxidant properties of HyFn and

fullerenols using bioluminescence enzymatic assay based on the system of coupled enzymatic reactions of bacterial luciferase and NAD(P)H:FMN-oxidoreductase. Suppressing(inhibition) of bioluminescence intensity of the assay system characterizes quantitatively a toxicity of a sample under investigation.The results show that HyFn is characterized by higher toxicity, as compared to fullerenols F1 and F2.The F1 was found to produce lower toxic effects,as compared to HyFn and F2. Basing on the experiments described, we chose the range of HyFn concentrations($< 0.0016 g\ L^{-1}$) providing the absence of its inhibiting effect for our further experiments – studying its antioxidant activity.The antioxidant activity of HyFn was studied in solutions of model inorganic and organic oxidizers:$K_3[Fe(CN)_6]$ and 1,4-benzoquinone. Antioxidant coefficients D^{OxT}were calculated,concentration intervals of antioxidant activity were determined. Values of $D^{OxT}$of HyFn appeared to be intermediate between those of the fullerenols. Hence,the comparison revealed the moderate antioxidant activity and higher toxicity HyFn as compared to the fullerenols.This result shows that the detailed research of biological activity of HyFn should be provided before its wide application in biomedicine.

1.02.10
Bioluminescence Cellular Assay As a Tool to Study Toxic and Antioxidant Properties of Nanoparticles
E.S. Kovel, FRC KSC SB RAS; L. Bondarenko, K. Kydralieva, Moscow Aviation Institute; A. Kicheeva, FRC KSC SB RAS; N. Kudryasheva, Federal Research Center Krasnoyarsk Science Center of the Siberian Branch of the Russian Academy o
Currently, a synthesis and application of nanoparticles get ahead of investigation of their properties. We studied biological activity of a series of carbon nanoparticles, i.e. fullerenols, Fs, with different number of oxygen substituents, as well as bare and modiffied magnetite (Fe_3O_4) nanoparticles (MNPs). It is known that study of Fs properties forms a perspective basis for drug development, while MNPs have been widely used as adsorbents to extract ecotoxicants from natural and waste waters. We used the physico-chemical approach based on the “structure-function” relations and allows for predicting the toxic and antioxidant properties of nanoparticles. The relations can be useful in further applied studies, from synthesis to medical or ecological applications. Additionally, the relations can help to minimize further routine experiments with organs and whole organisms, which are usually time-consuming, expensive, and less reproducible. The physico-chemical approach assumes the application of simple biological assays as models. We applied unicellular bioassay, which uses luminescence intensity as a test parameter. Luminescent registration provides high rates, low costs, and convenience of the bioassay procedures; it ensures simultaneous multiple analyses and, hence, proper statistical processing and reliability for the results of the investigations. All Fs produced toxic effect at higher concentrations >10 mg/L, suspensions of bare Fe_3O_4 provided toxic effects at higher concentration $>10^{-2}$ mg/L. This toxic effect of MNPs can be explained by a release of surface Fe^{2+} to media with its following oxidation to Fe^{3+} in water solutions. Modification of the magnetite surface with humic acids also did not protect the bacteria from the toxic effect, in contrast to the silica-modified magnetite. We have not detected antioxidant properties of MNPs, but Fs demonstrated antioxidant activity at lower and ultralow concentrations under conditions of an oxidative stress (i.e., in the solutions of 1,4-benzoquinone as a model oxidizer). Lower toxicity and higher antioxidant activity was determined in the solutions of Fs with fewer oxygen substituents. It was concluded that bioluminescence unicellular assay is a convenient tool to (1) to evaluate bioavailability of iron ions in natural water dispersions of magnetite, ferrihydrite, etc., and (2) to provide the pharmaceutical sciences with a basis for selection of carbon nanoparticles of proper biological activity. The work was supported by the grant of RFBR N18-29-19003mk.

1.02.13
Bioaccumulation and Adverse Effects of Metal Nanoparticles in Sediments
A. Thit Jensen, Roskilde University / Science and Environment; C. Mouneyrac, Université Catholique de lOuest / Université Catholique de lOuest; H. Selck, Roskilde University / Dept Science and Environment
Engineered metal nanoparticles (Me-ENPs) are released into the aquatic environment, where aggregation and agglomeration leads to sedimentation and accumulation in the sediment compartment. Benthic organisms may therefore be particularly at risk of Me-ENP exposure. However, only few studies have focused on bioaccumulation and effects in sediment-dwelling organisms. Here we address following questions: 1) Is bioavailability, uptake, internal distribution and toxicity of Me-ENPs in sediments different from that of aqueous metals (i.e., metal salt)? And 2) what is the main route of uptake: water or sediment? There is evidence that sediment-associated Me-ENPs are bioavailable and toxic to sediment-dwelling organisms. However, whether Me-ENPs are more or less bioavailable and toxic than the aqueous metal form is not straightforward. Furthermore, current data suggests that water-only setups do not provide results that are directly related those from sediment exposures. Thus, whether the findings from water only setups can be extrapolated to sediment exposures needs further investigation. To progress Me-ENP research and risk assessment, we highlight the need for a better understanding of Me-ENPs in complex compartments (e.g., sediment) and experimental setups appropriate for Me-ENP sediment studies. We specifically suggest focusing on mechanisms of bioaccumulation, internal distribution, toxicity endpoints, chronic studies, population-level effects, as well as interactions and behavior of Me-ENPs in natural environments with focus on the sediment compartment. Furthermore, we suggest that the sediment compartment should be investigated further, since findings from water only studies cannot be directly extrapolated to the sediment compartment.

1.02.14
Testing Strategy for Bioaccumulation Assessment of Nanomaterials Using a Freshwater Invertebrate Species
C. Schlechtriem, Fraunhofer IME / Department Bioaccumulation and Animal Metabolism; S. Kuhr, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Department Bioaccumulation and Animal Metabolism
The high production volume of ENMs may lead to high pressure on the environment and a scientific assessment of ENMs that bioaccumulate in organisms and biomagnify in food webs is necessary. Within the regulation of chemicals in several jurisdictions, such as the European regulation REACH, the bioconcentration factor is the standard endpoint. The bioconcentration factor is mostly determined by flow-through fish tests. Several risk assessment regulations allow the usage of data gained during tests using invertebrates and such data may allow a waiver of futher tests using vertebrates. A literature study has elucidated the potential of different aquatic invertebrate species to be used in laboratory bioaccumulation studies on ENMs (Kuehr et al. 2020). Amphipods were identified as the most promising species for ENM testing. Modified BCF and BMF values can be calculated that fulfil the requirements of endpoints needed for the bioaccumulation assessment of ENMs under regulations like REACH. A testing strategy representing a modified version of the tiered approach presented by Handy et al. (2018), was recently proposed including an assessment scheme with the aim of defining a “bioaccumulative” or “non bioaccumulative” grading for ENMs without using fish. In addition, decision criteria / endpoints that are more robust regarding the analytical challenges involved in ENM studies compared to the established endpoints were suggested (Kuehr et a. 2020). The poster will provide a summary of the tiered approach and describe the gaps and data requirements which will need to be closed in the future to allow the implementation of the testing strategy using amphipods in the regulatory bioaccumulation assessment of ENMs.

1.02.15
Developmental Phenotypes Induced by Titanium Dioxide Nanoparticles (Nano-Tio2) in Embryos of the Tropical Sea Urchin Lytechinus Variegatus (Lamarck, 1816)
L. Palmeira Pinto, A. Krupinski Emerenciano, University of São Paulo / Cell and Developmental Biology, Institute of Biomedical Science; E. Bergami, University of Siena / Physical, Earth and Environmental Sciences; W. Reina Joviano, A. Ribeiro Rosa, University of São Paulo / Cell and Developmental Biology, Institute of Biomedical Science; I. Corsi, University of Siena / Physical, Earth and Environmental Sciences; L. Marques dos Santos, Universidade Federal da Paraíba / Departamento de Biologia Molecular; J.M. Silva, University of São Paulo / Cell and Developmental Biology, Institute of Biomedical Science
Nanoparticles (NPs) are a major and growing environmental concerns for coastal marine environments. Among them, titanium dioxide (nano-TiO_2) are widely present in numerous products for everyday use as well as for human consumption, and can be occur at higher concentrations in marine coastal waters (< 1mg/L), where they represent a risk to the organisms. Sea urchins are considered excellent bioindicators of environmental and anthropogenic stressors, such as climate change and marine pollutants, respectively, especially during their embryonic development. Exposure to NPs in the early life stages mainly implies larval malformations and developmental arrest, which may compromise the recruitment of these

species. The present study aimed to evaluate the effects of nano-TiO_2 (0.005, 0.05, 0.5 and 5 µg/mL) on the fertilization and embryonic development of the sea urchin *Lytechinus variegatus*. The range tested also included low concentrations close to environmentally relevant scenarios. The toxicity of nano-TiO_2 was evaluated through spermiotoxicity and embryotoxicity tests, taking into account changes in fertilization and developmental rates, morphological (investigated by TEM - transmission electron microscopy) and morphometric parameters. A decrease in fertilization rate was observed only at the highest concentration (5 µg/mL). During the development of the gastrulae, nano-TiO_2 induced higher toxicity at the low environmentally relevant concentration of 0.005 µg/mL, with a large number of embryos with arrested development. In the development of larvae, the highest concentration was more toxic, with a large number of arrested development larvae (EC_{50} > 5 µg/mL). The measurements showed that nano-TiO_2 induced a decrease in the size of the post-oral arms of the larva pluteus at 28 hpf, especially at the highest concentrations (0.5 and 5 µg/mL). Large nano-TiO_2 agglomerates were observed around the larvae body. Taking into account that larvae are filter-feeding organisms, they could interact more easily with agglomerate-shaped NPs, resulting in a greater impact on development and growth. In conclusion, we showed the toxicity potential of nano-TiO_2 to the tropical sea urchin *L. variegatus*, with deleterious effects on fertilization rate and malformations and delays in the development of embryos and larvae, in concentrations ranging from environmentally- relevant to acute exposure scenarios.

1.02.16
Underlying Toxic Effects After a Short-Term Exposure of Rainbow Trout to Aluminosilicate Nanoparticles
E. Bernal, National Institute of Agricultural Research (INIA); M. Connolly, INIA-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria / Department of Environment and Agronomy; A. Valdehita, INIA -National Institute for Agricultural and Food Research and Technology / Department of Environment and Agronomy; M. Fernandez-Cruz, INIA - National Institute for Agricultural and Food Research and Technology / Environment
The use of aluminosilicate nanoparticles (AlSi NPs) within the aquaculture sector has significantly expanded and includes applications in water purification, wastewater treatment, as well as in feeding strategies to optimize growth or in fish feed to adsorb contaminants due to ion exchange and adsorption-desorption capacity. Because of the increasing use of AlSi NPs it is important to understand the impact on aquatic organisms. To our knowledge this is the first study addressing the acute toxicity and the induction of oxidative stress of AlSi NPs in fish. An *in vitro* and *in vivo* approach was used. To screen *in vitro* the toxicity of AlSi NPs (30-50 nm, from Torrecid, Spain), RTgill-W1 fish cell lines were exposed to a range of AlSi NPs concentrations (0,0002 mg/L to 2,000 mg/L) during 24 hours. The cytotoxicity was evaluated by the fluorimetric assays AlamarBlue, CFDA-AM and neutral red uptake (NRU), which measures the cell metabolism at mitochondrial level and the plasma and lysosome membrane integrities, respectively. To study the short-term toxicity in rainbow trout, a limit test following the OCDE Test Guideline 203 was performed. Seven fish were exposed to 100 mg/L of the AlSi NPs during 96 hours and the induction of the glutathione S-transferase (GST) and ethoxyresorufin- *O* -deethylase (EROD) activities were assessed in the liver and gill. The size of the AlSi NPs were characterised by dynamic light scattering (DLS) during the exposure period. In the cell culture medium AlSi NPs form big aggregates at concentrations higher than 200 mg/L. Those aggregates were bigger in aquarium water. The NRU assay couldn't be considered to study the cytotoxicity of AlSi NPs as interferences were observed. AlSi NPs were not cytotoxic to the RTgill-W1 cells at the mitochondrial or plasma membrane level. No deaths or abnormal behaviours were registered during the short-term exposure of fish. However, the EROD activity increased significantly in liver and the GST activity increased significantly in the liver and gills. The results indicate that although AlSi NPs do not produce a decrease in cell viability or mortality to fish *in vivo* sublethal effects were evidenced which should be considered in the long-term exposure to these nanoparticles.
Acknowledgements: INIA project RTA2015-00041-00-00. PEJ-2018-AI-AMB-10703, Sistema de Garantía Juvenil, CAM. Talent Attraction call of the Community of Madrid (2018-T2/AMB-11392, Mode 2, Young Doctor Recruitment).

1.02.17
Toxicity Assessment of Nanoparticulate TiO2 UV Filters Alone and in Binary Mixture With Organic UV Filters Using Fish Gill Cells (RTgill-W1)
N. Martin, University of Gothenburg / Department of Biological and Environmental Sciences; D. Slomberg, J. Labille, Aix Marseille University, CNRS, IRD, INRAE, Coll France, CEREGE, France; T. Lammel, University of Gothenburg / Department of Biological and Environmental Sciences
Since the 1950s health authorities advise people to use sunscreen to avoid harmful ultraviolet (UV) radiation, and sunscreen consumption is increasing ever since. Concomitantly, a cocktail of organic and inorganic UV filter substances contained in sunscreens has been, and is being, released in high amounts into aquatic environments. While there is a growing body of literature reporting adverse effects of traditional (organic) UV filter substances, there is still little knowledge on the toxicity of novel (inorganic) nanoparticulate UV filter substances on aquatic life, and on the combined effects of both UV filter classes when present as mixture. This study sought to determine the toxicity of TiO_2 nanoparticles (NP)-based UV filters with different surface chemistry (Eusolex® T-Avo: SiO_2-coated; T-Lite™ SF: $Al(OH)_3$/PDMS-coated; and Eusolex® T-S: Al_2O_3/stearic acid-coated) applied alone and as binary mixture with highly used organic UV filters (Octinoxate, Avobenzone, Octocrylene) to fish gill cells (RTgill-W1 cell line). The AlamarBlue, CFDA-AM and Neutral Red Retention (NRR) assays were used to assess effects on cellular metabolic activity, plasma membrane integrity and lysosomal integrity, respectively. In addition, transmission electron microscopy (TEM) was used to examine NP uptake by the cells. Our results show that all TiO_2 NPs were not cytotoxic at the concentrations tested (0.1-10 µg/ml; 24 h). The organic UV filters, however, showed dose-dependent cytotoxicity between 3.1 and 100 µM. For the mixtures, lower toxicity was generally observed compared to the corresponding single substance treatments, and the level of decrease depended on both NP-type and concentration. For some mixtures, the data show an increase in toxicity compared to the single substance treatments, yet experiments using a modified assay protocol suggest that this may be largely due to NP interference. TEM analysis showed that the hydrophilic T-Avo was endocytosed but the hydrophobic T-Lite SF and T-S were not. Our results suggest that nanoparticulate UV filters can adsorb organic UV filters co-existing in sunscreens and the environment to their surface and modulate their fate and toxicity. The degree of interaction with the organic UV filters as well as the uptake of the NPs/NP-chemical complexes by cells depends on the NPs' physico-chemical properties. More research is needed to shed lite on the interaction kinetics and the toxicological mechanisms underlying mixture effects.

1.02.19
Grouping Nanoforms With Differing Organic Surface Treatments Based on Coating Material Biodegradation Rates
R. Cross, UK Centre for Ecology & Hydrology / Pollution and Ecotoxicology; M. Matzke, UK Centre for Ecology and Hydrology; D. Spurgeon, Centre for Ecology & Hydrology; M. Diez-Ortiz, Leitat Technological Center; V. Gonzalez-Andres, LEITAT Technological Centre; M. Esponda, LEITAT Technological Centre / Human & Environmental Health & Safety; M.F. Esponda, LEITAT Technological Centre; C. Svendsen, CEH, Wallingford
Understanding how modulation of key extrinsic properties of nanoforms (NFs), through the application of different surface treatments, affects exposure and hazard is a priority to address within grouping efforts. One hypothesis to assess NFs with different organic surface modifications is to group according to the biodegradation of the organic surface treatment. This approach has been developed within GRACIOUS. The hypothesis contends that NFs with a similar core chemistry, but with different organic surface treatments may be grouped if the surface treatment is likely to be lost through biodegradation, and therefore no longer modulates fate, exposure and toxicity of the NF. To test this hypothesis, an IATA has been developed that includes an adaptation of a colorimetric carbon substrate utilisation assay for organic molecule biodegradation to screen for coating material biodegradation by wastewater activated sludge microbes. This assay has been tested with 20 common surface treatments including synthetic and natural polymers, non-ionic and ionic surfactants and stabilisers and benchmarked against OECD TG301 results. **Results** Results for common nanomaterial surface treatments from both the colorimetric screening assay and OECD TG301 identified ready, partial and non-biodegradable substances. Citric acid (readily biodegradable) and Polyvinylpyrrolidone (non-biodegradable) provided the upper and lower dynamic range of the assay. The carbon substrate utilisation assay was shown to be well suited to early tier screening, while OECD TG301F data provide confirmatory support and verification prior to movement to higher tiers. **Conclusions** This study demonstrates both the conceptual basis of an IATA for grouping nanoforms based on organic coating degradation and presents an

adapted screening assay that can be used as part of a tiered testing strategy associated with this IATA.

Eco- and Human Neurotoxicology

1.03.04
Lack of Methodological Consistency Impedes Interpretation of Developmental Neurotoxicity Larval Zebrafish Behavioral Assays
B. Hill, ORISE Research Participant - EPA; K. Britton, ORAU at US EPA; D.L. Hunter, J. Olin, M. Lowery, US EPA / ORD CCTE BCTD; S. Padilla, U.S. Environmental Protection Agency / ORD CCTE BCTD
There is a need for new consistent approaches in developmental neurotoxicity (DNT) screening due to the tens of thousands of chemicals requiring risk determinations. Many laboratories are using zebrafish (*Danio rerio*) as an alternative vertebrate model to conduct DNT tests, but without a standardized protocol for larval behavioral assays, comparison of results among laboratories is hampered. To determine if there is a consistent experimental design among laboratories, we conducted a robust literature review covering DNT studies for 48 chemicals focusing on zebrafish larval behavior assays as an endpoint. This allowed us to compare the similarity of the experimental protocols among laboratories and understand exactly which methodological information was commonly reported. Publications were gathered from PubMed via Abstract Sifter (Baker et al. 2017), screened for relevance, and critiqued by two independent reviewers. Our initial round of review focused on 65 unique methodological variables where chemical exposure occurred in early development and subsequent locomotor assays included a light/dark photoperiod transition. Our detailed review first revealed a troubling issue: often basic variables were unclear or not reported. There was not a single publication that reported 100% of our targeted variables. Only 62-88% of these variables were reported in the reviewed publications. Moreover, while there were some aspects of the experimental design that were consistent, no experimental design was the same among any of the publications. On a variable by variable basis, only one variable was the same among all papers selected: in all cases the chemical was introduced within the first 24 hours after fertilization, whereas pH of media was the least reported variable. An attempt was made to compare behavior results on a chemical by chemical basis, however this was further complicated by the inconsistencies within each experimental method which led to differences in statistical approaches and data reporting. Overall, there is not only a strong need for the development of a standardized testing protocol for larval zebrafish locomotor assays, but also a standardized protocol for reporting experimental variables in the literature. *This abstract may not necessarily reflect official Agency policy.*

1.03.05
An Inter-Laboratory Case Study to Determine the Added Value of the Zebrafish Light-Dark Transition Test to Predict Developmental Neurotoxicity
A. Alzualde, BBD BioPhenix S.L.; J.H. Hsieh, NIHNIEHS / NTP; L.D. Ellis, National Research Council of Canada / Aquatic and Crop Resource Development; V. Schiavone, ZECLINICS, S.L.; L. Truong, Oregon State University / EMT; J. Legradi, Vrije Universiteit Amsterdam; D. Rubbini, ZeClinics; C. Woodland, Government of Canada- Health Canada / Health Canada; N. Kluver, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology; K. Ryan, National Institute of Environmental Health Sciences; M. Behl, NIEHS-NIH; J. Terriente, ZECLINICS SL; A. Muriana, BioBide / R&D Department; R.L. Tanguay, Oregon State University / Environmental and Molecular Toxicology; M. Sachana, European Commission Joint Research Centre; A. Price, Joint Research Centre - EC / F3-Chemical Safety and Alternative Methods Unit-EURL ECVAM; B.N. Hill, Baylor University; S. Padilla, U.S. Environmental Protection Agency / ORD CCTE BCTD; T. Shafer, US EPA; E. Hessel, RIVM
Developmental neurotoxicity (DNT) entails one of the most complex areas in toxicology. Development of the central nervous system is a complex process involving many different events within strictly controlled time frames and therefore each event might create a different window of vulnerability to chemical exposure. OECD test guidelines for DNT (TG 426 and 443) are only occasionally carried out and the predictivity of these *in vivo* animal tests for human health effects may be limited. There is a high need for human-relevant *in vitro* models to assess DNT potential of chemicals. OECD is, therefore, building a guidance document containing a testing strategy to predict DNT. This testing strategy consists of a combination of *in vitro* tests encompassing the critical processes in brain development. The aim of this study is to investigate the added value of the zebrafish DNT behavioral model in this testing strategy. Up until 120 hours post-fertilization (hpf) zebrafish are not considered as experimental animals under the current European animal directive (2010/63/EU). The neurological system, the different neuron types, and neurotransmitters are well studied and well conserved among zebrafish and other species, including humans. The advantage of the zebrafish model in comparison to other *in vitro* assays is that whole brain development occurs within a relatively short period and effects of chemicals on brain development and behaviour can be tested. A group of experts agreed on a protocol for the light-dark transition test to predict DNT. At 120 hpf, zebrafish are tested in the light-dark transition test after chemical exposure from 6-120 hpf in a 96 well plate, 7 concentrations and 12 larvae per concentration. 28 known DNT compounds will be tested in five different laboratories. Data analysis will focus on locomotor activity (distance moved) of the larvae during the testing period. Benchmark dose analysis will be performed to determine the critical effect dose of each compound and for comparison of results across laboratories. Based on the results of this inter-laboratory case study, the robustness, biological domain and addition of the light dark-transition assay to predict DNT will be discussed and determined. The future goal is to add the zebrafish DNT assays to the OECD guidance document and to use this model to predict DNT. (This abstract does not reflect EPA policy).

1.03.09
Mechanistic Evaluation of Ethyl-Parathion Toxicity in Mammalian Exposures to the Neat and Dust-Incorporated Compound
K.A. Gust, U.S. Army Engineer Research and Development Center / Environmental Laboratory; S. Amar, Us Army Engineer Research & Development Center / Environmental Lab; D.R. Mattie, US Air Force Research Laboratory; R. James, T.D. King, D.M. Holtzapple, US Navy / Naval Medical Research Unit; M.A. Chappell, A.J. Kennedy, R.M. Styles, US Army Engineer Research and Development Center / Environmental Laboratory; R.R. Karna, Bennett Aerospace Inc. / Environmental Laboratory Sciences
The US Department of Defense (DOD) is increasingly concerned about the use of toxic industrial compounds (TICs) in improvised chemical weapons attacks. The present study is poised to provide important environmental context regarding the toxicological impacts of acute exposure to a TIC of high concern, the acetylcholinesterase-inhibiting organophosphorus (OP) pesticide, ethyl-parathion. Specifically, effects are being characterized for acute inhalation of the neat compound, but also for parathion that has been incorporated into the dust-fraction of soil representing exposure pathways relevant to the DOD mission, but also occupational exposures in agriculture. To accomplish this, mammalian inhalation exposures are being conducted in male Sprague-Dawley rats exposed to ethyl-parathion at 0 mg/m^3 (control), 1.0 mg/m^3, 10.0 mg/m^3, and 20.0 mg/m^3 as neat chemical exposures and incorporated into dust. The 4 hour exposure involves one group of animals examined immediately after exposure and a second group examined after 48-hours of recovery. Examinations will include collection of respiratory parameters as well as a battery of neurobehavioral tests to gauge effects on locomotor activity and coordination. Histopathology examination will be conducted on the primary target tissues, lung and brain, as well as examination of nasal airways, trachea and larynx. Organ weights will also be measured including, brain, liver, kidneys, and adrenal glands. Samples of brain, liver, and lung tissues will also be collected for transcriptomics and biochemical assays. Global transcriptomic expression will be examined using RNAseq assays to identify genes, pathways and signaling networks affected in the parathion exposures. Additionally, enzymatic tests will be conducted to quantify levels of acetylcholinesterase, acetylcholine, and additional targets identified by RNAseq analysis to integrate biochemical, tissue-level and whole-animal effects across the adverse outcome pathway (AOP) for acetylcholinesterase inhibition. The results will provide fundamental information about the toxicity of ethyl-parathion from an important yet unstudied exposure source, inhalation of the dust-incorporated compound.

1.03.10
Biomarkers of Neurotoxic Effects of Fipronil As a Model Biocide in Human Neuroblastoma SH-SY5Y Cells
S. Kaisarevic, D. Tenji, I. Vulin, T. Tomic, University of Novi Sad Faculty of Sciences / Department of Biology and Ecology; I. Teodorovic, University of Novi Sad / Department of Biology and Ecology
Fipronil is a highly effective neuroactive phenylpyrazole insecticide with primary mode of action attributed to its ability to block chloride channels of the $GABA_A$ receptors, resulting in neuronal hyperexcitation and death in target organisms. It is widely used in control of many agricultural and domestic pests, leading

to environmental contamination and exposure of nontarget organisms, including humans. In recent environmental studies, fipronil has been recognized as important contaminant in the aquatic environment. In this study, it was used as a model biocide, to contribute to database on neurotoxicity patterns which could be used in characterization of environmental contaminants. We investigated neurotoxicity profile of fipronil on human neuroblastoma SH-SY5Y cells. Selected concentrations (including environmentally relevant) were used for gene expression analysis (RQ-PCR) of biomarkers of neurotoxicity. The analysis was conducted for genes encoding some of the key elements in neurotransmitter pathways such as neurotransmitter receptors (nicotinic, muscarinic, dopamine, GABA receptors), acetylcholineesterase (AChE) and monoamine oxidase (MAO_A and MAO_B), as well as protein involved in exocytosis of neurotransmitters (synaptotagmin 10). The resulting neurotoxicity signature of fipronil is a contribution to the knowledge on mechanistic data on fipronil toxicity in human. The responsive biomarkers will be associated with the molecular initiating events (MIEs) or key events (KEs) from the adverse outcome pathways (AOP_S) available in the AOP-Wiki, to imply to possible outcomes of the fipronil exposure. Moreover, the disturbances in the expression of responsive genes will be discussed as possible biomarkers of neurotoxic effects of other environmental contaminants and mixtures of compounds with unknown mode of action. *Acknowledgements:* This research was supported by the Science Fund of the Republic of Serbia, PROMIS, Grant No. 6061817, BIANCO. The abstract content is the responsibility of the Faculty of Sciences University of Novi Sad, and it does not reflect the opinion of the Science Fund of the Republic of Serbia.

1.03.12

Cytotoxicity Induced by Pure Cylindrospermopsin in E17 Embrionic Mice Primary Neuronal Cells

A. Prieto, Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, Profesor García González n◦2, 41012 Seville, Spain / Toxicology; M. Hinojosa, University of Sevilla / Toxicology; C. Muñoz-Castro, University of Sevilla / Departamento de Bioquímica y Biología Molecular; M. Sánchez-Mico, Massachusetts General Hospital and Harvard Medical School / Department of Neurology; J. Vitorica, University of Sevilla / Departamento de Bioquímica y Biología Molecular; A. Jos, A.M. Cameán, Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, Profesor García González n◦2, 41012 Seville, Spain / Toxicology

Cylindrospermopsin (CYN) is an emerging cytotoxin produced by different cyanobacteria species such as *Aphanizomenon ovalisporum*. CYN has been shown to induce mainly renal and hepatic toxic effects in different species. However, it has been also reported to exert damage in the nervous system, in spite of not being considered a neurotoxin. The studies focused on its neurotoxic potential as well as their potential contribution to neurodegenerative diseases are very scarce. To our knowledge, no studies have been performed in primary neuronal cultures so far. The aim of this study was to investigate the cytotoxicity of pure CYN on mice primary neuronal cultures assessing the influence of concentration and exposure time. Thus, primary hippocampal neuronal cultures were obtained from E17 embryonic CD1 mice. The neuronal cultures were kept in neurobasal medium supplemented with B27 for 21 days prior toxin exposure. Concentrations of 0.25, 0.5 and 1 µg CYN/ ml were added to the neuronal cultures during 12, 24 and 48 hours of exposure. For the negative and positive control, normal medium with and without B27 were added respectively. For immunocytochemistry, primary (mouse anti-NeuN and rabbit anti-Microtubule associated protein 2) and secondary (Alexa fluor™ 546 donkey anti-rabbit Ig G and 488 donkey anti-mouse IgG) antibodies were added. After washing, cells were mounted on coverslips, observed with a Zeiss apotome epifluorescence microscope and quantified using ImageJ (FIJI). Two assays in duplicate were performed. After 12 hours of exposure, CYN only affected neurons exposed to the highest concentrations (0.5 and 1 µg / ml), reducing their viability up to 65% in both exposed groups compared to the negative control. However, CYN was shown to produce toxic effects in all exposed groups (0.25, 0.5 and 1 µg / ml) after 24 and 48 hours of exposure. At 24 hours, the observed viability was 83%, 59% and 30% compared to the control group, respectively. In addition, the lowest CYN concentration reduced neuronal viability to 30% after 48 hours of exposure. This study reports, for the first time, that CYN produces toxicity in primary neuronal cultures in a time and concentration dependent manner.
Acknowledgments: Spanish Ministerio de Economía y Competitividad (AGL2015-64558-R, MINECO/FEDER, UE) and Spanish Ministerio de Ciencia e Innovación for the project PID2019-104890RB-I00 MICINN.

Effects of Contaminants in Wildlife: Endocrine Disruption, Immunomodulation, Oxidative Stress and Other Biomarker Responses

1.04.01

Measurement of Telomere Length in the European Flounder From Seine Estuary: Investigating a Link Between Telomere Length, DNA Damage and Chemical Body Burden

F. Akcha, IFREMER / 44; C. Cahuc, J. Rouxel, C. Munschy, Y. Aminot, T. Chouvelon, IFREMER / Unité Biogéochimie et Ecotoxicologie; K. Mahe, IFREMER / Laboratoire Ressources Halieutiques; H. Budzinski, University of Bordeaux, CNRS / UMR 5805 EPOC, LPTC; a. Mauffret, IFREMER / Unité Biogéochimie et Ecotoxicologie

Estuarine and coastal marine systems receive complex mixtures of chemical contaminants that can display various adverse biological effects in exposed organisms, affecting the biodiversity and the functioning of these valuable ecosystems. The environmental, sanitary and socio-economic issues raised by chemical pollution drove European Water Policies to adopt the Marine Strategy Framework Directive (MSFD) in order to protect the marine environment. Among the descriptors of anthropic pressure defined within the MSFD, descriptor 8 is especially addressing chemical pollution thanks to the implementation of monitoring programmes. In addition to chemical analysis, pollutant effects are studied by measuring selected biomarkers in sentinel species. In this context and with the aim to propose new biomarkers, it appeared valuable to investigate in flatfish the impact of pollution on telomeres. Poorly investigated until now in ecotoxicology, telomeres protect the coding sequence of chromosome ends and play a key role in genomic stability. In human, telomere length (TL) has been proposed as a biomarker of cellular aging, cumulative stress exposure and life span. By adapting a q-PCR protocol, relative and absolute TL were measured in the blood of two-year-old flounders from the English Channel. DNA damage was also measured in the erythrocytes by the comet assay. Several trace metal elements in the liver, hydroxylated metabolites of polycyclic aromatic hydrocarbons (PAHs) in the bile, and polychlorobiphenyls (PCBs), organochlorinated pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), perfluoroalkyl substances (PFASs) and mercury in the muscle were also analysed to investigate correlations between contaminant levels and both DNA damage and TL. Results showed that the European flounder has short telomeres compared to other fish species. Differences in TL have however already been reported depending on the methodology used for TL measurement. Whatever considering relative or absolute TL, no correlation was observed with DNA strand breaks level and any of the measured contaminant concentrations. Because the number of samples was limited in this study, further investigations are required to state on a possible impact of chemical pollution on flatfish telomeres. This is also motivated by the correlations observed with some organochlorinated compounds (PCBs, DDT, OCPs) when decreasing statistical significance (from $\alpha = 0.05$ to 0.10).

1.04.07

Metal-Induced Effects on Physiology of Wild Red-Necked Nightjars (Caprimulgus ruficollis)

S. Espin, P. Sánchez-Virosta, University of Murcia / Department of Health Sciences, Area of Toxicology; J. Zamora-Marín, University of Murcia / Department of Zoology and Physical Anthropology, Faculty of Biology; M. León-Ortega, University of Murcia / Department of Ecology and Hydrology, Faculty of Biology; P. Jiménez, University of Murcia / Department of Health Sciences, Area of Toxicology; A. Zamora-López, University of Murcia / Department of Zoology and Physical Anthropology, Faculty of Biology; P. Camarero, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM) / Toxicology in wildlife; R. Mateo, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM); T. Eeva, University of Turku / Department of Biology; A. Garcia-Fernandez, University of Murcia / Health Sciences

Nightjars are considered human-tolerant species due to the population densities reached in strongly managed landscapes. However, the potential effects of metals on physiology, condition or fitness have not been studied in any nightjar species so far. This presentation shows how metal exposure affects physiology and condition in red-necked nightjar (*Caprimulgus ruficollis*) populations inhabiting three different environments in SE Spain: agricultural-urban, mining and control areas. In the mining-

impacted environment, nightjars showed decreased retinol and albumin, probably impaired by a combination of toxic metal exposure and low prey quantity/quality in that area. Moreover, they showed increased plasma tocopherol levels which may be a response to cope with metal-induced oxidative stress and lipid peroxidation. Blood concentrations of toxic elements (As, Pb, Cd and Hg) were negatively associated with Ca, P, Mg, alkaline phosphatase (ALP), total proteins and body condition index. This could lead to metal-related disorders in mineral metabolism and ALP activity that may potentially increase the risk of skeletal pathologies and consequent risk of fractures in the long term, compromising the survival of individuals. *Acknowledgments*: This work was supported by the *Fundación Séneca* (20031/SF/16). S. Espín was financially supported by *Fundación Séneca* and by *Ministerio de Ciencia, Innovación y Universidades* (IJCI-2017-34653). In loving memory of Prof. Richard F. Shore.

1.04.08

Metal-Driven Effects on Vitamins a, E and D3 in Birds: A Review on Avian Ecotoxicology

S. Espin, University of Murcia / Department of Health Sciences, Area of Toxicology; P. Sanchez Virosta, University of Turku / Biology

Vitamins A, E and D3 are fat-soluble nutrients involved in multiple physiological functions (e.g., immune function, vision, reproduction, growth and development). Some toxic elements (Pb, Cd, Hg, As) have been broadly studied in avian species and are well known for their accumulation capacity and deleterious effects, including alterations in vitamin levels. Publications reporting metal-induced effects on vitamins in birds are growing and, in some cases, results seem contradictory, making it difficult to interpret them. Therefore, a clearer view of the overall picture is needed. This communication presents a mini-review compiling relevant data and describing current knowledge on the effects of the most toxic elements (i.e., Pb, Cd, Hg, As) on vitamins A, E and D3 in birds. Although vitamins are diet dependent, they are strongly regulated and transformed in the organism, and metal-related disruption in their homeostasis may trigger disorders in different directions. Moreover, vitamin status and form *in vivo* is the result of complex interacting processes in the organism and metal exposure may produce cascade effects. Different potential factors that may contribute to the variable response of vitamins to metals in birds are also presented. Some final remarks and recommendations are provided for future studies. This communication shows an overview of the current knowledge in metal-induced alterations in vitamins of special concern for avian ecotoxicology, a research discipline facing important challenges in the coming years. *Acknowledgments*: S. Espín was supported by *Ministerio de Ciencia, Innovación y Universidades* (IJCI-2017-34653). In loving memory of Prof. Richard F. Shore.

1.04.09

Effects of Toxic Elements on Plasma Vitamin Levels in Nestling Eagle Owls (Bubo bubo)

P. Sanchez Virosta, University of Turku / Biology; M. León-Ortega, J. Calvo, University of Murcia / Department of Ecology and Hydrology, Faculty of Biology; P. Camarero, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM) / Toxicology in wildlife; R. Mateo, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM); M. Zumbado, O.P. Luzardo, University of Las Palmas de Gran Canaria / Toxicology Unit, Research Institute of Biomedical and Health Sciences; T. Eeva, University of Turku / Department of Biology; A. Garcia-Fernandez, University of Murcia / Health Sciences; S. Espin, University of Murcia / Department of Health Sciences, Area of Toxicology

Vitamins and carotenoids play essential roles in nestling growth and proper development, and are known to be affected by toxic metals. The main goal of this communication is to present how element exposure affects plasma vitamin and carotenoid levels in nestling Eagle owls (*Bubo bubo*) inhabiting three different scenarios of pollution (mining, industrial and control areas) in southeastern Spain. Our results show that local contamination in the mining area contributes to increased blood concentrations of Pb, As and Tl in nestlings, while diet differences between control and mining/industrial areas may account for the different levels of Mn, Zn, and Sr in blood, and lutein in plasma. Plasma tocopherol levels were increased in the mining-impacted environment, which may be a mechanism of protection to prevent toxic-element-related oxidative stress. Plasma α-tocopherol was enhanced by 20 and 56% at blood Pb concentrations ≥8 ng/mL and ≥170 ng/mL, respectively. Tocopherol seems to be a sensitive biomarker under an exposure to certain toxic elements (e.g. Pb, As, Tl). *Acknowledgments*: Funding was provided by *Fundación Séneca* (20031/SF/16) and University of Castilla-La Mancha (CGT13-0183). S. Espín was supported by *Fundación Séneca* (20031/SF/16) and by *Ministerio de Ciencia, Innovación y Universidades* (IJCI-2017-34653). P. R. Camarero benefited from a contract financed by the Spanish Ministry of Economy and Innovation and CSIC (PTA2017-14583-I). In loving memory of Eloy Pérez and Prof. Richard F. Shore.

1.04.10

Oxidative Stress and Lead Toxicity in Migrant and Resident Populations of Turkey Vulture (Cathartes aura)

A. Gresham, California State Polytechnic University, Pomona / Biological Sciences; M. Saggese, Western University of Health Sciences / College of Veterinary Medicine; P. Bloom, Bloom Biological Incorporated; A. Bonisoli-Alquati, California State Polytechnic University, Pomona / Department of Biological Sciences

Oxidative stress is a result of an imbalance between reactive oxygen species and antioxidants in the body. In birds oxidative stress is known to increase during migration, leaving individuals in a deficit for fat stores and antioxidants. The Turkey Vulture (*Cathartes aura*) is an obligate scavenger with populations ranging from Canada to South America. Some Turkey Vulture populations are resident, while others are migrants, and may experience higher oxidative stress and its health effects. Another stressor Turkey Vultures may face is lead (Pb) toxicosis, caused by ingesting Pb ammunition embedded in carcasses left behind by hunters. The State of California recently banned Pb ammunition for hunting purposes. Yet, migratory populations of Turkey Vultures are not necessarily protected due to potential Pb exposure outside of California's borders. Additionally, oxidative stress from costly migratory behavior might make migrating birds even more susceptible to Pb toxicosis. To study how Pb exposure and effects vary with migratory behavior, we are capturing vultures from migratory and resident populations in Southern California and quantifying blood Pb levels and plasma markers of oxidative stress. These biomarkers include total glutathione, the main intra-cellular antioxidant; protein carbonyls, a marker of oxidative damage to proteins; and ∂ALAD, an enzyme that is inhibited in the presence of Pb. We expect that (I) migrants will have higher blood Pb concentrations, and (II) higher oxidative stress because of the metabolic cost of migration. Additionally, we predict vultures with high blood Pb levels will have (III) lower ∂ALAD levels and (IV) higher oxidative stress, because limited antioxidant resources may be allocated to combat either Pb toxicosis or the metabolic results of flight, but hardly both. Our study will test the use of Turkey Vultures as sentinels of Pb toxicity, enabling us to monitor Pb in terrestrial ecosystems. It will also test the hypothesis that local environmental measures at local and regional scales inadequately address the conservation needs of long-distance migrants, whose ranges exceed state and country borders.

1.04.11

Circulating MicroRNAs As Potential Biomarkers of Pollution in Wildlife

A. Brand, Norwegian University of Science & Technology (NTNU) / Biology; M. Klaassen, Deakin University; C. Waugh, Nord University; V. Jaspers, Norwegian University of Science & Technology / Biology

Migratory shorebirds in the East Asian Australian flyway (EAAF) are facing significant conservation threats, e.g. habitat degradation, pollution and infectious disease exposure. Coincidentally, many of these shorebird populations are experiencing severe population declines. Although several studies report pollutant concentrations in shorebirds, the contribution of this stressor to their health and survival remains unclear. The detrimental effects of pollution exposure might further be exacerbated in these birds due to their energy-demanding annual extreme migrations, where lipophilic pollutants can redistribute in tissues and potentially cause toxicity. Recently, microRNAs have been identified as potential biomarkers of pollutant toxicity. MicroRNAs are a class of small noncoding RNAs, which are involved in the regulation of gene expression at the posttranscriptional level. MiRNAs can be detected in various biological matrices, including blood. They play an important role in intercellular communication, and therefore have great potential as minimally invasive biomarkers of effect. Alternations in microRNA expression have been associated with exposure to a wide variety of pollutants, including persistent organic pollutants and metals. Recent *in vitro* findings suggest that pollution exposure can alter disease susceptibility by modulating the expression of important immune-related miRNA pathways in birds, but this must be further explored. We therefore aim to investigate the potential of microRNAs as biomarkers pollution exposure in free-living migratory shorebirds. Targeted compounds (both legacy and emerging contaminants) in blood samples of EAAF migratory shorebirds

will be extracted and quantified. High-throughput sequencing technology will be used to identify microRNAs in paired blood samples. Pollution and microRNA data will be combined to investigate potential microRNA signatures of pollution exposure. The identification of associations between pollution exposure and specific microRNA profiles could (i) lead way to novel biomarkers of exposure and toxicity, as well as (ii) provide new insights into the molecular mechanisms that form the pathway to an adverse health outcome and (iii) offer a way of assessing pollution exposure effects in free-living birds of conservation concern. The first data will be obtained early in 2021 and we wish to present our preliminary findings during SETAC Europe 2021.

1.04.12

Impact Assessment of Point and Diffuse Contaminant Sources in Feral Fish in the Holtemme River Using Physiological and Molecular Biomarkers

B. Deutschmann, RWTH Aachen University / Institute for Environmental Research, Department of Ecosystem Analysis (ESA); M.T. Schmitz, Goethe University Frankfurt am Main / Evolutionary Ecology and Environmental Toxicology; N. Markert, RWTH Aachen University / Institute for Environmental Research, Department of Ecosystem Analysis (ESA); T. Backhaus, University of Gothenburg / Department of Biology and Environmental Sciences; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis; M. Brauns, Helmholtz Centre for Environmental Research UFZ / Department of River Ecology; M. Brinkmann, University of Saskatchewan / School of Environment and Sustainability and Toxicology Centre; T. Seiler, Hygiene-Institut des Ruhrgebiets / Directorate; P. Fink, Helmholtz Centre for Environmental Research UFZ / Departments of River Ecology and of Aquatic Ecosystem Analysis and Management; S. Beitel, J.A. Doering, University of Saskatchewan / Toxicology Centre; M. Hecker, University of Saskatchewan / School of the Environment & Sustainability and Toxicology Centre; Y. Shao, Chongqing University; T. Schulze, Helmholtz centre for environmental research - UFZ / Effect-Directed Analysis; M. Weitere, R. Wild, Helmholtz Centre for Environmental Research UFZ / Department of River Ecology; M. Velki, Josip Juraj Strossmayer University in Osijek, Department of Biology; H. Hollert, Goethe University Frankfurt / Department Evolutionary Ecology and Environmental Toxicology, Faculty Biological Sciences

It is possible to identify toxicity drivers in environmental mixtures of contaminants through approaches that integrate chemical monitoring and bioanalytical methods paired with mechanistic toxicology. Such approaches hence allow causal links of pollution to ecologically relevant endpoints. The present study applied a comprehensive set of biomarkers, including several biochemical endpoints and whole transcriptome analysis in wild brown trout (*Salmo trutta fario*). Trout were collected along a pollution gradient (characterized in a parallel study) in the Holtemme River, Germany, that receives effluents from two wastewater treatment plants (WWTP) and run-off from agricultural land use. A cumulative assessment of the acute and chronic ecotoxicity using the toxic unit approach indicated a high contribution of the pharmaceuticals diclofenac and carbamazepine to the overall chronic toxic stress. Compared to fish from the reference site, trout collected downstream of the WWTPs showed a significant increase in micronucleus formation, phase I and II enzyme-activities, and oxidative stress parameters. Due to the variability in individual biomarkers responses, we could not identify a "most impacted site". However, by integrating single biomarker responses into an Aggregated Biomarker Response (ABR) approach, the contribution of the two WWTPs to the observed toxicity could be clearly differentiated. The ABR results were supported by the chemical analyses and whole transcriptome data, which revealed alterations of steroid biosynthesis and associated pathways, including an antiandrogenic effect, as some of the key drivers of the observed toxicity. Overall, this combined approach of *in situ* biomarker responses complemented with molecular pathway analysis allowed for a comprehensive ecotoxicological assessment of the Holtemme River. This study provides evidence for specific hazard potentials caused by mixtures of agricultural and WWTP derived chemicals at sublethal concentrations. The use of aggregated physiological and molecular biomarker responses combined with chemical analyses enabled an evidence-based ranking of sites with different degrees of pollution according to toxic stress and observed effects.

1.04.13

Progress and Trends in Ecotoxicology of Amphibians and Reptiles in Latin America

O. Cruz-Santiago, Universidad Autonoma de San Luis Potosi / Centro de Investigación Aplicada en Ambiente y Salud (CIAAS-CIACyT); C.A. Ilizaliturri-Hernandez, Universidad Autonoma de San Luis Potosi / Centro de Investigación y Aplicación en Ambiente y Salud (CIAAS); D.J. Gonzalez-Mille, Universidad Autonoma de San Luis Potosi / Cátedras CONACyT; G. Espinosa-Reyes, Universidad Autonoma de San Luis Potosi / Centro de Investigación Aplicada en Ambiente y Salud (CIAAS-CIACyT); D. Godinez Bravo, Universidad Autonoma de San Luis Potosi / Facultad de Ciencias

This work reviews the advances in Ecotoxicology over the last few years, focusing mainly on the published works on amphibians and reptiles of Latin America. Amphibians are susceptible to environmental pollutants, making them good bioindicators of environmental health. In contrast, pesticide and metal accumulation processes have also been confirmed to be related to impaired reproduction and other effects on reptiles. Thus, these species' use as bioindicators allows us to have a clearer idea of the direct and indirect repercussions of environmental pollution. In this context, the objective of this study is to review the different studies that have been published in recent years (1985-2019), focusing mainly on the studies carried out on amphibians or reptiles, thus identifying the main gaps that exist for these two species in the field of ecotoxicology in Latin America.

We found 292 articles published on ecotoxicology of amphibians and reptiles in Latin America, of which 196 are studies carried out on amphibians and 98 are studies carried out on reptiles. According to the above, for amphibians, of 196 published studies, 57.14% belong to Argentina, 17.85% to Brazil, 12.24% to Mexico, 7.14% to Colombia, 3.06% to Costa Rica, 1.53 % to Chile, and 1.02% to Ecuador, and of 98 studies published on reptiles, 39% belong to Mexico, 31% to Brazil, 13% to Argentina, 7% to Costa Rica, 5% to Belize, 3% to Colombia, and 1% for both Chile and Panama.

Among amphibians, the most studied pollutant class has been pesticides with 43.38%, followed by metals, organochlorines, pH, radiation, nitrogen compounds, endocrine disruptors, and PCBs, respectively. The most studied pollutant class for reptiles are heavy metals with 42.71%, followed by organochlorine, pesticides, PCBs, and PAHs.

In general, we can conclude that there has not been a considerable advance and development of the studies carried out on the ecotoxicology of amphibians and reptiles in Latin America compared to other classes of vertebrates. However, we hope that in the coming years, the number of published studies will increase. In this context, we hope that this study can help educational institutions and research centers gradually develop more studies aimed at the classes of pollutants, taxonomic groups, and ecosystems neglected by most centers of research on amphibians reptiles.

Endocrine Disrupting Chemicals in Vertebrates: New Developments and Challenges

1.05.01

An In Silico Battery to Rule out Chemicals With No Endocrine Disrupting Potential on Known Targets

F. Bauer, Z. Todorov, P. Thomas, KREATiS

The number of chemicals to which humans and nature are exposed is increasing, leading to serious health issues reflecting the need for a widespread assessment of endocrine disrupting (ED) properties. Regulations have been updated or are under review, but the proposed methods are complex, time consuming and expensive, making rapid evaluation impossible. The potential for ED is primarily dependent upon the capacity of a chemical to form a ligand-protein complex. No complex, no ED modality. Thus, we propose an *in silico*, 3-step battery to deprioritise chemicals for which no ED potential on known targets can be detected for oestrogenic, androgenic, thyroidal and steroidogenic (EATS) modalities:1. iSafeRat® ED SAR, a structural alert (2D) scheme,2. OTS: a set of external structural alerts models,3. 3D molecular docking : a pipeline compiling and developing various molecular modelling software to assess the binding potency of chemicals with EATS targets.This battery will be complemented by a more precise 4D analysis, using molecular dynamics simulations.iSafeRat® ED SAR (presented at last SETAC EU annual meeting) has been updated using ToxCast *in vitro* assays, now including robust rules to identify substances with no potential for specific EATS endpoints. For instance, one of the structural alerts for progesterone receptor antagonism has been trained with a subset of 87 chemicals of which 100% tested negative.OTS battery includes 32 models for various endpoints. These models are validated internally so that the reliability of each alert can be assessed. A consensus prediction is given for each test substance based on the reliable alerts only.The first version of the molecular docking pipeline relies on experimental structural data for human EATS

proteins. Given the scarcity of these inputs, our strategy is to generate missing conformations of the ED targets with the help of molecular dynamics simulations. The first version of our pipeline will be tested beginning 2021.If the consensus of the three steps of our battery indicates that a substance has no disrupting potential on studied EATS pathways, it provides strong evidence to allow deprioritisation and to focus testing on substances with suspected endocrine activity.

1.05.02
Characterization of In Vitro Models of Thyroid and Developing Brain Towards Their Application for the Assessment of Thyroid Hormone Disruption
O. Brozman, J. Novak, Masaryk University, Faculty of Science / RECETOX; K. Hilscherova, Masaryk University, Faculty of Science, RECETOX / RECETOX
Anthropogenic and environmental endocrine disruptive pollutants have been studied for a long time. However, thyroid hormone system (THS) has been rather neglected so far, even though the dysregulation of THS had been linked with adverse health effects such as carcinoma development and (neuro)developmental changes. The reason for such insufficient THS knowledge is the lack of suitable high throughput-compatible bioassays. Nowadays, several initiatives are trying to close this gap. The H2020 ERGO project aims to develop a battery of *in vitro* bioassays and/or alternative approaches respecting the "3R" principle. An important step in such process is to characterize the relevance of *in vitro* systems and evaluate their suitability for studying different endpoints. The endpoints within THS have been prioritized based on Adverse Outcome Pathway (AOP) network and they cover processes on a level of hormone synthesis, transport, metabolization and signaling. Thyroid hormone regulation is complex, with different organs involved. Thus, in this study, two *in vitro* models with potential to assess the impact of THS disruptors on thyroid and developing brain are being characterized regarding their applicability to study effects on a range of molecular initiating events and key events relevant for AOP network. Human thyroid-derived Nthy-ori 3-1 cell line is used as the model for human thyroid, and human neural stem cell line NSC-H9 as a brain model at different levels of differentiation. To prove the suitability of these cell models, they have been characterized regarding the expression of the prioritized set of 18 genes, representing the molecular initiating events or key events based on AOP network being developed within ERGO. These included genes coding proteins involved in hormone synthesis (e.g. TPO, TSHR, IYD, DUOX and NIS), transport (MCT-8 and -10, OATP1C1), metabolism (e.g. DIO-1, -2 and -3) and receptor activation (e.g. TR-a and -b). The gene expression profile from the studied *in vitro* models was compared with the profile derived from human brain and thyroid gland *ex vivo* samples. The comparison provides an important background information for the development of the *in vitro* cell-based battery of bioassays within the ERGO project and demonstrates the applicability of the characterized cell models for the assessment of the disruption of THS. This research has received funding from the EU H2020 research and innovation programme under grant agreement No. 825753.

1.05.03
Development of Bioassays Battery for Screening Thyroid Hormone Disrupting Activity of Chemicals
r. liu, A. Martináková, J. Novak, Masaryk University, Faculty of Science / RECETOX; K. Hilscherova, Masaryk University, Faculty of Science, RECETOX / RECETOX
The interference of chemicals with thyroid hormone (TH) signaling can be linked with many adverse effects and health problems including impairment of early fish development, frog metamorphosis or neurodevelopmental disorders, thyroid neoplasms, thyroid autoimmune disorders and increased cardiovascular risk due to altered lipid metabolism in human. In the EU H2020 ERGO project, we are designing a battery of *in vitro* assays for screening of potential thyroid hormone disrupting chemicals (TDCs). The battery is designed based on Adverse Outcome Pathway concept (AOP) and the bioassays focus on prioritized molecular initiative events (MIEs) within the thyroid hormone AOP network covering TH synthesis, transport, signaling and metabolism. In TH synthesis, the MIE of interest is the thyroperoxidase (TPO) inhibition since this enzyme plays an important role in the synthesis. The interspecies differences are also compared through effects on TPO from human and rat and other species reported in literature. In TH transport, a thyroxine-transthyretin (T4-TTR) displacement was addressed since transthyretin is an important carrier of TH in the blood and plays a crucial role during neurodevelopment. Interaction of TDCs with TH receptor signaling was assessed using a human cell line stably transfected with reporter gene under control of TH receptor activity. For TDCs effect on metabolization of TH, aryl hydrocarbon receptor (AhR), which regulates genes implicated in chemical metabolism, was assessed using a human cell-based reporter gene assay. The battery is being validated on a set of 20 prioritized model chemicals with clear environmental exposure relevance. The set includes environmental pollutants, natural compounds and pharmaceuticals. While none of the chemicals activated AhR, several of them were able to activate TH receptor. Bisphenol A, propylthiouracil and benzophenone strongly inhibited TPO activity in both human and rat cell-based assay and very good cross-species concordance was demonstrated. Many chemicals also showed a strong inhibition of TTR binding. Thus, the effectiveness and sensitivity of the assays in the battery was demonstrated and after further validation, they could serve for AOP-based high throughput screening, prioritization and hazard assessment of TDCs. *The project has received funding from the EU H2020 research and innovation programme project ERGO (grant agreement No.825753).*

1.05.04
Screening Thyroid Hormone Disruption Mechanisms of Chemicals Using Hepatic Cell Lines of Human, Rat, and Zebrafish Origin, Along With Different Life Stages of Zebrafish In Vivo Model
B. Kwon, S. Chu, Seoul National University / Graduate School of Public Health; M. Kim, Y. Park, Seoul National University Hospital / Department of Internal Medicine; S. Kim, Baylor University / Department of Environmental Science; J. Lee, Helmholtz centre for environmental research - UFZ / Cell Toxicology; J. Kim, D. Ahn, Seoul National University / Graduate School of Public Health; B. Oh, Lee Gil Ya Cancer and Diabetes Institute, Gachon University College of Medicine / Department of Physiology; K. Choi, Seoul National University / Environmental Health Sciences
Many chemicals that are frequently detected in humans can disrupt thyroid hormone (TH) homeostasis and potentially lead to various adverse health outcomes. Significant knowledge gaps, however, are present on mechanisms of the TH disruption. The aim of this study is to understand the effects of several model chemicals on hepatic metabolism of THs using liver cell lines with different origins, i.e., human, rat, and zebrafish. For this purpose, human liver cell (Hep G2), rat liver cell (McA-RH7777), and zebrafish liver cell (ZFL) were used. In addition, zebrafish model was also used to understand the corresponding effects *in vivo*. Known or suspected thyroid disrupting chemicals (TDCs) were chosen as model chemicals, based on either epidemiological or experimental studies, and these chemicals include *p,p*'-DDE, PFOS, DEHP, BPA, BP-3, EHMC, and DINCH. After the exposure, which was 24 hr for Hep G2, and McA-RH7777; 48 hr for ZFL; 120 hr and 21 days for embryo-larvae and adult male zebrafish, respectively, transcriptional changes of genes related to hepatic metabolism, and for fish, TH, were quantified. Exposure to PFOS, BPA, and BP-3 significantly down-regulated *TBG* gene and up-regulated *PPARA* and *UGT1A1* genes in Hep G2 cells. In McA-RH7777 cells, exposure to EHMC significantly down-regulated *Ugt1a1*, *Dio1*, *Ttr*, *Sult1b1,* and *Ppara* genes. In ZFL cells, exposure to BPA caused significant up-regulation of *ugt1ab* gene. In addition, a marked increase pattern of *ugt1ab* gene was shown following exposure to *p,p'*-DDE, DEHP, or BP-3. Both embryo-larval and adult male zebrafish exposed to BP-3 or EHMC demonstrated a significant decrease of THs levels, along with transcriptional changes of several genes. The direction of changes related to hepatic metabolism, however, were different by life stages of the zebrafish: In zebrafish embryo-larvae, BP-3 and EHMC exposure significantly down-regulated *ahr2*, *cyp2aa1*, and *cyp3a65*. Regulatory change of *ugt1ab* gene was also differed by chemicals which were up-regulated by BP-3 and down-regulated by EHMC, indicating the different mode of action by THs hepatic regulation. In adult zebrafish liver, BP-3 and EHMC activated *pxr*, *ahr2*, *cyp1a*, *cyp2*, *cyp3a65*, *ugt1ab,* and *sult1 st5* genes, indicating the reduction of THs in adult male zebrafish might be due to enhanced TH excretion by hepatic metabolism. The results of this study show chemical-induced thyroid disruption may differ because of species difference in hepatic metabolism. Further experimental studies are warranted to validate our observations and to understand the effects of the chemical exposure to axes of thyroid hormone disruption other than the liver.

1.05.05
Thyroid Disruption in Zebrafish (Danio rerio) - a Case Study With Resorcinol
L. Behnstedt, University of Heidelberg / Aquatic Ecology and Toxicology, Centre for Organismal Studies; M.P. Fagundes, University of Heidelberg; L. Reger, University of Heidelberg / Aquatic Ecology and Toxicology,

Centre for Organismal Studies; I.B. Chabchoubi, University of Monastir, Higher Institute of Biotechnology of Monastir / University of Sfax, Environmental and Ecotechnology Engineering Laboratory; L. Golz, COS University of Heidelberg / Aquatic Ecology & Toxicology, COS; P. Pannetier, University of Heidelberg / Aquatic Ecology & Toxicology, COS; T. Braunbeck, University of Heidelberg / AquaTox Centre for Organismal Studies; L.A. Baumann, University of Heidelberg / Aquatic Ecology and Toxicology
Resorcinol is a dihydroxylated benzene compound used in rubber and wood industries, in hair dye production and as a dermatological pharmaceutical, amongst other uses. Recently no consensus was achieved for the classification of resorcinol as a substance of very high concern, despite its endocrine disruptive action in mammals. In the present study, thyroid-disruptive effects of resorcinol on zebrafish (*Danio rerio*) were compared to those of a pharmaceutical with a similar mode of action, propylthiouracil (PTU). Both of these substances are goitrogenic and inhibit thyroid peroxidase, an essential enzyme for the synthesis of thyroid hormones (THs). In mammals, resorcinol shows a lower potency to reduce the production of THs than PTU. Contrary, *in vivo* studies with transgenic zebrafish lines showed a comparable or greater potency of resorcinol to interfere with TH synthesis, which may be relevant, since resorcinol is found mostly in aquatic systems. As a starting point for the characterisation of resorcinol toxicity in fish, a light-dark locomotion test was conducted with 5 days old zebrafish embryos exposed to different concentrations of either resorcinol (10, 30 and 100 mg/L) or PTU (50, 100 and 200 mg/L). This test is used for the screening of anxiolytic / anxiogenic effects by pharmaceuticals in embryos submitted to abrupt light changes. During light phases, the swimming activity of the embryos is reduced, and changes from light to dark trigger an increase in swimming. In comparison with a water control treatment, both substances decreased embryo swimming during light phases. PTU induced a decrease in embryo swimming which was more intense than that induced by resorcinol at same mass concentration (100 mg/L). In contrast, resorcinol at 100 mg/L had a stronger inhibitory effect during the dark phase, inducing a 40 % swimming decrease from control values. Although reducing swimming in the dark phase, PTU produced a gradual decrease of this effect with increasing concentrations. As an additional thyroid-related observation, swim bladder inflation was also decreased after exposure to 100 mg/L resorcinol. Investigations on other thyroid-related endpoints are underway, namely thyroid follicle size and density, size and inflation of the swim bladder, eye morphology as well as photoreceptor composition and distribution. Funding by: European Union's Horizon 2020 research and innovation program, under grant agreement No. 825753 (ERGO).

1.05.06
Effects of Thyroid Disrupting Chemicals on the Development of Sensory Systems in Zebrafish (Danio rerio)
S. Volz, Syddansk Universitet / Biology; H. Holbech, University of Southern Denmark / Biology
The thyroid hormone system plays a vital role in vertebrate development. To date, the potential of chemicals to disrupt the thyroid hormone system is separately assessed for mammalian and non-mammalian vertebrates. The present study is part of the ERGO (EndoCrine Guideline Optimisation project, which aims at improving the hazard assessment of endocrine disrupting chemicals (EDCs). ERGO is expected to remove the currently existing separation between mammalian and non-mammalian EDC research and demonstrate the feasibility of a cross-vertebrate EDC assessment. To reach this goal, suitable endpoints for extrapolation from non-mammalian to mammalian vertebrates have to be identified and evaluated. The objective of the current study is to identify and evaluate endpoints that are relevant for the assessment of thyroid disruption in fish early life stages. To this end, two chemicals known to disrupt the thyroid hormone system via different mode of actions were selected, namely bisphenol A and amiodarone; while bisphenol A affects the synthesis of the thyroid hormone thyroxine (T4), amiodarone disrupts the conversion of T4 into the biologically most active thyroid hormone 3,5,3'-triiodothyronine (T3). Freshly fertilized zebrafish eggs are exposed to five concentrations of the respective test substance for eight days and morphological anomalies are documented on a daily basis. To investigate effects on the thyroid-dependent development of sensory systems, changes in swimming behavior following stimulation with either light of different wavelengths or odorants is measured eight days post fertilization. At the end of the experiment, zebrafish larvae are euthanized and further processed for histological analysis of the eyes and the olfactory system as well as for the measurement of whole-body thyroid hormone levels. While the experiments are currently still ongoing, a previous histological analysis of zebrafish exposed for sixty days showed that bisphenol A disrupted the eye development thereby inducing structural changes in the retina. The ongoing studies are expected to contribute to the identification of endpoints suitable for the assessment of thyroid disruption in fish early life stages. In a next step, these endpoints could to be evaluated regarding their potential to be used for extrapolation of EDC effects across vertebrate classes.

1.05.07
A New Standard for Endocrine Disruptor Testing in Fish - the Integrated Fish Endocrine Disruptor Test (iFEDT)
L.A. Baumann, University of Heidelberg / Aquatic Ecology and Toxicology; P. Pannetier, University of Heidelberg / Aquatic Ecology & Toxicology, COS; M.P. Fagundes, University of Heidelberg; L. Golz, COS University of Heidelberg / Aquatic Ecology & Toxicology, COS; E. Stinckens, University of Antwerp; L. Vergauwen, D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; J.E. Morthorst, University of Southern Denmark / Department of Biology; H. Holbech, University of Southern Denmark / Biology; T. Braunbeck, University of Heidelberg / AquaTox Centre for Organismal Studies
Various EU research projects deal with the optimization of aquatic vertebrate test systems for the identification of endocrine disruptors (EDs). Attempts are made to cover all relevant life stages, to identify EDs with different modes of action, to replace existing amphibian tests and to include thyroid- related endpoints in existing fish test guidelines(TGs). For this end, the EU tender project "Integrated Fish Endocrine Disruptor Test (iFEDT)" was designed to establish a new test system that combines and extends two existing fish test guidelines: the Fish Short Term Reproduction Assay (OECD TG 229) and the Fish Sexual Development Test (OECD TG 234). Propylthiouracil (PTU; 0 to 78.125 mg/L; n = 4) and Ethinylestradiol (EE2; 0 to 10 ng/L; n = 4) were tested as model substances for disruption of the thyroid hormone and the sexual hormone system, respectively. In addition to endpoints established in the existing OECD TGs (fecundity, growth, gonad histopathology, vitellogenin, etc.), various thyroid-related endpoints such as hormone levels, thyroid histopathology and (eye) development were assessed during the 85 days exposure period. Results from the first experiment with PTU reveal that PTU not only impairs the reproductive performance of adult fish and reduces the growth of juvenile fish, but also induces strong thyroid follicle proliferation in all life stages. Moreover, histopathology of the eyes demonstrates that this endpoint is very sensitive to thyroid disruption in younger life stages. The experiment with EE2 is ongoing. Based on the PTU results, the merged test design iFEDT already appears to be a promising tool to integrate various important ED modalities in fish into one assay.Funding: This study receives funding from the European Commission under contract no. No 07.0203/2018/794670/ETU/ENV.B.2 ("Development of a study protocol for regulatory testing to identify endocrine disrupting substances in biotic systems").

1.05.09
Zebrafish As a Model to Screen Thyroid Disruption
O. Jaka, BBD BioPhenix-Biobide / R&D Department; M. Mazón, BBD BioPhenix - Biobide / R&D; A. Muriana, BioBide / R&D Department; A. Puertas, I. Iturria, C. Martí, BBD BioPhenix-Biobide / R&D Department
There is an increasing concern about the environmental impacts of chemicals that can disrupt the endocrine system, which can result in adverse effects on developmental, reproductive, neurological, and/or immune functions in both humans and wildlife. The negative impact of these Endocrine Disrupting Compounds (EDCs) is becoming a real public health issue, therefore the necessity of tests to assess the potential risk of new chemicals before they are marketed is increasing. *In vitro* tools are inexpensive and scalable for high-throughput platforms, however, they pose low relevance to vertebrate species. On the other hand, working with adult animals is expensive and represents ethical issues. Small fish like zebrafish (*Danio rerio*) are an excellent alternative to *in vitro* and *in vivo* models. They offer a unique experimental system where screening assays can be performed at the whole animal level, being at the same time compatible with the 3R principles (Replacement, Reduction, and Refinement in animal testing). In this work, we present a three-phase assay to screen the endocrine disrupting potential of chemical compounds. First, thyroid disrupting (TD) chemicals are evaluated using the transgenic line tg(tg:mcherry), which expresses red fluorescence in the thyroid gland under the thyroglobulin promoter. The fluorescence of the reporter gene allows monitoring *in vivo* the upregulation of the thyroglobulin gene

expression as a compensatory reaction to thyroid gland disruption. The signal intensity is assessed *in vivo* to screen and characterize potential TD, either directly or after metabolization by the zebrafish embryo. Second, a gene expression analysis is carried out to characterize the thyroid disrupting effect by measuring three thyroid-related genes (*tsh*β, *tg*, and *tpo*). And third, the thyroid hormone (TH) levels of T4, T3, 3,5-T2, and 3,3'-T2 are quantified by liquid chromatography (LC/HRMS) in whole-embryo extracts. Our data show that reference compounds (such as potassium perchlorate and propylthiouracil), and environmental pollutants (such as benzophenone-2) induce an increase of the fluorescence of thyroglobulin in the tg(tg:mcherry) transgenic line, and overexpression of thyroid-related genes *tsh*β, *tg*, and *tpo*. These results were confirmed in TH level measurements by LC/HRMS. This screening methodology showed to be a sensitive and cost-effective assay to screen and evaluate potential EDCs chemicals.

1.05.10
Control Performance of Amphibian Metamorphosis Assays With Xenopus laevis
J.R. Wheeler, Shell International; R. Puglisi, Health and Environmental Sciences Institute (HESI); J.C. Wolf, EPL, Inc.; S. Glaberman, George Mason University / Biology; L.L. Lagadic, Bayer Ag / Environmental Safety; Z. Gao, Bayer Ag; C. Mitchell, Health and Environmental Sciences Institute (HESI); F. Sewell, NC3Rs; M.R. Embry, Health and Environmental Sciences Institute (HESI)
The amphibian metamorphosis assay (AMA) is an *in vivo* screen to assess potential interactions of chemicals with the amphibian thyroid system. Larvae are exposed for 21-days. At 7 and 21 days, individuals are assessed for development and growth metrics. Thyroid gland histopathology is performed at test termination. This poster presents data from 46 studies performed to satisfy test orders from the United States Environmental Protection Agency's Endocrine Disruptor Screening Program. Data Evaluation Records were used to collate the typical control variability and performance of test parameters in AMAs conducted in different laboratories. We assess whether available historical control data (HCD) can assist evidence-based interpretation of the endpoints. We investigate whether reliable HCD ranges could be developed for the core study endpoints and the associated validity and performance criteria. The data are discussed in terms of improving assay performance and interpretation.

1.05.11
Effects of Three Thyroid Hormone System Disruptors: Perchlorate, PTU and TBBPA, on the European Common Frog Rana temporaria
A. Aviles, Syddansk Universitet / Department of Biology; K. Hulgard, University of Southern Denmark / Biology; H.S. Hjort-Gregersen, University of Southern Denmark; A. Duus, University of Southern Denmark / Biology; B. Holbech, SDU / Biology; P. Bjerregaard, H. Holbech, University of Southern Denmark / Biology; J.E. Morthorst, University of Southern Denmark / Department of Biology
Amphibian populations have been decreasing over the past decades and the main reason is anthropogenic activities including chemical pollution. Amphibians are particularly sensitive to endocrine disrupting chemicals targeting the thyroid hormone system, because thyroid hormones control amphibian development and growth. The African clawed frog (*Xenopus laevis* and *tropicalis*) is a well-renowned model organism in many research areas including human disease and toxicity testing, and currently, endpoints characterizing thyroid-mediated modes of action are included in three Xenopus OECD test guidelines. However, the life cycle of Xenopus is 100 % aquatic which is unlike most European amphibians having both terrestrial and aquatic life stages. This project aims at comparing the sensitivity of Xenopus and the common frog by studying the effects of thyroid disrupting chemicals. Embryos were exposed from fertilization to metamorphic climax to: sodium perchlorate (11.9-426.5 µg/L), 6-propylthiouracil (PTU) (1.23-47.7 mg/L) and tetrabromobisphenol A (TBBPA) (18.59-349.62 µg/L), and the effects on developmental stage, biometric data, and thyroid and eye histopathology were investigated. The highest concentrations of PTU caused individuals to develop slower but increase in body size, showing that PTU inhibits the development but not the growth, and PTU also affected thyroid gland and thyroid cell morphology (e.g. thyroid gland size, and follicle cell height). Perchlorate and TBBPA did not affect the biometric measures, but the investigation of the effects on developmental stage and thyroid histopathology is ongoing. In fish, eye malformations (e.g. eye size and pigmentation) have been reported as possible new endpoints for thyroid hormone disruption, and these parameters are currently being investigated too.

1.05.14
Exposure to a Mixture of Ethinylestradiol and Levonorgestrel After Birth Disturbed the Gonad Development but Not Sex Differentiation of Guppy Fish Fry
R. Alves, M. Rocha, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto,Portugal.; E. Rocha, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto (U.Porto), Porto, Portugal
Countless human-made chemical compounds polluting the aquatic environments may negatively influence fish health, survival, development, growth, and breeding. Some pollutants are active ingredients of pharmaceutical drugs and include estrogenic and progestogenic compounds. Assays with zebrafish and other oviparous fish showed that those two endocrine disruptor chemicals might impact gonadal development and sex differentiation. The effects of exposures to mixtures of those two classes of compounds are more challenging and far less well known compared with what is known from single exposures. To improve the knowledge of the mixtures' impacts on fish, particularly on livebearers, a study was carried out using a combination of the estrogen ethinylestradiol (EE2) and the progestin levonorgestrel (LGN). The aim was to look for impacts of the early gonadal development of the guppy fish (*Poecilia reticulata*). As a first approach and up to 12 hours after birth, guppy fry were independently exposed to a mixture of o EE2 (500 ng/L) and LNG (500 ng / L) for 7 days and 21 days. The fish were sacrificed with an overdose of anaesthetic, their biometrics recorded, and processed for histology. Besides sexing the individuals by microscopy, we estimated the gonadal volumes by stereology and using the Cavalieri principle (n=6/sex/group). The results showed that the ratio of males to females was close to 1:1, and it did not vary between controls and exposed fish. Moreover, both experimental groups did not differ in body mass and length. Regarding the gonadal development (enlargement), EE2+LNG exposed females had bigger ovaries at both 7 and 21 days. In males, exposure to the mixture induced larger testes at 7 days; a difference that faded at 21 days. Further studies are being done to know how the exposed females ended up with more voluminous ovaries and how exposed males matched the controls at 21 days. Hypothetically, the latter fact could mechanistically result from stroma enlargement, oogonia proliferation, and/or hypertrophy of gametes. For now, we concluded that, under the tested conditions, a few days of exposure to EE2+LNG promotes structural changes in the early growth of both male and female guppy gonads, without impacts on sex differentiation and body growth. The present data justify further studies. Funding: ICBAS, FCT (UIDB/04423/2020, UIDP/04423/2020), and Project ATLANTIDA (NORTE-01-0145-FEDER-000040), by NORTE 2020 and PORTUGAL 2020, through ERDF.

1.05.18
Zebrafish As a Model for Chemical Induced Adipogenesis and Related Metabolic Diseases
M. Den Broeder, N. Habraeken, Utrecht University / Institute for Risk Assessment Sciences; L. Kamminga, Wageningen University / WUR Library; J. Kamstra, Institute for Risk Assessment Sciences Utrecht University / Institute for Risk Assessment Sciences; J. Legler, Utrecht University / Institute for Risk Assessment Sciences
Humans are widely exposed to endocrine disrupting chemicals (EDCs) and current research in has indicated that developmental exposure to specific EDCs, so-called metabolism disrupting chemicals (MDCs), may play a role in the latent onset of obesity and other metabolic disorders like type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD). Within the European H2020 project GOLIATH (Generation Of NoveL, Integrated and Internationally Harmonised Approaches for Testing Metabolism Disrupting Compounds) we aim to get a better understanding of molecular mechanisms of MDCs, and work towards integrated testing and assessment of this class of chemicals. Zebrafish is emerging as a valuable model organism to study human metabolic diseases. The goal of this study is to analyze the effects of early developmental exposure to chemicals on adipogenesis, and pancreas and liver development in the zebrafish (Danio rerio). We have developed a zebrafish model of adipogenesis and obesity, and use advanced imaging technology and fluorescent lipid stains to visualize the development of adipocytes in living larvae at 15 dpf. With this approach we have shown that developmental exposure to the biocide tributyltin stimulates adipogenesis, similar to previous reports in rodent models. Here, we will take this approach further and use transgenic fish lines to examine effects of MDCs on the endocrine and exocrine pancreas,

as well as lipid metabolism in the liver, in combination with a lipid stain to assess alterations in adipogenesis. This project (GOLIATH) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825489

1.05.19
Testosterone and Dihydrotestosterone Effects on Lipid-Related Genes in Brown Trout Primary Hepatocytes Suggest That Xenoandrogens May Disrupt Lipid Metabolism in Fish
C. Lopes, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto (U.Porto), Porto, Portugal / Microscopy; E. Rocha, I.L. Pereira, T. Madureira, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto (U.Porto), Porto, Portugal
The potential of androgens to disrupt lipid metabolism in fish has been seldom explored. A few studies indicated that these could affect the expression of lipid-related genes, particularly those related to lipogenesis, lipid transport, fatty acid uptake, and cholesterol metabolism. Studies in brown trout (*Salmo trutta* f. *fario*) primary hepatocytes exposed to model androgens (testosterone - T and dihydrotestosterone - DHT) showed that these could modulate the expression of crucial proteins in fish oogenesis, namely vitellogenin and zona pellucida proteins. Considering that oogenesis and the global reproduction process are regulated by sex-hormones and associated with lipid changes, it was here questioned if androgens may impact on lipid metabolic pathways and signaling. In this study, we exposed juvenile brown trout primary hepatocytes to six concentrations (1, 10, 100 nM, and 1, 10, 100 μM) of T and DHT, for 96 hours. We then assessed the exposure effects in the mRNA levels of lipid-related genes, including long-chain acyl-CoA synthetase 1 (Acsl1), involved in fatty-acid activation; apolipoprotein A1 (ApoA1), associated with lipid transport; fatty acid-binding protein 1 (Fabp1), linked to fatty acid intracellular transport; steroidogenic acute regulatory protein (StAR), important in cholesterol metabolism; lipoprotein lipase (LPL), involved in the uptake of fatty acids; peroxisome proliferator-activated receptor gamma (PPARγ), a regulator of adipogenesis; acyl-CoA oxidase 3I (Acox1-3I), from peroxisomal β-oxidation; and acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), the main enzymes that mediate fatty acid synthesis in *de novo* lipogenesis. Acsl1 mRNA levels were up-regulated from 100 nM to 100 μM of DHT, and by 10 and 100 μM of T, comparing with controls. FAS and ACC were also up-regulated by the highest (100 μM) concentrations of T and DHT, respectively. On the contrary, ApoA1 was down-regulated by DHT, at 10 and 100 μM, and by T, at 100 μM. PPARγ mRNA levels decreased after the highest concentration of both androgens, while Acox1-3I was down-regulated only by T, also at 100 μM. Fabp1, StAR, and LPL expressions were not altered. The findings suggest that androgens may be directing lipid metabolism for lipogenesis. Androgens may also be impacting on lipid transport. Overall, data support the potential of xenoandrogens to affect lipid metabolism in the fish liver. Funding: Supported by FCT and ERDF (UIDB/04423/2020 and UIDP/04423/2020) and ICBAS.

1.05.22
Effects of Endocrine Disruptors on Caged Juvenile Nile Tilapia (Oreochromis niloticus) in the Seven Lakes of San Pablo
M.V. Paraso, College of Veterinary Medicine / Department of Basic Veterinary Sciences; J. Dela Cruz, University of the Philippines Los Baños / Department of Basic Veterinary Sciences; M. Lola, University of the Philippines Los Baños / Department of Veterinary Paraclinical Sciences; R. Marcelino, University of the Philippines Los Baños / Institute of Statistics; A. Elepaño, J. Biscocho, M. Capistrano, C. Alburo, A. Pagkaliwagan, University of the Philippines Los Baños / Department of Basic Veterinary Sciences
The occurrence of endocrine-disrupting chemicals (EDCs) in surface waters has elicited concern due to their potential interference in the endocrine functions of vulnerable aquatic organisms. Small lakes such as the seven lakes of San Pablo have a lower absorptive capacity for pollutants, which could result in irreversible damage to the ecological balance. This study was conducted to assess endocrine disruption in juvenile Nile tilapia (*Oreochromis niloticus*) caged for 35 days in these lakes. The following endpoints were assessed in fish: vitellogenin (VTG) levels in skin mucus, hepatic VTG mRNA expression, and histopathological changes in the testis and the ovary. Water samples were also analyzed for levels of fecal coliform and known EDCs, estradiol (E2), and bisphenol A (BPA). Results provide evidence of endocrine disruption in fish as shown in the induction of VTG synthesis in the epidermal mucus, presence of VTG transcripts in the majority of liver samples, and microscopic lesions in gonads that are consistent with exposure to endocrine-active compounds. These biological responses were further supported by the detectable concentrations of E2 and BPA in water samples. Fecal coliform levels are suggestive of contamination of the lakes with sewage or nonpoint sources of human and animal excreta from which E2 most likely originated. These findings add the seven lakes of San Pablo to the growing list of freshwater systems contaminated with endocrine disruptors.

Environmental Epigenetics: Impacts of Stressors on the Epigenome, Short-Term and Long-Term Effects and Challenges for the Integration into Risk Assessment

1.06.02
DNA Methylation and Expression of Estrogen Receptor Alpha in Fathead Minnows Exposed to 17α-Ethynylestradiol
J. Fetke, Environmental Protection Agency / ORD/CCTE/GLTED/MIB, University of Cincinnati; J. Martinson, R. Flick, U.S. EPA / ORD/CCTE/GLTED/MIB; W. Huang, M. See, U.S. EPA / ORD/CTTE/GLTED/MIB; E. Pilgrim, U.S. Environmental Protection Agency / Center for Environmental Measurement & Modeling Watershed & Ecosystem Characterization Division; R. DeBry, University of Cincinnati / Department of Biological Sciences; A.D. Biales, U.S. Environmental Protection Agency / ORD/CCTE/GLTED
The gene expression response thought to underlie the negative apical effects resulting from estrogen exposure have been thoroughly described in fish. Although epigenetics are believed to play a critical role translating environmental exposures into the development of adverse apical effects, they remain poorly characterized in fish species. This study investigated alterations of DNA methylation of *estrogen receptor alpha* (*esr1*) in brain and liver tissues from male fathead minnows (*Pimephales promelas*) after a 2d exposure to either 2.5ng/L or 10ng/L 17α-ethynylestradiol (EE2). Changes in the patterns of methylation were evaluated using targeted deep sequencing of bisulfite treated DNA in the 5' region of *esr1*. Methylation and gene expression were assessed at 2d of exposure and after a 7 and 14d depuration period. After 2d EE2 exposure, males exhibited significant demethylation in the 5' upstream region of *esr1* in liver tissue, which was inversely correlated to gene expression. This methylation pattern reflected what was seen in females. No gene body methylation (GBM) was observed for liver of exposed males. Differential methylation was observed for a single upstream CpG site in the liver after the 14d depuration. A less pronounced methylation response was observed in the upstream region in brain tissue, however, several CpGs were necessarily excluded from the analysis. In contrast to the liver, a significant GBM response was observed across the entire gene body, which was sustained until at least 7d post-exposure. No differential expression was observed in the brain, limiting functional interpretation of methylation changes. The identification of EE2-dependent changes in methylation levels strongly suggests the importance of epigenetic mechanisms as a mediator of the organismal response to environmental exposures and the need for further characterization of the epigenome. Further, differential methylation following depuration indicates estrogenic effects persist well after the active exposure, which has implications for the risk posed by repeated exposures.

1.06.03
Identification of Differentially Methylated Regions in Zebrafish Eleutheroembryos Exposed to TBT
L. Navarro-Martin, Institute of Environmental Assessment and Water Research / Environmental Chemistry; J. Kamstra, Utrecht University / Institute for Risk Assesment Sciences (IRAS); M. Gual-Gimeno, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); R. Martinez, IDAEA-CSIC / Environmental Chemistrty; J. Legler, Utrecht University / Institute for Risk Assessment Sciences; B. Piña, Institute of Environmental Assessment and Water Research (IDAEA) Spanish Research Council (CSIC) / Environmental Chemistry
Environmental exposures to chemicals can affect the epigenetic status of ecotoxicologically relevant species of fish, plants and invertebrates. The prevalent hypothesis is to use the "epigenetic foot-print" as a tool to evaluate the exposure of a given organism to toxicants, with the ultimate goal of determine if epigenetic signatures characterized after early developmental exposures are maintained through time once the pollutant exposure

ceased, and if those can cause adverse effects on the exposed animals. Indeed, several publications have demonstrated that EDCs can act as epigenomic disruptors. However, the mechanism of action remains unclear. For that reason, further studies are needed to better understand the involvement of upstream mechanisms, such as epigenetics, and elucidate how EDCs act on epigenetic endpoints capable to regulate gene expression. Zebrafish has been recognized to be a good model species to study environmental epigenetics. In the present study we have exposed zebrafish eleutheroembryos from 2 to 5 days post fertilization to 3, 30 and 100 nM of TBT. Extraction of RNA and DNA was performed simultaneously from pools of 8 eleutheroembryos. RNA sequencing revealed that transcriptomic dysregulation of TBT suggested a general, rather unspecific toxicity pattern, since most of the affected pathways were related to cell viability and development. This was in concordance with morphometric results previously published that showed that the affected traits were more related to developmental delays rather than to a specific mode of action of TBT. However, steroid biosynthesis pathways that lead the synthesis of cholesterol and vitamin D_3 appeared as the modules that become affected at the lowest TBT concentrations. Furthermore, five replicate samples per condition were sequenced for control, 3 and 30 nM of TBT using whole genome bisulfite sequencing (WGBS). Results from this analysis will be integrated with transcriptomic data obtained. The ultimate goal of this study is to identify potential epigenetic biomarkers of exposure to EDCs and to better understand the modes of action of EDCs using holistic approaches.

1.06.05
Transgenerational Epigenetic Effects After Early Life Stage Exposure to Perfluoroalkyl Substances
M. Fallet, MTM Research centre Örebro University / Science and Technology; M. Di Criscio, Department of Organismal Biology, Uppsala University / Organismal Biology; P. Antczak, Centre for Molecular Medicine / University of Cologne; J. Rüegg, Uppsala University / Department of Organismal Biology; N. Scherbak, Orebro University / Life Science Centre; S. Keiter, Orebro University / MTM Research centre
Research shows that the individual lifestyle of an organism can influence its phenotype and potentially also the phenotype of its offspring. The different genetic and non-genetic components of the inheritance system and their mutual interactions are key mechanisms to generate inherited phenotypic changes. Once released into the environment, pollutants represent a serious threat to human and environmental health and their exposure may also affect subsequent generations through transgenerational epigenetic modifications. Thus, there has been growing concern that epigenetic modifications by pollutants mediate adverse health outcomes over several generations. Poly- and perfluoroalkyl substances (PFAS) are particularly investigated due to long and extensive use in firefighting foams, textiles, and paper products. Among PFAS, perfluorooctane sulfonic acid (PFOS) is the most studied as it is known to be persistent, bioaccumulative, toxic, and undergo wide transportation across all environmental media. Perfluorobutane sulfonic acid (PFBS) is considered less toxic and is now used in replacement for PFOS. However, preliminary results indicate the PFBS alter epigenetic marks; thus, further investigations are needed to study the impact of PFBS on organisms over generations. Therefore, we will study the transgenerational epigenetic inheritance of PFAS to increase our understanding of how these compounds impact on epigenetic mechanisms and related adverse effects. For that purpose, we induced a precocious (0-2hpf post-fertilization) and chronic (28 days) exposure of two PFAS: PFOS and PFBS, on zebrafish embryos. Subsequently, we will study the impact of this exposure on zebrafish transcriptome, metabolic activity, phenotype, and behavior on the exposed generations (F0) but also on the subsequent unexposed ones (F1-F2), to investigate the transgenerational effect of PFAS. Finally, we will look at the epigenetic and genetic components to decipher the molecular mechanisms involved in the transgenerational effects of PFAS.

1.06.08
Mechanistic Evaluation of Uv-Degraded NQ Toxicity Increase in Daphnia pulex: A Transcriptome Sequencing Approach
S. Amar, Us Army Engineer Research & Development Center / Environmental Lab; A.J. Kennedy, US Army Engineer Research and Development Center / Environmental Laboratory; L. Moore, US Army Engineer Research and Development Center; K.A. Gust, U.S. Army Engineer Research and Development Center / Environmental Laboratory
Mechanistic evaluation of UV-degraded NQ toxicity increase in *Daphnia pulex:* A transcriptome sequencing approachAuthors: -[1]Saroj K Amar – sarojkumaramar@gmail.com[2]Alan J Kennedy - Alan.J.Kennedy@usace.army.mil2[2]Lee C. Moores - Lee.C.Moores@usace.army.mil[2]Kurt A Gust – kurt.a.gust@usace.army.mil [1]Oak Ridge Institute for Science and Education[2]US Army, Engineer Research and Development Center, Environmental Laboratory
ABSTRACT: The US Army is developing Insensitive Munition (IMs) to replace fire and shock-sensitive traditional munitions. The Army's flagship IM formulation, IMX-101, performs very well as an IM and is being deployed in an increasing number of weapons systems. The increased use of IMX-101 has necessitated expanded characterization of potential environmental risks, which has revealed that the IMX-101 formulation component, nitroguanidine (NQ), has low toxicity in aquatic exposure, but becomes orders of magnitude more toxic once UV-degraded. The present study seeks to identify the toxicological mechanisms underlying the increased toxicity of UV-degraded NQ in Daphnia pulex using RNAseq-based transcriptome expression analysis. *D. pulex* were exposed to a UV-NQ degradation series where the NQ parent compound was degraded by 0%, 7%, 18%, 42% or 100%. Each UV-NQ degradation treatment was deployed in separate dose-series experiments. Additionally, we investigated the effect of NQ degradation product aging (8d aging) for the 18% and 42% NQ degradation product treatments. RNAseq-based global transcriptomics expression assays were conducted for the UV-degraded NQ exposures using Illumina HiSeq with an average of 36.8M reads per sample. Differentially expressed transcripts (DETs) linked with % UV degradation of NQ Vs. 0-NQ with 0%, 7%, 18%, 42% and 100% UV degraded NQ are 2389, 2483, 516, 2879 and 1237, respectively. A total of 1287 and 901 significant DETs were identified with aged 18% degraded and non-aged 18% degraded NQ, respectively, with 377 DETs found in common. A total of 2811 and 3560 DETs were identified with aged 50% degraded and non-aged 50% degraded NQ, respectively, where 1036 DETs were found in common. KEGG pathway and gene ontology (GO) enrichment analyses were conducted within each pairwise comparison of % UV-degraded NQ versus the paired 0mg/L NQ control. A total of 32, 39, 5, 45, and 15 significantly enriched KEGG pathways were identified across the 0%, 7%, 18%, 42% & 100% UV-degraded NQ treatments, respectively, as well as a broad suite of significant GO clusters. Evaluation of significant GO processes affected in *D. pulex* included drug metabolism and cellular macro-molecule metabolism while KEGG pathways for xenobiotic and drug metabolism by cytochrome P450 as well as fatty acid metabolism were significantly enriched across the UV-degraded NQ treatments. The results set is being mined to ID mechanisms of increased UV-degraded NQ toxicity. *Keywords: Transcriptomics profile, Daphnia pulex, UV radiation, Nitroguanidine, Toxicity pathways.*

Fish Model Species in Human and Environmental Toxicology

1.07.01
Effect of Broken Si-Based Solar Cell Modules on the Early Development of Zebrafish Embryo
L. Kim, J. Kwak, Konkuk University; P. Gautam, S. Jeong, Kunsan National University / Department of Environmental Engineering; Y. An, Konkuk University / Department of Environmental Heath Science
In the history of solar cell development, one of the traditional photovoltaic modules is silicon-based solar cell and they are divided with monocrystalline and poly-crystalline Si-modules in PV markets. The panels are installed usually in mountain and farm areas and exposed to weathering effects. Some panels could be damaged by accident then, the components of solar cells would be leaked to environment and especially in raining conditions, toxicants from panels would be flowed and exposed to aquatic environment. However, there are limited research about the ecotoxicity of solar cell panels. In this study, early-stage toxicity test was conducted with zebrafish under OECD TG No.236 with some modifications for assessing the effect of solar panel. The environmentally accidental situation of PV panels was imitated with artificial conditions. Two types of solar panels (monocrystalline and poly-crystalline Si-modules) were broken with same size and the leachate was made under Method 1311 (US EPA). Zebrafish embryos were exposed to each leachate and the abnormalities were measured. Monocrystalline Si-module leachate showed significant adverse effects in 5 % and poly-crystalline Si-module in 25% of leachate. The finding of this study suggests that damaged solar panels release the toxic components that could affect the early-stage development of fish in aquatic environments. *Acknowledgement. This work was supported by the Global Frontier R&D Program on Center for Multiscale Energy*

System funded by the National Research Foundation under the Ministry of Science and ICT, Korea (2016M3A6A7945504).This study was also funded by the Ministry of Environment (MOE) through the Graduate School of Specialization for Safe Management of Chemicals.

1.07.02

Evaluation of the Genotoxic Effect of Emerging Pollutants on Zebrafish Danio rerio

A. Sobrino-Figueroa, Universidad Autonoma Metropolitana / Hidrobiology; S. Alvarez-Hernandez, Universidad Autónoma Metropolitana-Iztapalapa / Applied Ficology Laboratory. Department of Hidrobiology; A. Perez-Rojas, Universidad Autonoma Metropolitana Iztapalapa / Limnology and geology Laboratory. Department of Hydrobiology

The emerging contaminants are chemical compounds, pharmaceuticals and mainly personal care products, which have been detected in concentrations of 0.0001 to 500 mg/L in the waters of treatment plants and in natural systems. There is limited information of the effects that these pollutants have on the organisms that inhabit aquatic systems, for this reason, in this study the oxidative and genotoxic effects of 14 products were evaluated (5 non-steroidal analgesics, 3 detergents, 2 toothpaste, 2 mouthwashes and 2 cloth softener) on the zebrafish. Bioassays with a duration of 10 days were carried out where adult zebrafish were exposed to a sublethal concentration (LC1) of these compounds. At the end of the exposure period, the degree of lipoperoxidation (Tbars) in the gills and the genetic damage (frequency of micronuclei) in peripheral blood were evaluated. In the results obtained, it was observed that there are significant differences in the degree of lipoperoxidation and genetic damage between the exposed organisms and the control group ($p < 0.05$). The most toxic product was a mouthwash (LC50 = 0.56 mg/L) and the least toxic was a toothpaste (> 1000 mg/L). Detergents and drugs were those that had the greatest oxidative effect. In the genotoxicity evaluation, the highest micronuclei frequency was observed in fish exposed to paracetamol (0.74%) (control 0.071%). The results indicated that all the products tested caused deleterious effects in the fish at sublethal concentrations.

1.07.04

Morphometric Analysis in Zebrafish Embryos to Predict Teratogenicity and Modes of Action in Mammals

S. Jarque, ZECLINICS, S.L. / R&D Services; M. Rubio-Brotons, ZECLINICS, S.L.; J. Ibarra, ZECLINICS, S.L. / R&D Services; V. Ordoñez, S. Dyballa, R. Miñana-Prieto, V. Schiavone, J. Terriente, ZECLINICS SL

To answer the growing demand for a fast, reliable and cost-effective methodology to detect the chemical substances toxicity (particularly new drugs and medications in Human Risk Assessment), several laboratories across the world are joining forces to develop, standardize, harmonize and promote Zebrafish as a new test method. In this scenario, we are acting on several fronts: participating in a large number of global and multi-laboratories initiatives but also boosting our internal R&D in order to validate it as preclinical alternative model and achieve soon the approval of international test guidelines. In the project presented here, we exposed zebrafish embryos from 0 to 96 h post fertilization to a battery of 31 compounds classified as teratogens or non-teratogens in mammals to prove its predictivity. The teratogenicity score was based on the measurement of 16 phenotypical parameters, namely heart edema, pigmentation, body length, eye size, yolk size, yolk sac edema, otic vesicle defects, otoliths defects, body axis defects, developmental delay, tail bending, scoliosis, lateral fins absence, hatching ratio, lower jaw malformations and tissue necrosis, using a high throughput automated software. Among the 31 compounds, 20 were detected as teratogens and 11 as non-teratogens, resulting in 94.44 % sensitivity, 90.91 % specificity and 87.10 % accuracy compared to rodents. These percentages decreased slightly when referred to humans, with 87.50 % sensitivity, 81.82 % specificity and 74.19 % accuracy, but allowed an increase in the prediction levels reported by rodents for the same compounds. Positive compounds showed a high correlation among all teratogenic phenotypes, pointing out at general developmental delay as major cause to explain the physiological/morphological malformations. A subsequent analysis based on deviations from main trends revealed potential specific modes of action for some compounds such as retinoic acid, DEAB, ochratoxin A, haloperidol, warfarin, valproic acid, acetaminophen, dasatinib, imatinib, dexamethasone, 6-aminonicotinamide and bisphenol A. The high degree of predictivity and the possibility of applying mechanistic approaches makes zebrafish a powerful model for screening teratogenicity.

1.07.05

Genome Editing in Rainbow Trout Cells Via CRISPR-Cas9 Ribonucleoprotein Delivery

M. Zoppo, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Department Environmental Toxicology; N. Okoniewski, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; S. Pantelyushin, J. vom Berg, University of Zurich / Institute of Laboratory Animal Science; K. Schirmer, Eawag / Environmental Toxicology

The advent of the CRISPR/Cas9 technology marked the beginning of a new era in the field of molecular biology, allowing the efficient and precise creation of targeted mutations in the genome of every living cell. This powerful technology holds great promise in the field of aquatic toxicology, where the CRISPR/Cas9 system can be utilized to interrogate genes involved in toxicity pathways on a cellular level. Since its discovery, different gene editing approaches based on the CRISPR/Cas9 technology have been widely established in mammalian cell lines, while on the other hand, limited knowledge is available on genetic manipulation in fish cell lines. In this work, a gene editing approach based on the CRISPR/Cas9 technology was developed in the rainbow trout (*Oncorhynchus mykiss*) intestinal derived cell line, RTgutGC. Ribonucleoprotein complexes, consisting of the Cas9 protein and a fluorescently labeled crRNA/tracrRNA duplex targeting the *cyp1a1* gene, were delivered via electroporation. A T7 endonuclease I (T7EI) assay was performed on FACS-enriched transfected cells in order to detect CRISPR-mediated targeted mutations in the *cyp1a1* locus, revealing an overall gene editing efficiency of 55%. Sanger sequencing coupled with bioinformatics analysis led to the detection of multiple insertions and deletions of variable lengths in the *cyp1a1* region targeted by CRISPR/Cas9 machinery. A new method for clonal cell line isolation based on the usage on cloning cylinders was developed in rainbow trout cell lines, allowing to overcome the genetic heterogeneity created by the CRISPR/Cas9 gene editing. Using this method, two clonal *cyp1a1* mutant cell lines, bearing a 101 or 1 base pair insertion disrupting the *cyp1a1* gene open reading frame, respectively, were identified. This is the first report of a clonal CRISPR/Cas9 gene-edited fish cell line in rainbow trout.

1.07.07

Effect of Trabectedin in the Bacterial Community of Danio rerio Larvae

E.P. Damasceno, Department of Biology & CESAM - University of Aveiro / Departament of Biology & CESAM; J. Oliveira, University of Aveiro / CESAM & Department of Biology; S. Lopes Peixoto, CESAM & University of Aveiro / Department of Biology; I. Henriques, University of Coimbra / University of Coimbra, CESAM & Department of Life Sciences, Faculty of Sciences and Technology, Calçada Martins de Freitas 3000-456 Coimbra, Portugal; A.M. Soares, Universidade de Aveiro / CESAM & Department of Biology; L.V. Lotufo, University of São Paulo; M.D. Pavlaki, University of Aveiro / Department of Biology; S. Loureiro, Universidade de Aveiro / Biology

Anticancer agents (AAs) are present in the aquatic environment due to inefficient removal by conventional wastewater treatment plants. Being among the most waterborne toxic compounds, AAs exposure may induce changes in the bacterial communities associated with human, animal hosts or aquatic environment. This study aimed to assess the effects of trabectedin, a cytotoxic AA, in the bacterial community of zebrafish 96 h-larvae (*Danio rerio*) and in the surrounding medium. For that, the zebrafish embryos were exposed to trabectedin (static exposure), in quintuplicate, each replicate was composed of 15 zebrafish embryos in a volume of 20 mL test medium, and the bacterial communities of both 96 h-larvae and exposure medium were investigated. For this purpose, a 16S rRNA gene PCR - Denaturing gradient gel electrophoresis (DGGE) analysis was used to evaluate changes in the structure and richness (number of bands) of the bacterial communities. Overall, the DGGE profiles of communities from non-treated exposure medium were different from the remaining samples (sharing less than 30% similarity). Non-treated larvae and trabectedin-exposed larvae grouped in the same cluster, sharing nearly 70% similarity. After trabectedin exposure, the bacterial communities of the exposure medium revealed a DGGE profile more similar to the bacterial communities of the zebrafish larvae (nearly 50% similarity) than to the non-treated exposure media (only 30%). Richness index revealed no significant differences towards the respective controls. Trabectedin affects differently the structure of the bacterial community associated to larvae and to the exposure medium. Further investigations are needed to evaluate potential effects of trabectedin on the bacterial communities and the implications regarding the host and services provided by the aquatic environment.

1.07.09

Behavioural Alterations in Zebrafish Eleutheroembryos Induced After Exposure to Acetamiprid and Nicotine

V. Ovcharova, University Koblenz Landau / Ecotoxicology; R. Von Hellfeld, University of Heidelberg / Aquatic Ecology and Toxicology, Centre for Organismal Studies; T. Braunbeck, University of Heidelberg / AquaTox Centre for Organismal Studies

Numerous anthropogenic chemicals including pesticides, metals, and pharmaceuticals have been identified to elicit neurotoxic effects in organisms, and − given their increased pressure on wildlife, human, and ecosystem health – there is an urgent need for testing of not only acute toxicity but also subtle adverse effects. Behavioural assays hold great capacity as a source of early warning signals and are considered specifically sensitive for chemicals with neurotoxic potential. As a test system, the zebrafish (*Danio rerio*) steadily wins acceptance, further strengthened by its complex and increasingly studied behavioural repertoire. The present study focused on acetamiprid, a representative of the strongly disputed class of systemic neonicotinoid agrochemicals, and nicotine, a model developmental neurotoxicant. In a modified version of the FET test (OECD TG 236), embryos were exposed to a broad range of concentrations, and behavioural changes were recorded following two experimental designs, covering an extended exposure window: (1) the spontaneous tail coiling (STC) assay at 21 - 47 hpf, and (2) the basal swimming activity assay at 83 - 119 hpf. STC represents the first motor behaviour of zebrafish embryos and is assumed to play a critical role in locomotion development. Whilst its analysis is frequently limited to specific time points coupled with a short assessment duration, the present study attempted to quantify the entire development of the coiling movement, employing a semi-automated setup. The investigation of locomotor development was complemented by the recording of swimming behaviour (distance travelled) under a prolonged exposure scenario. Changes in the ambient illumination were integrated to address potential stress responses, triggered by the sudden shift from light to total darkness. Although developmental aberrations could not be observed for any compound tested, there were significant alterations in behaviour: throughout embryonic development, nicotine caused a biphasic effect by stimulating coiling activity at 2.5 and 6.25 µM followed by a significant suppression at 12.5 and 25 µM. In contrast, acetamiprid gave no clear trend in behavioural changes in embryos, but more prominent alterations in activity were evident during subsequent larval stages. Upon exposure to 50 and 100 µM acetamiprid, hypoactivity was observed during dark phases and with respect to the overall distance moved. Although not as potent as nicotine, acetamiprid may thus also impair neurodevelopment, potentially leading to derailment from normal developmental pathways. These findings confirm the sensitivity of behavioural endpoints to discern subtle changes that might otherwise go unnoticed. However, underlying mechanistic relationships require further verification.

1.07.11

Multigenerational Impacts of Dietary Exposure to the Flame Retardant, BDE-99, in the Atlantic Killifish (Fundulus heteroclitus)

N.A. McNabb, University of California, Davis / Environmental Toxicology; B.W. Clark, U.S. Environmental Protection Agency / Atlantic Coastal Environmental Sciences Division (ACESD); M. Francoeur, H. Schrader, ORISE at U.S. Environmental Protection Agency / Atlantic Coastal Environmental Sciences Division (ACESD); S. Jayaraman, L.J. Mills, D.E. Nacci, U.S. Environmental Protection Agency / Atlantic Coastal Environmental Sciences Division (ACESD); A. Whitehead, University of California, Davis / Environmental Toxicology

Polybrominated diphenyl ethers (PBDEs) are a class of flame retardants that are persistent, bioaccumulative, and ubiquitous environmental pollutants linked to a variety of adverse health effects, including endocrine disruption and developmental neurotoxicity. Although maternal transfer of PBDEs has been documented, relatively few studies have investigated effects propagated across generations. Using Atlantic killifish (*Fundulus heteroclitus*) as a vertebrate model system, we are testing for multigenerational impacts of dietary exposure to a predominant PBDE congener, BDE-99. Adult wild-caught killifish were fed diets amended with two concentrations of BDE-99, 37.5 and 150 ng/g fish wet weight/day, for 64 days. Fish length and weight were measured at the start and end of the exposure period to monitor growth. To produce the F1 generation, exposed (F0) fish were manually strip spawned at five time points, and eggs were fertilized with subsets archived to quantify maternal transfer. At termination of the dietary exposure, the F0 adults were sampled, and tissues (brain, liver, gonads, and abdominal fat) were weighed and archived for molecular analyses. No significant differences in growth, tissue indices, reproduction, or fertilization rate were observed between treatments in the directly exposed generation. To evaluate neurological impairment in the offspring (F1 generation), locomotor activity was measured in response to alternating light/dark conditions at larval and juvenile time points. Hypoactivity was observed in some of the BDE-99 exposure lineages. At 4 months post-hatch, novel tank diving tests were conducted to assess anxiety-like behavior. Results suggest an anxiolytic effect in F1 fish parentally-exposed to BDE-99. Ongoing experiments will test for persistence of behavioral alterations in the F1 generation, as well as impacts propagated across generations from these parental exposures.

1.07.12

From Genes to Behaviour: Impact of Rare Earth Elements on Mobility, Neuronal Development and Gene Expression on Early Life Stages of Zebrafish (Danio rerio)

S. Piarulli, SINTEF Ocean; B. Hansen, SINTEF Ocean / Environment and New Resources; S. Vicario, University of Milano-Bicocca; F. Kermen, F.N. Fossum, Norwegian University of Science and Technology (NTNU); B. Kvæstad, SINTEF Ocean / Environment and New Resources; A. Booth, SINTEF Ocean / Environmental Technology; J. Farkas, SINTEF Materials and Chemistry / Environment and New Resources

Rare earth elements comprise a total of 16 elements (the 15 lanthanides and Yttrium: REY). Due to their unique physical-chemical properties, REY are essential for a wide range of human applications including new and traditional industries (e.g. renewable energy, automotive industries, metallurgy, textiles), agriculture and medical diagnostics. Rising REY production and use can lead to an increased release into the environment and represents a potential environmental concern. Despite the growing research interests, the effects of REY released from anthropogenic use remain significantly understudied in aquatic organisms and ecosystems. In this study we evaluated the impact of three REY (Nd, Gd and Yb) on early life stages of zebrafish (*Danio rerio).* Fish embryos (n=10 per 4 replicated treatments) were exposed for 6 days to nominally 5000, 500, 50, 5 and 0.5 µg L^{-1}. Mortality, hatching and larvae development were monitored daily. At day 6, larvae (n=3) from each exposure treatment were subjected to imaging analyses to evaluate their mobility and behaviour. Preliminary results showed no acute toxicity effect but a clear decrease in larvae mobility at increasing REY concentrations, with the highest exposures (5000 and 500 µg L^{-1}) causing the largest effect compared to control treatments. After the behaviour monitoring, larvae were sampled for subsequent neuronal development analyses. Further, additional individuals were used for transcriptomic analyses of genes involved in Ca^{2+} pathways and neuronal development. Given the existing knowledge gaps, results from this study are expected to provide advanced understandings on the ecotoxicological risks and impact associated to the rising REY levels in the aquatic ecosystems.

1.07.15

Determination of Organic Pollutants in Eel (Anguilla anguilla) by Liquid Chromatography Coupled With Tandem Mass Spectrometry (LC-MS/MS)

D. Vitale, University of Valencia; R. Alvarez-Ruiz, University of Valencia / Food and Environmental Safety Research Group (SAMA-UV), Desertification Research Centre (CIDE, UV-CSIC-GV),; Y. Pico, University of Valencia / Environmental Quality and Soil

Anguilla anguilla (Linnaeus, 1758) is critically endangered. The impacts of anthropogenic origin, including the presence of contaminants in the coastal ecosystem (comprising also wetlands and shallow lakes), affect the stages of the eel's life cycle. Studying the occurrence of these contaminants in eels it is possible to assess their exposition and safeguard them. This species has also been selected for their high content in proteins (7-15%) and lipids (5-20%) w.w., (Degani, et al., 1986). This poses a challenge for the development of extraction procedures. Eels were from a local market of Valencia city (Spain), where eels have an environmental and cultural interest, because of their presence in the Albufera National Park, where the rice farming and eels fishing are closely related. A multi-residue method was developed for the simultaneous extraction of 5 pesticides, 5 perfluoroalkyl substances (PFASs), 10 pharmaceuticals and 2 illicit drugs. Samples of liver and muscle of *Anguilla anguilla* spiked at three different levels (50, 250 and 500 ng/g) were analyzed after QuEChERs extraction with dispersive solid phase extraction (dSPE) employing Enhanced Matrix Removal (EMR-lipid), specially designed for lipid elimination. Analysis was performed via LC-MS/MS, using an Agilent 1260 Ultra-High-Performance Liquid Chromatograph (UHPLC), coupled to an Agilent 6410 Triple Quadrupole (QqQ) Mass Spectrometer (MS/MS), (Agilent Technologies,

Santa Clara, CA, USA). The extraction procedure achieved recoveries ranging 40-150 % and 40-160% in liver and muscle, respectively; showing the best results at 500 ng/g. Satisfactory repeatability (< 20%) and reproducibility (< 30%) and strong matrix effect (values ranging from -35 to 90%) were observed for most compounds. Furthermore, the validated method was tested in samples from a bioaccumulation study, where differences between the compounds accumulated in muscle and liver were detected. The validated method successfully extracts a wide variety of compounds in eel liver and muscle demonstrating the utility for future studies related to toxicology, metabolomics and occurrence monitoring of organic pollutants in *Anguilla anguilla*. ReferenceDegani, G., Viola, S., & Levanon, D. (1986). Effects of dietary carbohydrate source on growth and body composition of the European eel (*Anguilla anguilla* L.). Aquaculture, 52(2), 97-104.Acknowledgments This work has been supported by the Spanish Ministry of Science, Innovation and Universities and the ERDF (European Regional Development Fund) through the project CICLIC—subproject WETANPACK (RTI2018-097158-B-C31) and by the Generalitat Valenciana through the project ANTROPOCEN@ (PROMETEO/2018/155). R. Álvarez-Ruiz acknowledges the Spanish Ministry of Science, Innovation and Universities and the ERDF (European Regional Development Fund) for his FPI grant BES-2016-078612.

1.07.16

Investigative Monitoring With Zebrafish Embryo Test in the Urban Part of a River After a Fish-Kill Event in Central Italy

M. Carere, Italian Institute of Health; I. Lacchetti, Italian Institute of Health (ISS) / Department of Environment and Health; W. Cristiano, University of Torino / Department of Environment and Health; K. Di Domenico, Italian Institute of Health ISS / Department of Environment and Health; L. Mancini, Italian Institute of Health / Department of Environment and Health

The use of Effect Based Methods (EBM) is recommended in the context of the Common Implementation Strategy of the European Water Framework Directive (WFD). The European environmental agency also suggests their use for screening purposes. Therefore, Member States are obliged to use investigative monitoring programmes that aim to determine the reasons for exceedances or predicted failures in achieving environmental objectives if these reasons are not already known. Furthermore, such monitoring programmes also aim to determine the magnitude and impacts caused by accidental pollution. The use of Zebrafish embryos is emerging as a valid tool for investigation in support of chemical analysis to detect lethal and sub-lethal effects in waterbodies affected by point and diffuse pollution. This approach is beneficial for the authorities in applying the appropriate measures. The Tiber river is a very large river in Italy affected by several sources of pollution (agricultural, industrial, zootechnical) in the urban area of Rome. The river receives the discharges of wastewater treatment plants and the routinary pollution deriving by human activities (personal care products, pharmaceuticals, etc.). During the spring of 2020, a fish-kill event happened in the urban part of the river in the city of Rome, where thousands of fishes of different species have been found dead. The reasons of this event have been rapidly investigated by the local authorities by using chemical analyses. In this context, we have sampled the water column in 3 different sites in the urban part of the river after the event and used the Zebrafish embryo test in order to investigate the causes of the fish-kill. The preliminary analysis performed with the Fish Embryo Toxicity (FET) Test have detected embryological and neurological effects: in particular a percentage of embryos showed spine deformation (scoliosis or lordosis) and other individuals appeared with poor pigmentation. The swimming behaviour also appeared not normal in a percentage of cases. The effects detected can be caused by mixtures of pollutants or by specific emerging contaminants normally present in the Tiber river and not necessarily linked to the event. The causes of the kill-fish event can be also due to a large quantity of suspended solids leached after a flash-storm that happened the day before and every investigative study can be useful. The results of this research will contribute indeed to investigate the reasons behind this event and will help the local authorities to apply the preventive measures needed to avoid analogue events in future.

1.07.17

Impacts of Wildfires on Aquatic Ecosystems - Biomarker Responses of Gambusia Holbrooki in In-Situ Bioassays

A. Ré, Aveiro University & CESAM / Biology; A.T. Rocha, University of Aveiro / department of Biology & CESAM; I. Campos, Universidade de Aveiro / Environment; S.M. Marques, University of Aveiro / Department of Biology and CESAM; J. Keizer, University of Aveiro-CESAM / Department of Environment and Planning; F.J. Gonçalves, University of Aveiro / Department of Biology and CESAM; J. Pereira, University of Aveiro / Department of Biology & CESAM; N. Abrantes, University of Aveiro-CESAM / CESAM/DAO

In the Mediterranean countries of southern-Europe and particularly in Portugal, wildfires have occurred with high frequency and affecting large areas. After the wildfire, and during the first rainfall events, ashes rich on metal and polycyclic aromatic compounds can be mobilized from burnt areas to water systems. Since these compounds are persistent, toxic, mutagenic and carcinogenic, and tend to bioaccumulate along food webs, the mobilization and diffuse dispersal of these contaminants into aquatic ecosystems has received increasing attention from the research community. In fact, these xenobiotics may enter to the organisms and may accumulate in body tissues interacting to target molecules and causing damage that eventually result in disease, malfunction and death. The use of biomarkers in environmental assessment, particularly the biochemical biomarkers, can provide early detection of contaminant exposure at low toxicity levels and an early indication of the potential effects at high levels of biological organization. Hence, the present study using in situ assays and a battery of sub-cellular biomarkers, aimed to assess the impacts of post-fire contamination in the fish *Gambusia holbrooki*. For that, in a recently burnt area, fish were exposed 96h *in-situ* in four different sites: one upstream and other downstream the burnt area on a main river - Ceira river (RUS and RDS, respectively) and two sites within the burnt area in tributary streams (BS_1 and BS_2). Gills and liver were the tissues chosen because they are metabolically active tissues and tend to accumulate high levels of toxicants. Glutathione-S-transferase (GST), enzymatic antioxidants (GPX, GR), a non-enzymatic antioxidant (glutathione-GSH) were measured, the extent of lipoperoxidative damage was also monitored by quantifying the amount of malondialdehyde (MDA) by the TBARS assay and DNA damage was evaluated in erythrocytes nuclear abnormality by ENA assays. In general, the several biomarkers used reveal distinct responses between fire-impacted sites and the non-impacted site, allowing its differentiation. These results support the usefulness of using high relevant approaches and parameters, such as in situ assays and biomarkers to assess the exposure and effects of post-fire contamination in aquatic species such as fish.

1.07.18

Developmental Defects of In-Water Cleaning Wastewater on Resident Non-Target Organism

D. Shin, Korea Institute of Science and Technology; Y. Choi, Korea Institute of Ocean Science & Technology / Risk Assessment Research Center; M. Kim, Korea University of Science and Technology/Korea Institute of Ocean Science and Technology / Risk Assessment Research Center; Z. Soon, Korea University of Science and Technology / School of Ocean Science; J. Jung, Korea Institutue of Ocean Science and Technology (KIOST) / Risk Assessment Research Center

In-water cleaning operation can result in the release of antifouling system to the surrounding coastal environment. The International Maritime Organization (IMO) recommend that not only biosecurity but also contaminant risks are appropriately managed in accordance with the 2015 Guidelines. To define the toxic effects of wastewater from in water hull cleaning on resident non-target organisms, we investigated the chemical concentrations and developmental toxicity on embryonic flounder, which is an organism sensitive to chemical contamination. The dominant inorganic metal was zinc in robot in-water cleaning wastewater (RICW). Forty-eight hours after exposure, the frequency percentage of malformation began to increase in embryos exposed to 50-fold dilution of RICW. We performed transcriptome sequencing to characterize the toxicological developmental effects of RICW exposure at the molecular level. The results of the analysis revealed significantly altered expression of genes associated with muscle cell, muscle contraction, lens development in camera-type eye, and nervous system development (cutoff $P< 0.05$) in embryonic flounder exposed to RICW. Although the toxicity of the malformation defects in embryos exposed to RICW decreased through the robot filter, it was confirmed that the effects of genetic level continued due to dissolved contaminants. These findings provide a greater understanding RICW effluent management practices to reduce impacts on non-target coastal organisms.

Integrating New Approach Methodologies (NAMs) and Quantitative Adverse Outcome Pathways (qAOPs) from

Development to Deployment into Risk Assessment and Decision-Making

1.08.03

Gene Expression Impact of Fenoxycarb in the Aquatic Gasteropod Physella acuta

P. Caballero Carretero, M. Prieto Amador, National Distance Education University (UNED) / Mathematical and Fluid Physics; J. Martinez-Guitarte, UNED / Fisica Matematica y de Fluidos
Grupo de Biología y Toxicología Ambiental. Facultad de Ciencias. UNED, Madrid, Spain.Intensive agriculture requires pesticides to prevent damage to the cultures. Fenoxycarb is a carbamate insecticide with low toxicity for bees, birds, and humans. It is non-neurotoxic and mimics the juvenile hormone to prevent the maturation of insects. It is used to control pests such as flea, mosquito, cockroach, butterflies, moths, scale insects, and sucking insects on fields and stored products. The knowledge of the effect on other invertebrate groups different from insects is still low. A transcriptome project on *Physella acuta*, a snail that lives in freshwater ecosystems, has allowed us to identify genes covering the endocrine system, detoxification mechanisms, stress response, epigenetic modifications, DNA repairing mechanism, and energy metabolism. An array has been designed to evaluate the impact of fenoxycarb on this species. Adult snails have been exposed for one week to 0.01, 1, and 100 µg/L of fenoxycarb to evaluate the transcriptional activity by Real-Time PCR using the array. The transcriptional activity has been studied in each snail. The results obtained in this work provide the first approach at the molecular and cellular levels in gasteropods to elucidate the fenoyxcarb's impact in non-target organisms.Keywords: non-target organism, Real-Time PCR, carbamate.This work has been funded by the Ministerio de Ciencia, Innovación y Universidades (SPAIN), CTM RTI2018-094598-B-I00.

1.08.06

Biomarker Development for Neonicotinoid Exposure in the Soil Dwelling Folsomia candida

R. Bakker, Vrije Universiteit Amsterdam / Ecological Sciences; D. Roelofs, Vrije Universiteit / Ecological Sciences; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science
Pesticide risk assessment has traditionally been determined by exposing test animals in artificial soil to a single compound and measuring its toxicity on phenotypic endpoints such as survival, reproduction and growth. However, it is hard to extrapolate these results to field conditions as pesticide toxicity is altered by interaction effects with other pollution and physiochemical properties of the soil. Gene regulation, gene expression or protein abundance, could be used as biomarkers for pesticide toxicity even under varying mixture compositions and soil properties. Special interest goes out to neonicotinoids, insecticides with high toxicity to a range of soil invertebrates. Biomarker development is carried out in the soil ecotoxicological model *Folsomia candida* (springtails). First, time-resolved transcriptome and proteome data was obtained from springtails exposed to Imidacloprid, a neonicotinoid. We observed that the shift in gene expression patterns was most pronounced at 48 hours after the onset of exposure. Hence, for this time point, additional transcriptome and proteome data was obtained from springtails under combined exposure to Imidacloprid with the fungicide Cyproconazole. Simultaneously, we assessed the validity of biomarkers by studying their gene expression via real-time-quantitative PCR (qPCR) under mixture exposure of Imidacloprid with Cyproconazole and the known metabolomic inhibitor piperonyl butoxide (PBO). Combined, the results identify biomarkers for the development of rapid and cheap soil quality assessment such as insecticide detection. These biomarkers may provide policy makers with metrics of soil pollution that are biologically relevant, i.e. stress related gene regulation patterns, instead of dose limits derived from testing of single compounds in artificial soils.

1.08.07

Lithium Cobalt Oxide Nanosheet Exposure and Cross-Species Comparisons: Identifying Critical Pathways for Common Responses and Interspecies Differences

B. Curtis, N. Niemuth, E. Bennett, University of Wisconsin - Milwaukee / School of Freshwater Sciences; A. Mohaimani, A. Schmoldt, O. Mueller, University of Wisconsin, Milwaukee / Great Lakes Genomics Center; E. Laudadio, University of Wisconsin-Madison / Department of Chemistry; Y. Shen, J.C. White, Connecticut Agricultural Experiment Station; R.J. Hamers, Universtiy of Wisconsin-Madison / Chemistry; R.D. Klaper, University of Wisconsin, Milwaukee / School of Freshwater Sciences
Lithium cobalt oxide (LCO) is a complex metal oxide often utilized in lithium-ion batteries. The absence of a national (U.S.) lithium-ion battery recycling infrastructure and high projected production numbers for electric vehicles in the coming years provide a potential route of entry into aquatic systems through the leaching of spent battery waste. The magnitude of sensitivity to the same nanomaterial can vary over several orders of magnitude across different species. This work assesses molecular responses of three freshwater model species to LCO nanosheet exposure: *Danio rerio*, *Daphnia magna*, and *Chironomus riparius*. All three species (early life stages) were exposed for 48 hours to 1 and 10 mg/l nanosheets, and to a mixture of lithium and cobalt ions. Next-generation RNA-sequencing was utilized to assess changes to gene global gene expression across species and the resulting pathways that may be impacted were examined. Assessment of cobalt body burden via ICP-OES analysis was also carried out. Resulting global transcriptomic profile analysis revealed both molecular-level impacts that were shared across species, as well as those that were species-specific. Nano-specific impacts were observed across all species. *D. rerio* and *D. magna* showed more distinct responses between particle and ion exposures, while *C. riparius*, considered the least sensitive of the three, exhibited clear differences from control as a response to 10 mg/l LCO nanosheets and exhibited more similarities between control, 1 mg/l and ion-exposed organisms. *D. rerio* and *C. riparius* each exhibited low levels of cobalt body burden (0 – 0.02 µg/individual), while *D. magna* had the highest burden (up to 1.35 µg/individual). Affected pathways across species included expected mechanisms of nanotoxicity such as oxidative stress and impacts to energy metabolism, and impacts that are not as extensively discussed such as disruptions of neurological functions and cardiovascular system impacts. Examples of species-specific responses included histamine synthesis in *D. magna*, and androgen/estrogen/progesterone biosynthesis in *D. rerio.*

1.08.09

Chitin Synthase 1 Inhibition Leading to Mortality: Strategies for Evolving a Qualitative to a Quantitative Adverse Outcome Pathway

S. Schmid, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; Y. Song, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; K. Tollefsen, Norwegian Institute for Water Research (NIVA) / Computational Toxicology Program
Arthropods need to molt periodically in order to grow and develop. To complete this process, they depend on the synthesis of cuticular chitin, a *N*-acetyl-D-glucosamine polymer, which constitutes a large part of the arthropod exoskeleton. An enzyme, chitin synthase 1 (CHS-1), is responsible for catalyzing chitin polymerization and translocating the growing chitin chains through the cell membrane. The exoskeleton of an arthropod has several critical functions, such as protecting organisms from pathogens, and providing support for muscular functions. The latter is especially important during the process of molting, where the old exoskeleton is shed through a programmed sequence of muscular contractions. The high degree of conservation of chitin synthesis in arthropods makes this process an exploitable target for pest management initiatives. We recently developed a qualitative Adverse Outcome Pathway (AOP, aopwiki.org/aops/360) that links the inhibition of CHS-1 (molecular initiating event/MIE), through decreased cuticular chitin content and premature molting (key events/KEs) to mortality (adverse outcome/AO) in arthropods. To further develop this AOP into a quantitative AOP (qAOP), the KEs need to be quantified using standardized laboratory analytical techniques and the response-response relationships of the KEs characterized by mathematical/computational approaches. On this poster, different bioassays and approaches will be discussed to assist the development of the qAOP. Numerous different possibilities exist to assess the cuticular chitin content with different levels of throughput which are suitable for generation of quantitative data for this KE. The findings can be supported by histopathological approaches. Premature molting and increased mortality are readily characterized by an OECD 202 *Daphnia sp.* acute mortality test. Since CHS-1 is a large transmembrane enzyme it is difficult to isolate or to recombinantly express in cellular systems. However, one could characterize of the MIE by using crude protein extracts of *D. magna*. The assays will be standardized in a high-throughput format and regression-based approaches will be employed to characterize the response-response relationships. Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 859891 and supported by NIVAs Computational Toxicology Program (www.niva.no/nctp).

1.08.11

Quantification of an AOP Network for Effects of UV Radiation by Statistical and Bayesian Network Modelling

J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; Y. Cao, Orebro University / Clinical Epidemiology and Biostatistics, School of Medical Sciences; R. de Bin, University of Oslo / Department of Mathematics; K. Tollefsen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; Y. Song, Norwegian Institute for Water Research (NIVA) / Section for Ecotoxicology and Risk Assessment

An adverse outcome pathway (AOP) network has been developed to describe the adverse effect of UV-B radiation (AOP #327−330). This tentative AOP, which is the first AOP for a non-chemical stressor, is a complex network linking a molecular initiating event (MIE: cellular ROS formation) to an adverse outcome (AO: reduced survival of a crustacean), through eleven potential key events (KE). The AOP structure has been developed based on literature studies and experimental data, and quantified by experimental studies, where *Daphnia* individuals were exposed to UV radiation in 6 different dose-rates in the range 0 - 0.4 w/m2. All variables (the MIE, KEs, and AO) were measured with a minimum of three repeated measurements for each dose-rate. The resulting proposed AOP network consists of four potential pathways from the stressor to the adverse outcome, which has been quantified with equations fitted to the data using the EPA tool Benchmark Dose Analysis Software. The current study aims to further develop this quantitative AOP (qAOP) by a combination of statistical modelling and causal probabilistic modelling (Bayesian network), within the project RiskAOP (https://www.niva.no/en/projectweb/RiskAOP). Bayesian regression modelling is applied to quantify the key event relationships (KERs) as dose-response curves and the associated uncertainty based on experimental data. Additional statistical methods such as boosting and structural equation models will be explored to support the quantification of KERs. The fitted regression models are subsequently used to generate probability distributions for quantifying the KERs as conditional probability tables in the Bayesian network (BN) model. The fully quantified BN model can be used to further explore the properties of the qAOP. For example, sensitivity analysis of the BN-AOP model can be used for ranking the pathways of the AOP network according to the strength of their influence on the adverse outcomes. The BN-AOP can potentially be used both for prognostic inference (from the MIE to the AO) and vice versa for diagnostic inference (from the AO backwards via the KERs to the MIE). The approach demonstrated here – a combination of expert knowledge, experimental data, exploratory data analysis, and probabilistic modelling - is a promising strategy for the data-oriented selection of key AOPs and the identification of knowledge gaps associated with these AOPs.

New Methods and Tools for the Challenge to Emerging Contaminant Issue

1.09.02

Extended Ultra-Sensitive Method for the Determination of 12 Hormones and Estrogenically Active Substances With Gc/Ms-Ms

C. Sowa, Shimadzu Deutschland GmbH / Marketing Manager; S. MOREAU, Shimadzu Europa Gmbh / Product & Marketing Management

The European Union's monitoring list includes the estrogenically active substances estrone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2). For water monitoring, detection limits (LOD) of 400 pg/l for E1 and E2 and 35 pg/l for EE2 are required to be met. The stringency of these levels comes from the adverse biological effects of these substances at these concentration ranges. The requirements of the EU Water Framework Directive (EU-WFD) state that the total water sample must be analyzed without prior filtration[AS1] , which can lead to considerable problems due to matrix effects. In addition to the three estrogenically active substances mentioned in the EU-WFD, there are other compounds of interest[AS2] like Androsterone, Testosterone and Bisphenol F. In this new method, the entire suite of analytes of interest can be measured in a single run, to get a deeper look into the water sample and the contamination with hormones and estrogenically active substances, at the required sensitivity with extraordinary robustness.
[AS1]Filtration or Sample Cleanup? If no filtration, the problem would be solids clogging
[AS2]List a couple?

1.09.05

Uptake and Translocation of Nanoplastics Across Fish Intestinal Epithelia

G. Asmonaite, University of Gothenburg / Department of Biological and Environmental Sciences; D.M. Mitrano, ETH Zurich / Environmental Systems Science; T. Lammel, University of Gothenburg / Department of Biological and Environmental Sciences; J. Gallego, University of Gothenburg / Department of Marine Sciences; H. Sundh, B.C. Almroth, University of Gothenburg / Department of Biological and Environmental Sciences

The exposure to microplastics and nanoplastics to aquatic organisms is a growing (eco)toxicological issue, since ingestion of small plastic particles is regarded as a prominent route of exposure in aquatic organisms such fish. Knowledge concerning particle uptake and translocation from the intestinal tract to an organism, however, remains scarce, as reliable approaches and tools suited for qualitative and quantitative assessment of plastic particle uptake remain under development. The goal of the present study was to investigate the uptake and translocation of nanoplastics across fish intestinal epithelia and confirm the feasibility of using Palladium-doped polystyrene nanoparticles (Pd-PSNPs) to aid in the detection of plastics in model exposure systems. Herein, segments of proximal and distal intestines of rainbow trout were subjected to Pd-PSNPs exposure ex vivo, with subsequent quantification of Palladium as a proxy for nanoplastics by ICP-MS to assess uptake and transport of nanoplastics across the tissue. Furthermore, exposed sections of intestinal tissue were imaged using transmission electron microscopy to examine particle uptake. In this study, we demonstrated uptake and translocation of metal-doped nanoplastics across the viable fish intestine. Estimated particle translocation in fish intestines was low (< 0.1% 90 min-1). While we demonstrated that it is feasible to use metal-doped polystyrene particles to study particle uptake and translocation in fish, the quantitative estimation of nanoplastics' uptake over longer exposure times is required, as well as further experimental validation in vivo. With our work, we also highlighted remaining challenges and practical considerations in nanoplastics' uptake and translocation studies.

1.09.06

Associations of Iron Oxide Nanoparticles With Glyphosate Induces Toxicity on Liver Tissue and Cells of Poecilia reticulata, Followed by Recovery Trend in Pos-Exposure Period

J.d. Faria, Federal University of Goias / Lab. Celular Behavior; L. Nunes Guimarães, V. Costa da Silva, L. Kênida Lessa Martins, L. Braga Alonso, Federal University of Goias / Lab. Cellular Behavior; S.M. Teixeira Sabóia-Morais, Federal University of Goias / Department of Morphology

Nanotechnology is an increasingly explored field, due to the diversity of applications in areas such as agriculture, human and environmental health. In this universe, the citrate functionalized iron oxide nanoparticles (IONPs) are alternatives for the remediation of contaminated environments. On the other hand, glyphosate (GLY), the main component of herbicides (GBH) such as Roundup®, has a recognized toxicity on aquatic organisms. In this study, the toxicity of the co-exposure of IONPs associated with GBH / GLY and the potential for recovery - from post-exposure - of females of *Poecilia reticulata* were evaluated. Ten animals per group were exposed for 7, 14 and 21 days to treatments with iron ions (IFe) and associations of IONPs (0.3mg / L) to GLY (0.65mg / L) and GBH (0.65-1.30mgGLY / L), followed by post-exposure in reconstituted water for the same period. The histology, histopathological index and ultrastructure were evaluated in tissue and liver cells. All treatments shows hepatotoxicity and induces damage in liver tissue, so that circulatory disturbance (Rp1) and regressive alterations (Rp2) increased during exposure, followed by decreased and post-exposed recovery, in contrast, inflammatory response (Rp3) took longer than Rp1 and Rp2 and persisted until the end of post-exposure time on all treatments. The inflammatory response results on a high impact in the total histopathological index, that persists in damage despite the decrease on post-exposure time. In general, liver hepatotoxicity by association of IONP+GBH is higher than IONP+GLY and the highest GBH concentration caused inhibition of inflammatory response, beyond this, steatosis was found in all treatments signaling intense process of oxidative stress and lipid peroxidation. Ultrastructural analyses confirm histological assessments from disorganization of rugose endoplasmic reticule, mitochondria proliferation, lisossomal activity and increase of Disse space surface contact. So, this work concludes that the animals had an active immune response by the detoxification system in cells, so that the inflammatory responses was persistent on 21 days post-exposure and in parallel there is a recovery trend from the total histopathological index. On this way, this study contributes to knowledge about IONPs as an environmental alternative, incrementing the possibilities about its use and impacts.

1.09.07

Occurrence of Microplastics in Semi-Intensive Aquaculture and Potential Biochemical Impacts in Farmed Fish Related to ENvironmental and Anthropogenic Stressors

C. Rocha, University of Coimbra, MARE - Marine and Environmental Sciences Centre / Department of Life Sciences; J.C. Marques, University of Coimbra, MARE - Marine and Environmental Sciences Centre / Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal; A. Goncalves, MARE - Marine and Environmental Sciences Centre, University of Coimbra / Life Sciences

The overall characteristics of organisms are determined by the set of environmental factors to which they are subjected to, with factors derived from human activities being particularly important, as most produce nefarious impacts to the environment. Plastic pollution is currently among society's main concerns regarding human impact in ecosystems. Estuaries are particularly pressured by human activities, and aquacultures are in true expansion in such systems. Fish production is particularly important in Southern European countries, and some of these, as Portugal, rear fish in semi-intensive regimes, using estuarine water for the production tanks. The water is renewed roughly every two weeks, when the water kept in the tanks is released to the estuary, while water with lower matter load enters the tanks. As fish are a highly important food source and given the poor control of environmental conditions in semi-intensive regimes, there is an interest in understanding how plastic pollution may affect these systems. This study aims to understand the input of plastic in estuarine aquacultures and potential effects in farmed fish. Therefore, different matrices (water, sediment and fish) were sampled from rearing tanks of four Portuguese aquacultures, located in two estuaries – Mondego and Sado –, subjected to different polluting pressures. Results show: i) water released from tanks carries higher microplastic (MP) load, compared to incoming water; ii) water from the Sado estuary, with higher human pressure, presents a higher count of MP particles in both incoming and release water; iii) fibres are the main type of MP in water; iv) MP of higher density and various shapes are enclosed in sediment; v) fish tissues accumulate different types of MP, with fibres being the most abundant. FTIR analysis are being performed to identify the polymers in the samples, to set a link to the potential origin of the particles found – if transported to tanks by incoming water, or if the practice itself contributes to plastic input in estuaries. Biochemical analysis regarding fatty acid and carbohydrate content of fish were performed, with calculations ongoing, to assess the potential impact of the set of environmental and human-derived stressors, like MP, in the organisms' nutritional content. This study provides further supporting material to enhance strategies to food safety and security and economic development, highlighting the potential impacts to human diet and health.

1.09.08

Chironomus riparius Molecular Response to Polystyrene Microparticles

J. Kalman, National Distance Education University (UNED) / Biology and Environmental Toxicology; A. Garcia, UNED / UNEDLab; J. Martinez-Guitarte, UNED / Fisica Matematica y de Fluidos

Nowadays, plastics are present in many forms and ways in daily life. They have been used for decades and solved many problems. However, the same properties that make them useful also are a disadvantage for degradation and waste, being a severe problem at the environmental level. Yearly, tons of plastics reach the water and start a degradation process that involves mechanical and chemical processes. Consequently, the big fragments of plastics are broken into smaller pieces reaching, in the end, microscopic sizes. The particles less than 5 mm are known as microplastics and increase their presence in the environment, both from primary sources and by the degradation of massive plastic waste. Microplastics can be of different sizes, ranging from micrometers to millimeters and various compositions. In this work, we have analyzed the impact of commercial 5.09 µm polystyrene particles on the gene expression of *Chironomus riparius*, a dipteran with aquatic larvae. Characterization of the microparticles was carried out by NMR (nuclear magnetic resonance) and microscopy. Fourth instar larvae were exposed to three different concentrations of 5.09 µm microparticles (1X, 10X, 100X) for 24h, 48h, and 72h. The mRNA was extracted and used to perform a Real-Time PCR analysis with two arrays covering genes involved in hormonal regulation, stress response, energy metabolism, DNA repairing, and detoxification mechanisms. The differential expression has been detected in several genes showing that the microplastics could alter the cell's mRNA levels, disturbing the processes that are in progress. Additional research is needed to elucidate the extension of the impact and microplastics' ability to affect *Chironomus riparius*.

1.09.09

Ribosomal Proteins As Refence Genes in Real Time PCR for Ecotoxicology Analysis

M. Prieto Amador, P. Caballero Carretero, National Distance Education University (UNED) / Mathematical and Fluid Physics; J. Martinez-Guitarte, UNED / Fisica Matematica y de Fluidos

Real-time PCR attracts growing interest in ecotoxicology as it provides information at molecular and cellular levels about the mechanisms involved in the toxicity and the response to toxicants. The method is based on good reference genes that are not affected by the tested conditions. The reference genes, therefore, are essential to validate the result, but they are often not tested to confirm whether they are stable. Massive sequencing offers the possibility of studying the transcriptome of practically all the species, giving access to a vast number of genes that can be used as a reference. Ribosomal proteins are among the most used as reference genes, but no studies have shown their transcription behavior in the presence of toxicants, backing them as perfect reference genes. In this work, several ribosomal protein genes have been identified in the transcriptome of *Chironomus riparius*, a dipteran with aquatic larvae and a model species used frequently in ecotoxicology. The genes coding from the large and small ribosomal units have been identified and used in two arrays to analyze the impact of two pollutants, Bisphenol A and endosulfan, at the transcriptional activity. The reference genes were four non-ribosomal genes (actin, GAPDH, RNA polymerase II, and TBP). The results showed that treated larvae did not exhibit transcriptional modulation in the concentrations tested. Although only two toxicants have been tested and additional toxicants should be investigated, the present work backs the use of ribosomal protein genes as reference genes on this species.

1.09.10

Design of the Biological Module Based on Immobilized Bacterial Coupled Enzymatic System: NAD(P)H: Fmn-Oxidoreductase-Luciferase for Bioluminescent Sensors

I.G. Torgashina, Siberian Federal University / Biophysics; E. Esimbekova, Siberian Federal University / Department of Biophysics; V. Kratasyuk, Siberian Federal University / Biophysical

Bioluminescent enzyme systems based on bacterial luciferases offer a unique and general tool for assays of the many analytes in the environment, research and clinical laboratories and other fields. The goal of the study is to design a biological module of a bioluminescent biosensor based on immobilized into starch gel bioluminescent enzyme system. The sensor of such kind is suitable for environmental risk assessment provided an alarm detection and quantitative measurement of integral toxicity levels caused by chemical threats. The biological module for the enzyme-based biosensors included co-immobilized into dried starch gel bacterial coupled enzyme system: NADH:FMN-oxidoreductase and luciferase and their substrates (aldehyde and NADH). To design an active and sensitive biological module, the characteristics of the enzyme system NADH:FMN-oxidoreductase and luciferase immobilized together with NADH and aldehydes of different lengths of the hydrocarbon chain were investigated. Such aldehydes as decanal (C_{10}) and tetradecanal (C_{14}) were chosen as substrates of the luciferase. The maximum luminescence intensity of the biological module was much higher when we used C_{14} as the luciferase substrate. The sensitivity of the biological module consisting of enzyme system immobilized together with NADH and C_{14} to the copper sulphate used as a model toxicant is an order of magnitude higher than that of the enzyme system immobilized together with NADH and C_{10}. To ensure high activity and sensitivity of the bioluminescent sensor to the action of toxic substances, it is advisable to use C_{14} as a component of biological module. It is possible to increase the sensitivity of the biological module to the toxicants by decreasing of the enzymes concentration in it. The lowest value of IC50 for copper sulphate was equal 1.25 mg / L and was achieved with 0.2 µg of luciferase and $0.6x10^{-4}$ units of activity of NADH: FMN - oxidoreductase in the module. The designed biological module kept its activity and sensitivity to the toxicants for 2 months of storage at 4^0C. The reported study was funded by Russian Foundation for Basic Research, Government of Krasnoyarsk Territory, Krasnoyarsk Regional Fund of Science, to the research project № 20-44-242001.

1.09.11

Histological Evaluation of Sexually Mature Oreochromis mossambicus (Peters, 1852) Gonads After Sub-Acute Exposure to the Antiretroviral Drug Efavirenz

M. Nibamureke, M. Bopape, I. Wagenaar, University of Johannesburg / Zoology

The antiretroviral (ARV) drug Efavirenz (EFV) is widely administered to HIV patients in South Africa and has been detected in surface water around the country. There are concerns that the presence of pharmaceutical compounds, including ARVs, in water may negatively affect fish health at long term. This study was conducted in 2018 and its aim was to investigate the potential effects of sub-acute exposure to EFV on *O. mossambicus* gonads using histopathology as a biomarker. Seventy-two sexually mature *O. mossambicus* (red strain) were exposed to two different concentrations of EFV (10.3 and 20.6 ng/L). These concentrations were quantified from the Luvuvhu River in Limpopo Province during a field trip in 2016. The laboratory exposure lasted 28 days and a static-renewal system was used; the exposure medium was renewed every 96 hours. At the end of the exposure, the fish were killed, and the gonads were sampled and processed following standard histological protocols. A semi-quantitative histological assessment of the gonadal tissue was conducted using light microscopy and a histological scoring system. The non-parametric Kruskal-Wallis test was used to compare the gonad indices between exposed fish and the control groups. The preliminary results showed increased histological changes in male fish gonads with the fish exposed to EFV (20.6 ng/L) presenting higher testis indices compared to the control. However, there were no statistically significant differences in testis indices between the fish exposed to EFV and the control ($p > 0.05$). For female fish, also no significant difference was found in ovary indices between exposed fish and the control groups. With tap water being used as a medium, the study could not conclude with certainty if EFV had no negative effects on the fish gonadal tissue as the analysis of both the control medium and tap water revealed the presence of EFV up to 18 ng/L. These findings draw attention to the complex problem of pharmaceutical pollution in aquatic environments. They also highlight how problematic is testing the effects of some pharmaceuticals such as ARVs on aquatic organisms as these compounds are present in all types of water resources in Africa.

1.09.12
Regeneration Rate in Hydra Vulgaris Pallas, 1766 to Detect Aquatic Teratogenic Risks
G. Cesarini, University of Roma Tre / Science; F. Spani, Università Campus Bio-Medico di Roma / Departmental Faculty of Medicine and Surgery; M. Scalici, Roma Tre University / Department of Sciences
The evaluation and monitoring of chemical impacts affecting both the environmental and human health have been improved by using sentinel organisms, as is required by the European Water Framework Directive (2000/60/EC). The regenerative ability of *Hydra vulgaris* Pallas, 1766 a freshwater coelenterate (Cnidaria: Hydrozoa), was tested as early warning system to asses the teratogenic risk of freshwaters. The new eco-toxicological index, the Teratogenic Risk Index (TRI), is based on the regeneration rate and the aberration frequency of the columna after the cut of head and tentacles. This study tested the teratogenic risk of 9 rivers of the Central Italy surrounding by different land use. Among the 22 sampling sites investigated, 18% of them fell in class I (no risk), 68% in II (low risk), 9% in III (moderate risk), and 5% in IV (high risk). No sites fell in class V (very high risk). Results highlighted a general low teratogenic risk, focusing also on some criticisms. The exposure of *H. vulgaris* at the sampled water did not allow a complete regeneration of the head after 96 h for each site. Surprisingly, this dynamic it is also observed in the control tests. Among the 9 investigated rivers showed significant differences between average regeneration rates compared to the control. In particular, the River Tiber and River Ausente, showed a significantly higher average regeneration than control although they belonged to a low teratogenic risk class. In order to know the causes it is necessary to investigate more deeply with chemical analyses and toxicological tests to detect the substances present in freshwaters, however the increase of the regenerative rate in the river samples compared to the control induces to think to mechanisms of the tumour masses. Therefore, the *Hydra* assay needs to be further investigated in order to validate it as an early warning system not only for teratogenic substances but also for carcinogenic substances, which increase cell proliferation. For future studies using the Hydra assay, the feeding test after the 96 h of exposure provides an important tool for testing the proper functioning of the nervous system. In addition, histological analysis can be conducted to assess the presence of hyperplasia. The use of *H. vulgaris* provides a realistically assess of the emergent contaminant impacts with a one health approach.

1.09.13
Mitigation of Glyphosate Ecotoxicity by Vacuum-Uv Treatment
S. Bjergbakke Fogh, Aalborg University / Department of Chemistry and Bioscience; P. Roslev, Aalborg University / Biology and Environmental Science
N-(phosphonomethyl)glycine (glyphosate) is the most widely used active ingredient in non-selective broad-spectrum herbicide formulations. Glyphosate is approved for both professional and non-professional use and is frequently detected as a contaminant in surface waters and groundwater. The environmental toxicity of glyphosate to non-target organisms has been considered low but adverse effects have been observed in organisms from different trophic levels including bacteria, algae, crustaceans, amphibians, and fish. In this study, we examined whether Vacuum UV and UV-C treatment of glyphosate in drinking water can degrade this herbicide and decrease the toxicity to non-target organisms. VUV irradiation of water can generate different chemical radicals including hydroxyl radicals that can potentially degrade environmental contaminants. The UV mediated degradation of glyphosate was quantified by LC analysis, and changes in toxicity was measured using bioassays with the bacterium *Bacillus subtilis* and the green microalga *Raphidocelis subcapitata.* The bioassays were applied to integrate biological effect of all constituents in the water samples before and after UV treatment including transformation products. The results showed that exposure of glyphosate to VUV (185 nm) and UV-C irradiation (254 nm) significantly decreased concentrations of glyphosate and decreased the toxicity to aquatic test organisms as indicated by increasing EC50 values. This effect was observed in different drinking water types and at different UV doses (Joule/cm^2), and there was a direct positive correlation between irradiation dose and decreases in toxicity to the test organisms. Several reactive oxygen species were generated during the VUV and UV-C treatment and likely interacted with aqueous glyphosate. The less toxic degradation product aminomethylphosphonic acid (AMPA) and orthophosphate were among the transformation products observed after UV-C and VUV treatment. The study suggests that VUV and UV-C mediated photolysis can decrease concentrations of glyphosate and generate less toxic products with decreased overall toxicity to aquatic organisms.

1.09.14
Ecotoxicity of Azole Fungicides After Uv-C and Vacuum-Uv Irradiation
O. Del Puerto, Aalborg University; N.P. Gonçalves, Università di Torino / Dipartimento di Chimica; P. Calza, Università di Torino / Dipartimento di Chimica; A.B. Prevot, Università di Torino / Dipartimento di Chimica; P. Roslev, Aalborg University / Biology and Environmental Science
Azole fungicides have a variety of different applications to prevent the spread of fungal diseases. They are widely used not only in agriculture to control diseases in crops and vineyards, but also in medicine to treat human diseases. Due to their widespread use and environmental persistence, azole fungicides are frequently detected in wastewater, surface water and occasionally in groundwater. Their bioavailability to aquatic organisms has raised concern about potentially adverse effects on non-targeted organisms. UV treatment has appeared as a promising technology to remove unwanted chemicals from the water cycle and can potentially mitigate environmental impacts. Vacuum-UV (VUV) irradiation of water results in the in situ generation of reactive species, mainly hydroxyl radicals, able to react with a wide range of chemical pollutants. In this study, we investigated the potential of VUV (185 nm) and UV-C (254 nm) irradiation to degrade the fungicides tebuconazole (pesticide) and clotrimazole (pharmaceutical) in water matrices. The effects of monochromatic UV-C irradiation were compared with simultaneous irradiation by UV-C and VUV. The degradation of tebuconazole and clotrimazole and the associated transformation pathways were elucidated by HPLC and LC-HRMS analysis. Changes in toxicity during different UV regimes were examined using aquatic organisms from different trophic levels. The test battery included the luminescent bacterium *Aliivibrio fischeri*, the bioluminescent yeast *Saccharomyces cerevisiae* BLYR*,* the fungus *Fusarium graminearum,* the freshwater microalgae *Raphidocelis subcapitata*, and the crustacean *Daphnia magna.* These organisms were used to assess biological effect before and after UV exposure of parent compounds and degradation products. UV-C and VUV irradiation significantly decreased concentrations of the parent compounds and decreased the ecotoxicity by increasing the apparent EC values. A direct positive correlation was observed between the disappearance of the parent compounds and the measured decreases in toxicity to the test organisms. UV-C and VUV treatment was able to alleviate the ecotoxicity of tebuconazole and clotrimazole at concentrations below and above the mg/L level. The study showed that bioassays can provide key information about changes in water chemistry during photodegradation experiments

and that UV mediated photolysis of tebuconazole and clotrimazole can degrade these compounds and decrease the overall ecotoxicity.

1.09.15

Protein Model and Function Analysis in Quorum Sensing Pathway of Vibrio Qinghaiensis Sp-Q67

Z. Wang, Tongji University; S. Liu, Tongji University / State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering

Bioluminescence is the phenomenon of light emission and production in living organisms. For bioluminescent bacterial, there have been found in five main genera *Vibrio*, *Photobacterium*, *Aliivibrio*, *Photorhabdus*, and *Shewanella*. Bioluminescence in bacterial produced by bioluminescence reaction catalyzed by enzymes encoded by the core *lux* operon *luxCDABEG*. Moreover, the expression of the core *lux* gene is regulated by the quorum sensing (QS) pathway. At present, bioluminescent bacteria are mainly found in marine habitats. *Vibrio qinghaiensis* sp.-Q67 (Q67), as nonpathogenic bacteria, has been focused on since it has been widely used in environmental pollution monitoring and toxicity assessment. However, the lack of available crystal structure limits the elucidation of the structure of a functional protein of the QS system that regulates bacterial luminescence in Q67. In the study, 17 functional proteins were built through monomer and oligomer modeling based on 19 coding proteins in the QS system of Q67 by MODELLER. Except for failure LuxM without a suitable template, 16 functional proteins were successfully constructed. Among them, four functional proteins including LuxU, LuxO, LuxI, and LuxC were in monomer form, and other 12 proteins, including two heterologous oligomers, LuxPQ and LuxAB were in oligomer form. Furthermore, the relationship between the function and structure of 17 functional proteins were elucidated one by one according to the three functional classifications: autoinducer synthases and receptor, signal transmission protein (phosphotransferases, RNA chaperone and master QS regulator) and enzymes in bacterial bioluminescence reaction. This is the first time to analyze the whole process of bioluminescence regulation from the perspective of nonpathogenic freshwater bacteria at the molecular level. Also, it provides a theoretical basis for the explanation of applications of Q67 in which the luminescent inhibition is used as the endpoint.

1.09.17

Integration of Rare Earth Elements (REE) As Emerging Contaminants Into a Novel ICP-MS Method for the Analysis of Water Samples

T. Vincent, Thermo Fisher Scientific GmbH; C. Cojocariu, Thermo Fisher Scientific / CMD; D. Kutscher, Thermo Fisher Scientific GmbH / Applications; J. Renpenning, Thermo Fisher Scientific / CMD; S.M. Nelms, Thermo Fisher Scientific / marketing

Inductively coupled plasma mass spectrometry (ICP-MS) is the most widely employed technique for analysis of trace elements in environmental samples like surface waters and sediments. For the most common contaminants, such as chromium, arsenic, selenium, cadmium, mercury and lead, methods are well established for their analysis in analytical testing laboratories. In recent years, rare earth elements (REEs) have been observed as emerging contaminants despite their low occurrence in nature. Increased concentrations of gadolinium (Gd) have been reported in tap water[1] and river water collected close to medical facilities, due to its use as a contrast agent in MRI and computerized tomography (CT).[2,3] Other REEs have the potential to accidentally leach out into the environment from consumer electronics or from batteries disposed of incorrectly. Consequently, it is important to monitor REE levels in environment samples, and therefore most of these elements are included in regulated methods for the analysis of drinking and surface waters, such as ISO method 17294 which governs water analysis in the European Union. Quantifying REEs in such samples still comes with challenges, such as the ultra-low concentrations of REEs in water samples (typically $ng \cdot L^{-1}$), variable chemical composition of samples, and spectral interferences. Besides their potential to cause interferences on key analytes by formation of doubly charged interferences (i.e. $^{150}Nd^{++}$ on $^{75}As^{+}$)[4], lighter members of this group of elements can contribute to the resulting signal for the heavier homologs through oxide formation (e.g. $^{156}Gd^{16}O^{+}$ on $^{172}Yb^{+}$). This poster presentation describes how interference free, low level analysis of rare earth elements can be integrated into a fast, sensitive and robust multi-element ICP-MS method for the analysis of drinking and surface water samples. This analytical method was tested using water samples collected locally as well as applicable certified reference materials (CRMs).

References: 1. 1: N. Tepe et al., (2014) *Applied Geochemistry, 45,* High Technology Metals as emerging contaminants: Strong increase of anthropogenic gadolinium levels in tap water of Berlin, Germany, from 2009-2012 2. L. Telgmann et al., (2013) *Analytica Chimica Acta*, **764**, 1. Determination of gadolinium-based MRI contrast agents in biological and environmental samples 3. M. Birka et al., (2016) *Journal of Chromatography A*, 1440, 105. Tracing gadolinium-based contrast agents from surface water to drinking water by means of speciation analysis4. Thermo Fisher Scientific. Application Note 43285: Accurate determination of arsenic and selenium in environmental samples using triple quadrupole ICP-MS.

1.09.18

Extraction and Seasonal Characterisation of Selected Benzodiazepines in Wastewater Samples

S. Nzube, Cape Peninsula University of Technology (CPUT) / Department of Chemistry; V.S. Somerset, CPUT / Chemistry

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 lorezapam as benzodiazepines. Preliminary results have shown the diazepam concentrations to range between 1 to 7 ppm in seasonal wastewater samples analysed. Further results for seasonal characterisation are still being investigated in this ongoing study.

1.09.19

New Insights Into Associations Between TOrCs Biotransformation, Microbial Composition and Functional Potential in Biofiltration Systems

L. Cao, C. Wurzbacher, Technical University of Munich / Chair of Urban Water Systems Engineering; A. Wick, Federal Institute of Hydrology / G2 -Water Chemistry; D. Wolff, Bundesanstalt für Gewässerkunde / Aquatic Chemistry; R. Liguori, Parthenope University of Naples / Department of Science and Technology

Trace organic chemicals (TOrCs) such as pharmaceuticals, personal care products and pesticides, are of emerging concern due to their widespread occurrence in aquatic environments and the potential risk they pose to aquatic ecosystems as well as human health. Biofiltration processes can be applied for the removal of TOrCs both in wastewater and drinking water treatment systems. However, *the TOrCs biotransformation mechanisms as well as the underlying drivers behind the transformation processes still remain elusive. In this study,* we used laboratory batch studies to investigate the biotransformation of 51 TOrCs by various sand filter materials from wastewater and drinking water treatment plants. Chemical analysis was complemented by 16S rRNA and metagenome sequencing for assessing associations between the biotransformation rate constants, microbial composition and identified functional enzymes. *Differences in biotransformation rate constants were mirrored in differential microbial community composition, enzyme abundances and involved biotransformation pathways. Nitrospira was found to indicate the high biomass in wastewater biofiltration systems, and indirectly indicated the good biotransformation performance of TOrCs under eutrophic condition. Pseudomonas could indicate the slow biotransformation of TOrCs under oligotrophic condition. The up-regulation of enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase enzyme indicated the enhancement of TOrCs biotransformation. In general, this study provides new insights into so far rarely addressed associations between TOrCs biotransformation, biomass, microbial composition and functional potential, which will be used to suggest novel biological indicators for assessing the removal performance of TOrCs in biofiltration systems.* p margin-bottom: 0.1in; direction: ltr; color: #000000; line-height: 115%; text-align: left; orphans: 2; widows: 2 p.western font-family: "Liberation Serif", serif; font-size: 12pt; so-language: en-US p.cjk font-family: "Noto Sans CJK SC", sans-serif; font-size: 12pt; so-language: zh-CN p.ctl font-family: "Lohit Devanagari"; font-size: 12pt; so-language: hi-IN

1.09.20

Sampling Emerging Pollutants in Three Rivers of the Basque Country Impacted by Wastewater Effluents: How and What to Measure?

B. Gonzalez-Gaya, ESTACIÓN MARINA DE PLENTZIA. UPV/EHU / Analytical Chemistry; I. Garrido, ESTACIÓN MARINA DE PLENTZIA. UPV/EHU; J. Urra, University of the Basque country (UPV/EHU) / Chemical and Environmental Engineering; M. Olivares, A. Prieto, N. Etxebarria, O. Zuloaga, University of the Basque country (UPV/EHU) / Analytical Chemistry; J. Lechuga, University of the Basque country (UPV/EHU); E. Ruiz Romera, University of the Basque country (UPV/EHU) / Chemical and Environmental Engineering

The effects of urban, industrial and hospital wastewater discharges were evaluated on the occurrence of emerging contaminants (ECs) in three riverine basins impacted by wastewater treatment plants (WWTP) effluents. The objective was to identify ECs in water samples using different sampling techniques and targeted screening analysis with liquid chromatography coupled to high resolution mass spectrometry. In this study, an annual cycle of sampling campaigns were carried out; November 2019 and January, August, November 2020. Water samples were taken from three catchments in the Basque Country (North Spain) where three sampling points were stablished: upstream and downstream of the WWTPs discharge and in the point directly affected by the WWTPs effluents. Grab sampling was compared with passive sampling using diffusive gradients in thin films (DGT) with two sorbent types, XAD and HLB, for their suitability to screen ECs in rivers. Moreover, 24 h composite samples gathered directly at the WWTP installations were analyzed in order to know the composition of the effluents. An abiotic monitoring was carried out to evaluate the characteristics of the receiving environment and to detect possible correlations between organic pollutants and other physical/chemical variables such as temperature, pH, redox potential, dissolved oxygen, soluble anions, orthophosphate, nitrites, nitrates, ammonia and organic carbon. For the ECs analysis, 1L grab samples were extracted with triphasic cartridges (HR-X, WAX, WCX) while DGT devices packed with XAD and HLB sorbents and exposed for one week, were washed correspondingly. The targeted analysis with a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific Q Exactive Focus Orbitrap mass spectrometer, comprised more than 250 target compounds including pesticides, pharmaceuticals and personal care substances, food additives, plasticizers, water repellents and other ECs of interest. Preliminary results point to a dominance of the pharmaceutical compounds in the waters, which come mainly from the WWTP effluents, although other non-point sources for compounds like pesticides cannot be neglected. DGTs sorbent characteristics were determinant to favor the adsorption of the ECs and HLB resulted as the most efficient among the tested ones. It allowed detecting some pharmaceutical compounds even at higher concentrations than the grab samples, although the grabbed waters provided a higher number of observed compounds. ACKNOWLEDGEMETS: This work was financially supported by the Spanish Ministry of Science and Innovation through the RTI2018-093989-B-I00 project.

Extended submission 1 - Ecotoxicology and human toxicology: from molecules to organisms, from omics to in vivo

1.10.02

Beyond Apis mellifera -A Phylogenomics Approach to Identify Putative Detoxification Enzymes in Different Bee Species

J. Haas, University of Bonn / INRES; N. Exeler, B. Buer, M.T. Almanza, R. Nauen, Bayer CropScience

Insect and especially bee pollinator declines are a topic of great concern and public debate. Although reasons are manifold, the use of pesticides is under particular scrutiny as a major factor involved in decreases in bee pollinator diversity and abundance. Historically, bee pollinator risk assessment of crop protection products relies on the honey bee *Apis mellifera* as a model organism due to its importance, broad availability and standardized methodology. Recently, the question arose whether or not *A. mellifera* is a good surrogate species in risk assessment for the very diverse and complex clade of Anthophila. Although huge efforts are invested in developing new bioassays for different bee species, a problem remains: the practicability and/or validity of these test systems as well as the availability of rare species for laboratory testing. This gap in methodology can in parts be filled by using approaches utilizing the rapidly advancing field of genomics. Leveraging the increasing amount of published genomic data, we conducted a phylogenetic approach to identify close orthologues of the *A. mellifera* CYP9Q enzymes – a P450 subfamily previously shown to effectively detoxify bee-safe insecticides – in different bee species and families. After the functional expression of more than 20 candidate genes covering a diverse range of bee species we investigated their activity against a range of fluorescent model substrates to compare substrate profiles. We additionally tested their ability to metabolize different chemical classes of insecticides. These results provided insights into the comparability of detoxification systems between different bee species and families, helping us to better understand and assess the intrinsic toxicity of insecticides to bees covering a broad phylogenetic range. Our novel toxicogenomics-based phylogenetic approach is designed to complement existing methods for pesticide risk assessment by assessing the capacity of recombinantly expressed bee pollinator P450 enzymes known to confer pesticide selectivity, thus helping to biochemically address issues of ecotoxicological concern.

1.10.06

Zebrafish Toxicity, Uptake and Biotransformation Assessment of Tetracaine Exposure Under Different pH Values, Utilizing Embryotoxicity and Lc-Hrms

D.E. Damalas, E.I. Panagopoulou, E. Aleiferi, R. Aalizadeh, National and Kapodistrian University of Athens / Department of Chemistry; M. Schweizer, A. Schlösinger, University of Tuebingen / Animal Physiological Ecology; H.R. Köhler, University of Tubingen / Animal Physiological Ecology; P. von der Ohe, German Environment Agency / Section IV 1.3 Plant Protection Products; R. Triebskorn, University of Tuebingen / Animal Physiological Ecology; N. Thomaidis, National and Kapodistrian University of Athens / Department of Chemistry

The contamination of the aquatic environment has raised concerns in the scientific community and regulatory authorities, over the last decades. Given the large number of xenobiotics (such as pharmaceuticals), for most of them, there is a striking deficit in the literature concerning their adverse effects on aquatic organisms. Tetracaine (TCN) belongs to the category of ionizable organic compounds (IOCs). Therefore, its behavior such as the uptake, bioaccumulation and toxicity can be significantly altered by shifts in the ambient pH values. However, the influence of the different pH values on toxicity of IOCs has not been extensively evaluated in risk assessment studies. Zebrafish (*Danio rerio*) has emerged as a powerful model organism to study various aspects of developmental and cell biology as well as physiology, while it provides a promising alternative model for acute toxicological studies. The objectives of this study were (1) to assess to what extent TCN induces toxicity to zebrafish embryos. In addition (2), we evaluated the uptake and biotransformation processes of TCN by zebrafish and examined whether biotransformation data could be used in a complementary way to the concentration of the parent compound to interpret the induced toxicity. The final goal was to evaluate to which extent the pH is influencing CTR's uptake, potential bioaccumulation and biotransformation, as well as toxicity. More specifically, the zebrafish embryotoxicity assay was used to calculate the LC_{50} of TCN, as well as to evaluate potential sub-lethal endpoints (e.g. hatching rate). Exposure experiments were conducted at three different pH values (5, 6 and 8), to assess potential pH-dependent differences in an environmentally relevant pH range. Concerning the toxicokinetic part of the study, exposure water samples and zebrafish extracts were analyzed by RPLC and HILIC methods, in both positive and negative ionization mode, to cover the widest possible range of polarities, using LC-QTOF HRMS. Detection and identification of tentative TCN biotransformation products were performed through in-house developed suspect and non-target screening workflows. Finally, the biotransformation pathway of TCN in zebrafish embryos was proposed.

1.10.07

Chemical Warfare Agents in the Baltic Sea: Biotransformation Products and Their Toxicity

P. Vanninen, Finnish Institute for Verification of the Chemical Weapons Convention / Department of Chemistry, University of Helsinki; K.K. Lehtonen, Finnish Environment Institute / Marine Research Centre; H. Hakulinen, H. Heikkinen, H. Kiljunen, M. Kjellberg, Finnish Institute for Verification of the Chemical Weapons Convention / Department of Chemistry, University of Helsinki; A. Lastumäki, Finnish Environment Institute / Marine Research Centre; O. Michenkova, Finnish Institute for Verification of the Chemical Weapons Convention / Department of Chemistry, University of Helsinki; H. Niemikoski, Finnish Environment Institute / Laboratory Centre; A. Reunamo, Finnish Environment Institute / Marine Research Centre; R. Turja, Finnish Environment Institute, SYKE / Marine Research Centre

Chemical warfare agents (CWA) produced in mass quantities during World War I and II were disposed of by sea-dumping, a practice adopted by many nations in Europe and other countries worldwide. Studies carried out during the past 15 years have proven that corroded munitions containing CWA are leaking and therefore causing potential risk to the marine environment. Thus far research has focused on intact and primary degradation products of toxic phenylarsenic CWA and sulfur mustard. The objectives of the WARTOX project (2021-2023) funded by the Academy of Finland are to (a) investigate the mechanisms of phenylarsenic CWA and sulfur mustard biotransformation in Baltic Sea sediments and the main microbial groups responsible, (b) develop targeted chemical analysis methods for the identified biotransformation products, (c) study the metabolism of sulfur mustard in aquatic biota using in vitro models, and (d) assess toxicity and sublethal effects of the main biotransformation products of phenylarsenic CWAs in model species. Microbial community changes and biotransformation pathways are identified by amplicon sequencing, metagenomics and -transcriptomics in CWA contaminated sediments and spiked reference sediments. Biotransformation products are identified by using sophisticated structure elucidation methods in sediments and aquatic organisms. Fish cell line assays (RTL-W1) will be used to study cytotoxicity and cellular metabolism. Toxicity, bioaccumulation and various sublethal biological effects will be evaluated using standard invertebrate (*Daphnia* sp., *Lumbriculus variegatus*) and vertebrate (*Danio rerio*) biotest organisms. The expected results include an updated list of the target chemicals to be used in monitoring as well as toxicity thresholds for improved risk assessment of the dumped CWA.

1.10.09
The Effects of Multiple Stressors on Daphnia: A Meta-Analysis
S. Ladner, California State Polytechnic University, Pomona / Biological Sciences
Due to an increase in anthropogenic activity over the last century, environmental contaminants have become increasingly pervasive globally. A variety of those toxicants persist and bioaccumulate in freshwater ecosystems. While the effects of toxicants and natural stressors are often examined individually, their combined effects have not been readily studied. This is primarily because of the conceptual and logistical challenges of simultaneously analyzing multiple factors in a single study. The microcrustacean *Daphnia* is found in most freshwater ecosystems and is exposed to both natural and anthropogenic stressors. Its parthenogenic reproduction, rapid progeny development, and well-studied life history make it an ideal model organism to study stressors' combined effects. We conducted a meta-analysis of ecotoxicological studies that focused on the individual and combined effects of various natural and anthropogenic stressors. We categorized stressors, their mechanisms of action, and responses in various *Daphnia* species through this approach. Here, we make the case that exposure to multiple stressors might impact Daphnia's behavior and fitness in an antagonistic, additive, or synergistic way. We hypothesized that synergistic interactions are the most prevalent type, based on the principle that the time and energy available for organisms to combat stressors' effects are extremely limited—a response to one stressor, therefore trade-off against responses to another. We also propose that more studies use model organisms and explore the impact of multiple stressors in aquatic environments.

Advances in Behavioural (Eco)Toxicology-the Missing Link Connecting Proximate and Ultimate Effects of Chemical Contaminants and Environmental Changes

2.01.02
EthoCRED: A Framework to Guide Reporting and Evaluation of the Reliability and Relevance of Behavioural Ecotoxicity Studies
M.G. Bertram, Monash University / Biological Sciences; M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry; J. Allen, U.S. Environmental Protection Agency / Office of Research and Development Cincinnati; B.W. Brooks, Baylor University / Department of Environmental Science; Z. Dang, National Institute for Public Health and the Environment (RIVM); S. Duquesne, UBA, Federal Environment agency / Plant Protection Products; A. Ford, University of Portsmouth / Biological Sciences; F. Hoffmann, German Federal Institute for Risk Assessment / Chemical Safety; H. Hollert, Goethe University Frankfurt / Department Evolutionary Ecology and Environmental Toxicology, Faculty Biological Sciences; S. Jacob, German Environment Agency (UBA); W. Kloas, Leibniz-Institute of Freshwater Ecology and Inland Fisheries / Ecophysiology and Aquaculture; N. Kluver, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology; J.M. Lazorchak, U.S. Environmental Protection Agency / Office of Research and Development Cincinnati; M. Ledesma, Swedish Chemicals Agency; G. Maack, German Environment Agency / Ecotoxicological Assessment of Pharmaceuticals; S. Melvin, Australian Rivers Institute / School of Environment and Science, Griffith University; S. Mohr, German Environment Agency / IV; S. Padilla, U.S. Environmental Protection Agency / ORD CCTE BCTD; G.G. Pyle, University of Lethbridge / Biological Sciences; M. Saaristo, Monash University / School of Biological Sciences; R. Sahm, German Environment Agency (UBA); S. Scholz, Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology; E. Smit, RIVM / Centre for Safety of Substances and Products; J.A. Steevens, U.S. Geological Survey / Columbia Environmental Research Center; S.v. Berg, Wageningen University & Research / Aquatic Ecology and Water Quality Management; B.B. Wong, Monash University / School of Biological Sciences; M. Ziegler, University of Tuebingen / Animal Physiological Ecology; T. Brodin, Swedish University of Agricultural Sciences (SLU) / Department of Wildlife, Fish, and Environmental Studies
Behavioural analysis has been garnering significant attention as a broad indicator of sub-lethal toxicity, and has secured a place as an important sub-discipline in aquatic ecotoxicology. One of the most notable characteristics of behavioural research, compared to other established approaches in sub-lethal ecotoxicology (e.g. reproductive and developmental bioassays), is the wide range of study designs being used and the diversity of endpoints considered. At the same time, environmental hazard and risk assessment, which underpins regulatory decisions to protect the environment from potentially harmful chemicals, often recommends that ecotoxicological data be produced following accepted and validated test guidelines. These guidelines typically do not address behavioral changes, meaning that these, often sensitive, effects are not represented in hazard and risk assessments. Here, we propose a new tool, the EthoCRED evaluation method, for assessing the reliability and relevance of behavioural ecotoxicity data, which considers the unique requirements and challenges encountered in this field. This method, and accompanying reporting recommendations, are designed to serve as an extension of the 'Criteria for Reporting and Evaluating Ecotoxicity Data (CRED)' project. As such, EthoCRED can both accommodate the wide array of experimental design approaches seen in behavioural ecotoxicology, and is able to be readily implemented into regulatory frameworks in different jurisdictions to allow better integration of knowledge gained from behavioural testing into environmental protection. Furthermore, through our reporting recommendations, we aim to improve the reporting of behavioral studies in the peer-reviewed literature, and thereby increase their usefulness in chemicals regulation.

2.01.03
Frontiers in Quantifying Wildlife Behavioural Responses to Chemical Pollution
M.G. Bertram, Monash University / Biological Sciences; J.M. Martin, Monash University / School of Biological Sciences; E. McCallum, Swedish University of Agricultural Sciences; L.A. Alton, J.A. Brand, Monash University / School of Biological Sciences; B.W. Brooks, Baylor University / Department of Environmental Science; D. Cerveny, Swedish University of Agricultural Sciences / Department of Wildlife, Fish, and Environmental Studies; J. Fick, Umea University / Department of Chemistry; A. Ford, University of Portsmouth / Biological Sciences; G. Hellström, Swedish University of Agricultural Sciences / Department of Wildlife, Fish, and Environmental Studies; M. Michelangeli, Swedish University of Agricultural Sciences (SLU); S. Nakagawa, University of New South Wales / School of Biological, Earth and Environmental Sciences; G. Polverino, University of Western Australia / Centre for Evolutionary Biology; M. Saaristo, Monash University / School of Biological Sciences; A. Sih, University of California Davis / Department of Environmental Science and Policy; J. Sundin, Swedish University of Agricultural Sciences / Department of Aquatic Resources; H. Tan, Monash University / School of Biological Sciences; C.R. Tyler, University of Exeter / Biosciences College of Life and Environmental Sciences; B.B. Wong, Monash University / School of Biological Sciences; T. Brodin, Swedish University of Agricultural Sciences (SLU) / Department of Wildlife, Fish, and Environmental Studies
Recent research has demonstrated that animal behaviour is remarkably sensitive to disruption by chemical pollution, with widespread implications for ecological and evolutionary processes in wild animal populations. Despite this, conventional techniques applied to

understand impacts of chemical pollution on wildlife behaviour are often rudimentary—e.g. exposing individuals of a single species in isolation and measuring movement in a confined arena. Such approaches are largely inadequate in assessing effects of contaminants in highly complex and interconnected natural systems. The aim of this review is to push forward and guide the field of behavioural ecotoxicology, with an emphasis on harnessing recent experimental and technological advancements to increase mechanistic understanding and environmental realism. First, we discuss the utility of behavioural ecotoxicology and consider current limitations and future opportunities. Next, we examine how recent advances in analytical chemistry and exposure modelling can inform prioritisation of research questions, stressing the importance of hypothesis-driven approaches. We then argue for targeting behavioural endpoints with high ecological significance, and outline a range of concepts within ecology that have been broadly overlooked within ecotoxicology but, nevertheless, have the potential to provide unparalleled insights. Then, we present a suite of cutting-edge experimental methods and technologies available for quantification of animal behaviour in the lab and the field, over both short and extended temporal scales. We go on to outline developments in statistical sophistication necessary to evaluate behavioural impacts of contaminants occurring in complex systems. Finally, we assess challenges and opportunities in incorporating increased environmental realism into behavioural ecotoxicology. Given the rapid proliferation of behavioural ecotoxicology research in recent years, this review provides a roadmap of the major outstanding questions in this field, and highlights the need for increased cross-talk with other disciplines in order to find the answers.

2.01.06

Does an Anxiolytic Pharmaceutical Affect Fish Predator-Prey Interactions in the Wild? A Field Study Using Acoustic Telemetry

E. McCallum, Swedish University of Agricultural Sciences; G. Hellström, D. Cerveny, Swedish University of Agricultural Sciences / Department of Wildlife, Fish, and Environmental Studies; H. Baktoft, DTU AQUA National Institute of Aquatic Resources; A. Nilsson, K. Hulthén, L. Hansson, Lund University; C. Brönmark, Lund University / Dept of Biology; T. Brodin, Swedish University of Agricultural Sciences (SLU) / Department of Wildlife, Fish, and Environmental Studies

Pharmaceutical pollution has scientists and environmental managers around the world concerned about how these relatively novel pollutants will affect aquatic wildlife. Of the many pharmaceutical pollutants present in the environment, psychiatric pharmaceuticals such as anxiolytics, antidepressants, and mood-stabilizers are widespread and have great potential to profoundly impact animal behaviour, especially anti-predator behaviour and species interactions. However, few studies have explored how exposure to behaviour-modifying pharmaceuticals affects fish and their behaviour in natural habitats. Here, we exposed two freshwater fish species: roach (prey) and pike (predator) to an anxiolytic benzodiazepine (oxazepam) using novel, slow-release internal implants. We then tracked their movement in a natural pond using high-resolution acoustic telemetry where half the fish were exposed and the other half were unexposed. Our study will compare how exposed fish vary their activity, habitat-use, and shoaling behaviour (e.g., shoal size, proximity), and how these endpoints varied over the day night-cycle and the month-long exposure period. We will identify any pike predation events, quantify predator avoidance using nearest predator "neighbour" metrics, and identify how pike presence alters roach shoaling and activity responses. Field-based studies focusing on the behavioural effects of pharmaceutical pollutants are still rare, but are valuable for extending laboratory findings to the real world. Our findings add to a growing literature advocating for "upscaling" studies of ecotoxicology to include, among many things, more realistic and large-scale exposure settings such as natural freshwater habitats.

2.01.07

Spatial Use of an Agrarian Landscape by Linnets (Linaria cannabina) in Central Europe in Late Summer

C. Wolf, C. Gutiérrez-Expósito, B. Gießing, Tier3 Solutions GmbH; J. Hahne, Bayer AG Crop Science Division / Environment Safety; C. Vives-Ferrándiz Sánchez, Tier3 Solutions GmbH

The current EFSA Guidance Document on the risk assessment for birds and mammals for plant protection products specifies the linnet (*Linaria cannabina*) as a focal bird species for 10 different crops. Since linnets preferably forage on small seeds, this species can therefore be treated as an important representative of the granivorous diet guild. To evaluate the potential exposure of linnets by plant protection products data from wild linnets can be gathered. In order to support the assessment of the actual relevance of the utilisation of arable fields and their preference in comparison to other habitats, information on the spatial use of agrarian landscapes by linnets is of high interest. This spatially explicit information is especially interesting during late summer when the availability of volunteer seeds from recently harvested fields and freshly drilled seeds overlaps. For this purpose 21 linnets were continuously radio-tracked for a complete daylight activity period in an agrarian landscape in north-east Germany. For every change in the position of the bird, the geographic coordinates of its location were recorded and assigned to the habitat categories 'arable field' (further specified as 'current crop' or 'harvested field'), 'settlement' and 'natural habitat'. The duration of the birds' visit at each location was noted. The recorded locations were used to generate individual home ranges. The categorized habitats within these home ranges were mapped in order to quantify their availability. The time spent by each tracked individual in the specified habitats was calculated. The combination of the time-span that a tracked linnet spent in a given habitat and the spatial proportion of this habitat in its home range enables the calculation of the individual habitat preference index. The species specific index value was calculated as the mean of the single values obtained from the tracked individuals. Linnets spent most of the time in arable fields followed by natural habitat and settlement. However, within the summarised category 'arable fields' linnets spent on average more time in 'harvested fields' in comparison to 'current crops'. Considering the habitat availability, tracked linnets show also a clear preference for 'harvested fields' most likely due to the high abundance of volunteer seeds.

2.01.08

Focal Species Aerial Survey in ECOTOX Field Studies - a Near Future Technique?

O. Fuelling, Tier3 Solutions GmbH / Field study; C. Wolf, Tier3 Solutions GmbH; A. Rossbach, Tier3 Solutions GmbH / Field team

Non-target mammalian field studies in the context of plant protection product registration are focussing on small and medium sized mammals like mice, voles, rabbits and hares (EFSA birds & mammals guidance document 2009). Hares in particular are night active. A well-established method to count them utilises the fact that the hare's eyes reflect light from a strong spotlight at night. During the last decade, thermal imaging devices entered the civilian market and became more affordable and handier. Thermal imaging allows to detect and to observe the animals at night, without disturbance. However, spotlight counting and thermal image observations from the ground are limited by plant height and corrugations in the terrain that hide the animals. Furthermore, distance estimation is difficult at night no matter if using traditional spotlight method or a thermal imaging device. Both, hidden areas in the terrain and unknown distances make density estimation (i.e. individuals/hectare) difficult. Meanwhile drones have become a valuable tool for many surveying tasks even in field biology. Here we tested their usability of thermal imaging drones for counting hares in arable landscapes. The drone platform can be operated manually but much more interesting for our purpose is the feature of pre-programmed – so called – missions. This feature allows a precise selection of the study area, flight altitude, angle of front view of the cameras, overlap of survey corridors etc. Such features seem to make thermal imaging drones suitable for comparing rabbit or hare densities in different crops. First test-missions showed that hares and other wildlife didn't escape or evolve any obvious phobic behaviour when a drone was approaching, so that the technique has a low-invasive impact on the animals.

2.01.10

Fluoxetine Disrupts Collective Behaviour of Fish Across Ecological Contexts

M. Michelangeli, J.M. Martin, Monash University / School of Biological Sciences; J. Henry, RMIT University / Biosciences and Food Technology; J.A. Brand, Monash University / School of Biological Sciences; T. Brodin, Swedish University of Agricultural Sciences (SLU) / Department of Wildlife, Fish, and Environmental Studies; D. Wlodkowic, RMIT University / School of Applied Sciences; B.B. Wong, Monash University / School of Biological Sciences

Collective behaviours in wildlife, like schooling in fish, are key traits shaping ecological communities and demographic processes. Such grouping behaviours hinge upon universal interaction rules among group members that mediate both group movement and performance. Pharmaceutical contaminants likely have the capacity to disrupt collective behaviours via effects on individual sociality and cognitive abilities. Yet, very few studies have considered how the behavioural impacts of pharmaceutical contaminants might manifest at the group-level. Accordingly, we examined the impact of a pervasive antidepressant pollutant, fluoxetine, on the structure, movement and foraging performance of mosquitofish groups. We

repeatedly tested groups of fish across two ecological contexts both before and after exposure to one of three fluoxetine treatments – control (0 ng/L), low (30 ng/L), high (300 ng/L). Using high-throughput animal tracking software, we then quantified repeatable group-level behaviours, including group cohesion, leadership, movement and orientation. Our study (1) provides one of the first examples of how pharmaceutical pollution scales-up to effect group-level dynamics, (2) highlights the necessity for behavioral tests to consider the social context to more accurately predict the environmental risk of chemical pollutants for social-living species, and (3) demonstrates a robust methodological approach for testing ecologically-relevant behaviours across multiple levels of biological organisation.

2.01.13
Pharmacological Modulation of Phototactic Response of Daphnia magna to Fish. the Role of Cholinergic, GABAergic and Histaminergic Signalling
J. Bedrossiantz, IDAEA CSIC / Environmental Toxicology; L. De Meester, Katholieke Universiteit Leuven / Biology; C. Barata, CSIC / Environmental Chemistry; D. Raldúa, IDAEA-CSIC / Environmental Chemistry
Animal behaviour is closely related with individual fitness allowing animals to choose suitable mates or avoid predation. The central nervous system (CNS) regulates many aspects if not all of animal behaviour responses, therefore behavioural responses can be special sensitive to compounds with a neurodevelopmental or neurofunctional mode of action. Phototactic behavioural changes against fish in the freshwater crustacean Daphnia magna and/or its closest relative D. pulex have been the subject of many ecological and more recently genomic investigations. Individuals living in lakes with fish showed a heritable negative phototactic behaviour when sense the presence of fish. Genomic and pharmacological information indicate that the histaminergic, GABAergic and cholinergic neurological systems may be involved. However, reported results are not conclusive. The aim of this study was to identify which neurotramitter systems modulate the phototactic behaviour to fish kairomoines. We use a positive phototactic clone ($P_1$32,85) that shows a marked negative phototactisc after exposure to fish kairomones. Fish conditioned water was obtained with 8 cm juveiles of *Leuciscus idus* (1 fish/50 L). Treatments include up to 20 known agonists and antagonist of serotonergic, cholinergic, Dopaminergic, Histaminergic and GABAergic receptprs. A new custom designed vertical oriented two 50 mL chamber device with an apical white led light controlled by the Noldus software (Netherlands) was used. Changes in preferred area (bottom, middle vs upper areas) were analyzed using groups of animals upon exposure to 24 h to selected substances. Results indicated that agonists of the acetylcholine and GABA receptors and their equiefective mixture decreased the negative phototactic response to fish kairomones whereas antagonists of the above mentioned receptors together with histaminergic ones increased negative phototaxis. The analysis of the profile of neurotransmitters and related metabolites in exposed and unexposed D. magna individuals by high peformance liquit chromatography coupled with tandem mass spectromely indicated that most of the tested drugs had a similar pharmacological effects in Daphnia than in humans.

2.01.15
Linking Behavioral Response and Ecotoxicology in Decapod Crustaceans Using Activity Assays
F. Keitel-Gröner, NORCE - Norweigian research centre; S. Bamber, NORCE Norwegian Research Centre; R.K. Bechmann, NORCE Norwegian Research Centre / IRIS; T. Baussant, NORCE Norwegian Research Centre
The behavioral response of an organism exposed to stress depends on its biochemical and physiological conditions and therefore represents an acute cumulative reaction. Behavioral tests are noninvasive and considered of high ecological relevance. They cover a variety of activities, such as avoidance/escape, feeding, locomotion and mating. Escape behavior, for example, has been shown to be an early warning of acute exposure to toxic compounds in different (marine) organisms. Here, we present an activity assay setup to study decapod crustacean behavior in relation to various anthropogenic stressors. Further, we provide an overview of its application in our research. This work focus on the Northern shrimp, *Pandalus borealis*, an ecologically and economically important species of northern ecosystems along the Norwegian coast and the Arctic up to Svalbard. Activity in shrimp was logged using an infrared light beam system allowing simultaneous continous recording of individual shrimp held under subdued lighting conditions over several days. While the initial test chambers were designed to study locomotory behavior in adult specimens, the system was modified for use with early life stages as well. Larval stages exhibit positive phototaxy and this response to light is crucial in nature to put them close to their food and enhance their chances of successful growth and development. Hence, with this assay, the ability of larvae to respond to light following their exposure to various treatments can be recorded. We illustrate the behavioral response of Northern shrimp larvae and adult stages to a variety of anthropogenic stressors such as (accidential) petroleum discharges and anti-parasitic drugs used in salmon fish aquaculture. This research highlights the value of linking standard ecotoxicological endpoints and behavioral responses to further increase the ecological relevance.

2.01.16
What Is the Relationship of Behavioral Endpoints to Traditional Acute, Growth and Reproduction Endpoints for Several Inorganic and Pharmaceutical Chemicals Using ECOTOX
S. van den Berg, Wageningen University & Research / Environmental Risk Assessment; J.M. Lazorchak, J. Allen, U.S. Environmental Protection Agency / Office of Research and Development Cincinnati
Behavioral responses are an important endpoint in ecotoxicology due to the link they provide between effects found at a cellular level to impacts on the fitness of an individual. However, what has so far remained unclear is how behavioral endpoints relate to more traditional endpoints, like mortality, growth and reproduction. We perform a meta-analysis of the ECOTOXicology knowledgebase (ECOTOX), which is a comprehensive, publicly available knowledgebase providing single chemical environmental toxicity data on aquatic life, terrestrial plants and wildlife. Our meta-analysis reveals that at a broad level behavioral endpoints are not always the most sensitive endpoint for each taxa-chemical combination. To get a better insight on whether experimental and or research-quality parameters can help explain this unexpected result, we subsequently performed a more in-depth analysis of the data focusing on standard test species such as *Daphnia magna, Hyalella azteca,* and *Danio rerio*. We compared the behavioral endpoints of such commonly tested species with standard endpoints for the following chemicals: copper, zinc, cadmium and lead (heavy metals), ethynylestradiol (an estrogen), trenbolone (an androgen), fluoxetine, atenolol (both neurotransmitter modulators), sulfamethoxazole (an antibacterial), hydrochlorothiazide (anti-hypertensive), ibuprofen (anti-inflammatory), and gemfibrozil (lipid modifier). We compared the LD 50s, LC50s, IC10s and NOECs for both Behavioral endpoints and standard tests. The combination of both broad-level as well as an in-depth analysis of the ECOTOX database allows us to create a clear picture of why behavioral data is currently not used in risk assessment, and how we can make behavioral data acceptable for future regulatory use.

2.01.18
Environmentally Relevant Concentration of Caffeine - Effect on Activity and Circadian Rhythm in Wild Perch
D. Cerveny, Swedish University of Agricultural Science / Department of Wildlife, Fish, and Environmental Studies; P. Cisar, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters; T. Brodin, Swedish University of Agricultural Sciences (SLU) / Department of Wildlife, Fish, and Environmental Studies; J. Fick, Umea University / Department of Chemistry
We studied ecological consequences of widespread caffeine contamination by conducting experiment focused on changes in behavior traits of wild perch (*Perca fluviatilis*) after waterborne exposure to 10 µg L^{-1} of caffeine. We monitored fish swimming performance during both light and dark conditions to study effect of caffeine on fish activity and circadian rhythm. All individuals undergo three series of behavior trials – before exposure, after 24 hours of exposure, and after five days of exposure. We did not observe any effect of given caffeine concentration on fish activity under light or dark condition. Contrary to caffeine exposure, fish swimming performance was significantly affected by both light conditions and repeating of behavior trials. Individuals in both treatments swam significantly more during the light regimes and their activity was increasing with time as follows: before exposure< after 24 hours of exposure< after five days of exposure. We confirmed a great applicability of three-dimensional automated tracking system based on infrared sensor for conducting behavior experiments under completely dark conditions.

2.01.19
A Novel and Versatile Assay System to Assess Contamination-Driven Habitat Selection in Chemically Heterogeneous Landscapes: The Ultimate System for Non-Forced Ecotoxicology Tests
D. Salvatierra, A. Cordero-de-Castro, .

RodrÃguez-Ruiz, Institute of Marine Sciences of Andalusia (CSIC) / Department of Ecology and Coastal Management; A. Laissaoui, National Centre for Nuclear Energy, Science and Technology; D.C. Silva, University of Southern and Southeastern Paṙ / Institute of Xingu Studies; E. Espindola, University / Hydraulics and Sanitation; F. Mena, Universidad Nacional / Regional Institute for Studies on Toxic Substances (IRET); M. Banni, Higher Institute of Agronomy / Laboratory of Biochemical and Environmental Toxicology; M.A. Islam, Faculty of Fisheries, Sylhet Agricultural University / Department of Aquatic Resource Management; J. Blasco, Instituto de Ciencias Marinas de Andalucia (ICMAN-CSIC) / ECOLOGY AND COASTAL MANAGEMENT; C.V. Araujo, Institute of Marine Sciences of Andalusia (CSIC) / Department of Ecology and Coastal Management

Aquatic ecosystems are complex structures where the exchange of nutrients and biomass is crucial to their structure and functioning. As the environmental complexities of ecosystems have been such a challenge to ecotoxicology, the effects of contaminants have been assessed at the individual level under quite a reductionist environmental scenario. In this sense, the scenario traditionally simulated in the ecotoxicity experiments is characterized by a forced exposure in a confined environment. This practical and easy-to-use scenario has helped ecotoxicology to understand the risks of contaminants and mechanisms involved for organisms and ecosystems, but with no consideration of the spatial connectivity that could exist among contaminated and uncontaminated ecosystems. In the last two decades, some non-forced multi-compartmented exposure systems have been developed aiming to simulate chemically heterogeneous environmental scenarios, throughout which organisms can move. The most recent assay system, called HeMHAS (Heterogeneous Multi-Habitat Assay System), is made of fixed compartments connected by gates contained in a single module. However, the fixing of the compartments supposes a limitation of the system. Hence, the current work aims to present a novel and versatile version of the HeMHAS that allows a more complex simulation of the environmental heterogeneity. This system comprises several independent square habitats (compartments), which can be connected using small bridges with rotating doors. The doors inside the bridges can be manually or automatically operated. The habitats simulated in each compartment can be connected and organisms can move freely among them. The methodological advance offered by the new HeMHAS allows the simulation of uncountable scenarios of contamination in rivers, lakes and estuarine areas. The current work shows how this system could be integrated into the ecotoxicological studies to clarify how contaminants might: (i) affect the spatial distribution of populations in a chemically heterogeneous landscape, (ii) increase the loss of local biodiversity and disrupt the functioning of the ecosystems due to evasion of organisms, and (iii) change the ecological niche of avoiders/invaders due to the contamination-driven habitat selection. This novel system is a complementary tool to be used in ecotoxicology to understand the impact of contamination on the ecosystems from a spatially broader and connected environmental perspective.

2.01.20

Impact of Contaminants on the Spatial Distribution of Aquatic Species in a Non-Forced Heterogeneous Exposure Scenario: An Approach Based on the Spatial Avoidance

D. Salvatierra, . RodrÃguez-Ruiz, A. Cordero-de-Castro, Institute of Marine Sciences of Andalusia (CSIC) / Department of Ecology and Coastal Management; E. Ramos-Rodṙguez, University of Granada / Department of Ecology and Institute of Water Research; G. Parra, University of Jȧn / Department of animal biology, plant biology and ecology; C.V. Araujo, Institute of Marine Sciences of Andalusia (CSIC) / Department of Ecology and Coastal Management

Drugs and pesticides are chemicals that are continuously generated and poorly removed at wastewater treatment plants. Apart from the fact that they degrade slowly, they are becoming pseudo-persistent contaminants and may have a biological impact on aquatic ecosystems. Irgarol and caffeine are two examples of contaminants of emerging concern. Irgarol is a water soluble pesticide that is widely used as an antifouling agent in paint and has proven to cause serious damage to organisms. Similarly, caffeine has been considered a pharmaceutically active compound (PhAC) of environmental concern due to the large consumption of it throughout the world. In addition to these two compounds, the metal cadmium, a traditional reference element in ecotoxicology, was used as a test substance in the current study. Although the role of the contamination in the loss of biodiversity is commonly linked to the mortality or the deleterious effects on the reproduction, a contaminated ecosystem could suffer a reduction in biodiversity due to the chemical repellency that force the displacement of organisms to other habitats. In the current study, we assessed the repellent potential of irgarol, caffeine and cadmium to two freshwater species, a cladoceran *Daphnia magna* clone and the shrimp *Atyaephyra desmarestii*. The potential of contaminants to cause repellency was studied in a multi-compartmented non-forced exposure system (HeMHAS) that simulates contamination gradients. The displacement (fleeing or not) of organisms among those gradients indicates the repellency of the contaminant. In general, organisms were exposed to a gradient of the chemicals that varied from 0.01 ɔg/L to 10 mg/L. Exposure occurred in the dark and during a period no longer than 24 h. Successive observations were made at intervals to check for possible delay in the ability to detect aversive concentrations and the avoidance response. *D. magna* was not able to avoid caffeine, but did do so with irgarol and cadmium. *A. desmaresti* detected all tested contaminants, avoiding caffeine and cadmium and was attracted to irgarol. These results indicate that a single contaminant could have differential effects on organisms belong to different trophic levels, so the detected responses might affect the entire trophic web.

2.01.21

Behavioural Toxicity of Di-2-Ethylhexyl Phthalate (DEHP) and Dibutyl Phthalate (DBP) to a Marine Amphipod

B. Green, University of Lagos, Akoka / Institute of Marine Science, Department of Biological Sciences; M. Tenório Botelho, University of São Paulo / Biological Oceanography Department; M.O. Parker, L. Grinsted, University of Portsmouth; A. Ford, University of Portsmouth / Biological Sciences

Phthalates are commonly used as plasticizers to increase the flexibility and durability of plastics across a wide variety of personal and household product. These phthalates are well known for their numerous toxic effects including endocrine disruption, reproduction and developmental abnormalities in both human and animals. There is growing evidence that DEHP as an endocrine disrupting chemical (EDC) interfering with ecdysteroid signalling in crustaceans. Standard toxicity studies have consistently shown a decrease in reproduction, life span and development when exposed to these chemicals. However, limited studies have been carried out on the effect of phthalates using behaviour as an endpoint. Therefore, this study set out to evaluate the effect of DEHP and DBP on behaviour and moulting using marine amphipod *Echinogammarus marinus* for a 14-days exposure period. Animals were exposed to DEHP concentration of 0.5, 5, 50, 500 and 5000µg/l. The nominal concentration is verified by analysis to confirm actual concentration exposed to the animals. The behaviour of these animals will be tracked using DanioVision and the EthosVision video tracking software to record the velocity, distance and activity state of the exposed animals in the behavioural chamber. During the 14-day period, moulting and mortality are observed daily. The results from this experiment will establish the extent to which phthalates can influence the behaviour of amphipods.

2.01.22

Is Spatial Navigation in Echolocating Bats Affected by Organophosphate Pesticides?

N.I. Sandoval Herrera, University of Toronto Scarborough / Ecology and Evolutionary Biology; P. Faure, McMaster University / Psychology, Neuroscience & Behaviour; K.C. Welch, University of Toronto Scarborough / Biological Sciences

Bats are potentially exposed to pesticides by eating contaminated insects in croplands. Commonly used pesticides such as organophosphates (OPs) are neurotoxic for non-target vertebrate species and even low doses can impair essential processes such as locomotion and cognition. These neurotoxic effects are usually sublethal and can therefore be difficult to study using traditional toxicological assessments. Behavioral studies are a promising alternative to evaluate sublethal effects of pesticides on wildlife. Echolocating bats often develop individual stereotyped flight patterns as they become familiar with a novel space. We evaluated bats' ability to memorize and navigate a new space by comparing the consistency of flight in bats exposed and unexposed to pesticides. We orally dosed captive big brown bats (*Eptesicus fuscus*) with an environmentally relevant concentration of Chlorpyrifos, a common insecticide, and tracked their flight behavior while exploring a tent. We evaluated the similarity of flight trajectories within and between trials, time spent in flight, and landing frequency. We also quantified cholinesterase (ChE) activity in brain and plasma as biomarkers of potential neurotoxicity. Preliminary results suggest there is increased variability of flight trajectories in bats exposed to Chlorpyrifos within trials, and an increase in landing frequency compared to unexposed bats. Exposed bats presented a 60% reduction of ChE

activity in the brain. These results support the sensitivity of behavior as a biomarker of toxicity and as a tool to elucidate potential ecological implications of anthropogenic stressors on wildlife.

Advantages of Using Invertebrates in Ecotoxicology: Challenges and Opportunities for Environmental Risk Assessment and Endocrine and Neuro-Endocrine Research

2.02.01

Optimising the Synchronisation of the Mayfly Cloeon Dipterum for Use in Chronic, Higher-Tier Laboratory Testing

Z.L. Jones, Cambridge Environmental Assessments (CEA) / CEA; H. Schuster, M. Brown, Cambridge Environmental Assessments (CEA) / Ecotoxicology; J. Smith, Cambridge Environmental Asessments (CEA) / Ecotoxicology; N. Taylor, Cambridge Environmental Assessments (CEA) / Ecotoxicology

The pre-adult stages of mayflies (Ephemeroptera) reside in freshwater habitats such as ponds, streams, rivers and lakes. The mayfly species, *Cloeon dipterum* is highly abundant throughout the British Isles and across Europe. They are often used as a non-standard test species in acute laboratory studies for plant protection products (PPPs). Recent studies have found that, in some instances, mayfly larvae are more sensitive to PPPs than other benthic macroinvertebrates. As such, there is a need for the development of methods for the chronic testing of mayfly species, such as *C. dipterum*. During experimental laboratory trials, we have developed methods for measuring effects on survival, growth, physiology and emergence. An integral issue in developing such methods using field caught organisms has been the intrinsic variance when recording the time to emergence. Reducing this variation is key to ensuring the reproducibility of experimental data going forwards. Here, we present our developmental work with laboratory rearing of mayfly larvae. The main objective of this work is to define the ideal length of a chronic laboratory study alongside the start size of *C. dipterum* larvae to ensure a successful emergence endpoint and to enable the recording of sub lethal, developmental and physiological endpoints.

2.02.03

Ecotoxicology Testing Experiences With EPT Taxa

M. Brown, H. Schuster, Cambridge Environmental Assessments (CEA) / Ecotoxicology; Z.L. Jones, Cambridge Environmental Assessments (CEA) / CEA; J. Smith, Cambridge Environmental Asessments (CEA) / Ecotoxicology; N. Taylor, Cambridge Environmental Assessments (CEA) / Ecotoxicology

EPT taxa (Ephemoptera; mayflies, Plecoptera; stoneflies and Trichoptera; caddisflies) are used as biological indicators of water quality and have been shown to be sensitive to environmental pollutants such as PPP's. These taxa are also particularly vulnerable as their long aquatic larval stages mean that individuals are potentially exposed to PPPs in water bodies for extended time periods. The life histories of EPT taxa make them inherently problematic for ecotoxicology testing as they are generally low in abundance in the natural environment and typically have a 1 to 2 year life cycle. This means that full life cycle culturing in the laboratory is labour intensive and costly, and the development of standardised tests using lab-cultured organisms would be very challenging to achieve. It is possible to perform laboratory toxicity tests using field collected organisms, selecting organisms based on size or life stage. This approach often results in higher variability in comparison to using lab-cultured organisms, due to the lack of synchronicity associated with using field collected organisms of a particular size range. It can also require additional work to determine the most sensitive life-cycle stage(s) to use in toxicity tests. It is also difficult to assess long term effects on emergence and reproduction in the laboratory, due to the lengthy generation times of these taxa, though surrogate sublethal endpoints can be measured e.g. growth, feeding. Mesocosm studies offer an alternative approach, however EPT taxa are often considered to be under-represented in static systems. However, there are some E and T taxa that thrive in static water habitats, such as the mayfly *Cloeon dipterum* and Caddisfly taxa Phryganeidae. One of the major challenges for mesocosm studies is achieving consistently high abundances of organisms to allow sufficient statistical power to detect significant differences. EPT taxa often yield low abundances and highly variable data, which can result in uncertainties in the detection of treatment-related effects. Here we present our findings for determining the optimum experimental conditions to provide improved endpoint measures for EPT taxa in (i) laboratory systems, including techniques for the measurement of sub-lethal endpoints with species where it is not possible to test full life cycles and (ii) static mesocosms by comparing the abundances of EPT taxa in historical control data with mesocosms of different design.

2.02.04

Life-History Responses of the Non-Biting Midge Chironomus riparius Exposed to Mixtures of Fungal- and Bacterial-Based Insecticides

M.D. Bordalo, University of Aveiro; A.L. Machado, University of Aveiro / CESAM & Department of Biology; J. Pestana, CESAM & University of Aveiro / Biology

As microbial insecticides are being increasingly used as environmentally friendly alternatives to synthetic insecticides, it is crucial to understand their effects in the aquatic environment. These compounds may reach freshwater ecosystems through runoff or direct spraying and therefore different microbial insecticides may occur simultaneously. The evaluation of the potential effects of such mixtures represents an important challenge, namely regarding the exposure of non-target species. The aim of this work was to assess the joint effect of two microbial insecticides on the life-history traits of the non-biting midge *Chironomus riparius.* These chironomid larvae were exposed to single and combined treatments of a fungal-based insecticide (active ingredient *Beauveria bassiana* - Bb) and a bacterial-based insecticide (active ingredient *Bacillus thuringiensis* subsp. *israelensis* - Bti).Effects on life-history traits differed according to the bioinsecticides, as these compounds have distinct modes of action. Larval growth, emergence rate, time to emergence and adult weight were significantly affected by single or combined exposures of Bb and Bti.Larval growth declined with increasing Bb concentrations while no effects were observed for larvae exposed to Bti, even when combined with Bb. Both Bb and Bti decreased adult emergence, however no interaction between the two compounds was observed. A significant delay in emergence was observed on adults exposed to Bb, but this was not reflected in differences in adult females' weight. Bti alone did not alter development time and female adult weight nor when combined with Bb. Interestingly, an interaction was found for the combined exposure to Bb and Bti on time to emergence and weight of adult males.Since chironomids are one of the most dominant invertebrates in freshwaters and important prey for higher trophic levels, consequences along trophic chains and at ecosystem level should not be disregarded. This study highlights the importance of assessing the effects of the combined application of different microbial insecticides, reinforcing the need for the risk assessment of these compounds in aquatic ecosystems.

2.02.05

Effects of Wildfire Ashes on Chironomus Riparius: A Molecular Approach

A. Muñiz-González, UNED / Mathematical Physics and Fluids; I. Campos, University of Aveiro / Department of Environment and CESAM; A. Re, F.J. Gonçalves, University of Aveiro / Department of Biology and CESAM; N. Abrantes, University of Aveiro-CESAM / CESAM/DAO; J. Martinez-Guitarte, UNED / Fisica Matematica y de Fluidos

Wildfires have become a major environmental problem during the last years, partly due to the climate change and overall temperature rise. In Mediterranean regions such as Portugal the number of fires is increasing every year, becoming more remarkable in the spring-summer seasons. There has been growing concern about adverse impacts on soils and water ecosystems hence on their flora and fauna. Wildfire are a diffuse source of pollution to the aquatic systems, mainly by ash overland flow, damaging the ecosystems at different levels. Among the several compounds attached to ashes, the presence of certain trace metals and polycyclic aromatic hydrocarbons (PAH) make them potential toxic to aquatic life. Albeit some previous studies have observed effects in the survival and growth of aquatic organisms due to ash exposure, however no effects have been tested at molecular level on aquatic biota. Trying to fill the gap of knowledge about the molecular alterations derived from this fundamental environmental problem, *Chironomus riparius* as basal food chain organism, was selected as model organism. For that, IV instar larvae were exposed to aqueous extract of ash (AEA) (12.5%, 25%, 50%, 75% and 100%) from low and high severe wildfires for 72h. The altered mRNA levels were analyzed by Real time PCR, employing an array with 40 genes related to essential routes in invertebrates. The results showed effects on endocrine system, detoxification, stress, oxidative damage reponse and DNA repairing genes to a greater or lesser extent at all concentrations tested. This strong alteration on gene expression even at the low concentrations (12.5%) emphasizes the strong effect of wildfires on aquatic organisms. In consequence, wildfire ashes could be able to modify the growth and development of *C. riparius* as was

evidenced by the strong disruption on genes related to essential hormones. Moreover, the activation of essential systems such as detoxification or stress could limit its future response to external aggressions. The main advantage from this study is the early detection effects preventing future damage to Chironomids populations and highlights the need to combine the molecular and physiology effects in the ecotoxicology evaluation. Overall, wildfire ashes showed to be a notable environmental disruptor to *C. riparius* even at this short exposure time.

2.02.06

Development and Evaluation of Molecular Endpoints in Laboratory and In Situ Toxicity Tests for Routine Sediment Quality Assessment With Chironomus riparius

R. Beauvais, Centre Ecotox; B. Ferrari, Ecotox Centre CH

Effect-based monitoring approaches are important to assess sediment quality, but are still rarely undertaken. Among them, however, *in situ* exposure bioassays enable to increase the ecological relevance of ecotoxicological testing, especially when assessing key endpoints like reproduction. Different *in situ* devices allow exposing chironomids from larval to adult stages, assessing growth, emergence and reproduction of the individuals. Chironomids are key organisms of the macrozoobenthos used worldwide for bioavailability, toxicity or food chain transfer studies of sediment-linked chemicals. In addition, molecular endpoints (e.g. gene expression, enzymes activity, energy allocation budgets) are believed to be sensitive markers to be used as early-warning signal of the traditional effects like growth or reproduction inhibition in sediment quality monitoring. In this context, the aim of our work is to complement standard and well-established bioassays with *Chironomus riparius* with molecular endpoints, namely gene expression biomarkers, in both laboratory and *in situ* experiments. As a preliminary experiment, we exposed 4th instar larvae of *C. riparius* for 3 hours to increasing concentrations of copper (0.05, 0.5 and 5 mg/L Cu, as Cu sulphate), based on lethal concentration LC50 obtained in our laboratory. After exposure, half or the larvae were snap-frozen on dry ice and stored at - 80°C before further analysis. The remaining larvae were left in contact with the spiked water and clean sand until emergence occurred. While emergence rate was 90-100% in control, 0.05, and 0.5 mg/L Cu, only 30% larvae survived until emergence at 5 mg/L Cu. A differential gene expression is therefore expected in larvae that were exposed to 5 mg/L Cu compared to control larvae but not excluded in lower exposure concentrations. This hypothesis will be tested using both retro- transcription quantitative PCR and whole-transcriptome sequencing. *Chironomus sp.* being among the most used benthic organism in ecotoxicology, with this project we hope to gather information on key adverse effects at different levels of biological organization, combining laboratory studies and field approaches, in line with the adverse outcome pathway framework.

2.02.08

Evaluation of Physiological State of Indigenous Mussels Mytilus galloprovincialis in Ecological State Assessing of Recreational Potential of Sevastopol Bays

T. Kuznetsova, Saint-Petersburg Scientific Research Center for Ecological Safety Russian Academy of Sciences / Lab.Bioelectronic Methods for Geoecological Monitoring; S. Kholodkevich, Saint-Petersburg Scientific Research Center for Ecological Safety; M. Kirin, Institute of Biology of the Southern Seas Russian Academy of Sciences

The goal of the study was to test the innovative method of ecosystem state (health) assessment developed earlier by the authors in the regional program for assessing environmental quality of aquatic ecosystems in coastal marine recreational areas of Sevastopol city (the Black Sea, Crimea). The data were obtained during the study in a number of recreational areas of Sevastopol city. The assessment was carried out by testing the physiological state of the bivalve Mytilus galloprovincialis Lam. inhabiting 5 sites of concern, using the functional load method based on the analysis of mussel's heart rate (HR) measured by optic-fiber bioelectronic system before, under and after standardized functional loading (50% decrease of water salinity). It had been established that the functional state of mollusks from different sites varied, depending upon the bays with different anthropogenic pressure. Our results revealed significantly higher values of HR recovery time (HRrec) in Grafskaya Bay compared to the values for mussels from reference Kazach'ya Bay. Seasonal variations in cardiac responses to load were also revealed. Thus, it was shown that mussels may serve as an indicator of excessive pollution of coastal waters by the objects that discharge insufficiently treated wastewater, including household wastewater. The method applied in this study together with the assessment of biochemical markers of chemical stress (oxidative stress indicators) in tested mussels may be effectively used for detection of early signs of alterations in the health status of aquatic organisms and contribute to ensuring the ecological safety of recreational water areas, serving as an infobase for the development of science-based environmental management decisions. The reported study was funded by RFBR and Government of the Sevastopol according to the research project № 18-44-920010 — "Assessment of the recreational potential of the waters of the Sevastopol region by bioindication methods". Keywords: ecological state of coastal water areas; functional state of animals; heart rate of molluscs, Black Sea

2.02.16

Linking Transcriptomic Responses of Gammarus pulex to Pesticide Pollution: A Pilot Study in Agriculture Areas in Southern Sweden

P.A. Inostroza, University of Gothenburg / Department of Biological and Environmental Sciences; S. Hassan, Karolinska Institutet / Dept. of Neurobiology; H. Aronsson, University of Gothenburg / Department of Biological and Environmental Sciences; T. Backhaus, University of Gothenburg / Department of Biology and Environmental Sciences

The expansion and intensification of agricultural areas and consequently the application of pesticides are recognised as one of the major drivers affecting aquatic biodiversity in stream ecosystems. Pesticide stress, although seasonal, is capable of changing the diversity and abundance of macroinvertebrate organisms jeopardising relevant ecological functions (e.g. breakdown organic matter). On the other hand, in order to cope with this seasonal pressure macroinvertebrates make use of their phenotypic plasticity up- and down- regulating their gene expression. Macroinvertebrate communities represent one of the Biological Quality Elements (BQEs) according to the EU Water Framework Directive. Therefore, they are extensively used as biological indicators to assess water quality. However, a thorough understanding of whether pesticides drive gene expression responses in wild macroinvertebrates is still absent. Through the application of an ecotoxicogenomic approach, we aim to shed light on identifying expression profiles associated with pesticide pressure and specifically linking those responses to known pesticide´s mode of action (MoA). The study was conducted in Southern Sweden, where the main agricultural activities take place. The studied area is a recognised pesticide hotspot and it is part of Sweden's national pesticides monitoring program. Three streams, one with low agricultural pressure "reference" and two running through intense agriculture areas were sampled. *Gammarus pulex*, a non-model ecotoxicological specimen but widely studied to understand the effect of pesticide in the field, was sequenced using RNA sequencing (TagSeq) technique. Our study informs on pesticide fingerprints, quantitative changes in expression levels between the sampling sites, and gene-MoA relationships among exposed populations to pesticides in distinct agricultural landscapes. Besides, the study gathered critical environmental information about mechanisms driving changes of genomic functions in freshwater populations.

2.02.18

Evaluation of Toxicity of As, Cd, Cr, Cu, Hg, Ni and Pb Metals on Six Species of Freshwater Cladocerans

A. Sobrino-Figueroa, Universidad Autonoma Metropolitana / Hidrobiology; S. Alvarez-Hernandez, Universidad Autónoma Metropolitana-Iztapalapa / Applied Ficology Laboratory. Department of Hidrobiology; A. Perez-Rojas, Universidad Autonoma Metropolitana Iztapalapa / Limnology and geology Laboratory. Department of Hydrobiology

In this work, an evaluation of the toxic effect of metals: Arsenic, Cadmium, Chromium, Copper, Mercury, Nickel and Lead, which are found in concentrations above the LMP (Maximum Permissible Limits) in aquatic systems of the Valley of Mexico, on 6 species of freshwater cladocerans: *Daphnia exilis, D. pulex, Cerodaphnia dubia, Simocephalus mixtus, Moina macrocopa*, y *Chydorus sp.* species that inhabit aquatic environments in Mexico and *Daphnia magna* organism that is used in toxicity tests of effluents that are discharged into aquatic systems (NMX-AA-087). The objective of this work was to compare the sensitivity of native cladocera species with *Daphnia magna*. Static bioassays (48 hrs) were carried out where the organisms were exposed to 5 concentrations of each toxic, plus a negative control. The LC50 (lethal concentration 50) was determined and a comparison of the LC50 obtained was made to compare the sensitivity of the species. The most toxic metal for cladocerans was copper and the least toxic was arsenic and nickel. Native species were more sensitive to metals compared to *Daphnia magna*. The species most sensitive to metals was *Daphnia exilis*. Because the LC50 values for the metals Cd, Cu, Cr and Ni are

lower than those set by NOM 001-Semarnat for discharges in aquatic systems, it is important to continue conducting research and monitoring to detect responses that indicate possible damage to the populations of these organisms, to avoid irreversible deterioration in the medium and long term.

2.02.20
Analysis of Toxicity Data of Terrestrial Non-Target Arthropods
M. Steiner, Agroscope Wädenswil / Research group Ecotoxicology; J.F. Blom, Agroscope Wädenswil / Competence Division for Plants and Plant Products - Ecotoxicology Group; O. Daniel, Agroscope; M. Lehto, Agroscope / Research group Ecotoxicology
Drift of plant protection products (PPP) can affect terrestrial non-target arthropods (NTA) in off-crop habitats. Currently, the risk assessment of terrestrial non-target arthropods (NTA) begins with toxicological endpoints determined by glass plate studies using the standard test species *T. pyri* und *A. rhopalosiphi*. If these first tier studies indicate a higher risk or if an active substance is expected to have a general higher risk potential for NTA, additional species and extended studies or field studies are required. The aim is to refine the risk assessment with a closer consideration of natural conditions. A limitation of this approach is the exclusive use of beneficial arthropods for the testing. Up to now it is unclear, whether the current risk assessment predicts effects for off-crop NTA sufficiently well. We analysed the currently available data submitted by companies during the authorisation process of plant protection products and identified specific aspects of uncertainty or assumptions that need to be validated in the current risk assessment approach for off-crop habitats. To this end, we compared toxicological endpoints of standard test species and other beneficial arthropods for Tier 1 and Tier 2 studies. Furthermore, we investigated the relationship between the mode of action of insecticides and their toxicity. Finally, we reviewed the available off-crop field studies for NTA and discussed the results in relation to the assumptions of the current off-crop risk assessment for terrestrial NTA.

Adverse Effects of Chemicals on Host-Associated and Free-Living Microbial Communities

2.03.01
Microbial Population Dynamics in a Model Sewage Treatment Plant and the Effect of Gold Nanoparticles
K. Schlich, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecotoxicology; C. Diaz Navarrete, B. Gomez Pizzaro, K. Hund-Rinke, Fraunhofer IME / Ecotoxicology
Adequate functioning of a sewage treatment plant (STP) is essential to protect the downstream aquatic environment (ECHA 2017) and information on degradability of chemicals and their toxicity on sewage sludge microorganisms is required. An environmental realistic higher tier test is a STP simulation test as described in OECD 303A (2001). For nanoparticles it is also used to study their sorption behavior to sewage sludge. But information is limited on the influence of synthetic sewage on the microbial community with potential modifications of sludge floccules. One objective was to study the impact of the test conditions as described in OECD 303A and of gold nanoparticles (AuNPs) on the microbial diversity of natural sewage sludge. AuNPs were used as case study due to their rising medical applications and therefore increasing probability to reach the sewer and STP. Furthermore, we investigated whether considering the functional properties of a STP (DOC elimination, nitrification) is sufficient or whether the microbial community structure provides additional information for a hazard assessment. We used four model STPs with a denitrification, nitrification and settling tank. The effect of AuNPs on the STP function was investigated by (i) microbial activity by DOC elimination, and (ii) nitrification by measurements of ammonium, nitrite and nitrate in the effluent. In addition, the changes in the structural community were assessed by DNA-sequencing. The community structure changed significantly only comparing the fresh sludge gathered from the municipal STP and the sludge fed with synthetic sewage afterwards. Tests carried out in two seasons (summer, winter) revealed a comparable community structure in both tests. The AuNPs dispersant did not influence the STP function. Dosing 10 days AuNPs into the STP lead to significantly lower nitrite concentration but subsequent higher nitrate concentration in the effluent. This faster conversion was not reflected in the data on the microbial community structure. Results of the case study with AuNPs showed that an additional consideration of the change in the structural microbial community does not provide additional insights for a risk assessment. However, the assessment of the structural community by RNA-based approaches or for other pollutants could show differences and both approaches should still be applied to receive further information. This project has received funding from the EU's Horizon 2020 (BIORIMA).

2.03.02
Effect of AgNPs in the Functional and Structural Microbial Community Using ARISA and Next Generation Sequencing
B. Gomez Pizzaro, Fraunhofer IME / Ecotoxicology; K. Schlich, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecotoxicology; K. Hund-Rinke, C. Diaz, Fraunhofer IME / Ecotoxicology
Chemicals must undergo risk assessment studies before being marketed. Among others their effect on soil microflora is of interest. There are doubts that the standard approaches (e.g. OECD 216/217) are sufficient to assess their risk to terrestrial environment. A discussion is ongoing about including microbial biodiversity more closely by the European Food Safety Authority (EFSA). For the assessment of microbial activity, three to four replicates are considered to be sufficient (according OECD test guidelines), however there is not much information in this regard when using metagenomics data. Our experience with metabarcoding of eDNA extracted from suspended particular matter (SPM) has shown high variability among replicates, suggesting homogeneity problems. Therefore, there is the need to establish a validated methodology to provide reliable data for evaluation. This study aimed to evaluate the performance of two fingerprinting methods, using eDNA extracted from RefeSoil 01A (www.refesol.de) which is used for OECD tests. A study case using silver nanoparticles (AgNPs) in concentrations of 0.56 and 1.67 mg/kg dry matter soil was conducted for 28 days, supplementing results on the effect of AgNPs in the standard OECD tests (Schlich et al, 2015). Samples were taken at day 0, 7 and 28 and evaluated regarding the effect of the AgNPs on the microbial population in soil using: automated ribosomal intergenic spacer analysis (ARISA) and next generation sequencing (NGS). Furthermore we also aimed to evaluate the reproducibility of the results obtained from extraction (DNA or RNA) replicates of both techniques, regarding the structural communities (DNA-based approach) and the functional community (RNA-based approach). Additionally, the possibility of pooling the genetic material before each analysis as is a common practice used for reducing costs was evaluated. The results have shown a high reproducibility in the community profiles among the replicates and the pooled samples, obtained in both ARISA and NGS analysis. It was possible to observe significant changes in the functional community over time at the different sampling times, which could not be related to AgNPs. This changes were not observed in the structural community. While there were effects in the standard ecotoxicological OECD tests, there is no effect of the AgNPs, in the concentrations applied, neither on the structural nor the functional community.

2.03.03
Assessment of the Effects of Ashes From Wildfires in Bacterial Isolates From Skin Microbiome of Rana iberica
L. Coelho, M. Afonso, University of Aveiro / Biology; F.J. Gonçalves, University of Aveiro / Department of Biology and CESAM; I. Campos, Universidade de Aveiro / Environment; F. Jesus, University of Aveiro / Department of Environment and Planning & CESAM; D. Serpa, CESAM & Department of Environment and Planning, University of Aveiro; S.M. Marques, University of Aveiro / Department of Biology and CESAM
Amphibians are one of the vertebrate classes with the higher number of species threatened. Among the various reasons, one contributing greatly is the spread of infectious diseases, being the skin one of the most commonly affected organs. Typically, amphibians secrete in their skin a complex number of antimicrobial peptides that act as a barrier for some pathogens. Adding to this, various studies have showed that bacteria in the skin microbiome contribute also for the immunity of the animals. Bacteria present in the skin microbiome are not only dependent on the conditions provided by the animal but are also influenced by the environment. This means that changes in the environment may affect the bacteria in the microbiome, which in turn can affect the susceptibility of animals to diseases. One of the events that greatly affects local environments are wildfires. These have been increasing in frequency and in intensity and the post-fire effects on wildlife are not yet fully understood. This is particularly true for animals highly dependent on water courses, which are common recipients of post-fire runoffs. In this study our goal is to perceive if cultivable bacteria from *Rana iberica* skin are affected by post-fire runoffs. With this in mind, we have sampled in October the skin of five *Rana iberica* adult animals from an uncontaminated site. Initially, bacteria were inoculated in R2A plates and,

based on morphological traits, colonies were selected and isolated in TSA plates. As a result, more than 60 isolates were obtained. Simultaneously, ashes resulting from wildfire were collected to test their effects on the bacterial isolates. The identification of bacteria and the effects of the post-fire runoffs are discussed, including chemical composition of the runoffs, as well as the potential effect concerning the efficacy of the Rana iberica immune system.

2.03.04
Effect of Pharmaceutical Compounds on the Microbiome From a Multi-Species Aquatic Setup Mesocosm
A. Herruzo, University of Córdoba / Department of Biochemistry and Molecular Biology. CeiA3; M. Sendra, CSIC - Spanish National Research Council / ECOLOGY AND COASTAL MANAGEMENT; I. Moreno, Institute of Marine Science of Andalucia (CSIC); J. Blasco, Instituto de Ciencias Marinas de Andalucia (ICMAN-CSIC) / ECOLOGY AND COASTAL MANAGEMENT; C. Michán, J. Alhama, University of Córdoba / Department of Biochemistry and Molecular Biology. CeiA3
Every year, tens of thousands of tons of pharmaceutical chemicals are produced and, due to aging societies, this amount is quickly increasing. During their manufacture, use and disposal, these products are released into the environment producing exotoxicological effects on nontarget organisms. Pharmaceutical active substances have been found in most environmental matrices, and mostly in surface waters. Microbial communities can participate in their biodegradation or even produce transformation products that are more persistent or harmful. Nevertheless, little work has been performed on the impact of pharmaceutical active substances on the environmental microbiome and ecosystem funcitons. In this work, the effect of 2 antibacterial (ciprofloxacin and flumequine) and 2 anti-inflammatory (ibuprofen and diclofenac) drugs at 2 different concentrations (5 and 50 ug/L) was assessed using a metagenomic approach in a multispecies aquatic setup system. Microorganisms were identified by DNA sequencing of their 16S rRNA, and the alpha diversity was determined at the family level using the Shannon–Wiener´s (H′) index. The presence of these products did not affect the global diversity of the microbial communities, and most of the significant changes detected were increments, at all the taxonomic levels studied, upon exposure to the drugs. In general, anti-inflammatory drugs produced less alterations that were also of a small magnitude, with top increments in the Bartonellaceae family and in the Shaewanellaceae family upon exposure to ibuprofen and diclofenac, respectively. Exposure to the antimicrobial flumequine generated the highest alterations in the microbiome, increasing the levels of several families as Acidimicrobiaceae, Hyphomicrobiaceae, Moritellaceae, Mycobacteriaceae, Paenibacillaceae, Parachlamydiaceae and Sphingomonadaceae.**Acknowledgments:** Project (MINECO CTM2016-75908-R, MICINN PID2019-110049RB-100). Fundings to BIO187 group (PAIDI, UCO). Predoctoral contract of A.M. Herruzo (Plan Propio, UCO). SCAI (UCO).

2.03.07
Bacterial Bioindicators for Reporting Contamination in a Coastal Soil/River/Lake Continuum
Z. Bourhane, University of Pau and Pays de lAdour; A. Lanzén, Marine Research Division, AZTI-Tecnalia; C. Cagnon, Université de Pau et des Pays de lAdour / IPREM CME UMR5254 CNRS; O. Ben Said, Faculté des Sciences de Bizerte; F. Coulon, Cranfield University / School of Water Energy and Environment Cranfield Water Science Institute; . Borja, Azti-Tecnalia; C. Cravo-Laureau, UniversitÃ de Pau et des Pays de lAdour / IPREM CME UMR5254 CNRS; R. Duran, Pau University / Biology
The anthropogenic pressure in coastal areas increases dramatically with the exploitation of environmental resources. Biomonitoring coastal areas is crucial to determine the impact of pollutants on bacterial communities in soils and sediments, since they provide important ecosystem services. However, relevant biomonitoring tools allowing fast determination of the ecological status are yet to be defined. Microbial ecology approaches provide useful information for developing such microbial monitoring tools reporting on the effect of environmental stressors. Chemical and microbial molecular approaches were combined in order to determine microbial bioindicators for assessing the ecological status of soil and river ecosystems around the Ichkeul Lake (Tunisia), highly impacted by human activities. Samples were collected along soil/river/lake continuums in three stations around the Ichkeul Lake influenced by different human activities at two seasons (summer and winter). Contaminant pressure indexes (PI), including polycyclic aromatic hydrocarbons, alkanes, organochlorine pesticides, and metal contents, showed significant differences in the contamination level between the stations with seasonal variation. Bacterial communities were characterized by 16S rRNA gene metabarcoding. Although microgAMBI indexes, determined from the sequencing data, were in accordance with contaminant contents, they were not sufficient to fully explain the PI. Therefore, further microbial indicators are still to be defined. The comparison of bacterial communities revealed the specific microbial assemblage for soil, river and lake sediments, which were significantly correlated with contaminant contents and PI. Such observation offers the possibility to define a relevant set of bioindicators for reporting the effects of human activities on the microbial communities structure. Additionally, PICRUSt analysis revealed that the microbial bioindicators harbour functional potential for the transformation/degradation of pollutant. Therefore, such microbial bioindicators might constitute useful monitoring tools for the management of microbial communities in coastal areas in perspective of ecosystem recovery. **Keywords:** Contamination, Biomonitoring, Bacterial communities, Microbial bioindicators.

2.03.09
Assessment of the Effects of Aashes From Wildfires in Bacterial Isolates From Skin Microbiome of Salamandra salamandra
M. Afonso, L. Coelho, University of Aveiro / Biology; F.J. Gonçalves, University of Aveiro / Department of Biology and CESAM; I. Campos, Universidade de Aveiro / Environment; S.M. Marques, University of Aveiro / Department of Biology and CESAM; F. Jesus, University of Aveiro / Department of Environment and Planning & CESAM; D. Serpa, CESAM & Department of Environment and Planning, University of Aveiro
Presently most amphibian species are declining and many of them are considered threatened. Besides habitat lost, one of the most relevant contribution for this amphibian crisis is the spread of infectious diseases, being the skin one of the most commonly affected organs. Typically, amphibians produce and secrete in their skin antimicrobial peptides that act as a barrier for some pathogens. The action of the peptides can be many times complemented by bacteria in the skin microbiome. Bacteria present in the skin microbiome are not only dependent on the conditions provided by the animal (e.g. mucus production and antimicrobial peptides secreted) but are also influenced by the environment. This means that changes in the environment may affect the bacteria in the microbiome, which can have a profound impact in the susceptibility of animals to diseases. Wildfires affect deeply the ecosystems and are an increasing phenomenon, both in frequency and in intensity, and the post-fire effects on wildlife are not yet fully understood. This is especially true for amphibians, either more dependent on the terrestrial or aquatic compartment. When concerning more terrestrial species, such as the fire salamander (*Salamandra Salamandra*), that spend much time in refuges such as burrows, fires deeply affect their habitat, which might affect their microbiome and their resistance to pathogens. In this study we aim at perceiving if wildfires can affect, indirectly, cultivable bacteria from S. salamandra skin. With this in mind, we have sampled in November the skin of five *S. salamandra* adult animals from an uncontaminated site. Initially, bacteria were inoculated in R2A plates and, based on morphological traits, colonies were selected and isolated in TSA plates. As a result, more than 40 isolates were obtained. Simultaneously, ashes resulting from wildfire were collected to test their effects on the bacterial isolates. The identification of bacteria and the effects of the post-fire runoffs are discussed, including chemical composition of the runoffs, as well as the potential effect concerning the efficacy of the S. salamandra immune system.

2.03.10
Evaluation of the Inhibition of Bacteria (Aeromonas hydrophila) Exposed to ENvironmental Concentrations of Ciprofloxacin
L. Ângelo, Federal University of Paraná / Postgraduate Program in Ecology and Conservation, Department of Pharmacology.; T.T. Braga, K. Nogueira, Federal University of Paraná / Department of Pathology; H.C. Silva de Assis, Federal University of Paraná
Bacterial resistance to antibiotics is currently one of the most relevant public health problems worldwide, with concerning clinical and economic consequences. This study aimed to evaluate the exposure of the environmental concentrations of ciprofloxacin (CIP) to *Aeromonas hydrophila.* Ciprofloxacin is one of the most widely used antibiotics and *A. hydrophila* is one of the most common bacteria in aquatic environment. The Minimum Inhibitory Concentration (MIC) (µg/ml) of the bacteria was determined before (time 0), during

(28° and 52° day), and at the end of exposure to CIP (70° day) using a standard broth microdilution method. It was identified bacterial resistance to CIP as a significant increase in the MIC for mutant strains compared to control strains. The *in vitro* resistance study was carried out with the exposure of the bacteria daily to three concentrations of CIP, simulating concentrations found in surface water (1µg/L), wastewater (10µg/L), and pharmaceutical effluents (100µg/L) with 70 serial transfers and in triplicate. In addition to the MIC determination, the bacteria were subjected to an exposure test high CIP concentration, on the tenth day of exposure they were exposed to 100 µg / L, and on the sixtieth day they were exposed to 200 µg / L. The results showed that: (i) the concentrations of 1 and 10µg/L were subinhibitory and were able to induce *A. hydrophila* resistance to CIP. Additionally, the concentration of 100 µg/L was inhibitory; (ii) after the tenth day of exposure, the bacteria exposed to 1 and 10 µg/ L were able to tolerate 100µg/L of CIP, a concentration 100 and 10 times higher, respectively; (iii) after the sixtieth day of exposure, the bacteria tolerated 200µg/L of CIP; (iv) the MIC determination throughout the experiment revealed a positive correlation between the exposure time and the MIC ($r = 0.998$). Therefore, the longer the exposure time to CIP, more tolerant the bacteria became to the antibiotic. Finally, (v) the mutation behavior of the bacteria of both groups (1, and 10µg/L of CIP) was very similar ($R^2 = 0.996$), but with a proportion 32 times higher for bacteria exposed to 10µg/L of CIP. The concentrations of antibiotic that can induce bacterial resistance, as well as the time it takes are a concern. These data represent an alert to the indiscriminate use of antibiotics in human and veterinary medicine and in agricultural activities and the destination environmentally correct of these types of drugs.

2.03.11
Do Antibiotic Plant Protection Products Select for Clinically Important Antibiotic Resistance?
L.M. Murray, University of Exeter Medical School / Environment and Sustainability Institute; L. Zhang, University of Exeter / The European Centre for Environment and Human Health; A. Boxall, University of York / Department of Environment and Geography; J. Snape, AstraZeneca UK Ltd. / Global Sustainability; W. Gaze, University of Exeter / Medical School; A.K. Murray, University of Exeter
Agricultural activities require the direct application of plant protection products (PPPs) such as antibiotics to crops and as a result, soil. Antibiotics are applied alongside other PPPs and in some cases manure which may contain resistant bacteria, antibiotic residues and other co-selective agents. This cocktail of chemicals could result in the development and spread of resistance genes between environmentally and clinically relevant bacteria. Concerns have been raised previously about the high concentrations of antibiotics directly released into the environment by pharmaceutical waste at levels of mg/L. These areas have been termed "hotspots" and bacteria at these sites have elevated levels of resistance. Despite this, the direct application of crop agriculture antibiotics to soils at mg/L has received little attention. The selection pressure exerted by antibiotic PPPs and how they impact antimicrobial resistance is a novel research area and will be addressed in this presentation. A reduction in growth of a bacterial community has been validated as a good proxy for selection for resistance, this approach is called the SELECT method. Antibiotic PPP lowest observed effect concentrations (LOECs) were determined using the SELECT method. Results from these experiments determined the concentration range for long term evolution experiments. QPCR for 16S and *intI1* (a marker for resistance) was performed on exposed communities and metagenomic analyses were conducted to determine the effects of antibiotic PPP exposure on clinically relevant resistance genes. Results from these experiments will provide information on the effects of crop antibiotics on environmental AMR, contributing to understanding of AMR from a "One Health" perspective. This understanding is of great importance, particularly with regards to human exposure to environmental reservoirs of resistant bacteria.

Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment

2.04.01
Validating the Use of Definitive Screening Design to Conduct Metal Mixture Effects Assessments With a Marine Microalgae
C. Eastabrook, G.S. Caldwell, Newcastle University / School of Natural and Environmental Sciences; E. Jarosz, Norwegian University of Life Sciences NMBU; Y. Song, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; H. Teien, Norwegian University of Life Sciences NMBU / Centre for Environmental Radioactivity (CERAD); K. Tollefsen, Norwegian Institute for Water Research (NIVA) / Computational Toxicology Program
Marine algae are the foundation of marine ecosystems; however, the stability and balance of algae communities—particularly in coastal waters—is under threat from increased chemical and nutrient runoff, exacerbated by climate change. To best inform risk assessments and regulatory guidelines, effect assessments for these stressors need to be conducted both as singular and realistic mixtures. However, combined effects assessments can be difficult to conduct, with resource, time and financial limitations. Many of these limitations can be mitigated with a Design of Experiments approach; for example, a three-stressor mixture effect assessment would only require 15 experimental runs. This study aimed to: (1) characterise individual effects of three trace metals—copper, nickel and zinc chlorides—on the marine microalgae *Tetraselmis suecica*; and (2) conduct a combined toxicity assessment for the three metals. Experimental studies were conducted for seven days. Effective concentrations (EC_{50}) of the adverse outcome growth inhibition were calculated from concentration response curves for single stressors. Endpoints included *in situ* reactive oxygen species (ROS) formation (H_2DCFDA probe) and photophysiology (Maxi Imaging PAM). Metal concentrations were measured using ICP-MS. Mixture experiments were designed using single metal EC_{10}, EC_{50} and EC_{80} values, following a Definitive Screening Design (DSD). *T. suecica* growth was most inhibited by zinc (7d EC_{50} 0.18mgL^{-1}), followed by copper (7d EC_{50} 0.75mgL^{-1}) and nickel (7d EC_{50} 2.29mgL^{-1}) when exposed singularly. ROS formation was highest with $CuCl_2$, increasing significantly after 24 hours by 2.75-fold (2.5mgL^{-1}) and 3.75-fold (5mgL^{-1}) compared to controls. Combined toxicity studies identified copper as having the significant main effect ($P < 0.05$) on *T. suecica* growth. Combined experimental data showed only a slight increase in ROS after 24 hours, with the highest increase of 2.1-fold occurring in a high copper and nickel but low zinc mixture. This present study assesses metal interactions and comparisons between predictive and measured data to determine whether this DSD approach of combined effects assessment could be suitable and more widely implemented.

2.04.02
Determining the Effects of LiCoO2 Nanosheets on the Net Carbon Biomass Production of Raphidocelis subcapitata
E. Ostovich, University of Wisconsin, Milwaukee / School of Freshwater Science; E. Laudadio, University of Wisconsin-Madison / Department of Chemistry; C. Green, UW-Madison / Department of Chemistry; M. Spehlmann, N. Van Ee, UW-Milwaukee / School of Freshwater Sciences; R.J. Hamers, Universtiy of Wisconsin-Madison / Chemistry; R.D. Klaper, University of Wisconsin, Milwaukee / School of Freshwater Sciences
In the ever-growing field of nanotechnology, more and more consumer products are starting to incorporate nanomaterials into their design. Thus, understanding the environmental impacts of these nanomaterials is increasingly important. LiCoO2 (LCO) is a type of complex metal oxide nanosheet that is used as a cathode material for lithium ion batteries and is found in many consumer electronics and high-end vehicles. Since it's produced at high quantities of environmental significance while lacking infrastructure for recycling and disposal, and contains high valence metals with high reactivity and known inherent toxicity, this makes LCO is an emerging contaminant of concern. Thus, it will be important to study what types of adverse outcomes could manifest from the exposure of LCO to the environment. *Raphidocelis subcapitata* is a prevalent species of freshwater microalgae that, as a primary producer, is important for the overall health and sustainability of freshwater ecosystems. As such, it is also a relevant bioindicator species for the environmental and toxicological responses to LCO exposure. The assimilation of CO_2, by primary producers, is necessary to support all life on Earth as consumers in the environment rely on this net primary production of carbon biomass as a source of chemical energy. However, widespread contamination of nanomaterials, like LCO, could potentially interfere with this process due to their highly catalytic properties and inherent toxicity. This presentation will discuss how an exposure of LCO to freshwater ecosystems could potentially affect the net carbon biomass production of primary producers, like *R. subcapitata*, and what this means in terms of ecosystem measures.

2.04.04
Combined Effects of Gamma Radiation and Depleted Uranium on Aquatic Macrophyte Lemna minor
L. Xie, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; H. Teien, Norwegian University of

Life Sciences NMBU / Centre for Environmental Radioactivity (CERAD); Y. Song, Norwegian Institute for Water Research (NIVA) / Section for Ecotoxicology and Risk Assessment; K. Solhaug, Norwegian University of Life Sciences (NMBU) / Faculty of Environmental Sciences and Natural Resource Management; K. Tollefsen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment

Aquatic plants are often exposed to multiple environmental stressors in the natural environment. These stressors usually have different properties and thus exert their toxicity through different mechanisms. However, how elevated ionizing radiation modulates the chemical effects of depleted uranium on aquatic plants is poorly understood. The presented study aims to characterize the combined effects and fundamental mechanisms of ionizing radiation (γ-radiation) and radionuclides (depleted uranium, DU) toxicity in aquatic plants. In this study, the aquatic macrophytes *Lemna minor* was chosen as a model species to assess the effects of g-radiation (10, 20 and 40 mGy/h), DU (UO2(CH3COO)2.2H2O), 0.2-20 mg/L) and their combination. After exposure for 7 days with single and combine stressors, primary effects such as oxidative stress (ROS formation) and secondary physiological responses such as photosynthetic performance (PSII efficiency) and mitochondrial membrane potential (MMP) were studied to characterize how g-radiation and DU cause combined effects in *L .minor*. The combined effects (additivity and non-additivity) of the stressors were determined using a two-way analysis of variance (2W-ANOVA). However, g-radiation at 39.9 mGy/h and all concentrations of DU caused significant interactions (synergism and antagonism) on growth and PSII efficiency in L. minor, thus suggesting that target-specific and exposure-dependent deviations from additivity may occur in *L. minor*. Additional endpoints and statistical models will be implemented to provide the mechanistic understanding and analytical strategies to identify the most relevant toxicity pathways for the single and multiple stressors tested. The results are anticipated to aid future cumulative hazard and risk assessments for multiple stressors such as ionizing radiation and radionuclides.

2.04.05

Single and Combined Effects of Glyphosate and Flazasulfuron Soil Residues on Medicago Sativa L., a Non-Target Cover Crop Species

C. Soares, Faculty of Sciences of University of Porto; A. Cachada, CIIMAR - University of Porto / Department of Biology; F. Fidalgo, GreenUPorto & Faculty of Sciences, University of Porto; R. Pereira, Faculdade de Ciências da Universidade do Porto / Biology

Glyphosate (GLY), the active ingredient of several plant protection products (PPPs), is the most applied herbicide worldwide. However, due to herbicide resistance, farmers need to employ mixtures of agrochemicals in order to maximize weed control. Within this context, flazasulfuron (FLA) is a herbicide commonly applied together with GLY, namely on vineyards, to prevent the development of weeds. Thus, when unravelling the environmental impacts of PPPs, it is important not only to study their single effects, but also those of their mixtures. Therefore, the main goal of this study was to compare the ecotoxicity of GLY and FLA, alone or in combination, in the growth of an important non-target species, *Medicago sativa* L., a cover plant. For this purpose, and based on the recommended application doses (GLY – 20 mg kg^{-1}; FLA – 275 µg kg^{-1}), a set of multiple concentrations of GLY (0, 6, 9, 13, 20 and 30 mg kg^{-1}) and FLA (0, 81, 122, 183, 275 and 413 µg kg^{-1}) were tested in batches of an artificial soil (70% sand, 20% kaolin and 10% peat). For the mixture model, 275 µg kg^{-1} FLA was mixed with the above referred concentrations of GLY, given its high relevance and also the variability of its levels found in agricultural soils. After 14 d of growth, seed germination was not significantly affected by any of the tested herbicides, alone or in combination. Yet, plant growth was severely repressed by both PPPs, especially FLA, whose action arrested plant development almost completely even at the lowest tested concentration (81 µg kg^{-1}). On the contrary, GLY only impaired shoot and root elongation and biomass production at relatively high levels (≥ 9 mg kg^{-1}). When the two herbicides were mixed, data pointed towards the prevalence of FLA phytotoxicity, with significant changes in root length, shoot height and biomass production upon exposure to all GLY treatments combined with FLA (275 µg kg^{-1}). However, the degree of inhibition registered was higher than that observed for the single treatments, suggesting a synergic action between the two herbicides. Overall, results showed that FLA is potentially more toxic to non-target plants than GLY, impacting plant growth at lower concentrations (µg kg^{-1} *vs* mg kg^{-1}), with toxic effects at doses lower than the recommended when comparing with GLY. Moreover, under co-exposure, the phytotoxicity of FLA was even more notorious, suggesting an amplification of the effects when GLY is also present.

2.04.06

Algae TKTD Modelling for Tier 2C Risk Assessment: Ring Testing the Validation Studies

C. Rendal, Syngenta / Product Safety; R. Ashauer, R. Baetscher, Syngenta Crop Protection AG / Environment; T. Preuss, Bayer Ag / Environmental Safety; I. Sims, Syngenta / Product Safety; J. Witt, Bayer Ag / Environmental Safety; E. Bruns, Bayer AG, Division Bayer CropScience / Ecotoxicology

The use of TKTD modelling in European risk assessments 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. While an important research tool, this model was deemed not ready for use in risk assessment due to the lack of a robust and ring tested methodology for generating calibration and validation studies (EFSA, 2018). This work directly addresses these concerns. We present a small ring test across a series of independent laboratories, testing two approaches for generating validation studies for modelling the effect on algae populations of time-variable exposures. These methods include a flow-through system that is light and temperature controlled (Weber et al., 2012), and a method based on static tests where algae cells are transferred between static exposures with alternating exposure and recovery phases using a process of centrifugation/filtration and resuspension. This work will result in the development of guidance and recommendations for the uniform generation of robust data for the model validation required to support TKTD modelling with unicellular micro algae. Test procedures investigating the effects of variable exposure patterns on unicellular algae will allow the implementation of tier 2C according to the Aquatic Guidance Document (EFSA, 2013) in the primary producer risk assessment for algae. It is the expectation that the results of this study will enable the more widespread use of algae TKTD modelling in EU risk assessment.

References: EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2018. Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms EFSA Journal 2018;16(8):5377 DOI: 10.2903/j.efsa.2018.5377 EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. https://doi.org/10.2903/j.efsa.2013.3290 Weber D, Schaffer D, Dorgerloh M, Bruns E, Gorlitz G, Hammel K, Greuss T and Ratte H, 2012. Combination of a higher-tier flow-through system and population modeling to assess the effects of time-variable exposure of isoproturon on the green algae Desmodesmus subspictatus and pseudokirchneriella subcapitata. Environmental Toxicology and Chemistry, 31, 899–908.

2.04.07

Aquatic Macrophyte Growth Season in Central and Northern EU and the Implications for Aquatic Macrophyte Risk Assessments for Herbicides

H. Crosland, Cambridge Environmental Assessments (CEA) / Ecotoxicology; A.C. Brooks, Cambridge Environmental Assessments / Ecotoxicology Risk Assessment; M. Hackett, In-between / Ecotoxicology and Risk Assessment; T. Preuss, Bayer Ag / Environmental Safety

Under Regulation (EC) 1107/2009 (EC 2009), the potential risks to aquatic organisms must be assessed for proposed uses of plant protection products (PPPs) that may result in exposure to the environment. For herbicidal PPPs, aquatic macrophytes are often the most sensitive taxa. For some herbicidal modes of action, adverse effects on aquatic macrophytes are only observed when the plants are actively growing. For risk assessment of such substances, it is useful to know if application timings would result in surface water exposure of aquatic macrophytes during periods in which they are actively growing (and therefore could potentially result in effects). However, ecotoxicity studies on aquatic macrophytes are most commonly performed whilst plants are actively growing in order to identify effects more clearly. Resulting toxicity endpoints may therefore be over conservative for cases where exposure of PPPs will not co-occur with active plant growth. A comprehensive literature search was performed, using both a systematic and manual approach, with the aim of identifying the main active growing period during the year for macrophytes in natural freshwater bodies. The focus was on plant species present in climates relevant to the Central and Northern Zones of the EU. The results of the systematic and manual searches were rapidly screened to

identify those references that contained data pertinent to the aim of the literature review. A full evaluation was then performed on all potentially relevant references. Reliability was assessed using the principles of the Klimisch scoring system. As part of the full evaluations, growth periods were identified for each macrophyte species studied. Finally, all extracted growth periods were considered together to determine an overall active growth period for aquatic macrophytes in C/N-EU. Here we will present a summary of this literature review, including the active growth period identified for aquatic macrophytes in Central and Northern EU Zones. A discussion will be made of the potential impact of such factors as climate, water body type, and taxonomy on the start of the growth period.

2.04.09
Evaluation of Typha Spp. (Cattails) As Toxicity Test Species for the Risk Assessment of Stressors on Emergent Macrophytes
V. Sesin, Trent University / School of the Environment; C.M. Davy, Ontario Ministry of Natural Resources and Forestry / Wildlife Research and Monitoring Section; J.R. Freeland, Trent University / Department of Biology
Macrophytes play an important role in aquatic ecosystems, and thus are often used in ecological risk assessments of contaminants. Risk assessments for macrophyte populations or communities are commonly based on inferences drawn from standardized toxicity tests conducted on floating non-rooted *Lemna* species, or submerged-rooted *Myriophyllum* species. These tests follow strict guidelines to produce reliable and robust results to inform regulatory decisions and environmental legislation. However, results and inferences from these tests may not be transferrable to emergent macrophytes due to differences in morphology and physiology. Emergent macrophytes of the genus *Typha* L. are increasingly used for assessing phytotoxic effects of environmental stressors, although standardized testing protocols have not yet been developed for this genus. In this review we evaluate the potential to develop standard protocols to use in toxicity tests for *Typha* spp. based on six selection criteria: ecological relevance to the ecosystem; ecological relevance to the exposure scenario; availability of plant material; ease of cultivation; uniform growth; and appropriate and easily measurable toxicity endpoints. *Typha* meets the first four criteria, and we identify knowledge gaps that limit evaluation of the remaining two criteria. We provide suggestions for addressing these gaps, and we summarize the experimental design of ecotoxicology studies using *Typha*. We conclude that *Typha* spp. have strong potential as future standard test species for ecological risk assessments of contaminants to emergent macrophytes.

2.04.11
Application of RSCABS on Plant Condition Scoring
J.R. Arnie, Eurofins EAG Agrscience Services, LLC / Plants and Invertebrates; G.U. du Hoffmann, Eurofins EAG Agroscience Services, LLC / Operations; J.W. Green, JohnWGreen-ecostats.com / Data Science and Informatics; T.A. Springer, Springer Ecotox Consulting; J.C. Wolf, EPL, Inc.; H.O. Krueger, Eurofins Agroscience Services / Science and Reg Affairs
Recent regulatory guidance requests the estimation of ECx values based on visual injury (also referred to as visual phytotoxicity or plant condition score) for non-target terrestrial plant toxicity tests where a dose-response relationship is observed (EFSA Technical Report, June 2019). These visual injury scores are a numerical values assigned to assess the general health of each plant 21 days post application. The visual injury scores collected in these studies are on the ordinal scale of 0%, 10- 30%, 40-60%, 70-90%, and 100% and statistics performed on replicate averages. Unlike apical endpoints such as biomass or shoot height, visual injury is a qualitative parameter and highly subjective, dependent on the perception of the scientist observing the plants. Additionally, damage assessment criteria are not commonly defined amongst laboratories. Visual injury scores are similar statistically to severity scores in histopathology conducted with fish or amphibians in standardized bioassays evaluating potential endocrine disruptors. Severity scores for histopathology data are assessed on the ordinal scale of 0 through 5 and are statistically evaluated using the Rao-Scott Cochran-Armitage by Slices (RSCABS) test due to its high power and ability to maintain individual scores. RSCABS is recommended as a more appropriate statistical test since it takes into account variability within and overdispersion between the replicates and a monotonic concentration-response that is anticipated where a true phytotoxic effect is observed. This session will focus on the application of this unique statistical test to plant visual injury data and interpretation of the results into biological meaningful conclusions. This recommended method is a powerful tool for illustrating the dose-response allowing for sensible endpoint derivatization that is meaningful in risk assessment over the ECx or in coordination with the ECx in the context of the data type.

2.04.14
Algae TKTD Modelling in Tier 2C Risk Assessment: An Applied Example With Moving Time Windows
C. Rendal, Syngenta / Product Safety; R. Ashauer, Syngenta Crop Protection AG / Environment
The use of TKTD modelling in regulatory risk assessment is increasingly popular, especially since the 2018 EFSA opinion on TKTD modelling announced that several established models are ready for use in risk assessment. With careful adherence to the guidelines laid out by EFSA we present a stepwise approach to calibration, and validation of the SAM-x algae model for regulatory submission in Tier 2C. Our implementation demonstrates how the use of moving time windows across full FOCUS exposure profiles can generate thousands of virtual laboratory mimic simulations that seamlessly predict the effects of time variable exposures across a full FOCUS profile while maintaining the laboratory conditions of the standard OECD growth inhibition test. Thus, every virtual laboratory test has a duration of 72 hours, with OECD medium and constant light and temperature conditions. The only deviation from this standard test setup is the replacement of constant exposure conditions for FOCUS defined time variable concentrations. In accordance with the EFSA guidelines we use a EP50 of 10 as a threshold, meaning that if any segment of the FOCUS profile causes 50% growth inhibition when the exposure profiles is magnified by a factor of 10, the exposure can no longer be considered low risk. This case study brings to life the simple framework laid out by EFSA for the use of TKTD modelling in tier 2C risk assessment from experimental calibration and validation to the final assessment of whether a given exposure can be considered low risk.

2.04.15
Growth and Biochemical Changes in the Aquatic Macrophyte Lemna gibba Exposed to Ashes of Wildfires of Strawberry Trees
A. Goncalves, MARE - Marine and Environmental Sciences Centre, University of Coimbra / Life Sciences; A. Mesquita, Department of Biology & CESAM - University of Aveiro / Department of Biology and CESAM; F.J. Gonçalves, University of Aveiro / Department of Biology and CESAM; J.C. Marques, University of Coimbra, MARE - Marine and Environmental Sciences Centre / Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal; N. Abrantes, University of Aveiro-CESAM / CESAM/DAO
Nowadays, wildfires are a social and environmental worry worldwide due to its many adverse effects. These events increased in many regions in the last years still the impact of these phenomena on freshwater systems and at the biochemical profile of aquatic species remains poorly studied. By this, the present study aims to determine toxic and biochemical effects of ashes wildfires of high severity of the strawberry tree *Arbutus unedo* at the aquatic macrophyte *Lemna gibba.* It was determined the growth inhibition and the changes at the fatty acids and carbohydrates profiles of the duckweed species after exposure to a range of concentrations of ashes wildfires of *A. unedo*: 25%, 50%, 75% and 100%, plus the negative control, for 7 days, at 23±2ºC, with continuous light. The results showed a decrease on the growth rate with the raise of ash wildfires concentrations. Regarding to the biochemical results, an increase of fatty acids and polysaccharides concentrations where observed compared to the negative control. The increase on the lipids and carbohydrates content may be due to the ability of *L. gibba* to perform fatty acid and sugar molecules biosynthesis under stress conditions due to the presence of appropriate enzymes. The upregulation of such enzymes may contribute to an increase of metabolic rate and thus to fatty acid and polysaccharides increase. Similar toxic and biochemical results were observed to other aquatic species under thermal stress, which highlights the adaptation of the organisms to stress conditions.

Emerging and Legacy Contaminants in Wildlife: Biomonitoring, Exposure, and Effects

2.05.03
Understanding PFAAs Exposure in a Generalist Seabird Species Breeding in the Vicinity of a Fluorochemical Plant
A. Lopez Antia, Universiteit Antwerpen / Biology; M. Kavelaars, W. Müller, University of Antwerp / Department of Biology, Behavioural Ecology and Ecophysiology Group

(BECO); L. Bervoets, University of Antwerp / Department of Biology, Systemic Physiological and Ecotoxicologal Research (SPHERE); M. Eens, University of Antwerp / Department of Biology, Behavioural Ecology and Ecophysiology Group (BECO)
Perfluoroalkyl acids (PFAAs) are a group of chemicals that have been produced for more than five decades. Despite their ubiquity, PFAAs dynamics in the environment and the factors that determine wildlife exposure are not well understood yet. In this study, we examined PFAAs exposure in chicks of a generalist seabird species, the lesser black-backed (LBB) gull (*Larus fuscus*), breeding in The Netherlands, in the neighborhood (49 km away) of a fluorochemical plant. This breeding area is connected to the fluorochemical plant by the Scheldt river, which may transport the contamination downstream. In order to study the pathways of PFAAs exposure, we measured how chicks' PFAAs burden varied with age, sex, and body condition. In addition, we related PFAA concentrations to chicks' diet using stable isotope signatures. For this purpose, we studied plasma PFAA concentrations in 1-week and 4-week-old gull chicks. Four PFAA compounds were detected in the plasma of LLB gull chicks. Measured concentrations (mean±SD) of PFOS (166±138 pg/µL) and PFOA (7.3±8.5 pg/µL) were generally high compared to other seabird species but were highly variable between individuals. Results suggest that maternal transfer plays a significant role in determining chicks' PFAAs burden, and that there are variable sources of exposure for PFOS and PFOA during post-hatching development. On the other hand, the temporal individual patterns found in the chicks sampled at both sampling times, where PFOA levels increased and PFOS decreased in most of the chicks, suggest that the relative importance of the alternative source (or sources) of exposure, compared with maternal transfer, was higher for PFOA than for PFOS. The association between PFOS and specific stable isotopes (i.e. $\delta^{15}N$ and $\delta^{13}C$; both $p \leq 0.004$) suggests a higher exposure to PFOS of birds with a predominantly marine diet. We also found that males' condition (but not females') was positively associated with PFOS plasmatic concentration ($p<0.001$), probably due to the indirect effect of being fed a high quality (marine) diet which appears PFOS rich. Yet, exact exposure source(s) for PFOA remain(s) unclear. Given that PFOS concentrations measured in some chicks surpassed the toxicity reference value calculated for top avian predators, continued monitoring of exposure and health of this gull population, and other wildlife populations inhabiting the area is highly recommended.

2.05.04
Mercury Concentrations in Diurnal and Nocturnal Raptors From Southeastern Italy
S. Espin, P. Sánchez-Virosta, E. Sánchez-Vega, University of Murcia / Department of Health Sciences, Area of Toxicology; A. Gargiulo, Università degli Studi di Napoli Federico II / Medicina Veterinaria; P. Gómez-Ramírez, P. Jiménez, University of Murcia / Department of Health Sciences, Area of Toxicology; P. María-Mojica, University of Murcia / Wildlife Rehabilitation Center of Alicante, Generalitat Valenciana, Alicante, Spain; I. Navas, University of Murcia / Department of Health Sciences, Area of Toxicology; I. Valverde, Universidad de Murcia (Q-3018001-B) / Toxicology and Forensic Veterinary Service, Faculty of Veterinary, Department of Health Sciences.; A. Garcia-Fernandez, University of Murcia / Health Sciences
Mercury (Hg) is a persistent, bioaccumulative and toxic metal that biomagnifies in food chains and is associated with several adverse effects on wildlife. Little is known about the bioaccumulation and biomagnification potential of Hg for terrestrial food chains and new information about Hg levels in terrestrial species is needed. The main aim of this study is to evaluate the Hg exposure in adult diurnal and nocturnal raptors from Wildlife Rehabilitation Centres (WRC). For this purpose, Hg concentrations were analyzed in liver and kidney samples of 43 individuals received in the WRC "Federico II of Naples" between April and November 2016. The study species were common buzzard (*Buteo buteo*), Eurasian sparrowhawk (*Accipiter nisus*), little owl (*Athene noctua*) and barn owl (*Tyto alba*). The results showed a positive correlation between liver and kidney Hg concentrations ($r = 0.96$, $p < 0.001$, $n = 43$), and significant lower concentrations in barn owls compared to the other raptors studied. Although diet habits are typically one of the main factors affecting differences in Hg concentrations, the species studied shared similar characteristics. Potential differences in Hg contamination in the area where the birds came from could explain this result. In addition, although age data was not available, barn owls showed length and weight values lower than the mean body measurements reported in adults of this species, suggesting that they were juveniles. Thus, those lower Hg levels in barn owls could also be related to those individuals being younger, usually showing lower concentrations than adults. In general, Hg concentrations found may be considered low and below those related to lethal or sublethal effects in different raptors species. This study provides new data on Hg concentrations in terrestrial birds, since most studies are focused on marine environments. *Acknowledgements*: Séneca Foundation (MASCA'2014 19481/PI/14 and 20031/SF/16) and *Ministerio de Ciencia, Innovación y Universidades* (IJCI-2017-34653). In loving memory of Prof. Richard F. Shore.

2.05.05
Metals and Elements in Adult and Nestling Tawny Owls From Central Norway
I.B. Kroglund, Nord University / Faculty of Biosciences and Aquaculture; T. Ciesielski, Norwegian University of Science and Technology / Biology; J. Østnes, Nord University / Faculty of Biosciences and Aquaculture; K. Borgå, University of Oslo / Department of Biosciences; V. Jaspers, Norwegian University of Science & Technology / Biology
Ingvild B. Kroglund[a], Tomasz M. Ciesielski[b], Jan E. Østnes[a], Katrine Borgå[c], Veerle L.B. Jaspers[b] [a] Faculty of Biosciences and Aquaculture, Nord university, Norway [b] Department of Biology, Norwegian University of Science and Technology, Norway [c] Department of Biosciences, University of Oslo, Norway Keywords: Biomonitoring, metal contamination, stable isotopes, diet Human activities have disturbed the geochemical cycles of many elements, resulting in increased abundance of bioavailable forms of metals and other elements in the environment. The aim of the present study was to investigate the concentrations of a wide range of essential and non-essential elements in an avian top predator, the tawny owl *(Strix aluco)*, in central Norway. Since tawny owls reside in a restricted territory throughout the year, each individual is expected to reflect the elemental concentrations in their territory, and thus can be used to detect possible elemental pollution in their local environments. The concentrations of 62 elements were analyzed in tawny owls using blood and feather samples from adult females (n=28 and n=72, respectively) and their nestlings (n=35 and n=61, respectively). Samples were collected from 45 different territories during three subsequent field seasons (2016-2018). Feathers were washed in a five-step washing procedure prior to the element analysis in order to remove external contamination. Both blood and feather samples were analyzed for elemental concentrations using an inductively coupled plasma mass spectrometry (ICP-MS). The concentrations of most elements were higher in feathers of adults compared to nestlings. Twenty-two elements (Al, As, Au, Ba, Ce, Co, Cr, Dy, Er, Fe, Hg, La, Mn, Nd, Pr, S, Sm, Sr, U, V, Y and Yb) were positively correlated between adult and nestling feathers. Most of these elements are characteristic for lithic particles (e.g. soil particles), therefore the correlations indicate possibility of external contamination in spite of thorough, multistep washing procedure. On the other hand, two elements were positively correlated between adult and nestling blood (Cd and Cs), and three elements were positively correlated between adult blood and nestling feathers (As, Hg, and Se). Several of these correlations could be due to transfer from the mother into the egg, and/or be a result of common diet. Stable isotope analysis will be performed to reveal the dietary composition of adults and nestlings and to evaluate to what extent the diet affects the concentrations of toxic metals in feathers.

2.05.06
Analysis of Veterinary Pharmaceuticals in Avian Scavengers by a Multi-Class Method for Quantification in Small Amounts of Plasma
P. Gomez-Ramirez, University of Murcia (Q-3018001-B) / Sociosanitary Sciences; G. Blanco, MNCN-CSIC / Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, 28006 Madrid, Spain; A. Garcia-Fernandez, University of Murcia / Health Sciences
Among the most common veterinary pharmaceuticals, antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs) are cause of concern for human and animal health. It is well known that veterinary antibiotics have greatly contributed to the spread of antibiotic resistant bacteria, and there is increasing evidence of their occurrence in wildlife, including vultures; while diclofenac and other NSAIDs were identified as a threat for *Gyps* vultures after the population collapse in South Asia due to the consumption of carcasses of cows treated with diclofenac. The risk for scavengers may be increased when they feed on livestock carcasses provided at supplementary feeding stations (SFSs), as these are often stocked with recently medicated animals. Because these scavengers may be simultaneously exposed to several drugs, analytical methods to evaluate exposure potential should enable detection and quantification of as many different compounds as possible, preferably from small sample

volumes. After testing four different extraction methods, the most viable was a simple extraction, using methanol and 100 µL of plasma, that allowed quantification of 7 antibiotics (tetracycline, oxytetracycline, ciprofloxacin, enrofloxacin, nalidixic acid, trimethoprim, sulfadiazine) and 5 NSAIDs (meloxicam, flunixin, carprofen, tolfenamic acid, phenylbutazone). When the method was applied to 29 Eurasian griffon vulture (*Gyps fulvus*) nestling samples, enrofloxacin and tolfenamic acid were most commonly detected (69% and 20%, respectively). All antibiotics, except ciprofloxacin and oxytetracyclin were detected in at least one sample. However, in the case of NSAIDs, only tolfenamic acid was detected. Five out of the six individuals with residues of this NSAID also presented traces of enrofloxacin, while two of them also had a third compound (one with tetracycline and the other with nalidixic acid). The presence of these compounds can be explained by the use by vultures of SFSs provided with pork and chicken carcasses. To our knowledge, this is the first study to detail the exposure assessment of different classes of veterinary pharmaceuticals in scavenger birds with the simultaneous detection of antibiotics and NSAIDs in the same individuals. Aknowledgements: Ministerio of Economía y Competitividad (CGL2010-15726), Toxicology and Forensic Veterinary Service, U. Murcia, Fundación Séneca (20945/PI/18). This communication is dedicated to Prof Richard F. Shore.

2.05.07
Direct and Rapid Determination of Glyphosate in Hare Gastric Content by Uhplc-Ms/Ms

M. Martinez-Haro, IREC-Instituto de Investigación en Recursos Cinegéticos / Department of Life Sciences; J. Chinchilla, Institute for Game and Wildlife Research, IREC (CSIC-UCLM-JCCM) / Wildlife Toxicology; P. Camarero, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM) / Toxicology in wildlife; J. Viñuelas, Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla La Mancha (IRIAF) / CIAPA-Marchamalo; P. Acevedo, Institute for Game and Wildlife Research, IREC (CSIC-UCLM-JCCM) / SaBio IREC; M. Crespo, Labiana Life Science, S.A.; R. Mateo Soria, IREC (CSIC- UCLM) / Wildlife Toxicology

Glyphosate is the most used herbicide worldwide. It is a small and highly polar pesticide whose physicochemical properties make its analytical determination difficult. Here, a method based on high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) was developed for the determination of glyphosate in gastric content samples from Iberian hares (Lepus granatensis), an herbivorous species, strongly associated to agrosystems. The method involves direct analysis of samples without sample derivatization. The procedure was validated by intra e inter-day accuracy and precision studies with gastric content samples spiked at 1, 5, 10, and 30 ppb, and with internal standard. Finally, the performance of the method was tested on field samples of hunted from pesticides treated and free-pesticides areas (n=76 and 25, respectively), as well as from found dead hares (n=10). Satisfactory recoveries were achieved within the range of 77% to 113% using both primary and secondary quantitative ion transitions (RSD ≤ 20%). Positive samples ranged 9-24% in the case hunted animals from pesticides treated areas, and 40% in animals found dead. No residues were detected in animals from free-pesticide (organic farmland) areas. In practice, the developed methodology may be particularly useful in the context of research and other works related to agrosystem species.

2.05.10
Individual Prey Specialization Drives PCBs in North Atlantic Killer Whales

A. Remili, McGill University / Department of Natural Resource Sciences; R.J. Letcher, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health Division; F.I. Samarra, Marine and Freshwater Research Institute; R. Dietz, C. Sonne, Aarhus University / Department of Arctic Environment; J. Desforges, McGill University / Department of Natural Resource Sciences; G.A. Vikingsson, Marine and Freshwater Research Institute; D. Blair, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health Division; M.A. McKinney, McGill University / Department of Natural Resource Sciences

Inter-individual variation in diet is an important, but often overlooked aspect of population feeding ecology, and may affect intra-population variation in contaminant exposure. Here, we quantified the range of blubber concentrations of an extensive suite of legacy and new persistent organic pollutants (POPs) in the blubber of Icelandic killer whales (*Orcinus orca*) to assess intra-population variation and identify the main factors responsible, as well as possible implications for risks posed by these contaminants. Polychlorinated biphenyl (PCB) concentrations were >300 times higher in the most contaminated individual than in the least contaminated (428.6 versus 1.3 $mg.kg^{-1}$ lw). The most important factor explaining variation in contaminant concentrations was the diet-type, followed by sex. Mixed-diet whales, feeding to a certain extent on marine mammals, showed POP concentrations up to nine times higher than fish-feeding whales. The contaminant levels of these mixed-diet killer whales exceeded all known thresholds for adverse health effects, whereas levels in the fish-feeding individuals did not. These findings show that striking differences in contaminant accumulation can occur within populations exhibiting individual prey specialization and demonstrate that the ecology of individuals must be understood to accurately forecast how contaminants may threaten the long-term persistence of the world's ultimate marine predator.

2.05.12
Organophosphate Esters in Edible Fish From the Mediterranean Sea

B. Sala Solà, IDAEA-CSIC / Environmental Chemistry; J. Giménez Verdugo, ICM-CSIC; J. Fernández Arribas, IDAEA-CSIC; E. Lloret Lloret, M. Coll, ICM-CSIC; E. Eljarrat, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Research Council (CSIC) / Department of Environmental Chemistry

European anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) are two of the most important small pelagic fish species for the commercial fisheries of the Northwestern Mediterranean Sea. In the last decades, there has been an important decline in sardines and anchovies' populations in all the Western Mediterranean Sea. Amongst several hypotheses to explain these trends, the increase in pollution may be playing an important role. In this sense, plastic pollution is an increasing issue for the marine ecosystem with the Mediterranean Sea being a remarkably impacted area [1]. Marine organisms are able of ingesting plastics and microplastics with potential perturbations at different physiological levels [2]. Beyond the ingestion of plastic itself, the accumulation of plastics additives and pollutants and the capacity of some of those to bioaccumulate and/or biomagnify, is an increasing concern. In this study, we included anchovy and sardine together with one of its potential predators, European hake (*Merluccius merluccius*), to compare its concentration of plasticizers across different areas along a latitudinal gradient of the Western Mediterranean Sea ("Cap de Creus", "Delta de l'Ebre", "Golf de València" and "Cartagena") and investigate potential bioaccumulation and/or biomagnification of the organophosphate esters (OPEs). OPEs were detected in all the samples, with levels ranging from 2.02 to 73.4 ng/g wet weight (ww) in sardines, nq to 34.7 ng/g ww in anchovies and 0.29 to 6.97 ng/g ww in hakes. Ten out of fifteen OPEs analyzed were detected in sardine and anchovy samples and seven out of fifteen in hake. The distribution of OPEs was fairly similar for both small pelagic fish but contrasted with hake. Furthermore, comparison between the levels found in hake *versus* those found in sardine and anchovy seem to indicate that no OPE biomagnification occurred.[1] De Haan W.P., Sanchez-Vidal A., Canals M., Shipboard N. 2019. Floating microplastics and aggregate formation in the Western Mediterranean Sea. doi:10.1016/j.marpolbul.2019.01.053.[2] Gabriel L., Barboza A., Lopes C., Oliveira P., Bessa F., Otero V., et al. 2020. Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage , and human health risks associated with ingestion exposure. doi:10.1016/j.scitotenv.2019.134625.

2.05.13
Polychlorinated Biphenyls Are Associated With Reduced Testes Weights in Harbour Porpoises (Phocoena phocoena)

R. Williams, Zoological Society of London; D.J. Curnick, Institute of Zoology, Zoological Society of London, Regents Park, London NW1 4RY, UK; P.D. Jepson, Zoological Society of London; S. Jobling, Brunel University / Institute of Environment, Health and Societies

Polychlorinated biphenyls (PCBs) are highly toxic and persistent aquatic pollutants that are known to bioaccumulate in a variety of marine mammals. They have been associated with reduced recruitment rates and population declines in multiple species. Evidence to date documents effects of PCB exposures on female reproduction, but few studies have investigated whether PCB exposure impacts male fertility. Using blubber tissue samples of 99 adult and 168 juvenile UK-stranded harbour porpoises (*Phocoena phocoena*) collected between 1991 and 2017, here we show that PCBs exposures are associated with reduced testes weights in adults with good body condition. In animals with poor body condition, however, the impact of PCBs on testes weights was reduced, conceivably due to testes weights being limited by nutritional stress. This is the first study to investigate the relationship between PCB contaminant burden and testes weights in

cetaceans and represents a substantial advance in our understanding of the relationship between PCB exposures and male reproductive biology in cetaceans. As testes weight is a strong indicator of male fertility in seasonally breeding mammals, we suggest the inclusion of such effects in population level impact assessments involving PCB exposures. Given the re-emergent PCB threat our findings are globally significant, with potentially serious implications for long-lived mammals. We show that more effective PCB controls could have a substantial impact on the reproductive health of coastal cetacean species and that management actions may need to be escalated to ensure adequate protection of the most vulnerable cetacean populations.

2.05.16

Does Microplastic Ingestion Affect Fatty Acid Composition in the Japanese Quail?

E. McCann Smith, Norwegian University of Science & Technology (NTNU) / Biology; Z. Bartosova, Norwegian University of Science & Technology (NTNU) / Department of Biology; V. Jaspers, Norwegian University of Science & Technology / Biology; M. Wagner, Norwegian University of Science and Technology / Department of Biology; L. Monclus, Norwegian University of Science & Technology (NTNU) / Department of Biology

Plastics are pervasive pollutants in the environment and birds are among the most highly exposed groups of animals. High amounts of plastics and microplastics (MPs: < 5 mm) have been found in bird stomachs, which can result in altered physiology and starvation. Several studies in invertebrates have shown MPs to decrease lipid accumulation, and recent research has shown that polystyrene MPs influence lipid metabolism and fatty acid composition in zebrafish. Triglycerides and fatty acids are integral for energy storage and use in birds, especially in fledglings. However, very little research has focused on how MP ingestion affects fatty acid composition in birds. In the present experiment, we orally exposed Japanese quail (*Coturnix japonica*) to two different size classes of MPs to determine their effect on the composition and abundance of fatty acids in the liver. Fifty-six quails were randomly divided into four groups (n=14) including one control and three treatment groups exposed to either < 125 µm MP powder, 3 mm MP pellets, or a combination of the two size classes. The MP consisted of a mixture of polystyrene, polyethylene, and polypropylene with doses ranging from 25 mg to 75 mg per exposure. Starting at one week old, quails were fed MPs every third day for six weeks. After six weeks, the quails were sacrificed and liver samples were collected. Livers were flash frozen in liquid nitrogen and stored at -80 ºC until further analysis. After hydrolysis and extraction, fatty acid composition was analyzed using supercritical fluid chromatography with mass spectrometry (SFC-MS). The results of this ongoing study will be presented at the conference.

2.05.17

Analysing Avian Reproduction Studies Via Bioenergetics Modelling: DEB - TKTD for the Bobwhite Quail and the Mallard

B. Goussen, M. Trijau, IBACON GmbH / Ecological Modelling; R. Brain, J.D. Maul, Syngenta Crop Protection Inc. / Environmental Safety; N. Galic, Syngenta Crop Protection LLC / Environmental Safety

Current environmental risk assessments addressing chronic exposure of chemicals to birds is based on complex and controlled reproduction laboratory experiments. These experiments produce many response variables that are usually separately analysed which hampers a better understanding of the effects of the tested chemical as well as extrapolation to field conditions. In addition, behavioural responses are rarely considered. A way to better understand the avian reproduction data is to use mechanistic modelling, such as Toxicokinetic – Toxicodynamic (TKTD) modelling. Specifically, Dynamic Energy Budget (DEB) models coupled with TKTD modules (DEB-TKTD) are the leading approach to assess sublethal effects of chemicals on individuals by making holistic use of all data available through integration in a unique framework. In their current state, DEB models do not include the necessary flexibility to accurately capture the variety and specificity of data from avian reproduction studies. Here we present a tailored DEB-TKTD model able to overcome this challenge. We tested our model on 10 avian reproduction studies conducted on 5 different pesticides. The new implementation accurately captured the growth and reproduction observed in these studies. In addition, with this implementation, possible behavioural and direct physiological effects of the pesticides were quantified separately and in combination. This approach has the potential to make better use of existing and future data sets and provides means for a more accurate extrapolation of effects to birds from pesticide exposures in the field.

2.05.18

Land Use in Habitats Affects Metal Concentrations in Wild Lizards Around a Former Lead Mining Site

S.M. Nakayama, R. Doya, H. Nakata, Hokkaido University; H. Toyomaki, Hokkaido University / Laboratory of Toxicology; J. Yabe, The University of Zambia / Veterinary Medicine, Paraclinical Studies; K.M. Muzandu, University of Zambia; Y.B. Yohannes, A. Kataba, Hokkaido University / Faculty of Veterinary Medicine; G. Zyambo, The University of Zambia; T. Ogawa, Y. Uchida, Hokkaido University; Y. Ikenaka, M. Ishizuka, Hokkaido University / Faculty of Veterinary Medicine

Several studies have described environmental hazards preceding city development. Examples are environmental contamination in developing countries that is caused by poorly managed electronic waste-recycling facilities, dumping grounds, and mining sites. Until remediation is complete, humans and animals continue to be exposed to toxic substances. Moreover, even if the environment is not suitable for people to live in since contamination sources often correspond with local economic drivers, social communities and economic activities continue to flourish and fuel urbanization regardless. Therefore, appropriate city planning should be conducted before mass construction begins, so that people can receive the benefits of urbanization while their exposure to environmental pollutants is mitigated. We investigated the potential effects of different land use and other environmental factors on animals living in a contaminated environment. The study site in Kabwe, Zambia, is currently undergoing urban expansion, while lead contamination from former mining activities is still prevalent. We focused on a habitat generalist lizards (Trachylepis wahlbergii). The livers, lungs, blood, and stomach contents of 224 lizards were analyzed for their lead, zinc, cadmium, copper, nickel, and arsenic concentrations. Habitat types were categorized based on vegetation data obtained from satellite images. Multiple regression analysis revealed that land use categories of habitats and three other factors significantly affected lead concentrations in the lizards. Further investigation suggested that the lead concentrations in lizards living in bare fields were higher than expected based on the distance from the contaminant source, while those in lizards living in green fields were lower than expected. In addition, the lead concentration of lungs was higher than that of the liver in 19% of the lizards, implying direct exposure to lead via dust inhalation besides digestive exposure. Since vegetation reduces the production of dust from surface soil, it is plausible that dust from the mine is one of the contamination sources and that vegetation can reduce exposure to this.

2.05.20

Assessing the Impacts of Pesticide Exposure on Farmland Birds in England

P. Vijendra, University of Sussex / Evolution, Behaviour and Environment; D. Goulson, School of Life Sciences, University of Sussex

Pesticides have played a significant role in the agricultural landscape, by protecting crop from damage, and improving and increasing yields. However, there is strong evidence for their negative impact on ecosystems. Pollinators have significantly declined, predatory bird populations have only recently recovered from the lethal effects of organochlorines, and aquatic systems remain contaminated from long-term exposure. Extensive research has led to lethal pesticides to be banned from agricultural use but there have since been more toxic pesticides introduced, and the long-term effects of these are often unknown. Using bird abundance data and pesticide usage data for England, we conducted a spatio-temporal analysis to understand the changes in population of farmland bird species over 23 years and whether any of the associations were explained by exposure risks. The data will help us understand the risk posed by pesticides to farmland birds and identify the species most threatened by pesticide use.

2.05.21

Persistent Organic Pollutants in Feathers of the Greater Rhea (Rhea americana), a Near-Threatened Flightless Bird of the Pampas Grasslands

A. Lèche, Universidad Nacional de Cordoba; E. Gismondi, LEAE - University of Liege / Laboratory of Animal Ecology et Ecotoxicology; M. Martella, J. NAVARRO, Universidad Nacional de Cordoba

Persistent Organic Pollutants (POPs) are still globally distributed and can exert different effects on ecosystems. Little is known about the occurrence of these contaminants in terrestrial birds from South America. In this study, POPs were assessed for the first time in a flightless herbivorous species from the Pampas grasslands, the Greater rhea (*Rhea americana*). Concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs), were determined in 18 samples of feathers from free-ranging and captive individuals inhabiting four sites with different land use in central Argentina. Among the 16 POPs tested in those

feathers, 6 PCBs (28, 52, 101, 138, 153 and 180) and 8 OCPs (α-HCH, β-HCH, γ-HCH, p,p'-DDE, p,p'-DDD, o,p'-DDT, p,p'-DDT and HCB) were quantified. No PBDEs were detected. Total concentration of POPs was higher in populations living in an intensive crop production area (Agriculture: 159 $ng.g^{-1}$ and Farm: 97.53 $ng.g^{-1}$) compared to the population in an urban area (Zoo: 45.86 ng/g) and an agroecosystem with extensive rearing of livestock (Cattle rearing: 36.77 $ng.g^{-1}$). PCBs were the most abundant pollutants in all the populations studied. Lower chlorinated CB 52 and CB 101 were the principal PCB congeners detected, representing at least 70% of the total quantified. All populations studied showed a DDE + DDD / DDT ratio > 1, indicating a historical application of this insecticide. This study provides a new contribution to the scarce data on POP concentrations in South American bird species. Further investigations are needed to evaluate their potential effects on the health individuals and populations.

2.05.22

Presence of Organic Micropollutants in Greater Flamingo From Ebro Delta Natural Park

M. Dulsat-Masvidal, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); A. Bertolero, Associació Ornitològica Picampall de les Terres de l'Ebre; R. Mateo Soria, IREC (CSIC- UCLM) / Wildlife Toxicology; S. Lacorte, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Research Council (CSIC) / Environmental Chemistry

Ebro delta is a wetland of international importance for water bird conservation. In a relatively small surface, it comprises a great diversity of habitats and species: a total of 343 species of birds have been observed in the delta, from which 100 species breed regularly and 15 occasionally (1). Over the last 150 years the extensive wetland reclamation for rice cultivation has resulted in the loss of 65% of the natural habitats (2). The agricultural activities and the runoffs inputs from industries and wastewater treatment plants are an important source of organic contaminants to Ebro delta. In the recent years the presence of pesticides, pharmaceuticals, perfluorinated compounds and organophosphorus compounds has been reported in soils, sediments and water from Ebro delta. These organic contaminants can be bioaccumulated and transferred along the food chain affecting avifauna. The accumulation of pollutants in Yellow-legged gulls and Audouin's gulls from the delta Ebro have been reported in the recent years (3). The Greater Flamingo (*Phoenicopterus roseus*) is an emblematic species of the Ebro Delta Natural Park, since it is the only place where it breeds in Catalonia and one of the few stable places in the western Mediterranean. Conserving the species in one of the main objectives of the Natural Park, implying an accurate monitoring program. However, polluted sediments and water can affect flamingos and induce potential adverse effects on reproduction and survival, although this has never been assessed before. In this study, we have analysed for the first-time a near one hundred of contaminants, including pharmaceuticals, perfluorinated compounds, organophosphorus flame retardants and pesticides in blood from chicks of greater flamingos with aim to value their potential impact in the conservation status of the species. Our results suggest ovo- transfer of pollutants and sediment ingestion as a source of contamination of flamingo's flagging's. 1. Bigas D, Curcó A. Llista patró dels ocells del delta de l'Ebre. Parc Natural del Delta de l'Ebre, Generalitat de Catalunya [Internet]. 2015. Available from: http://parcsnaturals.gencat.cat/web/.content/home/delta_de_lebre/coneix-nos/centre_de_documentacio/fons_documental/publicacions/revistes_i_butlletins/noticiari_ornitologic/Nova_Edicio_llista_patro_ocells_Delta2015_web.pdf 2. Prado P, Alcaraz C, Benito X, Caiola N, Ibáñez C. Pristine vs. human-altered Ebro Delta habitats display contrasting resilience to RSLR. Sci Total Environ [Internet]. 2019;655:1376–86. Available from: https://doi.org/10.1016/j.scitotenv.2018.11.318 3. Zapata P, Ballesteros-Cano R, Colomer P, Bertolero A, Viana P, Lacorte S, et al. Presence and impact of Stockholm Convention POPs in gull eggs from Spanish and Portuguese natural and national parks. Sci Total Environ [Internet]. 2018;633:704–15. Available from: https://doi.org/10.1016/j.scitotenv.2018.03.081

2.05.24

A Novel Role for Natural Science Collections in European Contaminant Monitoring

P. Movalli, Naturalis Biodiversity Center; G. Cicero, University of Palermo; G. Ramello, Museo Civico di Storia Naturale di Carmagnola; G. Sbokos, Natural History Museum of Crete; K. Vlachopoulos, University of Thessaly; R.W. Dekker, Naturalis Biodiversity Center; S. Espín, University of Murcia / Department of Health Sciences, Area of Toxicology; A. Garcia-Fernandez, University of Murcia / Health Sciences; P. Gómez-Ramírez, University of Murcia / Department of Health Sciences, Area of Toxicology; P. Hosner, Natural History Museum of Denmark; S. Islam, D. Koureas, Naturalis Biodiversity Center; J. Kristensen, Natural History Museum of Denmark; S. Van der Mije, Naturalis Biodiversity Center; P. Sánchez-Virosta, University of Murcia / Department of Health Sciences, Area of Toxicology; O. Krone, Leibniz Institute for Zoo and Wildlife Research / Department of Wildlife Diseases; M. Leivits, Estonian University of Life Sciences / Institute of Veterinary Medicine and Animal Sciences; R. Shore, Centre for Ecology & Hydrology / Lancaster; A. Vrezec, National Institute of Biology / Department of Organisms and Ecosystems; L. Walker, UK Centre for Ecology & Hydrology; C. Wernham, British Trust for Ornithology; A. Lopez-Antia, University of Antwerp; R. Lourenco, Universidade de Evora / Mediterranean Institute for Agriculture, Environment and Development; R. Mateo, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM); A. Badry, German Environment Agency (Umweltbundesamt); T.I. Fuisz, Hungarian Natural History Museum; M. Guiraud, Muséum National d'Histoire Naturelle; U. Johansson, Naturhistoriska Riksmuseet; M. Pavia, University of Torino, Italy / Museo di Geologia e Paleontologia; O. Pauwels, Institut Royal des Sciences Naturelles de Belgique; G.M. Pereira, UK Centre for Ecology & Hydrology / Centralised Chemistry; T. Topfer, Zoological Research Museum Alexander Koenig; R. Väinölä, Finnish Museum of Natural History; D. Vangeluwe, Institut Royal des Sciences Naturelles de Belgique; N.A. Alygizakis, Environmental Institute; A. Androulakakis, National and Kapodistrian University of Athens; A. Cincinelli, University of Florence; W. Drost, Federal Environment Agency (UBA) / Chemicals; G. Gkotsis, National and Kapodistrian University of Athens / Laboratory of Analytical Chemistry, Department of Chemistry; N. Glowacka, Environmental Institute; J. Koschorreck, Umweltbundesamt / Inland Surface Waters; T. Martellini, University of Florence; M. Nika, National and Kapodistrian University of Athens / Laboratory of Analytical Chemistry, Department of Chemistry; V. Nikolopoulou, National and Kapodistrian University of Athens; J. Slobodnik, Environmental Institute; N. Thomaidis, National and Kapodistrian University of Athens / Department of Chemistry; G. Treu, German Environment Agency (Umweltbundesamt); N. Sarajlić, Ornithological Society Naše ptice; G. Duke, University of Oxford / Environmental Change Institute

Natural science collections (NSCs) increasingly engage in a wide range of applied research. Monitoring of contaminants in biota is relatively novel for NSCs, but has potential for high environmental, social and economic impact, which can in turn enhance the value of collections. Tens of thousands of chemical substances are released into Europe's environment and the tonnage of toxic substances used increases annually. Their increasing ubiquity carries high costs for wildlife and human health. EU regulations seek to address this challenge and the European Green Deal aims for a non-toxic environment with better monitoring of environmental pollutants and of the effectiveness of chemicals legislation. Yet regulators struggle to assess and manage chemical risks, given the vast number of substances involved and the lack of data on exposure and hazards. Raptors, as apex predators, are particularly well suited to contaminant monitoring. The European Raptor Biomonitoring Facility (ERBFacility) aims to put in place a distributed 'Facility' that brings together field ornithologists, collections and analytical laboratories, for the gathering, storage and analysis of raptor samples to deliver data at pan-European scale on contaminants in raptor tissues. Such data can usefully inform the prioritisation of substances for risk assessment, provide early warning of emerging contaminant problems, and throw light on the effectiveness of chemical risk management measures. This poster focuses on ERBFacility work to develop a distributed European Raptor Specimen Bank, including: (a) a review of existing collections of frozen raptor carcasses/tissues; (b) a protocol for NSCs for gathering, processing and storing of raptor carcasses/tissues for contaminant monitoring; (c) guidance for shipment of samples; (d) a European raptor specimen database aligned with the Distributed System of Scientific Collections (DiSSCo) Research Infrastructure and linked to the LIFE APEX tissue sample catalogue and contaminant databases. ERBFacility is already identifying and sourcing relevant raptor samples from collections across Europe for a pan-European proof of concept study and for pan-European demonstration studies under the LIFE APEX project. These studies are expected, inter alia, to aid EU agencies in the prioritisation of substances for persistence, bioaccumulation and toxicity (PBT) assessment. Eventual savings in terms of reduced impacts on human and wildlife health could be substantial.

2.05.25

A Schematic Sampling Protocol for Contaminant Monitoring in Raptors

S. Espin, University of Murcia / Department of Health Sciences, Area of Toxicology; J. Andevski, Vulture Conservation Foundatio; G. Duke, University of Oxford / Environmental Change Institute; I. Eulaers, Norwegian Polar Institute / Ecotoxicology; P. Gómez-Ramírez, University of Murcia / Department of Health Sciences, Area of Toxicology; G. Hallgrimsson, University of Iceland / Faculty of Life and Environmental Sciences; B. Helander, Swedish Museum of Natural History / Environmental Research & Monitoring; D. Herzke, NILU - Norwegian Institute for Air Research / FRAM Centre Tromso; V. Jaspers, Norwegian University of Science & Technology / Biology; O. Krone, Leibniz Institute for Zoo and Wildlife Research / Department of Wildlife Diseases; R. Lourenco, Universidade de Evora / Mediterranean Institute for Agriculture, Environment and Development; P. María-Mojica, University of Murcia / Wildlife Rehabilitation Center of Alicante, Generalitat Valenciana, Alicante, Spain; E. Martínez-López, University of Murcia / Department of Health Sciences, Area of Toxicology; R. Mateo, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM); P. Movalli, Naturalis Biodiversity Center; P. Sánchez-Virosta, University of Murcia / Department of Health Sciences, Area of Toxicology; R. Shore, Centre for Ecology & Hydrology / Lancaster; C. Sonne, Aarhus University / Department of Arctic Environment; N. van den Brink, Wageningen University / Dept of Toxicology; B. van Hattum, University Amsterdam / Dep. Environment and Health; A. Vrezec, National Institute of Biology / Department of Organisms and Ecosystems; C. Wernham, British Trust for Ornithology; A. Garcia-Fernandez, University of Murcia / Health Sciences

As a key part of the European Green Deal, the EU Chemicals Strategy aims for a non-toxic environment. A key challenge in this respect is to apply biomonitoring data in toxicological and ecotoxicological risk assessment to inform better management of chemicals in Europe. Raptors (birds of prey, owls and falcons) are widely used as sentinel species in monitoring programs and are particularly well-suited to contaminant monitoring. Delivering relevant contaminant data at pan-European scale requires the development of a pan-European network that brings together fieldworkers, sample collections and analytical laboratories, and of frameworks for the gathering, storage and analysis of raptor samples, together with related capacity-building and sharing of best practice. This challenge is being addressed by the COST Action *European Raptor Biomonitoring Facility* (https://erbfacility.eu/) building on the precursor Networking Programme *Research and Monitoring for and with Raptors in Europe* (EURAPMON; http://www.eurapmon.net/). This poster introduces a schematic sampling protocol for contaminant monitoring in raptors. The protocol provides best-practice guidance in a format accessible to both professionals and volunteers. The protocol aims to enhance sampling capabilities across Europe, ensure appropriate quality of samples and facilitate harmonization of procedures to maximize the reliability, comparability and interoperability of resulting contaminant data. The protocol starts with each matrix type, including sample types that are collected both during active (samples from captured birds and monitored nests) and passive (samples from dead birds and deserted nests) sampling. For each sample type (whole blood, plasma, serum, deserted or addled eggs, feathers, preen oil, regurgitated pellets, prey remains, gastric content and internal tissues), a specific protocol offering additional information is provided (e.g., collecting procedure, volume/mass of sample needed for contaminant monitoring, container types to conserve the sample, transport and storage conditions). The full protocol can be found online (https://doi.org/10.1007/s13280-020-01341-9).

Acknowledgments: European Raptor Biomonitoring Facility COST Action (CA16224) is supported by COST (European Cooperation in Science and Technology). Silvia Espín is supported by *Ministerio de Ciencia, Innovación y Universidades* (IJCI-2017-34653).

† This communication is dedicated to the memory of Prof. Richard F. Shore.

2.05.26

Use of Raptor Chemical Monitoring Data to Assess Effectiveness of EU Chemical Management Measures; the Impact of Pooling Liver Samples on Power to Detect Change in Contaminant Concentrations at Country Scale

P. Movalli, Naturalis Biodiversity Center; N.A. Alygizakis, Environmental Institute; A. Badry, German Environment Agency (Umweltbundesamt); R. Buij, Wageningen University & Research / Wageningen Environmental Research; A. Cincinelli, E. De Beni, University of Florence; R.W. Dekker, Naturalis Biodiversity Center; W. Drost, Federal Environment Agency (UBA) / Chemicals; G. Duke, University of Oxford / Environmental Change Institute; G. Gkotsis, National and Kapodistrian University of Athens / Laboratory of Analytical Chemistry, Department of Chemistry; N. Glowacka, Environmental Institute; H. Jansman, Wageningen Environmental Research / Team Animal Ecology; J. Koschorreck, Umweltbundesamt / Inland Surface Waters; T. Martellini, University of Florence; M. Nika, National and Kapodistrian University of Athens / Laboratory of Analytical Chemistry, Department of Chemistry; V. Nikolopoulou, National and Kapodistrian University of Athens; E.D. Potter, NERC Centre for Ecology & Hydrology / Lancaster; S. Santini, University of Florence / Department of Chemistry; C. Sarti, University of Florence; R. Shore, Centre for Ecology & Hydrology / Lancaster; J. Slobodnik, Environmental Institute; N. Thomaidis, National and Kapodistrian University of Athens / Department of Chemistry; G. Treu, German Environment Agency (Umweltbundesamt); N. van den Brink, Wageningen University / Dept of Toxicology; S. Van der Mije, Naturalis Biodiversity Center; L. Walker, UK Centre for Ecology & Hydrology

Raptors as apex predators are suitable sentinel species for contaminants in the environment. In particular, monitoring of contaminants in raptors may be used to assess the effectiveness of chemical management measures and to evaluate the effectiveness of chemicals regulations more generally in their aim of protecting environmental (and human) health. We analysed approximately 70 buzzard livers from The Netherlands, and 70 from the UK, spanning the period 1996-2020. Livers from The Netherlands were analysed for PCBs, PBDEs (including Deca-BDE) and Dechlorane Plus, those from the UK for anticoagulant rodenticides and Hg. EU chemical management has restricted the use of these substances in recent years/decades. If these management measures are effective, one would expect to see a decline in these substances in raptors following introduction of measures. We report on chemical residue concentrations found and how these change over time, prior to and following the introduction of chemical management measures. We simulate the impact that within-year pooling of samples (of 2, 3, 4 or 6 individuals) has on the provision of representative country-scale data for the detection of changes, over time, in average residue concentrations, with a view to assessing the power of monitoring with pooled samples. Power here means the size of change that can be detected with a certain statistical probability over a specified time period. We examine trade-offs between extent of sample pooling, number of pooled samples that are analysed, and projection of the magnitude of change in environmental concentrations that would be detectable if monitoring were continued for 5 and 10 years. Knowledge on the power of monitoring with pooled samples has the potential to inform the design of cost-effective monitoring (with raptors) of the effectiveness of chemical management measures.

2.05.28

Detecting Pollutants in Tropical Areas: Wildlife Conservation Requires an Ecosystem Health Assessment in Madagascar

W. Cristiano, University of Torino / Department of Environment and Health; C. Giacoma, University of Turin / Department of Life Sciences and Systems Biology; M. Carere, Italian Institute of Health; M. Gamba, University of Turin / Department of Life Sciences and Systems Biology; L. Mancini, Italian Institute of Health / Department of Environment and Health

The releases, emission, and discharge of chemical substances into the environment is a challenging issue that threatens the ecosystems by affecting both wildlife and human health. Especially relevant is the exposure of living organisms to emerging pollutants whose effects on natural populations are little unknown overall. Such substances represent new threats for wildlife worldwide and might interact with other compounds previously discharged into the ecosystems over the years causing a “cocktail effect”. The risks for the health of wildlife are particularly high in global biodiversity hotspots, such as Madagascar. This country hosts a huge amount of endemic species most at risk of extinction. Successful conservation projects must include the implementation of measures aiming to mitigate the exposure of local wildlife to emerging and legacy pollutants. However, the knowledge of the real effects of the chemical contaminants is often lacking. The goal of this study is to help in filling this knowledge gap in the southern part of the Alaotra-Mangoro region, Eastern Madagascar. This territory is characterised by a dense pristine tropical rainforest that gradually gives way to rural areas subjected to multiple potential sources of pollution, including farming and mining. Furthermore, this area represents a major tourist destination because of its unique fauna among which stand some of the most flourishing lemur populations. In order to gather information on the direct and indirect impacts of chemical

pollution on wildlife, experimental methods including chemical, physiological, ecotoxicological, and biological analyses on environmental and animal samples, i.e. water, sediments, macroinvertebrate, and lemurs will be applied. The study area will be divided into three zones along a gradient of anthropogenic disturbance: the city of Moramanga, the village of Andasibe, and the Analamazaotra Special Reserve, that is a part of Andasibe-Mantadia National Park. The results obtained through different analyses may be an asset in the data evaluation to understand the pressures and the sources through which the chemical substances, including emerging pollutants, occur in wildlife. The expected outputs may provide the first evidence of anthropogenic disturbance due to chemical pollution in the study area and will improve the policy environmental actions by helping in the identification of the threats to animal health.

2.05.29
The Effects of Copper and Benzo(A)Pyrene on Distinct Freshwater Species
M.O. Rodrigues, University of Aveiro / Department of Biology and CESAM; N. Abrantes, University of Aveiro-CESAM / CESAM/DAO; F.J. Gonçalves, University of Aveiro / Department of Biology and CESAM; A. Goncalves, MARE - Marine and Environmental Sciences Centre, University of Coimbra / Life Sciences
Freshwater systems are one of the most threatened ecosystems mainly due to anthropogenic activities (e.g. hydrological system alterations, pollution, introduction of invasive species and the overexploitation of biological resources). The input of contaminants in freshwater systems can derive from illegal discharges (e.g. agriculture wastewater, industrial effluents) and/or chemical run-off provided from terrestrial environments. These pressures can result in biodiversity and ecosystem services reduction. Metals and polycyclic aromatic hydrocarbons (PAHs) are among the most common contaminants found in water systems. They are characterized by their high persistence, toxicity and tendency to bioaccumulate. In addition, they have been reported to be carcinogenic and mutagenic. Thus, these pollutants are considered of high concern. Hence, this work attempted to evaluate the impacts of a metal (i.e. copper) and a PAH (i.e. benzo(a)pyrene) on three standard freshwater species (i.e. *Lemna minor*, *Raphidocelis subcapitata*, *Daphnia magna)*. The results revealed that the invertebrate *D. magna* was the most sensitive species to both contaminants in opposite to the macrophyte *L. minor*. In fact, besides no growth inhibition was found when *L. minor* was exposed to high concentrations of benzo(a)pyrene, a growth stimulus was even observed at low concentrations. Regarding the toxicity of both contaminants to the three species, benzo(a)pyrene showed to be more toxic than copper. Since the tested organisms play a crucial role in freshwater systems, occupying a basal position in the trophic chain, our results reinforce the concerns related with metals and PAHs contamination in aquatic systems and the importance to protect their ecosystems.

Impact of Chemical Pollutants in Food Webs within and across Ecosystem Boundaries

2.06.04
A Meta-Community Approach to Decipher the Role of Caddisfly Traits and Habitats in Hg Accumulation and Transfer
P. Marle, University of Geneva / Department F.A. Forel for Environmental and Aquatic Sciences and Institute for Environmental Sciences; W. Liu, University of Geneva / Department F.A. Forel for Environmental and Aquatic Sciences; P. Timoner, E. Castella, University of Geneva / Department F.A. Forel for Environmental and Aquatic Sciences and Institute for Environmental Sciences; V. Slaveykova, University of Geneva / Department F.A. Forel for Environmental and Aquatic Sciences
Accumulation of Hg in aquatic invertebrates depends on various environmental and biological factors, including physiology, ecological traits and habitats. Here, we present a new meta-community approach to explore the influence of species traits, such as adult body size, larval feeding type and micro-habitat, as well as larval macrohabitat (main river channel vs. floodplain water bodies) on the concentration of Hg accumulated [THg] in assemblages of adult caddisflies. We analyzed [THg] in 157 light-trapped adult caddisflies in a floodplain sector of the French upper Rhône River and used linear mixed effect models to decipher the role of species traits and habitats in Hg accumulation. Results showed that [THg] in individuals associated with floodplain macrohabitats was lower than in those associated with the main river channel. Variation of [THg] between species was best explained by the larval feeding type, whereas the contributions of larval micro-habitat and adult size were minor. We provide a first demonstration of the potential of an entire caddisfly assemblage for the assessment of contamination in a large river floodplain with high taxonomic resolution. We discussed the implications of our results in view of the possible role of caddisflies as vectors of Hg to riparian predators. The outcomes of the study have broad implications regarding the transfer of freshwater Hg towards terrestrial food webs and more generally for large river contaminant research and management.

2.06.05
The Transfer of Current Use Organic Pesticides From Aquatic to Terrestrial Ecosystems Via Insect Emergence
A.P. Roodt, University of Koblenz Landau, iES Landau, DFG Graduiertenkolleg Systemlink / Ecotoxicology; N. Röder, University of Koblenz-Landau, Germany / iES Landau, DFG Graduation College SystemLink; S. Pietz, Institute for Environmental Sciences, University of Koblenz-Landau / DFG Graduation College SystemLink; S. Kolbenschlag, University Koblenz Landau / iES Landau, DFG Graduation College SystemLink; K. Schwenk, University Koblenz-Landau, Germany / iES Landau, DFG Graduation College SystemLink; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment; R. Schulz, Institute for Environmental Sciences, University of Koblenz-Landau
Emerging merolimnic insects are important subsidies to terrestrial food webs in riparian systems. The developing aquatic insect larvae are exposed to, and thus contain body burdens of, a broad range of organic pesticides when inhabiting agricultural streams. Information on the fate of current use organic pesticides after the metamorphosis from larvae to adults, and the resulting body burdens, is, however, lacking. A better understanding of the properties of organic pesticides, such as the lipophilicity, which facilitate their transfer into the adjacent terrestrial ecosystems via this vector is required in order to evaluate the risks for exposure and biomagnification in terrestrial food webs.We measured the body burdens of nine current use organic pesticides, with log octanol/water partition coefficients (log Kow) between 2.5 and 5, in the late stage larvae and emergent adults of Chironomus riparius during a 30 day laboratory exposure experiment. The pesticides were applied at the start of the experiment, as formulation products, to the aqueous phase over a range of concentrations. The resulting insect body burdens were measured by high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS).All nine pesticides were detected in the late stage larvae with generally higher concentrations when compared to the adults. Eight of the nine pesticides were also detected in the adults. Body burdens in the adults were not strongly correlated with the log Kow values of the pesticides. The results indicate that metabolic processes which take place during metamorphosis are likely more important in determining which pesticides are transferred to the terrestrial riparian zone, and ultimately pose a risk for bioaccumulation in the terrestrial food web.

2.06.07
Specific Mosquito Control Agent? Reduction of Non-Target Organisms Chironomidae and Odonata Observed in a Semi-Field Mesocosm Treated With Bacillus Thuringiensis Var. Israelensis
V. Gerstle, DFG-Grk. SystemLink iES Landau, Institute for Environmental Sciences University of Koblenz-Landau / iES Landau, DFG Graduation College SystemLink; M. Gerken, A. Manfrin, University of Koblenz-Landau, Germany / iES Landau, DFG Graduation College SystemLink; S. Kolbenschlag, University Koblenz Landau / iES Landau, DFG Graduation College SystemLink; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment; C.A. Brühl, University of Koblenz-Landau / iES Landau, DFG Graduation College SystemLink
Bacillus thuringiensis var. *israelensis* (Bti) is a widely used biocide to control mosquito populations in wetlands. Despite being considered to specifically act on larvae of target organisms, i.e. mosquitoes, Bti was shown to reduce density of non-target chironomids in laboratory and field studies. Since chironomid larvae are a key food source in aquatic food webs, it is hypothesized that a possible reduction of chironomid larvae by Bti may also cascade on the aquatic food-web altering structure and composition of benthic communities. In this experiment, we investigated changes in the benthos community structure and composition in eight floodplain mesocosms (ponds; 18 x 4 x 0.3 m), from which four were treated with twice the field rate of VectoBac WDG (i.e., three applications in six weeks), while the other four remained untreated and were used as a control. Sampling was

conducted three weeks after the last Bti application and was comprised of six sampling points per pond, i.e. three sampling points located in macrophytes and three in gravel to investigate the influence of habitat types on the benthos community structure. Organisms were identified to species (Ephemeroptera, Odonata) or family level (Diptera, Coleoptera, Mollusca). Regardless of the treatment, habitat type 'macrophytes' showed a higher species diversity as well as a ~4-fold increase in the total number of sampled individuals compared to habitat type 'gravel' ($p < 0.0001$). In macrophytes, Chironomidae were the most affected group with a significant ($p < 0.03$) reduction of ~ 60%, 40%, and 80% for the chironomid subfamilies Chironominae, Tanypodinae and Orthocladinae, respectively. Furthermore, in ponds exposed to Bti, the total number of Odonata was reduced by ~ 35% in macrophytes (not significant), whereby first or second instar larvae were most affected up to 60%. The reduction of Odonata larvae may be the consequence of an interrupted food web either due to the substantial reduction of chironomid abundance and/or possible cannibalism occurring in older larval stages of Odonata, most likely depending prey availability. We conclude that Bti reduces the number of Chironomidae larvae and the effects can cascade on other groups of the benthic community in higher trophic levels. We believe this is particularly true in small stagnant water bodies where many aquatic predators depend on chironomids as a key food source.

2.06.08
Effects of the Mosquito Control Agent Bacillus Thuringiensis Var. Israelensis on Natural Aquatic Insect Communities - a Semi-Field Floodplain Study
S. Kolbenschlag, University Koblenz Landau / iES Landau, DFG Graduation College SystemLink; V. Gerstle, DFG-Grk. SystemLink iES Landau, Institute for Environmental Sciences University of Koblenz-Landau / iES Landau, DFG Graduation College SystemLink; N. Röder, University of Koblenz-Landau, Germany / iES Landau, DFG Graduation College SystemLink; J. Eberhardt, L. Bosch, University of Koblenz-Landau / iES Landau, DFG Graduation College SystemLink; C.A. Bruehl, University of Koblenz-Landau / iES, Institute for Environmental Sciences; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment
The bacterium *Bacillus thuringiensis* var. *israelensis* (Bti) is widely used as a mosquito control agent assuming a taxa-specific toxic mode of action against larvae. However, studies investigating (sub-)lethal effects on non-target organisms, especially on non-biting midges (Chironomidae), raised concerns about the selectivity of Bti. As chironomids are an important link between freshwater and adjacent riparian food webs, changes in the population development of this and other aquatic insect species point to adverse implications on higher and also lower trophic levels. Therefore, we investigated the emergence of insects (particularly chironomids) in Bti-treated and control ponds (21 m x 5.80 m, n = 6). During April and May 2020, Bti, i.e. Vectobac WDG, was applied three times at the highest rate recommended in Germany ($2.88 * 10^9$ ITU/ha) aligned with an artificially generated flooding of the ponds to mimic the common application scenario of Bti. The emergence of insects in each pond was collected by six floating traps (0.33 m²) which were emptied twice a week from April to mid-June. Thereafter, monitoring was continued until the end of July with a reduced sampling frequency (i.e. once per week). Emerged insects were identified to family level and their abundance was determined. Our results indicate a short-time emergence-promoting effect of Bti on chironomids after the first two applications. We assume an accelerated emergence of late-stage larvae to reduce energy-costly defence or repair mechanisms. Also, the abundance of Bti-resistant species might have increased. However, we observed a distinctly reduced number of emerged chironomids after the third and thus last application compared to untreated ponds. Most likely, early-stage larvae, being more susceptible to Bti, largely died during the first and the second application of Bti. Their loss then only became apparent weeks later as lacking emergence. In accordance with several other studies, this initial assessment of our results points to a negative impact of Bti on non-target insects such as Chironomidae. More detailed and additional analyses are planned to substantiate these findings even over multiple years of exposure. It is important to highlight that this experimental study can be considered as one of the most ecologically relevant efforts assessing for the impact of well-controlled Bti exposures, ultimately supporting risk evaluations of Bti applications under current application recommendations.

2.06.09
Trophic Upgrading: Is Chironomus riparius Able to Compensate Adverse Effects of Aquatic Stressors for Riparian Consumers?
S. Pietz, University Koblenz-Landau / iES Landau, DFG Graduation College SystemLink; L. Strotkötter, University of Koblenz-Landau, Germany / iES Landau, DFG Graduation College SystemLink; S. Kolbenschlag, University Koblenz Landau / iES Landau, DFG Graduation College SystemLink; N. Röder, University of Koblenz-Landau, Germany / iES Landau, DFG Graduation College SystemLink; A.P. Roodt, University of Koblenz Landau, iES Landau, DFG Graduiertenkolleg Systemlink / Ecotoxicology; K. Schwenk, University Koblenz-Landau, Germany / iES Landau, DFG Graduation College SystemLink; R. Schulz, University of Koblenz-Landau / iES Landau, Institute for Environmental Sciences; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment
Aquatic emerging insects represent an important subsidy of riparian food webs driven by their high nutritious value (e.g., the presence of long-chain polyunsaturated fatty acids, long-chain PUFAs). However, anthropogenic and natural stressors such as biocontrol agents or pesticides and low food quality can impair insect development with potential consequences for their nutritious value for riparian consumers. In this context, we examined in a first step the interacting effects of a biocontrol agent (*Bacillus thuringiensis israelensis*, Bti), organic (a mix of eight fungicides and three herbicides, PMix) or inorganic (copper, Cu) pesticides and two food qualities (TetraMin or *Spirulina*) on the biomass and nutritious value (measured as fatty and amino acid composition) of *Chironomus riparius* (Diptera: Chironomidae). The adult chironomids were, in a second step, fed to adult spiders (genus *Tetragnatha*) to determine potential impacts on the spiders' growth and physiology over three weeks. Food quality and chemical stress moderately but significantly reduced the biomass of adult *C. riparius* by approximately 10% and 5%, respectively. However, none of the stressors individually nor their combination affected spiders' growth. Instead, their growth rate (23–62% increase of biomass, not significant) suggests that chironomids' nutritious value, independent of the stressors, is sufficient to maintain the basal energy demands of spiders. Our analyses of the fatty and amino acid composition are still pending but will shed light on the underlying mechanisms or describe physiological responses.

2.06.10
Do Chemical Contaminants in Streams Decrease the Flying Activity and Foraging Behaviour of Riparian Bats?
M.F. Huszarik, University of Koblenz-Landau, iES Landau, Institute for Environmental Sciences / Ecosystem Analysis; N. Röder, University of Koblenz-Landau, Germany / iES Landau, DFG Graduation College SystemLink; E.L. Fernandes, University of Koblenz-Landau; M. Link, University of Koblenz-Landau / iES Landau, Institute for Environmental Sciences; R. Schulz, Institute for Environmental Sciences, University of Koblenz-Landau; M.H. Entling, University of Koblenz-Landau / iES Landau, Institute for Environmental Sciences
Pesticides and waste water pollution can reduce the number and diversity of flying insects emerging from streams. Bats are one of several terrestrial predators that strongly rely on emergent aquatic insects as prey. Thus, poor stream water quality can have a cascading effect on bats and decrease their foraging activity. However, the indirect effects of both pesticides and waste water contaminants on bats in natural areas have not been thoroughly investigated. Our study aims to examine the changes in bat activity, species diversity, and foraging behaviour at streams in response to pesticide and waste water pollution. We recorded bat calls at 16 forested stream sites in south-western Germany over 13 weeks. The streams varied in the proportion of urban and agricultural land in their catchments upstream of the study sites, and represented a gradient of exposure to pesticide and waste water contamination. Bat activity, diversity and foraging were measured by recording bat calls one night per week with acoustic loggers. Emergent aquatic insects were also collected with floating emergence traps, and malaise traps were used to collect and measure the proportion of aquatic to terrestrial flying insects. Additionally, the stream water was sampled weekly and the concentration of 95 current-use pesticides, as well as 4 pharmaceutical indicators for waste water were measured with HPLC-MS/MS. We expect that sites with high levels of pesticides and waste water contaminants will have lower emergent insect biomass and diversity, resulting in lower bat activity, species diversity and foraging behaviour. We expect this to be particularly true for bat species specializing in riparian habitats, such as Daubenton's bat *Myotis daubentonii*, the soprano pipistrelle *Pipistrellus pygmaeus*, and the common pipistrelle *Pipistrellus pipistrellus*. The results of this study will increase our understanding of how the effects of pesticides and waste water contaminants in streams extend

from the aquatic into the terrestrial ecosystem and alter food web interactions with riparian predators.

2.06.12
Assessment of the Effects of 2 Antiparasitic Drugs in Aquatic Organisms of Different Trophic Levels
L. Aboites Espinosa, Universidad Autónoma Metropolitana-Iztapalapa / Health Sciences; A. Sobrino-Figueroa, Universidad Autonoma Metropolitana / Hidrobiology
Drugs have become major sources of contamination of aquatic systems, because they are substances used in large quantities, which enter aquatic systems through the discharge of wastewater. Mebendazole is an anthelmintic that has embryotoxic and teratogenic effects in mice and Metronidazole is a drug used as a microbiocide and antibacterial that has genotoxicity as side effects. Since the sequelae of these compounds in aquatic species are not completely known, the objective of this work was to evaluate the toxic, genotoxic and neurotoxic effects of the drugs Metronidazole and Mebendazole in organisms of 3 trophic levels: the microalgae *Monoraphidium pusillum*, the cladoceran *Cerodaphnia dubia* and the zebrafish *Danio rerio. A*cute bioassays were performed with a duration of 96 hours for the microalgae and fish and 48 hours for the cladocerans where the LC50 was determined. Subsequently, sublethal tests were carried out with the LC40 and LC10 concentrations for the microalgae where the concentration of chlorophyll, carotenes, phenols and degree of lipoperoxidation were evaluated. In the tests with cladocerans and fish, LC10 and LC1 were tested and the degree of lipoperoxidation, the activity of AchE, the content of macromolecules and the genetic damage were evaluated. Toxicity tests indicated that the organism most sensitive to drugs was microalgae. In sublethal tests it was observed that both drugs alter chlorophyll concentrations, increase the production of carotenes and phenols. Mebendazole had an oxidative effect on the microalgae. In the bioassays with cladocerans and fish, it was observed that both drugs cause an increase in the levels of lipoperoxidation. Mebendazole caused a neurotoxic effect in cladocerans and fish. Both drugs caused genotoxic damage but acumulative effect was observed with Metronidazole test. The 2 drugs had deleterious effects at sublethal concentrations.

Insect Decline - the Contribution of Multiple Stressors on Landscape Level

2.07.04
Effect of Landscape Heterogeneity and Oilseed Rape Coverage on the Red Mason Bee Osmia bicornis
A. Misiewicz, Institute of Nature Conservation, Polish Academy of Sciences / Institute of Nature Conservation; . MikoŅajczyk, A. Bednarska, Polish Academy of Sciences / Institute of Nature Conservation
One of the main causes of pollinators decline observed in recent years is the intensification of agriculture. The agricultural landscape structure has changed substantially in the last few decades, shifting from small-scale farms with diverse cropping system towards large-scale monocultures where landscape heterogeneity disappears. Although many large-scale monocultures are not attractive to pollinators due to the lack of floral resources, some mass flowering crops, such as oilseed rape, may be important for them. Such crops, however, are treated with a range of pesticides and thus may affect pollinators using the contaminated nectar and pollen as food for themselves and the larvae. We studied how the landscape structure and oilseed rape coverage (ORC, % land cover) in the vicinity of the nest of the solitary bee *Osmia bicornis* affect population parameters and sensitivity of bees to an insecticide. In spring 2019, 12 *O. bicornis* nests, each with the same number of cocoons and nesting tubes, were located on the perimeters of oilseed rape fields of different size to represent different ORC (6-65%) within a circle area of 500 m radius around the nest. The local landscape structure around the nest was additionally characterized by 15 different landscape characteristics which were then reduced to two axes using Factor Analysis: F1 characterized the dataset primarily according to more ¢_urban" landscape features, whereas F2 captured the prevalence of "arable lands". The nests were left in the field for the entire flowering period of oilseed rape and used to measure life history parameters of the bees: number of cocoons, average cocoon mass, adult emergence rate and sex ratio. In addition, susceptibility of the emerged bees to insecticide Dursban 480 EC and their dry mass were tested after topical exposure with LT50 as an endpoint. Significant negative relationship of the emergence rate ($p=0.048$, $R^2= 34\%$) with ORC was found. Similar relationship was found for the LT50 ($p=0.009$, $R^2=51\%$) showing that females from sites with higher ORC were more sensitive to the insecticide. Dry weight of females increased with increasing ORC ($p=0.03$, $R^2=39\%$), whereas dry weight of males increased with F1 ($p=0.04$, $R^2=36\%$), i.e. in landscapes dominated by buildings, built-up area and peripheral vegetation but at the same time with high share of meadows and orchards. The research was founded by National Science Centre grant SONATA (2017/26/D/NZ8/00606).

2.07.05
Plant-Pollinator Interactions Along an Urban and Agricultural Gradient
A. Thompson, Martin Luther University of Halle-Wittenberg; T. Knight, Martin Luther University of Halle-Wittenberg / Biology
Observing plant-pollinator interactions in a habitat is a way of creating a plant-pollinator visitation network. These networks can be used to determine the structure of the plant and pollinator community and estimate stability of the interactions observed. These interactions are decreasing at an alarming rate because of anthropomorphic changes. Previous studies have looked at plant-pollinator networks along either urban or agricultural gradients, however our studied looked at changes in networks along both urban and agricultural land-use gradients. When looking at these networks along both gradients, we can observe and compare how patterns or metrics of the networks change with increasing or decreasing land-use. We observed 14 plant-pollinator visitation networks at sites varying in degrees of land-use. We did not find a significant difference in diversity of plant or insect communities along the gradients. However, we observed a disparity in composition of plant and insects; sites with higher urban and semi-natural percentage had similar composition of plants, whereas, sites with higher agricultural and semi-natural percentage share similar pollinator composition. We also found an association between insect family and site. This research helps us understand and compare how land-use changes impact pollinator and plant communities at both urban and agricultural gradients.

2.07.06
Development and Application of an IBR Index to Detect the Ecotoxicological Stress Induced by Anthropogenic Contaminants in Apis mellifera
S. Casini, University of Siena / Scienze Fisiche della Terra e dellAmbiente; T. Campani, University of Siena / Department of Physical, Earth and Environmental Sciences; A. D' Agostino, University of Naples "Parthenope" / Department of Management and Quantitative Studies; A. Di Noi, University of Siena / Department of Life Sciences; I. Caliani, University of Siena
A rapid decline of *Apis mellifera,* a keystone pollinator of wild plant species and agricultural crops, was recorded worldwide in recent years. The massive use of pesticides in agriculture associated with pollution generated by other human activities and the presence of parasites, can cause toxicological effects in bees including a decrease of the immune defenses, contributing to the collapse of the colonies. To adequately monitor the sublethal effects caused by contaminants in these pollinating organisms it is necessary to develop and apply highly sensitive and integrated ecotoxicological investigation methods. In this study a set of ecotoxicological biomarkers was developed and applied to monitor the effects of environmental contaminants on honeybees. Laboratory treatments with fungicides and trace elements were carried out as well as field sampling of colonies in rural and urban areas. The multidimensional perspective provided by the use of a large set of biomarkers was used to develop, for the first time in honeybees, a biomarker response index (IBRv2), which included biomarkers of neurotoxicity (AChE and CaE), genotoxicity (NA assay), metabolic alteration (ALP, and GST) and immune system (LYS, plasmatocytes). The IBR approach provides a simple tool for a general description of honeybees ecotoxicological health status, combining the different biomarker responses. It was able to outline different stress conditions in honeybees treated with or exposed to different contaminants. The results obtained also reinforce the idea that it is necessary to carry out more in-depth investigations on fungicides widely used in agriculture, which must be tested on non-target organisms and with sensitive methods for the determination of sublethal effects.

Multiple Stressor Effects in Aquatic Organisms and Ecosystems under a Changing Climate

2.08.01
Influence of Wildfire Severity on the Ecotoxicity of Ashes to Aquatic Life Stages of Anurans
I. Ferreira, University of Aveiro / Department of Biology; N. Abrantes, University of Aveiro-CESAM / CESAM/DAO; I. Campos, Universidade de Aveiro / Environment; I. Domingues, University of Aveiro / Biology

Department & CESAM; I. Lopes, University of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies
The predicted increase in temperatures and the occurrence of heat waves and droughts, will potentiate the frequency and severity of wildfires in Southern Europe. Fire severity depends on many factors and on their interactions, namely on temperature, geomorphology, fuel characteristics, meteorological conditions, among others. This, in turn, will influence the amount of ashes produced during the fire and their chemical composition. Ashes produced by forest fires are a complex matrix composed of organic and inorganic compounds, which are of great environmental concern due to their high toxicity, environmental persistence and tendency to bioaccumulate. This work intended to assess the influence of wildfire severity on the ecotoxicity of aqueous extracts of the generated ashes (AEA) on aquatic early life stages of two anuran species: *Pelophylax perezi*, and autochthonous species of the Iberian Peninsula and Southern France, and *Xenopus laevis*, a species original from Sub-Saharan Africa, that is listed as a model species to perform ecotoxicity assays by several standard guidelines. For that, embryos of *X. laevis* and tadpoles of the two species were exposed for 96 hours and 14 days, respectively, to serial dilutions (26.9 to100%) of AEA of a medium severity (MS) and a high severity (HS) wildfire. Endpoints at the individual (mortality, hatching rates, malformations, developmental stage, body length and weight) and sub-individual (total carbohydrates, lipids, proteins, electron transport system, catalase, lipid peroxidation, total glutathione, glutathione S-transferase and acetylcholinesterase) level were monitored. Chemical analyses were performed to the two AEA. Obtained results revealed different metal contents of LMS and HS AE: As, Co, Mn, Ni, Pb, and Ni were higher in MS while Cd was higher in HS. The two types of ashes induced similar effects in embryos of *X. laevis*. However, tadpoles of *X. laevis* showed a higher lethal sensitivity to MS. At sublethal levels, a different pattern was observed, with HS inducing higher effects for *P. perezi* and *X. laevis* tadpoles. For some of the analysed endpoints, *X. laevis* revealed to be the most sensitive species, thus suggesting that it may be used as a model species to assess the impacts of wildfires in aquatic stages of amphibians. Overall, these findings show that wildfire severity plays and important role on the chemical composition of the produced ashes and subsequently on their ecotoxicity to biota.

2.08.02
Effects of Ashes From Forests With Different Species Composition on Aquatic Stages of Amphibian
D. Santos, Department of Biology, University of Aveiro; N. Abrantes, University of Aveiro-CESAM / CESAM/DAO; I. Campos, Universidade de Aveiro / Environment; I. Domingues, University of Aveiro / Biology Department & CESAM; I. Lopes, University of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies
Recent increases in the frequency and severity of wildfires in the Mediterranean Basin have been associated with the occurrence of several ecological impacts in adjacent lotic and lentic aquatic systems. The post-fire rain events promote the ashes overland flow into freshwater ecosystems leading to its contamination. The increased loading of particulate matter in the water, caused by the entrance of ashes, alters its physical and chemical parameters (e.g., turbidity, nutrient levels, inorganic and organic contaminants) that, in turn, may adversely affect the biota inhabiting these areas. Aquatic life stages of amphibians are particularly exposed to this type of contamination, namely tadpoles that feed from organic matter at the surface of sediments, were ashes may settle. With this in mind, it is hypothesized that ash-runoff may cause lethal and sublethal effects on aquatic life stages of amphibians. Furthermore, it is also hypothesized that the plant tree cover (e.g. Pine forest, Eucalyptus forest) of the burnt forest may influence the ecotoxicity of the ashes produced during the wildfire. To answer these hypothesis, ecotoxicity assays with embryos and tadpoles of the amphibian's species *Xenopus laevis* (a model species) and *Pelophylax perezi* (autochthonous species of the Iberian Peninsula and Southern France) were carried out. Several apical (e.g. malformation, hatching rate, developmental stage, body length, weight) and biochemical endpoints (e.g. activity of enzymes related with oxidative stress and neurotoxicity, and other markers related with the energetic metabolism) were assessed for each life-stage and species after being exposed to serial dilutions of aqueous extracts of ashes (AEA) originated from a pine forest (PF) and eucalyptus forest (EF) wildfire. The chemical characterization of the two types of AEA was also performed. Ashes originated from PF caused a higher mortality in embryos of *X. laevis* and tadpoles of *P. perezi* than those of EF. However, EF ashes were the ones exerting a higher lethal toxicity to the tadpoles of *X. laevis*. Regarding the sublethal effects, overall, PF ashes induced more effects in the two species. Tadpoles of *X. laevis* showed a higher sensitivity to the AEA than those of *P. perezi*, suggesting being an adequate model species for the risk assessment of ashes derived from wildfire to amphibians. Further, the obtained results showed that short-term exposure to ash-load runoffs may compromise amphibian's populations.

2.08.03
On the Track of Wildfire Ashes Ecotoxicity to Freshwater Biota: The Role of Forest Tree Species
C. Venancio, University of Coimbra / Centre for Functional Ecology CFE; N. Abrantes, University of Aveiro-CESAM / CESAM/DAO; I. Campos, University of Aveiro / Department of Environment and CESAM; I. Lopes, University of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies
Mediterranean regions are naturally prone to wildfires. Ashes originated from forest fires may, enter adjacent aquatic systems affecting the water quality. Depending on the tree cover (e.g., pine or eucalyptus plantations), the potential toxic effects of the generated ashes may be substantially different due to distinct chemical composition. Hence, this work intended to evaluate the ecotoxicity of ashes from two distinct origins a maritime pine forest (PF) and an eucalyptus forest (EF), to freshwater biota. For this, adults of the cnidarian *Hydra viridissima* and embryos of the fish *Danio rerio* were exposed to a set of dilutions of aqueous extracts of the two types of ashes (AEAs:1.56 to 100%), plus a control (consisting in the culture medium of each species). The following endpoints were monitored: survival and morphological traits were evaluated in both species, while for *D. rerio* hatching, heartbeat rate and larvae length were also measured. Preliminary results indicate that PF ashes did not induce lethal effects (survival rates were ≥ 83.3%) or severe injuries on the embryos and larvae of *D. rerio*. Despite that, the heartbeat rate (measured at 48h as the number of beats per 15 seconds interval) was found to be significantly decreased at PF ashes dilution from 1.56% onwards: the average number of heartbeats was of 35.0 in the control condition, while it was reduced to 21.6 at 100% of PF ashes. Moreover, the hatching rate was found to be anticipated with increased dilutions percentages (over 75% from 3.13% onwards) in relation to control conditions (50%) at 48h; however, at 96h those differences disappear. Larvae were found to be smaller at intermediate dilutions (12.5%). The same tendency is expected with EF ashes. Assays with *H. viridissima* are still running, but considering that this is a highly sensitive species to chemical contamination, it might be hypothesized that effects not previously reported might stand out and place this invertebrate secondary consumer as a sentinel species regarding freshwater contamination wildfire-driven. Though no lethal effects were observed, ashes may t interfere with the normal development of freshwater organisms, impairing their performance. Research on different sublethal endpoints are then necessary to develop reliable analysis of potential risks of post-fire contamination to aquatic biota.

2.08.08
Elevated Water Temperature Increases Differential Gene Expression Induced by a Pesticide Mixture in Brown Trout (Salmo trutta)
A. Voisin, Oekotoxzentrum Eawag-EPFL; R. Beauvais, Centre Ecotox; M. Fasel, B. Ferrari, I. Werner, Ecotox Centre CH
Increased water temperature and input of pollutants represent a multi-stressor scenario common to many streams flowing through agricultural areas. This can negatively affect fish populations. However, effective methods to measure adverse effects in resident fish are limited. Application of transcriptomics to ecotoxicology offers a promising tool to detect sublethal effects of different stressors. In this context, our work focuses on the understanding of molecular events that occur in brown trout when exposed to pesticides, to elevated temperature or both. For this, we exposed juvenile brown trout at 12 or 15 °C in the presence or absence of a model pesticide mixture in the laboratory. After 14 days, we dissected the fish and snap-froze brain and liver tissues in liquid nitrogen. After RNA extraction and purification, we submitted both brain and liver samples to whole-transcriptome sequencing to identify new potential biomarker genes. Our results to date show that temperature is a key environmental parameter affecting molecular responses in the brain of the brown trout. A small increase in temperature from 12 to 15°C induced the dysregulation of up to 8000 genes, involved in many metabolic pathways. Interestingly, we observed molecular effects of exposure to pesticides mainly at the higher water temperature, suggesting that multiple stressors exposure enhances the effects of pesticides on gene expression in brown trout. In

the future, the impact of a third stressor, a parasite that causes proliferative kidney disease, on the transcriptome response to pesticides exposure will be evaluated. Candidate biomarker genes will then be gathered onto a gene chip and tested in a pilot study to develop an early-warning diagnostic tool to assess field-caught fish's health under a changing climate.

2.08.09
Ultraviolet B Modulates Stress Responses of Lemna minor to Gamma Radiation at Multiple Levels of Biological Organization
L. Xie, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; Y. Song, Norwegian Institute for Water Research (NIVA) / Section for Ecotoxicology and Risk Assessment; K. Petersen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; K. Solhaug, Norwegian University of Life Sciences (NMBU) / Faculty of Environmental Sciences and Natural Resource Management; O.C. Lind, Norwegian University of Life Sciences / Faculty of Environmental Sciences and Natural Resource Management; D.A. Brede, Norwegian University of Life Sciences / Centre for Environmental Radioactivity; B. Salbu, Norwegian University of Life Sciences (NMBU) / Centre for Environmental Radioactivity (CERAD); K. Tollefsen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment
Climate change, such as global warming, has been shown to have important implications for future stratospheric ozone depletion, which determines the intensity of UV radiation at the Earth's surface. In the aquatic ecosystem, UV radiation and ionizing radiation may co-occur and pose cumulative hazards to aquatic organisms such as macrophytes. However, the combined effects and underlying mechanisms of different types of radiation in aquatic plants remain poorly understood. The present study aims to provide in-depth understanding of how chronic (7 days) exposure to a single irradiance (0.5 W m^{-2}) of ultraviolet B (UVB) radiation modulates gamma (γ) radiation (14.9, 19.5, 43.6 mGy h^{-1}) induced stress responses in the aquatic macrophyte *Lemna minor*. In contrast to a majority of combined effect studies that focus on either molecular or apical endpoints, the present study used a suite of bioassays to quantify stress responses at multiple levels of biological organization. The combined effects (additivity, synergism, antagonism) of the stressors were determined using a combination of two-way analysis of variance (2W-ANOVA) and a modified Independent Action (IA) model. The single and combined effects of the stressors, and the potential causality between toxicological responses were further visualized by assembly of putative toxicity pathways. The results show that exposure to γ-radiation or UVB alone commonly induced oxidative stress and programmed cell death (PCD), and impaired oxidative phosphorylation (OXPHOS) and photosystem II (PSII) in *L. minor*. γ-radiation also activated antioxidant responses, DNA damage repair and chlorophyll metabolism, and inhibited growth at high dose rates (≥20 mGy h^{-1}). Additive effects of the two stressors were predominantly identified for antioxidant gene expression, energy quenching in PSII, frond size and frond weight for all dose rates, whereas antagonistic effects were observed for DNA damage, PCD, oxidative stress, OXPHOS and chlorophyll metabolism. Synergistic inhibition was observed for reproduction (fronds number) for all dose rates. The present study provides substantial mechanistic knowledge, quantitative understanding and novel analytical strategies to decipher combined effects at different levels of biological organization and is anticipated to facilitate future cumulative hazard and risk assessment of multiple stressors.

2.08.10
Cation Composition Influences the Toxicity of Salinity to Freshwater Biota
C. Venancio, University of Coimbra / Centre for Functional Ecology CFE; K. Cahon, CESAM & Department of Biology, Campus Universitário de Santiago, University of Aveiro, 3810-193 Aveiro, Portugal.; I. Lopes, University of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies
Causes of salinization on coastal regions worldwide are manifold. Whether associated with global climate change patterns and the subsequent sea level rise or related to several man-driven activities, increased ionic content of freshwater may lead to impacts at different levels of biological organization. So far, an extensive background has been constructed around salts as surrogates for natural seawater (SW). Sodium chloride (NaCl) is the most used and studied since both ions are the most representative of seawater. Although in less proportion, other major ions (e.g., calcium, magnesium, potassium) may represent a different toxic outcome but due to insipient data sets, no proposal has been delineated for them with regard to being more suitable surrogates for SW than NaCl. This work aimed at: i) evaluating the lethal and sublethal toxicity of different salts (calcium chloride-$CaCl_2$, potassium chloride-KCl, and magnesium chloride-$MgCl_2$) to freshwater ecologically relevant species (*Raphidocelis subcapitata*, *Daphnia magna*, *Brachionus calyciflorus*, *Hydra viridissima*); ii) comparing data obtained within this study with data collected for NaCl; iii) evaluating their propose as suitable surrogates of SW. Results indicated that amongst the three tested salts ($MgCl_2$, $CaCl_2$, KCl), the monovalent cation was the most toxic at lethal and sublethal levels for all the species whilst, $MgCl_2$ was the salt inducing the least toxicity. The LC_{50}s for KCl ranged from 0.28 g Cl^-/L (in *H. viridissima* at 96h) to 1.70 g Cl^-/L (in *B. calyciflorus* at 24h). The differences between $LC_{50,96h}$-KCl and $LC_{50,96h}$-$MgCl_2$ in *H. viridissima* reached 15-fold. Regarding the EC_{50}s values, for KCl ranged from 0.27 g Cl^-/L (in *H. viridissima* at 96h) to 0.82 g Cl^-/L (in *B. calyciflorus* at 48h), representing a 3-fold difference. The salt $CaCl_2$ presented intermediate toxicity to the tested species. Comparing KCl with data from the literature regarding NaCl toxicity, the latter seems to induce more toxicity than the former. However, due to the lack of correspondence between some species no sound conclusions can be drawn. Results highlight the need for further research to enrich salts database aiming to seek and propose for more suitable surrogate salts allowing to predict effects of salinization at a broader extent.

2.08.15
Impacts of Seaweed Exudates on Mytilus galloprovincialis Under Warming Temperature
H. Vieira, University of Aveiro; A.C. Rodrigues, Department of Biology & CESAM - University of Aveiro / CESAM & Biology; S. Pires, J. Oliveira, R.J. Rocha, University of Aveiro / CESAM & Department of Biology; M.D. Bordalo, University of Aveiro
Coastal areas are frequently exposed to several abiotic and biotic factors that challenge their biodiversity and ecosystem functions and services. Increase of seawater temperatures due to climate change have been promoting the survival and spreading of several non-indigenous species. In Europe, the red macroalga *Asparagopsis armata* Harvey (Bonnemaisoniales, Rhodophyta) originated from the Pacific Ocean is classified as an invasive species, being established in few regions of the Portuguese coast. This seaweed is known to produce a wide range of secondary metabolites that have primarily been seen as a chemical defense against consumers and epiphytic bacteria. Although these compounds act as surface mediators, they may have toxic effects towards native rocky coastal species. The mussel *Mytilus galloprovincialis* is a widespread and abundant filter-feeding bivalve along the Atlantic rocky shores of the Iberian Peninsula with ecological and high economic relevance. In this sense, the aim of this study was to evaluate biochemical responses related to oxidative stress status and metabolism of *M. galloprovincialis* after a short exposure (96 h) to algal exudates of *A. armata* under a warming temperature scenario. Results showed that exudation by *A. armata* and the increase in seawater temperature have impact in different enzymatic biomarkers in *M. galloprovincialis*. No cellular damage was observed in *M. galloprovincialis* gills, probably due to the action of antioxidant defenses such as . catalase and total glutathione that were able to counteract the action of reactive oxygen species (ROS) produced by the secondary metabolites and the rise in seawater temperature. However, damage caused by exposure to the exudates was observed in the digestive gland and muscle tissues, especially at the proteins level. On the other hand, the presence of exudates combined with warmed seawater did not change the energy consumption of the exposed mussels. Nevertheless, potential disruption of neurotransmission processes in mussels exposed to the exudates was observed. In sum, a short exposure to *A. armata* exudates caused changes in the oxidative stress status and neurophysiology of the mussels, with a tendency to an increasing toxic action under increased seawater temperatures. Thus, under a climate change scenario it may be anticipated that longer exposures to the combined action of both stressors may compromise *M. galloprovincialis* development and population dynamics.

Soil Ecotoxicology: New Methods and Novel Applications in Environmental Risk Assessment

2.09.03
The Treasure Is in the Soil: A Discussion on Risk Assessment Schemes for Soil Organisms in Latin America
F. Casallanovo, Syngenta Crop Protection AG / Product Safety; A. Cione Buchviser, F. Tincani, Syngenta Crop Protection / Environmental Safety
Soil organisms are key contributors to soil

health, since they are responsible for generating and maintaining a significant portion of the ecosystem services in this compartment, *e.g.* soil quality and composition, carbon and nutrient cycling, decomposition *etc*. The maintenance of soil health and the ecological services provided by these organisms is of interest of farmers. Regarding pesticide use in agriculture, it is necessary to evaluate the effects on soil organisms to identify possible risks and develop strategies both to prevent or mitigate them. To accomplish this goal, a robust environmental risk assessment (ERA) must be able to identify possible concerns and their impact by considering the exposure and the sensitivity of non-target organisms. It will also help to define strategies, to protect soil organisms and the ecological services they provide. In Latin America, the regulatory frameworks in most countries are evolving from a hazard-based analysis to an ERA-based analysis. In this sense, in the literature, a risk assessment scheme was suggested considering specific regional criteria for tropical environments by making a tiered approach where the degree of complexity increases as the evaluation progresses. We have applied this approach to 9 off-patent pesticides, equally divided in fungicides, insecticides and herbicides. At the first Tier, the exposure was calculated using a generic equation, commonly used by regulatory agencies such as the US-EPA and the UK HSE, which yields an Estimated Environmental Concentration in soil (EEC_{soil}). Nine pesticide labels were assessed to select the most critical rate in order to estimate the EEC_{soil}. As for the hazard component, the ecotoxicological data was retrieved from a public data bank and the level of concern (LOC) was set to 1.0, as used by some regulatory agencies. The outcome indicated that only 1 molecule failed the assessment. Therefore, for this pesticide, the exposure was further refined, in a second Tier, by using the UK Soil Calculator tool to yield a refined EEC_{soil}. The result in this Tier was below the LOC indicating no unacceptable risks. We understand that this is an initial exercise and an expansion of this assessment, including more pesticides, is recommended to allow a more comprehensive analysis showing the cases where pesticides fail at Tier I. In such cases, escalation to higher tiers would be necessary in order to evaluate possible mitigations and indicate safe agronomical uses.

2.09.06

Derivation of Draft Canadian Groundwater Quality Guidelines for the Protection of Environmental and Human Health

J. Cermak, T. El-Fityani, D.J. Spry, Environment and Climate Change Canada / National Guidelines and Standards Office, Science and Risk Assessment Directorate

The Canadian Council of Ministers of the Environment (CCME) is the federal, provincial and territorial interjurisdictional forum responsible for the development of Canadian environmental quality guidelines. CCME's Contaminated Sites Working Group has derived draft Canadian groundwater quality guidelines for the protection of environmental and human health for approximately 100 organic chemicals following CCME's *Protocol for the Derivation of Groundwater Quality Guidelines for Use at Contaminated Sites* (2015) (the *Protocol*). The draft guidelines were primarily derived by using existing benchmarks (e.g., Canadian water quality guidelines) and back-calculating a groundwater concentration using fate and transport models. They were developed to maintain specific uses of groundwater (e.g., irrigation or drinking water) and to protect receptors in environments that may directly or indirectly come into contact with contaminated groundwater due to contaminant migration (e.g., surface water bodies or vapour intrusion into basements). Two soil types (fine and coarse), four land-use categories (agricultural, residential, commercial and industrial) and multiple different exposure pathways and receptors were considered in the development of these guidelines. The *Protocol* allows for a tiered approach when applying the guidelines to account for site-specific conditions. This presentation describes the derivation of the groundwater guidelines, their use and the tiered approach to guideline implementation.

2.09.08

Integrated Biological Approach to Characterize and Discriminate Contaminated Wasteland in the Context of Its Requalification to an Ecological Use

N. Manier, D. Boulant-Cirederf, INERIS; O. Faure, Ecole des Mines Saint-Etienne / UMR CNRS EVS EMSE Géosciences et Environnement; J. Nau, F. Druez, EODD; N. Pucheux, INERIS

Urban and industrial activities led to the discharge of a wide range of hazardous chemicals in soils. Among abandoned wasteland, those whose soil functions were not altered beyond repair may ends up hosting transitional ecosystems with ecological values. However, there is a lack of screening methodologies to characterize and categorize among these sites. The main concern is to differentiate the soils/sites which are potential candidates for immediate upgrading to an "ecological usage" (e.g. biodiversity reserve) from those that require a comprehensive ecological risk assessment (which is sometimes difficult to applied for economical and/or technical reasons). In this context, the project TIPOMO (transfer and index of concern: tools for valuing medium-contaminated wasteland) aims at building an "index of concern" that allow the categorization of polluted soils hosting transitional ecosystems. This index will be calculated by considering the levels of soil contamination and its ability to trap polluting substances (characterization of bioavailability fraction). This method will be tested and validated in real case study. To do so, an integrated approach based on pollution characterization, and biological endpoints (both *ex situ* and *in situ*) were applied on 16 soil composites collecting on 8 sites mainly contaminated with HAP and/or hydrocarbons and/or metals. The biological endpoints were characterized by using a battery of ecotoxicity tests to assess the soil hazard and the potential pollutant bioavailability. Effect on species representative of bacteria (*i.e.* dehydrogenase inhibition activity in *Arthrobacter Globiformis*), mesaufauna (*i.e.* Growth and reproduction test of the nematodes), macrofauna (*i.e.* earthworms behavior and acute toxicity test) and higher plants (*i.e.* oats and turnip) were studied. The accumulation of HAP, hydrocarbons and metals were determined *ex situ* in earthworms. In addition, the aquatic ecotoxicity of the hydrosoluble fraction of pollutant were investigated on freshwater microalgae and microinvertebrate. Finally, *in situ* ecological criteria (i.e. plant inventory) and pollutant transfer in plants were investigated on each site. The poster will present the proposed approach as well as the first results and their interpretation regarding the categorization of the investigated sites.

2.09.09

A Framework of Sub-Lethal Ecotoxicological Effects in Eisenia fetida for Ecological Risk Assessment of Sewage Sludge

L. Sturba, M. Turano, G. Liberatori, University of Siena / Department of Physical, Earth and Environmental Sciences; M. Vannuccini, University of Siena / Physical earth and environmental science; I. Corsi, University of Siena / Physical, Earth and Environmental Sciences

Sewage sludge (SS) is the solid by-product of urban and industrial wastewater treatment. According to Directive 2008/98 / EC of the European Parliament, all methods of recycling and management of sludge must be preferred to landfilling. The cheaper alternative to cope with increasing sewage sludge amounts, is cropland deposition as fertilizer, indeed it is the most widely used at European level. However, this management method represents a threat for soil quality because could lead to soil contamination with mixtures of both organic and inorganic potentially toxic substances. There is a growing need for an accurate risk assessment of sewage sludge toxicity, able to easily detect not only contamination levels, but also the effects on biota and soil ecosystem. Soil quality is not related only to the degree of pollution but is commonly defined as "the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health" (Doran and Parkin, 1994, 1996)". In this perspective, multiple *in vivo* tests, based on the OECD 207 method, were performed with *Eisenia fetida* specimens exposed to various sludge samples dilution in LUFA soil. In addition to classical lethal endpoints (mortality and growth), in order to increase ecological relevance of ecotoxicty testing, some sub-lethal biological responses were investigated which include a battery of cellular and histological biomarkers. In particular, CAT and GST activities in SS exposed earthworms revealed an increase of oxidative stress even at higher dilution (1:30 SS: LUFA), up to damage to cell membranes (lipid peroxidation, MDA) and DNA (micronuclei) at lower dilution (1:10 SS: LUFA). In addition, AChE inhibition has proven to be a useful tool to assess exposure to neurotoxic compound which could be below analytical detection limits in SS. Histopatological alterations documented were also predictive of gastrointestinal tract damage and could be associated to exposure to heavy metals. Sub-lethal effects along with the evaluation of lethal endpoints in *E. fetida* could thus represent suitable tools to assess ecological risks associated to sludge application as amendants in soil.

2.09.10

Phosphogypsum for Soil Amendment - Ecotoxic Effect of High Calcium Content to the Soil Earthworms (Eisenia fetida)

L. Goncalves, GreenUPorto - Sustainable Agrifood Production Research Center & Department of Biology, Faculty of Science, University of Porto, Rua do Campo Alegre s/n, Porto, Portugal; S. Bouguerra, GreenUPorto &

Faculty of Sciences, University of Porto / Dept Biology Faculty of Sciences of the University of Porto; J. Lourenco, University of Aveiro; O. Hentati, M. Ksibi, Higher Institute of Biotechnology of Sfax, Route de Soukra km 4, PO. Box 1175, 3038 Sfax, Tunisia; R. Pereira, Faculdade de Ciências da Universidade do Porto / Biology

Phosphogypsum (PG) is the by-product (waste) of the world phosphate ore industry. PG is a harmful waste contributing to global environmental and economic problems. PG primary component is calcium sulphate dehydrated ($CaSO_4 \cdot 2H_2O$ or gypsum) or hemi-hydrated ($CaSO_4 \cdot 1/2H_2O$ or bassanite) which account for aprox. 95% by weight of PG composition. PG has been used as soil fertilizer in several countries during many years for calcium, phosphate and sulfate enrichment. More recently concerns are emerging related to the high-level of metals and radionuclides in PG, but few studies addressed the possible noxious effect of calcium. The main objective of this study was to assess the contribution of calcium to PG toxicity to soil invertebrates. To attain this objective, we determined the impact of PG (0.0, 0.6, 0.9, 1.3, 2.0 and 3.0%) and the equivalent content of CaO in amended OECD artificial soil to the terrestrial earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae) used as model species. The assessment was based on evaluating weight-loss, genotoxic effect (using the comet assay), impacts on nervous system (by measuring acetylcholinesterase activity) and oxidative stress biomarkers (catalase and glutathione-S-transferase activity and lipid peroxidation) after 28 d of exposure, besides effects on worms reproductive activity. This study reported important significant inhibition of worm's fertility in soils amended with lowest % of PG followed by suppression of reproductive function at % higher than 0.9%. Significant reduction of reproductive activity was also observed in soils amended with Ca treatments confirming that Ca content in PG greatly contribute in decreasing the fertility of worms. In addition, both PG and Ca contribute to important oxidative damage in *E. fetida* and severely affected the integrity of genetic information by inducing DNA damage even in soils amended with lowest proportions. These results reinforce that observed toxicological effects were greatly related to the high content of calcium in PG and were not exclusive on metals and radionuclides contents. Considering that earthworms play a major role in maintaining soil functions and services, this study gives rise to serious concerns about the consequences of fertilizing agricultural soils with calcium-rich waste such PG.

2.09.11

Green Strategies for Sustainable Horticulture: Application of Biochar for Nitrate Reduction in Surface Water and Groundwater

D. Losacco, National Research Council (CNR); C. De Palma, De Palma Thermofluid s.r.l. - Industrial projects division; C. Massarelli, Italian National Research Council / Water Research Institute; V. Uricchio, Italian National Research Council / Water Research Institute / Water Research Institute (IRSA); P. Cotugno, A. Tursi, University of the Studies of Bari Aldo Moro / Department of Biology; V. Ancona, Water Research Institute - Italian National Research Council / Water Research Institute

The growth of horticultural species is closely related to the availability of nitrogen (N), whose physiological role is of great importance. Considering both the demand for nitrogen for agricultural yield and the easy leaching of nitrogen compounds, the amount of this soil nutrient is carefully assessed for implementing suitable fertilization programs. Excessive use of nitrogen fertilizers causes nitrate pollution with quality loss of drinking water aquifers and eutrophication phenomena. In recent years, the slow release of nitrate by the application of carbon-rich amendment (*i.e.* biochar) has been suggested as a potential strategy for increasing the bioavailability of nitrogen nutrients and reducing nitrate leaching. Biochar is a promising sustainable tool to improve the yield of horticultural crops and reduce the use of mineral fertilizers in agroecosystems. The incorporation of biochar along with controlled doses of nitrogen mineral fertilizers could have a potential for mitigation of leached nitrates in surface water bodies and groundwater. The aim of this work is to assess the effect of different levels of N in soil with and without amendment of virgin wood biochar on crop yield, quality, and release of nitrates into the soil. The main objective is the characterization of plant materials of *Brassica oleracea L. var. botrytis*, in different doses of nitrogen and treatments with biochar, by analysis of Fourier Transform Infrared spectra. In this study, cauliflower was grown in mesocosms using two types of fertilization regimes: (1) dose of conventional inorganic fertilizer with and without biochar, (2) dose of high inorganic fertilizer with and without biochar. Soil biological and physico-chemical properties and, the quantification of nitrogenous compounds in the percolation water, are studied in samples collected 10 days after each fertilization and at the end of the experiment (complete ripening of the corymb). Biomass samples are characterized by ATR-FTIR spectroscopy. The experimental approach tested in this work intends to evaluate the biochar capabilities in preventing the loss of nitrates in horticultural systems, reducing the demand for nitrogen fertilizers, and improving agricultural yield.

2.09.12

Climate Change in Africa and Species Response to Heat Stress: A Bibliometric and Spatial-Based Information Assessment

O.A. Idris, North West University (Potchefstroom Campus) / Zoology; P.A. Opute, University of Benin, Benin City, Nigeria / Department of Animal and Environmental Biology; I.R. Orimoloye, Centre for Environmental Management, University of the Free State, Bloemfontein, South Africa; M. Maboeta, North-West University - Potchefstroom Campus / Unit for Environmental Sciences and Management

The impact of climate change over decades increases the likelihood of many species to undergo genetic alteration or even becomes extinct. Vegetation and belowground organisms such as earthworms are more vulnerable to the intensified impact of climate change due to possible lack of genetic plasticity and limited mobility. Organisms are inter-dependable in eco-systems, hence, this study focused on the impact of climate change, using the soil condition in Africa, vegetation responses and the overview of the physiological effects of climate change on species through a bibliometric study and remote sensing information. A bibliometric study from 1999 to 2019 on climate change-related literature, collected on Web of Science and Scopus database platform reveals a trend of an overall rapid increase in publication on climate change in Africa, with South Africa occupying a leading position in all the studied parameters. The spatial-based information on soil moisture, temperature and photosynthetic activities of vegetation studied with Fraction of Absorbed Photosynthetically Active Radiation (FAPAR), reveals there is an increase in drought in Africa with the most impacted countries being; Mauritania, Mali, Burkina Faso, Niger, Chad, Sudan, and Somalia. Most Africa countries, especially the above-mentioned need to urgently invest in support programs that aid tree planting, soil amelioration and increase irrigation for agriculture, to ease the impact of climate change. Based on the reviewed literature, the Earth's soils are the buffer for greenhouse gas (GHG), therefore, the study concluded that better land management and agricultural practices could help to combat global warming and climate change.

2.09.14

Zn Concentration Decline and Apical Endpoints Recovery of Earthworms (E. andrei) After Removal From an Acidic Soil Spiked With Coated ZnO Nanoparticles

M. Fernández, INIA-National Institute for Agricultural and Food Research and Technology / Laboratory for Ecotoxicology. Department of Environment and Agronomy; A. Obrador, Universidad Politecnica de Madrid / Chemical and Food Technology Department, CEIGRAM,; P. Sanchez-Arguello, INIA - National Institute for Agricultural and Food Research and Technology / Environment; C. GarciaGomez, INIA / Dpto. Environment and Agronomy

ZnO nanoparticles (NPs) can reach soil in both deliberate and non-deliberate ways, which leads to contamination. Notwithstanding, knowledge about ZnO NPs impacts on earthworms inhabiting these soils is limited, and gaps appear in the recovery of damaged functions after their migration to unpolluted environments. To estimate these impacts, earthworms (*Eisenia andrei*) were exposed firstly to different concentrations of ZnO NPs coated with (3-aminopropyl) triethoxysilane (cZnO NPs) (20, 250, 500, 1000 mg Zn kg^{-1}) for 28 days in an agricultural soil with an acidic pH. This soil was chosen because the low pH enhances metal availability. Subsequently, earthworms were placed in the same unpolluted soil to study the depletion of Zn accumulated and the recovery potential of the affected functions for another 28-day period. In the exposure phase, ecotoxicological responses were dose-dependent and related to mortality and growth at the two highest doses (500 and 1000 mg Zn kg^{-1}), and also to the reproduction function from 250 mg Zn kg^{-1}. Fertility (mean offspring per cocoon) was more sensitive parameter than fecundity (mean cocoons production). Zn uptake increased with cZnO NPs in soil, but it was efficiently regulated. The coating did not show significant effects compared to the control. After the recovery period, the Zn concentration values in earthworms exposed to treated soil were similar to the control regardless of the initially Zn accumulated. All animals increased their weight with a significantly greater gain in those previously exposed to 500 and 1000 mg Zn kg^{-1}. Reproductive capacity returned to the control values in the animals pre-exposed to 250 mg of

Zn kg^{-1} as cZnO NP. In the earthworms pre-exposed to the two highest doses, growth and fertility were stimulated compared to the control when placed in clean soil, but not fecundity. Fecundity was recovered, by up to 60% of that the control. Despite the fertility stimulation, the reproduction recovery at the two highest concentrations in terms of the total hatchlings number did not reach the control figures after 28 days cleaning time, but probably would for in longer times. These findings are very interesting because by avoiding nasty environments due to their capacity for moving, surviving earthworms have demonstrated being able to continue their cycle life when they are moved to an acceptable habitat.

2.09.15

Peat Does Matter for Soil Invertebrates - Theory and Data

T. Schmidt, IES Ltd / Ecotoxicology; B. Hodapp, Innovative Environmental Services IES Ltd / Ecotoxicology; S. Hoger, Innovative Environmental Services (IES) Ltd

According to the EC regulation No 1107/2009, invertebrate soil organisms need to be tested if the potential of exposure to a plant protection product is identified. So far, four laboratory test systems are in use testing population endpoints for one representative species of earthworms, springtails and mites as well as one functional endpoint of a soil microflora community. The need for expanding the number of test species by additional systematic groups (e.g. fungi, isopods), for implementing semi-field test designs and for paying more attention to the impact of soil properties to the availability of plant protection products is under intense discussion in the recently published "Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms" (EFSA Journal 2017;15(2):4690) by EFSA. In the current standard laboratory testing artificial soil consisting of defined amounts of quartz sand, kaolin and peat is recommended. Peat provides the organic carbon and is considered to affect the biological effects of plant protection products regarding their specific potential to adsorb and therefore not to be available to soil organisms. Accordingly, the soil risk assessment established factors to correct effect values for soils with high organic carbon. In this poster presentation, effects of a model compound on earthworm and soil mite reproduction are shown following the respective OECD guidelines 222 and 226 but tested with artificial OECD soils containing different peat contents. The differences in toxicity between the soil systems are discussed and the suitability of the standard correction factor is examined. Artificially created soil may represent a robust framework for detecting possible effects on soil organisms, but the use of natural and/or regional relevant soil could have the potential for more relevant and realistic exposure setups and effect determinations.

2.09.17

Scaling for Bioavailability: A Step Towards More Ecologically Relevant Risk Assessment for Soil Invertebrates

B.G. van Hall, Vrije Universiteit Amsterdam / Animal Ecology; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science; M. Bottoms, Syngenta

Lipophilic organic chemicals have the potential to adsorb to the organic matter (OM) fraction of soils, reducing their bioavailability to soil organisms. As such, the European Food Safety Authority (EFSA) mandates that when an active substance has a Log Kow > 2, the endpoints derived from first-tier earthworm tests performed in OECD artificial soil containing ~10% OM should be divided by a factor of 2 to correct for the lower soil OM contents in agricultural field soils (~5% OM). These corrected endpoints should be used in the subsequent risk assessments. However, the correction factor also applies to studies with springtails and soil mites, although these are usually performed with OM contents similar to those found in European agricultural soils. Additionally, the correction factor also applies when reducing the OM content in earthworm studies. The use of a correction factor of 2 is based on results of acute toxicity tests with earthworms, which makes its application to longer-term tests on earthworm reproduction questionable. Therefore some experts consider the correction factor of 2 as not scientifically underpinned, while others consider it not conservative enough. The goal of this research therefore is to improve our understanding of the relationship between the bioavailability to soil invertebrates of organic chemicals, especially pesticides, and soil organic matter content. To this end, we performed a literature review which focuses on obtaining toxicity data from studies that investigated the toxicity of organic chemicals to soil invertebrates in soils containing different OM contents. The obtained data will be used to answer the following questions: 1) What is the relationship between toxicity endpoints and soil OM content, and is this relationship the same for lethal (LC_{50}) and sublethal (EC_{50}) endpoints? 2) Is this relationship influenced by chemical properties of the compounds (i.e. Log Kow, pKa)? And 3) Is the relationship similar for different groups of soil invertebrates (i.e. hard-bodied vs soft-bodied)? The answers to these questions will provide input for targeted experimental research to further underpin the use of a correction factor for differences in soil organic matter content. Final aim is to provide regulators with a sound basis for increasing the accuracy and ecological relevance of chemical risk assessments.

2.09.18

Multi-Dimensional Modelling of the Effects of Pesticide Application Strategies on a Collembolan Species in the Soil

N. Sherborne, Syngenta / Environmental Safety; J. Dodd, J.C. Rumkee, Syngenta; C. Brown, University of York / Environment Department; R. Ashauer, Syngenta Crop Protection AG / Environment

Collembolan species are integral to healthy soil ecosystems and are rightly protected in risk assessment (RA) guidelines for the application of plant protection products (PPP). Standard regulatory methods are often simplistic and apply conservative approximations in place of sub-processes to allow for a more computationally tractable model. Broadly, this works well, but does not accurately describe some bespoke scenarios. Exposure resulting from a PPP application strategy depends on several factors related to the properties of the substance and the biology of exposed species. Modelling can revolutionise our understanding of how these factors determine the effects of stress and therefore improve management practice. For example, targeted applications such as in-furrow treatments give rise to horizontally heterogeneous concentrations that standard regulatory models do not capture. Movement and avoidance behaviour of soil organisms such as collembola also affects their exposure. These factors may contribute to significantly different effects at the population level than simple models would predict. In response, this poster presents the formulation, calibration and validation of a coupled mathematical model that describes the interaction between environmental fate, ecotoxicological exposure and effects. The model combines an updated version of the multi-dimensional fate model known as 2DROPS with an individual based model (IBM) of collembola. 2DROPS provides spatially and temporally explicit predictions of the fate and transport of a PPP in soil, both vertically and horizontally. This detailed concentration profile informs the exposure of the collembola as they move through the soil. At the core of the IBM is a dynamic energy budget (DEB) model with a toxicokinetic-toxicodynamic (TKTD) submodule which translates the exposure to lethal and sublethal effects. Using a DEB-TKTD approach improves the realism and applicability of the model beyond typical methods based on EC50 values or dose-response curves. The model species is *Folsomia candida*. However, since DEB is a general mechanistic model similar species will differ only in parameter values, not model structure. We go on to discuss the opportunities for its use in RA. We find that by combining modern modelling techniques with different application strategies, refinements and management practices can be assessed to the level of detail required.

2.09.19

Reduction in the transfer of persistent organic pollutants from soils to laying hens with biochar and activated carbon amendments

N. El Wanny, Université de Lorraine - UdL; M. Delannoy, Université de Lorraine - UdL / INRA URAFPA; M. Baroudi, Lebanese university / Faculty of Public Health-Section III; A. Fournier, Université de Lorraine - UdL / INRA URAFPA; C. Feidt, University of Lorraine - UdL / INRA URAFPA

Polychlorinated Biphenyls (PCBs), Dioxins, Furans (PCDD/Fs), and Chlordecone (CLD) are Persistent Organic Pollutants (POPs) polluting soil which acts as a reservoir. Due to involuntary ingestion of soil by free-ranged animals, they may transfer to animal food stuffs as they are highly bioaccumulative. In this frame, the amendment of contaminated soil with porous matrices like biochars and Activated Carbons (ACs) has been suggested as promising technique for trapping these organic contaminants in soil in order to reduce their bioavailability in animals. In this study, the efficiency of six carbonaceous matrices was assessed (3 biochars and 3 activated carbons). Three distinct samples of artificial soil were produced according to OECD guidelines 207 (1984) one contaminated by CLD only, the second contaminated by a mix of PCBs, Dioxins, and Furans (1 DL-PCB, 6 NDL-PCBs, 6 PCDDs, 5 PCDFs), and the third sample remained uncontaminated. Each soil sample was amended by 2% (by weight) of one of the six biochars or ACs. The relative bioavailability of pollutants was then measured in forty-eight laying hens aging 22-weeks (n=3 per treatment of each soil) and fed on a daily basis with 80 g

of pellets per 2kg of BW containing 10% of soil. After 20 days of oral exposure, the CLD concentration in liver and eggs of hens were analyzed by LC-MS/MS and PCB or PCDD/F ones by CPG-HRMS. A slight decrease in the concentration of contaminants was observed with the biochar amendments, while two of the activated carbons significantly reduced the concentrations of pollutants in the liver and eggs ($p< 0.001$). Furthermore, the significant reductions obtained with the two efficient activated carbons were compound dependent as follows: PCDD/Fs > DL-PCB with reduction of 74% to 78% of PCDD/Fs in liver and 83% in eggs. For DL-PCB (PCB77), this decrease ranged from 42 to 55% in liver and 53% to 66% in eggs. Only a slight reduction of CLD and NDL-PCB concentrations was obtained with these activated carbons. Thus, this study allows to highlight: (i) the ability of activated carbons amendments in highly reducing the bioavailability of soil organic contaminants to laying hens and (ii) the dependence of the contaminant reduction on both nature of the pollutant and characteristics of the matrix.

2.09.20
Neodymium Toxicity (Inorganic and Organometal) in Relation to Bioavailability in Soil
J. Princz, P. Boyd, Environment and Climate Change Canada / Biological Assessment and Standarization Section; H. Lemieux, Environment and Climate Change Canada / Biological Assessment and Standardization Section; J. Velicogna, Environment and Climate Change Canada / Environment and Climate Change Canada; R.P. Scroggins, Environment and Climate Change Canada / Biological Assessment and Standardization Section
Lanthanides are naturally present within soils. However, increased development of green technologies incorporating lanthanides, mining and refining operations, as well as leachates from discarded technology, contribute to increased loadings to the soil environment. Various rare earth elements (REEs) have been identified as priorities for risk assessment under the Government of Canada's Chemicals Management Plan, many of which soil hazard data is lacking. To address these concerns, the fate and toxicity of different neodymium (Nd) substances (organic and inorganic) in two soil types were explored. The experiments were designed to select substances that could contribute to a read-across framework, and ascertain whether the moiety-based assessment framework is applicable to organic-metal complexes. Recently, OECD (2015) released guidance based on the necessity of a strategy for the assessment of organic-metal complexes that do not fit within the metal moiety-based framework. As a result, the toxicity of two inorganic soluble salts (Nd-chloride and Nd-sulphate), as well as an organometal (Nd 2-ethyl-hexanoic acid) were assessed using three soil invertebrates: the earthworm, *Dendrodrilus rubidus*, the collembolan, *Proisotoma minuta*, and the oribatid mite, *Oppia nitens*. Measures of toxicity were based on bioavailable (0.01 M $CaCl_2$ extractable) and total metal, assessed over the course of 6 months. The toxicities based on total metal provide a comparative basis for species sensitivity relative to information available in the literature. However, the analysis of the bioavailable metal should provide additional information on whether the observed toxic trend for the organometal is a result of the metal moiety, or a combination of other sources (e.g., organic moiety, parent compound). Analyses over time will also provide a measure of how bioavailability and toxicity changes with aging of the substances in soil. The results will be presented and discussed in context to how these measures provide insight into the interpretation and influence of toxicity between substances, soils and time.

2.09.21
Application of the H14 "Ecotoxic" Property of the EU Waste Directive for North African Mine Waste Using Ecotoxicological Tests in Order to Consider Its Management Limits
J. Nouairi, Research Unit of Analysis and Process Applied to the Environment, Higher Institute of Applied Sciences and Technology University of Monastir, Tunisia; R. Pereira, Faculdade de Ciências da Universidade do Porto / Biology; V. Nogueira, Faculty of Sciences and CIIMAR - Porto University; S. Bouguerra, GreenUPorto & Faculty of Sciences, University of Porto / Dept Biology Faculty of Sciences of the University of Porto
Fine-grained waste materials from the abandoned Lakhouat Pb–Zn mining district (Government of Siliana, Northern Tunisia) contain environmentally significant amounts of potentially toxic elements (PTEs), such as Zn (94420 mg kg^{-1}), Pb (28040 mg kg^{-1}) and Cd (733 mg kg^{-1}). Mineralogical studies identified PTEs carriers' primary sulfides (galena, sphalerite, and pyrite) accompanied by sulfates (gypsum), calcite, and minor clay minerals. The hydrological network in the region is very dense, represented by numerous small dry rivers outside the rainy seasons. Wadi Remir, the most important one, intercepts the tailings from the dams to its confluence with Wadi Siliana, upstream part of the Mejerda River (the biggest supplier of freshwater in Tunisia). Surrounding soils are mainly occupied by annual crops or cereal cultivation. The remaining soils are occupied by olive groves. The aim of this study was the assessment of Lakhouat Pb-Zn mine wastes (MW) as ecotoxicologically hazardous or non-hazardous by the application of the hazard criterion (H14"ecotoxicity") protocol. The assessment was based on determining the ecotoxicity of soil amended with different percentages of MW (0.0, 3.3, 4.9, 7.4, 11.1, 16.7 and 25%) and of elutriates dilution obtained from MW – Medium culture (0.0, 13.2, 19.8, 29.6, 44.4, 66.7 and 100%) to a battery of terrestrial species (*Eisenia fetida*, *Folsomia candida*, *Lycopersicon esculentum* and *Triticum aestivum*) and aquatic species (*Lemna minor*, *Vibrio fischeri*, *Raphidocelis subcapitata* and *Daphnia magna*), respectively. The results revealed that terrestrial species were greatly impacted by the MW substrates, and that both plants species were the most sensitive. The MW was also highly toxic to all aquatic species, given that the luminescent bacteria *V. fischeri* and the duckweed *L. minor* reacted less strongly (EC20: 0.8% and 5.5%, respectively). In addition to the important role of intrinsic MW properties, the ecotoxicological characterization of this MW provide an estimation of the harmful impact on terrestrial and aquatic species and give rise to the serious concerns about the consequences to the surrounding environment. Such information's are needed to decide how the treatment or recovery process of this MW must be adapted to minimize the environmental impacts.

2.09.22
How Does a Widely Used Neonicotinoid Affect the Soil Arthropod Community Under Natural Field Conditions?
H. Konestabo, Department of Biosciences, University of Oslo / Department of Biosciences; T. Birkemoe, Norwegian University of Life Sciences (NMBU) / Faculty of Environmental Sciences and Natural Resource Management; H. Leinaas, University of Oslo / Department of Biosciences; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science; S. Sengupta, Department of Bioscences, University of Oslo / Department of Bioscences; K. Borgå, University of Oslo / Department of Biosciences
Agricultural soils generally have a low biodiversity, and a functional soil mesofauna is especially important for decomposition and nutrient cycling processes in such soils. The use of pesticides to protect crops often affects non-target organisms vital to ecosystem functioning. While in standard ecotoxicological risk assessments organisms are tested under optimal laboratory conditions to isolate the effects of the toxicant, it is difficult to extrapolate these effects to populations in their natural ecosystems, where interactions with other environmental variables occur in a complex manner. The awareness of importance of multiple stressors in ecosystem management is increasing, and field studies offer the opportunity of measuring effects in natural communities, thus evaluating biological responses under realistic scenarios. However, the response of any ecological community is a complex result of numerous direct responses as well as indirect effects of trophic interaction, which are in turn affected by the same environmental variations. This may challenge the realism of generalization beyond the system studied. As a step towards developing a controlled field validation method using mesocosms as Terrestrial Model Ecosystems (TMEs), we enclosed intact soil cores from an agricultural field in mesocosms with lids to limit or enable migration of mesofauna. The mesocosms were dosed with the insecticide imidacloprid and placed back in the field for 20 days. Imidacloprid concentrations of 0, 0.1, 1 and 10 mg/kg dry soil were chosen, a range which was most likely to have differential effects on the mesofauna. The abundance of both surface-living and soil-living springtails decreased in a concentration-dependent manner, with a total reduction of ~80% in the two highest concentrations, and a reduction of 12% at a more ecologically relevant concentration of 0.1 mg/kg. Surface and litter-living springtails were affected the most by imidacloprid exposure, whereas the effect was less pronounced in soil-living springtails. In contrast, neither predatory nor saprotrophic mites showed a reduction in numbers with imidacloprid exposure, concurring with effects on mites reported in the literature. We show how the soil arthropod community composition and abundance were affected by exposure to imidacloprid under natural conditions, and discuss the feasibility of a field mesocosm setup for predicting the fate of toxicant-exposed natural soil communities.

2.09.23
Impact of Nonylphenol on Redox Enzymes of Soddy-Podzolic Soil Microbiota
A. Russu, Saint-Petersburg Scientific Research Center for Ecological Safety Russian Academy of Sciences; T. Zaytseva, I. Kuzikova, St.

Petersburg Federal Research Center of the Russian Academy of Sciences (SPC RAS), Scientific Research Centre for Ecological Safety of the Russian Academy of Sciences
Endocrine disruptor nonylphenol (NP) is widely used in different manufactures and it harmfully affects on living organisms. In present study the impact of this compound on non-target spieces – microbiota of soddy-podzolic soils of Leningrad region, Russia - was investigated. In model experiment soil was contaminated with 300 mg NP/kg dry soil. Nonylphenol causes oxidative stress in many microorganisms, although activities of redox enzymes, such as dehydrogenase and catalase, in contaminated with NP samples were on the control level during 30 days of soil incubation. After 90 days of experiment the stimulation of both enzymes activities was observed – by 66% for dehydrogenase and by 14% for catalase activity. At the same time the number of heterotrophic bacteria increased by 5 times, compare with control variant, so this can be the cause of stimulation of redox enzymes activity.

Soil Function and Biodiversity: Impacts and Resilience under Stressed Environments

2.10.01
Soil Biodiversity in vinEyard Agro-Ecosystems - BEST
M. Piombino, A. Ferri, Corteva agriscience; C. Vaj, Corteva agriscience / The Agriculture Division of DowDuPont; M. Montagna, G. Magoga, M. Panella, M. Brunetti, Università degli Studi di Milano / Dipartimento di Scienze Agrarie e Ambientali
Soil is a fundamental component of the Earth's biosphere since it provides necessary ecosystem services for humans. Soil operates as a vital living system sustaining microbe-plant-animal lifecycles, promoting air and water quality. Soil is also fundamental in supporting human activities such as agriculture, which in turn can affect soil proprieties and consequently its quality. It is known that appropriate agricultural practices such as reduced tillage and incorporation of crop residues into soil improve its quality, while inappropriate and intensive practices decrease it. Vineyards, as the majority of agro-ecosystems, are nowadays facing emerging challenges primarily regarding the balance between the request of an enhanced productivity and an increased environmental sustainability, including the conservation of soil biodiversity and consequently of its functions. With the project soil Biodiversity in vinEyard agro-ecoSysTems – BEST, benefitting of the state of the art of next generation sequencing technologies, through a DNA metabarcoding approach targeting reference DNA markers (16s rRNA, ITS, 18S rRNA), the biodiversity of Bacteria, Fungi and Metazoa of vineyards soils across Italy will be characterized. Most used DNA metabarcoding pipelines will be adopted (e.g. Dada2 in Qiime2) coupled with last versions of reference databases Silva and Unite for achieving organisms' identification. Taxa composition, biodiversity levels (Shannon and Pielou indices) and functional role of taxa will be analyzed. The vineyards soil biodiversity will be linked with the quality of grapes, the plants productivity and with the adopted agronomic practices, accounting also for soil physical-chemical properties and environmental bioclimatic variables, in order to define vineyards management addressed to improve the soil functional biodiversity. The results achieved with BEST will provide fundamental insights for linking vineyards productivity with agricultural sustainability, preservation of soil biodiversity and ecosystem services.

2.10.02
From Seed to Food: The Role of Innovative Agriproducts in Assisting Food Production and Soil Protection
C.C. Malheiro, Department of Biology, University of Aveiro / Biology; G. Leite, University of Aveiro / CESAM & Department of Biology; M. Prodana, University of Aveiro / CESAM & Departament of Biology; A.M. Soares, Universidade de Aveiro / CESAM & Department of Biology; R. Morgado, University of Aveiro / CESAM & Department of Biology; S. Loureiro, Universidade de Aveiro / Biology
Soil is a non-renewable resource on which life depends. Nowadays, inadequate anthropogenic practices are impacting and preventing soil to function as expected. Some of those practices are related to agricultural food production systems and, therefore, expected to escalate due to the increase of global human population. The rise in global food demands pose challenges for sustainable agriculture, threatening soil ecosystems and imperilling the delivery of ecosystem services. For instance, the misuse of fertilizers is one of the major causes of ecosystem services deterioration. Therefore, it is necessary to develop sustainable methods to achieve productivity demands, without compromising environmental integrity and public health. This study aims to evaluate the effectiveness of innovative agriproducts in the productivity of several plant species (e.g. tomato, rocket, corn), through germination and growth tests. Soil enzymes will be also assessed, as a proxy of nutrient cycling and soil quality. Two types of innovative agriproducts were selected: i) two different forms of layered double hydroxides (LDH) nanomaterials (Zn-Al LDH, Mg-Al LDH) with potential use as carriers of fertilizers and slow release matrix; ii) an organic fertilizer, derived from the digestion of vegetable residues by the black soldier fly larvae. All products are at development stage, in partnership with portuguese R&D companies, Smallmatek Lda and Ingredient Odyssey Lda. To assess plants productivity, two experimental procedures were selected, using modified versions of the Phytotoxkit (MicroBioTestsInc., Belgium) and of the ISO 11269-2:2012 guideline. At the end of the Phytotoxkit test, all plants were measured and weighted (root and shoot length, fresh and dry weight). As for the growth test, besides the morphological endpoints, biochemical parameters related to productivity (pigment analysis, anti-oxidant capacity) will be analyzed. Overall, the Phytotoxkit test allowed a rapid, cost-effective evaluation of the agriproducts effect, through direct visualization. As for the ongoing plant growth tests, so far it is possible to see that the aplication of the innovative fertilizers is reflecting in higher plant productivity, when looking to the plants development and morphology. Soil enzymes will be assessed at the end of the experiment and changes in patterns dependent on the nutrient cycle are expected.

2.10.04
How Collembola Population Changes in the Presence of Copper: A Comparison Between Nano and Non-Nano Copper Compounds
D. Nunes Cardoso, CESAM, University of Aveiro / CESAM & Department of Biology; M. Prodana, University of Aveiro / CESAM & Departament of Biology; A.M. Soares, Universidade de Aveiro / CESAM & Department of Biology; S. Loureiro, Universidade de Aveiro / Biology; R. Morgado, University of Aveiro / CESAM & Department of Biology
In ecotoxicology, especially when using soil invertebrates, standardized tests most frequently evaluate individual-level endpoints related to key life-history traits (e.g., survival and reproduction of an organism). However, knowing that the primary aim of ecotoxicology is the prediction of the effects of a specific contaminant on natural population dynamics, population growth tests appear as a more integrative solution to understand the effects of stressors on populations. Following that, this study uses an innovative population growth test with the springtail *Folsomia candida* as model species, assessing and comparing the effects of different copper-based compounds. Copper-based pesticides are among the most used agrochemicals worldwide, revolutionizing crop protection and also remaining important in both conventional and organic farming. Therefore, and following the urge for a more comprehensive evaluation on the effects of novel copper-based nano pesticides, population growth experiments were conducted using Lufa 2.2 soil spiked with the same six concentrations (ranging between 300 and 2400 mg Cu kg^{-1} soil) of copper hydroxide ($Cu(OH)_2$) and copper oxide nanoparticles (nCuO). These compounds have markedly different dissolution behaviors, which affects their time-dependent bioavailability in soil. Five juveniles with five days were exposed to contaminated soil for 16 weeks, with 11 sampling times where the organisms were counted (adults and juveniles) and their size measured, when possible. Within this, it was possible to follow the population growth of collembolans in different nano and non-nano copper-based compounds and respective concentrations. Results revealed the higher toxicity induced by $Cu(OH)_2$ compared with nCuO, with strong impairments on collembolans reproduction and consequent population dynamics. More pronounced effects were also observed earlier in time when in the presence of $Cu(OH)_2$ in soils. During different sampling occasions, growth and survival seemed to be less sensitive endpoints than reproduction in the presence of copper in the soil. Besides the comparison between both copper nano and non-nano compounds, the results of both collembolans population growth tests and standardized reproduction tests will be compared and discussed, highlighting strengths and limitations of each, and the need for more ecologically relevant approaches for a more accurate risk assessment of stressors in soils.

2.10.05
Effects of Nano and Non-Nano Copper Formulations to Soil Organisms: A Mesocosm Approach
R. Morgado, M. Prodana, D.N. Cardoso, University of Aveiro / CESAM & Department of Biology; C.C. Malheiro, Department of Biology, University of Aveiro / Biology; S. Peixoto, University of Aveiro / Departament of Biology & CESAM; J. Neves, Universidade de Aveiro / CESAM & Department of Biology; C.S. Santos, Ghent University (UGent) /

Terrestrial Ecology Unit (TEREC) - Department of Biology; Z. Khodaparast, University of Aveiro / Biology; M.D. Pavlaki, University of Aveiro / Department of Biology; P. Monteiro, University of Aveiro / CESAM & Department of Biology; S. Rodrigues, University of Aveiro / CESAM & Department of Chemistry; S.P. Lopes, University of Aveiro / Department of Chemistry & QOPNA/LAQV-REQUIMTE; I. Henriques, University of Coimbra / CESAM & Department of Life Sciences, Faculty of Sciences and Technology; G. Pinto, University of Aveiro / CESAM & Department of Biology; S. Rodrigues, Universidade de Aveiro / CESAM & Department of Environment and Planning; S. Loureiro, Universidade de Aveiro / Biology

Copper-based pesticides are among the most used agrochemicals worldwide. Repeated applications resulted in serious issues of soil contamination in agroecosystems, raising concerns about long-term sustainability. To tackle this, copper (Cu) nanopesticides have been developed, whose benefits include controlled release of Cu ions for sustained and targeted fungicidal effect. These mechanisms are expected to allow cuts in application, reducing soil contamination while improving crop protection. However, new technologies often entail unforeseen risks, and few attempts have been made to assess their higher-tier effects.In this work, we designed a soil mesocosm experiment to evaluate how conventional and nano Cu-based compounds affect the structure/functioning of soil ecosystems and to understand their bioaccumulation. One conventional and three nano-based compounds were tested, each at 50 mg Cu/kg soil, alongside a negative control: 1) ionic $Cu(OH)_2$, 2) Kocide3000®, 3) $nCu(OH)_2$, 4) nCuO. The experiment was conducted in acclimatized carts containing 20 cm soil cores. A model community composed by wheat (*Triticum estivum*), earthworms (*Eiseinia andrei*), isopods (*Porcellionides pruinosus*), and mealworms (*Tenebrio molitor*) was added. Three replicates per treatment were destructively sampled at days 14 and 28. Organisms and plants were recovered, weighted/measured, and used for Cu bioaccumulation or energy reserves.Ionic $Cu(OH)_2$ significantly reduced wheat's root and shoot fresh weight and shoot length at day 28, compared to control. No significant differences were found in these parameters for any nano form. None of the compounds seemed to affect survival of earthworms and mealworms. Mealworms' growth was reduced by $Cu(OH)_2$ and $nCu(OH)_2$, whereas in earthworms, weight loss was generalized across treatments, more pronounced in $Cu(OH)_2$ and control at day 14 and nCuO and Kocide at day 28. Cu toxicokinetics were described using the one-compartment model. Similar patterns were observed for mealworms and earthworms, but higher elimination rates were generally found in the latter. The main difference was detected in nCuO, for which toxicokinetics indicated a very low uptake rate by earthworms, but the same was not found for mealworms. Both species agreed with the negligible Cu elimination rates after nCuO exposures, contrary to the remaining compounds. This difference may be related to the slower dissolution rate of nCuO in soil compared to the remaining materials tested.

2.10.06
The Investigation of the Species-Specific Detoxification Mechanisms in Cadmium Exposed Eisenia andrei, E. Fetida and Their Hybrids

F. Vandenbulcke, LGCgE, Universite de Lille 1 / LGCgE; A.B. Rorat, Univ Lille1 / LGCgE EA4515 Univ Lille1 and LSVF EA 4483 Univ Lille2; M. Jaskulak, Czestochowa University of Technology; L. Kurianska-Piatek, Jagiellonian University / Institute of Environmental Sciences; S. Hofman, J. Bigaj, Jagiellonian University / Department of Comparative Anatomy, and Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research; B. Plytycz, Jagiellonian University / Institute of Zoology

Eisenia fetida and *Eisenia andrei* are closely related earthworm species, capable of asymmetrical hybridization. Those lumbricid earthworms are ubiquitous and highly resistant to a variety of environmental stressors, such as heavy metals. Differences occur in the immune reaction of those invertebrates and may thus differ in hybrids between both species. This might affect their survival in hostile environments. Identification of the origin of the mitochondrial plasmid and of the nuclear genome in hybrids (either *E. andrei* or *E. fetida*) combined with measures of the gene expression of mitochondrial as well as nuclear genes involved in detoxification processes allow to gain insight in the defense mechanisms. The main aim of the present study was to investigate the cadmium-related defense mechanisms at transcriptomic level in genetically defined *E. fetida*, *E. andrei* and their hybrids exposed for 2 and 7 days to Cd-polluted soil in mesocosms (x=425 ± 45.66 mg/kg). The level of gene expression of glutathione S-transferase (*gst*), lysenin (*lys*), metalothionein (*mt*), superoxide dismutase (*sod*) and phytochelatine synthase (*pcs*) was assessed in extruded coelomocytes, i.e.the immune cells present in earthworm coelomic cavity, and related to the expression of two stable house-keeping genes – actin (*act*) and ribo13 (*r13*). No important differences in *gst* and *pcs* expression were noted. Lysenin expression was down-regulated following the Cd exposure, while the expression of *mt* and *sod* increased after 2 days in *E. fetida* and hybrids and after 7 days in *E. andrei*. The expression of mt was slightly higher in hybrids compared to pure species. In sum, capacity of cadmium bioaccumulation and detoxification mechanisms is more efficient in interspecific hybrids than in the pure species. Consistently, upregulation of *mt* and *sod* expression was fastest in hybrids, slower in *E. fetida* and the slowest in *E. andrei*. Hypothetically *E. fetida* and *E. andrei* differ in their mechanisms controlling expression of stress-related genes and immunity-connected genes thus further studies of these genes and their interactions in hybrids are necessary.The study was supported by NCN grant (2016/23/B/NZ8/00748).

2.10.08
Using Earthworm Avoidance Behaviour to Assess the Toxicity of Mancozeb and Copper Oxychloride Under Varying Soil Temperature and Moisture Content

P.A. Opute, University of Benin, Benin City, Nigeria / Department of Animal and Environmental Biology; H. Kaka, Northwest University, Potchefstroom / Unit for Environmental Sciences and Management; M. Maboeta, North-West University - Potchefstroom Campus / Unit for Environmental Sciences and Management

Increasing global warming is presently attracting attention leading to new scenarios in toxicity studies through the combination of different stress factors: chemical stress and thermal stress. This study aimed to predict the toxicity of mancozeb and copper oxychloride to earthworms under different climate change scenarios. *Eisenia Andrei* earthworms were maintained in a combination of three temperatures (20 ^{0}C, 22 ^{0}C and 25 ^{0}C) and three soil moisture contents (30%, 50%, and 60% WHC) in OECD artificial soil and simultaneously spiked with three concentrations of mancozeb (MnZn 44 mg/kg, MnZn 850 mg/kg, MnZn 1250 mg/kg), copper oxychloride (Cu 200 mg/kg, Cu 500 mg/kg, Cu 1000 mg/kg) and a mixture of both fungicides (Cu+MnZn 200+44 mg/kg, Cu+MnZn 500+850 mg/kg, Cu+MnZn 1000+1250 mg/kg) for 48 hours following the ISO earthworm avoidance test guideline using the two-chamber design. Response results from the earthworm avoidance behavioural tests showed a varied distribution for both fungicides from lower to a higher concentration. Relatively free movement of worms covering the entire test area was observed in the lowest concentration of mancozeb (i.e. MnZn 44 mg/kg) at both varying temperatures and soil moisture, without displaying a preference for a particular area. The net response of the worms was significant at higher temperatures and reduced moisture content (i.e. at 25 ^{0}C and 30% WHC). Earthworms exhibited 100% avoidance in the highest concentration of the binary mixtures irrespective of the temperature or soil moisture. These findings suggest the relevance of the avoidance test as a suitable screening method showing first tendencies of the influence of climate change-related stress on the toxicity of fungicides and its implication on the habitat function of soils.

2.10.09
The CLIMTOXFUN Project: A Multidisciplinary Approach to Evaluate the Ecotoxicity Risks of Metal-Polluted Soils From Semiarid Mediterranean Regions Under Forecasted Climate Change Scenarios

M. Gonzalez-Alcaraz, Technical University of Cartagena / Agronomic Engineering; J. Álvarez-Rogel, Technical University of Cartagena / Agricultural Engineering

CLIMTOXFUN is a research project funded by the Séneca Foundation of the Regional Government of Murcia (Murcia Region, Spain) within the framework of the Saavedra Fajardo program (21127/SF/19). The project, which began in June 2020 and will last for three years, is based at the Technical University of Cartagena. The project aims at assessing how and to what extent the ecotoxicity risks of anthropogenic metal-polluted soils of the semiarid Mediterranean region may be affected under forecasted climate change scenarios, simulated by climate factors combinations, using soil invertebrates and soil processes as bioindicators, and their relation to changes in soil physico-chemical and microbiological parameters. To achieve this goal, a multidisciplinary study is being carried out with field soils from the southeast of the Iberian Peninsula affected by metal pollution of different anthropogenic origing like mining, agriculture and industrial activities. Ecotoxicological (invertebrates' performance) and functional (carbon and nitrogen mineralization) aspects are being considered to evaluate the response of these polluted soils to

climate factors combinations that represent the current climate conditions and the predictions of the Intergovernmental Panel on Climate Change (IPCC) for the year 2100. The climate factors that are taken into account are atmpospheric CO_2 cocentration, air temperature and soil moisture content. The results of the project will enable a deeper knowledge of the response of terrestrial ecosystems affected by anthropogenic metal pollution under the current and forecasted climate conditions in the semiarid Mediterranean region, one of the most vulnerable to the impacts of climate change according to the IPCC projections. This will help to improve our ability to predict the consequences of climate change in antropogenic degraded environments, which, in turn, will allow a better adaptation and/or mitigation of the effects of this global phenomenon. The poster presents the outline of the project.

2.10.11

Toxicity Assessment of the Field Soil From an Abandoned Mine Using Soil Nematode

T. Lee, D. Kim, Konkuk University; Y. An, Konkuk University / Department of Environmental Heath Science

Abandoned mines continue to cause soil pollution even after activities have ceased. Therefore, risk assessment for abandoned mines is a necessary process for proper management of contaminated areas. In this study, toxicity assessment using soil nematodes was performed on contaminated soil from abandoned mines. The soil samples were collected from around of minehead area and waste rock-pile area. The soil on opposite slope of the same mountain was used as the reference soil. The soil leachate was extracted by deionized water at 24 hours. Nematodes were exposed in the soil for 24 hours and in the leachate for 72 hours to confirm the reproduction. As results, the reproduction of the nematodes exposed in the soil of waste rock-pile area decreased. Analysis of the composition of the leachate suggests that the toxicity of Zn and As among the heavy metals was expressed in soil of waste rock-pile area. In order to improve the reliability of the soil risk assessment, toxicity assessment using various types of soil organisms will be needed for contaminated soil from abandoned mines**.**

Acknowledgement- This study was funded by the Ministry of Environment (MOE) through the Graduate School of Specialization for Safe Management of Chemicals.

2.10.12

Long- Term Effects of AgNPs on Soil Bacterial Community: A Single Species Microcosm Approach

S. Lopes Peixoto, CESAM & University of Aveiro / Department of Biology; J. Oliveira, R. Morgado, University of Aveiro / CESAM & Department of Biology; I. Henriques, University of Coimbra / University of Coimbra, CESAM & Department of Life Sciences, Faculty of Sciences and Technology, Calçada Martins de Freitas 3000-456 Coimbra, Portugal; A.M. Soares, Universidade de Aveiro / CESAM & Department of Biology; S. Loureiro, Universidade de Aveiro / Biology

Silver nanoparticles (AgNPs) are widely used in consumer products due to their antibacterial properties. The release of AgNPs into the terrestrial environment is inevitable, with expected effects on the soil bacterial community (SBC). The presence of soil invertebrates stimulates microbial communities in soil and therefore, upon contamination, may induce changes in responses of this SBC to the contaminant. Our study aimed to explore the effects of AgNPs on the SBC and if these effects change in the presence of a terrestrial isopod (*Porcellionides pruinosus*) over-time. Additionally, $AgNO_3$ was assessed as an ionic control. The LUFA 2.2 soil was spiked with the two silver forms [5 µg of Ag/Kg of soil, equivalent to 6 x PEC (Predicted Environmental Concentration)], with and without *P. pruinosus*. Soil sampling (n=3 replicates per condition) occurred at day 42 and 56. The SBC was analyzed by Denaturing Gradient Gel Electrophoresis (DGGE; 16S rRNA gene) in terms of the structure, richness, diversity and evenness indexes. Overall, the structure of SBC was affected by Ag exposure (AgNPs or $AgNO_3$), after both exposure periods. The isopod presence affected the SBC structure but an attenuation over-time occurred in relation to contamination. At day 42, the structure of the SBC in the presence of *P. pruinosus* was distinct from the remaining treatments (only 55% similarity). Nonetheless, regardless of the presence of *P. pruinosus*, the Ag-exposure affected the structure of the SBC dependently of the silver forms, with the highest richness detected in SBC exposed to AgNPs. Day 56 revealed the same effects on the SBC structure but no differences were found between silver forms (in the absence of isopods). A significant decrease in evenness was found for SBC exposed to silver toward the control. In the presence of *P. pruinosus*, a significant increase in the diversity was found for SBC exposed to $AgNO_3$; while a significant decrease in richness was observed for SBC exposed to AgNPs. This study provided evidence that long-term exposure to AgNPs affect the SBC structure. *P. pruinosus* contributes to an attenuation of the impact of Ag contamination on the SBC structure (day 56).

2.10.13

Copper-Based Nanomaterials Alter the Soil Microbiome at the Functional and Structural Level - a Mesocosm Study

S. Lopes Peixoto, CESAM & University of Aveiro / Department of Biology; R. Morgado, University of Aveiro / CESAM & Department of Biology; D. Nunes Cardoso, CESAM, University of Aveiro / CESAM & Department of Biology; M. Prodana, University of Aveiro / CESAM & Departament of Biology; C.C. Malheiro, Department of Biology, University of Aveiro / Biology; J. Neves, Universidade de Aveiro / CESAM & Department of Biology; C.S. Santos, Ghent University (UGent) / Terrestrial Ecology Unit (TEREC) - Department of Biology; Z. Khodaparast, University of Aveiro / Biology; M.D. Pavlaki, University of Aveiro / Department of Biology; P. Monteiro, University of Aveiro / CESAM & Department of Biology; S. Rodrigues, University of Aveiro / CESAM & Department of Chemistry; G. Pinto, University of Aveiro / CESAM & Department of Biology; S. Rodrigues, Universidade de Aveiro / CESAM & Department of Environment and Planning; I. Henriques, University of Coimbra / University of Coimbra, CESAM & Department of Life Sciences, Faculty of Sciences and Technology, Calçada Martins de Freitas 3000-456 Coimbra, Portugal; S. Loureiro, Universidade de Aveiro / Biology

Copper-based nanoproducts are nowadays applied in agroecosystems to improve crop productivity. However, their frequent application might result in the accumulation of nanomaterials (NM) in terrestrial compartment, and consequently, the soil microbiome may be affected. We aimed to study the impact of different copper-based NM [$nCu(OH)_2$, Kocide and nCuO] on soil microbiome. Soil mesocosms were set up considering the predicted copper concentration for agricultural soils: 50 $mg(Cu)kg^{-1}$. The $Cu(OH)_2$-i was included in this study as an ionic control. Microbial analysis was conducted considering the effects on soil microbiome at functional (enzymatic activity, carbon substrate utilization) and structural levels (16S rRNA gene-based metagenomic analysis), after 0, 14 and 28 days of exposure. Results indicate that copper-based NM impacted the soil microbiome at both structural and functional levels. Effects were more pronounced after 28 days of exposure, with significant decreases in dehydrogenase, arylsulfatase and urease activities, suggesting negative impacts on microbial-mediated carbon, sulfur and nitrogen cycling. On the other hand, carbon substrate utilization was increased in communities exposed to copper, but only significantly different from control in soils spiked with nCuO. The soil microbiome exposed to different copper formulations presented a significantly different structure (based on Operational Taxonomic Unit abundance) compared to the control, exhibiting at least 43% of dissimilarity. Although we did not detect significant differences between copper forms, in both enzymatic activity and OTU-level community structure, compositional differences at the class level were detected. Thus, a significative increase of the abundance of Gemmatimonadetes [$nCu(OH)_2$ (+1%)], Clostridia [Kocide (+0.2%)]; and a significative decrease of Acidobacteriia [$Cu(OH)_2$-i (-6%), Kocide (-4%), nCuO (-7%)] and Flavobacteriia [$Cu(OH)_2$-s (-2%)] were observed in exposed soils toward the control. *In silico* functional metagenomic analysis (based on Piphillin tool) suggested that the copper-tolerance effect may be related to the activation of resistance mechanisms to expel copper ions from the bacterial cell (efflux pumps). This study demonstrates that different copper-based NM, at the environmental-relevant concentration, significantly impact the soil microbiome, possibly affecting the soil ecological role involving the C and S and N cycles.

2.10.14

Soil Microbiome Linked With Bioattenuation of Petroleum Hydrocarbon Contaminants in Gio Community, Niger Delta, Nigeria

C.B. Chikere, University of Port Harcourt / Microbiology; A.U. Okoye, Africa Centre of Excellence in Oilfield Chemicals Research, University of Port Harcourt / Environmental Microbiology; G.C. Okpokwasili, University of Port Harcourt / Department of Microbiology

The aim of this study was to evaluate microbial diversity profile of chronically oil-polluted soil microbiome linked with hydrocarbon degradation. Polluted soil was collected from Gio Community, Ogoni, Niger Delta at depths of 0-0.5m (Gio polluted surface soil [GPS]), 1m (Gio polluted subsurface soil [GPSS]) (North, East, West, and South) respectively and Gio unpolluted soil (GUPS) to serve as the reference soil. Gas chromatographic (GC-FID) analyses for total petroleum hydrocarbons (TPH) were 36,775.65ppm, 14,100.0ppm, 480.67ppm for GPS, GPSS and GUPS respectively. Polycyclic aromatic hydrocarbons (PAHs) determined were

12,200.3ppm, 3,300ppm, and 23 ppm for GPS, GPSS and GUPS respectively. The samples were analyzed using 16S rRNA amplicon sequencing on Illumina MiSeq platform to understand both the core soil microbiome and functional structure of the microbes. The following PAHs, benzo[a]anthracene, benzo[a]pyrene, benzo[e]pyrene, chrysene, fluoranthene, fluorene, phenanthrene, benzo[b]fluoranthene, indeno[1,2,3-c,d]pyrene, anthracene, benzo[g,h,i]perylene, and pyrene that were detected are of environmental concern as they are mutagenic, carcinogenic and listed as a group 1 carcinogens by the International Agency for Research on Cancer (IARC). Taxonomic distribution of bacteria in the oil-polluted surface and subsurface soils revealed the core microbiome within the class of Epsilon proteobacteria were mainly *Propionibacterium*, *Bacteroides*, *Pseudomonas*, *Sphingobacterium*, *Acholeplasm*, *Stenotrophomonas*, *Arenimonas, Brachybacterium. Sulfurimonas* and *Arcobacter.* These Group IV hydrogenases containing biodiversity are known for reducing nitrate, oxidizing sulphur and hydrogen and may strongly be linked with biological activities in the impacted soil and bacterial microbiome associated with bioattenuation of the hydrocarbons.

2.10.16

Study of Two Operational Criteria of a Pilot-Scale Composting System As a Proposal for the Management of Organic Solid Waste in the Municipality of Izúcar De Matamoros

J.H. Cardenas, Universidad Tecnologica de Izucar de Matamoros / Departamento de Investigación y Desarrollo; L. Morales Morales, Universidad Tecnologica de Izucar de Matamoros / P. E. Ingenieria en Procesos Alimentarios; R. Ponce Ortíz, Universidad Tecnologica de Izucar de Matamoros / P. E. Agricultura Sustentable y Protegida

In the municipality of Izúcar de Matamoros Puebla, 79 tons of garbage are generated daily, of which only 59 are collected. The garbage collected is stored in a landfill and approximately 60% of the waste collected is organic. Organic waste represents an alternative for the recovery of the fertility of agricultural soils, considering that agriculture is one of the main economic activities in the region, the treatment of this type of waste would be a way to solve two problems that affect the population: the volume of garbage generated and the low fertility of the soils. The objective of this work was to study two operational factors of the composting process: % manure (% E) and aeration frequency (FA): for this, nine systems were built based on an experimental design of two factors and three levels per factor. % E: 10, 25 and 50% and for FA: daily, twice a week and weekly; 20 L plastic containers were used, in these a volume of fresh manure was deposited according to the experimental design, and it was filled with crushed organic waste up to a maximum particle size of 2 cm; The output variable was the germination index for this, after twenty days of operation, this parameter was determined and if it was greater than 80%, it was assumed that the composting process had finished. The highest process temperatures were obtained with aeration twice a week and 50% manure, average pH values of 8.86 were obtained without observing a relationship with the studied factors; the germination percentages were higher than 90% for the compost extracts with weekly aeration and% E of 25%, however, no significant differences were observed for both factors. It is concluded that composting is a simple and straightforward technique for the treatment of organic waste that could be implemented in some rural communities of the municipality since strict control of the operational parameters is not necessary.

2.10.17

Evaluation of Composting Products Using Freshwater Microalgal Biomass for Soil Environment

C. Ahn, KICT / Department o Land, Water and Environment Research; s. lee, J. Park, Korea Institute of Civil Engineering and Building Technology (KICT) / Department of Land, Water and Environment Research

In this study, we attempted to manufacture a new type of algal soil ameliorant by composting discarded freshwater microalgal biomass. Additional additives (sawdust, pearlite, olicake, etc.) were mixed for efficient composting. Four types of ASCs with different mass ratios exhibited clear aerobic microbial reactions in the thermophilic phase. During the maturation process, the fertility index increased as well as macronutrients (NPK) as well as secondary macronutrients (Ca, Mg). In this result, Increasing the fertility index can not only contribute to improving soil productivity in terms of macronutrients, but also increase micronutrients when mainly using freshwater microalgal biomass. Therefore, green cmpounds or biostimulants, which are assumed to have originated in microalgae, are expected to not only improve the soil environment but also improve the physiological function of plants. As a result of long-term analysis, when loose and amorphous organic matter increased, it was judged that green waste including microalgal biomass was converted to matured SOM over time. These results assume that green waste, including algal biomass, can be utilized as a functional material capable of bioremediation and bioaccumulation if properly composted.

Unravelling the Occurrence and Impact of Multiple Stressors of Natural and Antropogenic Origin Pollutants in Polar Regions

2.11.06

Microplastics in Arctic and Antarctic Marine Food Webs: A First Assessment

J.D. Blumenroeder, The University of Hull / Energy and Environment Institute; C. Waller, The University of Hull; D.K. Barnes, British Antarctic Survey, Natural Environment Research Council; M. Johnson, The University of Hull

Microplastics have reached even the most remote regions on the planet including Arctic and Antarctic seas, continental shelves and coasts. At lower latitude, studies have shown negative impacts of microplastic exposure on a variety of organisms with potential adverse implications for the food chain, but little is known of high latitude foodweb impacts. While a small but increasing number of studies have investigated microplastic concentrations in Antarctic waters and sediments, only a handful have reported plastic particles and fibres in Antarctic marine fauna. With high densities of suspension feeders and long meal processing times, but only a short annual feeding window, there are good reasons to hypothesize that microplastic could pose a more significant problem at higher latitudes. Plastic pollution has the potential to become an additional stressor in a hotspot of physical change and thus could increase the vulnerability of polar biota. This study aims to create a holistic picture of microplastic ingestion by plankton and benthic invertebrates in the polar regions. A key question is which traits (such as feeding type, functional group, taxa, trophic level, and habitat) influence the rate of microplastic ingestion, and therefore their vulnerability/sensitivity towards plastic pollution? It is also important to elucidate whether microplastics accumulate along remote marine food webs. In addition, this study aims to investigate if the structural integrity of polymers changes through the ingestion by polar organisms. This will help to understand the impact and environmental consequences of microplastics in the marine environment. Given the importance polar invertebrates play for a significant part of the global marine food web, it is also imperative to understand what effect microplastics have in these biomes and what the possible consequences for higher tropic levels and ecosystem services are. Samples of water, plankton, sediment and benthic invertebrates were taken along three fjords emerging from glacier retreat along the Antarctic Peninsula. Similar samples were also collected at the Burdwood Bank just north of the Southern Ocean, as well as south of Svalbard, Norway, in the Arctic. A first analysis of water samples confirms the presence of microplastic in all sampled Antarctic fjords. The polymer composition of found plastic particles and fibres will be identified using Fourier-Transformed Infra-Red spectroscopy (FTIR).

2.11.07

Nanoplastics As a Further Stressor for Sub-Antarctic Pteropods Already Tackling Ocean Acidification

C. Manno, V. Peck, British Antarctic Survey; I. Corsi, E. Bergami, University of Siena / Physical, Earth and Environmental Sciences

In the Southern Ocean, plastic pollution is emerging as a further ecosystem stressor in addition to major environmental challenges. This region is considered a 'bellwether' for ocean acidification (OA), likely one of the most serious environmental threats of this century. The smallest plactics, nanoplastics, may pose a far more serious threat to marine species compared to microplastics as nanoplastic concentrations already exceed toxicity-thresholds in areas of high plastics accumulation. Although the degree of nanoplastic pollution in the Southern Ocean is yet to be determined, nanoplastics are predicted to be as pervasive as their bulkier counterparts as point source emissions of plastic debris are likely a source of nanoplastic contamination also. Shelled pteropods (holoplanktonic gastropods) are considered a sentinel for OA due to their aragonite shells, a relatively soluble polymorph of calcium carbonate, being particularly vulnerable to changes in seawater carbonate chemistry. Previous studies have investigated the combined impact of OA and other stressors (i.e., freshening, warming) on pteropods, showing that these organisms act as excellent target species to identify thresholds in response to multi anthropogenic stressors. Here we investigate the single and combined effects of nanoplastics and OA on the survival of the

Sub-Antarctic pteropod *Limacina retroversa* from the Scotia Sea in the Atlantic sector of the Southern Ocean. Organisms were exposed to surface charged polystyrene nanoparticles (PS NPs) and/or pCO_2 manipulated seawater under controlled laboratory conditions to investigate mortality and shell alterations. We found that the exposure to nanoplastics may compromise the ability of pteropods to counteract OA stress, resulting in a negative effect on their survivorship. Our results provide an insight on the crucial importance of assessing plastic pollution within the context of climate change in order to consider the full impact of this emerging environmental stressor on Southern Ocean marine ecosystems.

2.11.08

Plastic Pollution in Antarctic Terrestrial Ecosystems: An Underestimated Contamination With Manifold Implications for Fragile Polar Soil Communities

E. Bergami, E. Rota, University of Siena / Physical, Earth and Environmental Sciences; T. Caruso, University College Dublin / School of Biology and Environmental Science; W. Shim, S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; G. Birarda, L. Vaccari, Elettra–Sincrotrone Trieste / SISSI-Chemical and Life Science branch; A. Krupinski Emerenciano, L. Palmeira Pinto, W. Reina Joviano, University of São Paulo / Cell and Developmental Biology, Institute of Biomedical Science; V. Venuti, University of Messina / Mathematical and Computer Sciences, Physical Sciences and Earth Sciences; V. Crupi, University of Messina / Chemical, Biological, Pharmaceutical and Environmental Sciences; J.M. Silva, University of São Paulo / Cell and Developmental Biology, Institute of Biomedical Science; M. González-Aravena, Chilean Antarctic Institute / Scientific Department; I. Corsi, University of Siena / Physical, Earth and Environmental Sciences

Antarctica has been depicted as the region least affected by plastic pollution worldwide, with low levels of floating plastics reported in the Southern Ocean and marine biota. Current research focuses primarily on the occurrence and distribution of plastic waste in the Antarctic marine environment and its resources, by assuming that land contamination only occurs in isolated areas. However, compared to continental Antarctica, the Antarctic Peninsula and Maritime Antarctica are by far the most intensely anthropized portions, which may hide plastic accumulation areas, not detected by large-scale surveys. Here we present an overview of the extent of plastic contamination in ice-free areas of the Fildes Peninsula on King George Island (South Shetland archipelago) and its interaction with Antarctic soil communities. Stranded macroplastics (> 1 cm) found on the Fildes Peninsula included polymeric foils, fishing buoys, paint flakes and food packaging, with some of the materials dated more than 37 years old and probably ascribed to local contamination. Their distribution was mainly related to the prevailing West wind, type of substrate and human activities. ATR-FTIR analysis revealed their polymeric nature as high-density polyethylene (HD-PE), polypropylene (PP), polystyrene (PS) and resins. Aged items of expanded polystyrene (EPS) foam were found at each site visited, including an Antarctica Specially Protected Area (i.e., ASPA 125). Chemical analysis undergone on EPS fragments collected from three different sites revealed high concentrations of hexabromocyclododecanes (HBCDs) from 323 to 567 μg/g (d.w.), a brominated flame retardant additive for polymers. The majority of the EPS was colonized by microalgae and, in some cases, by mosses, lichens and microarthropods, while low colonization was found in the other plastic items. μ-FTIR spectroscopy revealed the presence of micro-sized EPS fragments ingested by collembolans (*Cryptopygus antarcticus*) and adhering to the cuticle of moss mites (*Alaskozetes antarcticus*), with potential redistribution of microplastics through the soil profile and transfer to their common predators. Our findings are evidence of a widespread presence of plastic debris in ice-free areas in the Maritime Antarctic region. Such level of contamination should not be underestimated, as it can be an important source of multiple stressors for Antarctic soil communities, which already face major changes related to climate change.

2.11.09

A Stress Ecology Study for Establishing a Baseline Data on Health Status of the Adélie Penguins (Pygoscelis adeliae) Population Breeding in Mid Victoria Land, Ross Sea, Antarctica

s. simonetti, S. Olmastroni, University of Siena; N. Fattorini, University of Milan; I. Corsi, University of Siena / Physical, Earth and Environmental Sciences

Adélie penguin is considered a keystone species of the Antarctic ecosystem and has shown high sensitivity to habitat changes. Around 38% of its global population inhabits the Ross Sea. Among the Southern Ocean, this marine region is still considered pristine, but in the last decades it has experienced increased anthropogenic pressure (i.e. resource exploitation, pollution and climate changes). Although the Adélie penguin have been long monitored, still few studies are currently reporting on their health status and consequently on their ability to cope with increasing levels of disturbance. Since changes in habitat quality is likely to induce effects on wildlife physiology, useful proxies which can provide an early alert on changes on individuals and assess the potential impact of environmental stressors are highly demanded. Hematic parameters obtained from the analysis of blood smears (i.e. erythrocytes nuclear abnormalities (ENAs) and leukocytic profile) have been recognized as valuable indicators of birds' immune status and largely employed also in penguins' studies being also non-invasive and easy to perform. A preliminary study was conducted during the 2014-2015 summer season using blood smears from Adélie penguins breeding in one colony located in Mid Victoria Land. Sampling were then repeated during the 2017-18 summer season with the aim to establish a baseline for the Adélie penguin's population of Mid Victoria Land, breeding at three different sites: Edmonson Point, Adélie Cove and Inexpressible Island. In all penguin's blood smears analysed from the three colonies, several ENAs were found. In particular micronuclei (MN) accounted for less than 10% of ENAs while nucleus with tail was the most abundant (>20%). Heterophils were the most common white blood cells (43%) in agreement with the generic avian leukocytes profile. A low number of lymphocytes were detected resulting in a higher heterophils to lymphocytes ratio (H:L). Our results indicate ENAs and H:L are confirmed as reliable indexes of penguin's immune condition and besides possible environmental stressors they may also reflect individual adaptation according to age, sex, body condition or different stages of the breeding season. These baseline data may be used as reference indicators of penguin's health status in future monitoring and conservation studies to assess population and ecosystem health in a changing environment.

2.11.10

Mercury Risk Evaluation, Risk Management and Risk Reduction Measures in the Arctic (ARCRISK)

H. Braaten, Norwegian Institute for Water Research NIVA; C.B. Gundersen, E. Hovland Steindal, NIVA - Norwegian Institute for Water Research; J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; E. Yakushev, Norwegian Institute for Water Research; G. Christensen, Akvaplan-niva AS; J.L. Kirk, Environment and Climate Change Canada / Aquatic Contaminants Research Division; H. Hintelmann, Trent University; N.L. Frolova, Lomonosov Moscow State University / Department of Geography; P. Terentjev, Kola Science Centre of the Russian Academy of Sciences / Institute of North Industrial Ecology Problems; S. Roberts, Environment and Climate Change Canada; W.G. Landis, Western Washington University / Institute of Environmental Toxicology and Chemistry

The project "Risk evaluation, risk reduction and risk management action plans for mercury in the Arctic – a circumpolar management approach" (ARCRISK) has been developed to address mercury pollution in the Arctic. The main objective is to develop an action plan with targeted risk reduction measures for mercury releases from key sources to land and water in the Arctic. The ARCRISK project team consists of experts from nine research organisations in Canada, Norway, Russia and USA. An inception workshop was held in Oslo in March 2020 to consolidate the team and collectively develop the project framework. ARCRISK was developed in the backdrop of the ratification of the Minamata Convention (MC) on mercury that entered into force in 2017, with the objective to "*protect the human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds*". Whereas emissions to air gained significant attention throughout the negotiations of the convention, less attention was bestowed to mercury releases to land and water. External stressors such as climate change, increased industrialization, and urbanization may further exacerbate the situation e.g. by influencing the natural mercury cycling and should therefore be taken into consideration when assessing mercury releases. Concurrently with the ongoing processes under the MC it is crucial to obtain a better understanding of the presence and magnitude of mercury release sources to the Arctic, and the risk associated with these releases, especially to locals and indigenous inhabitants. The aim of ARCRISK is closely aligned with the purpose of the Arctic Contaminants Action Program (ACAP); to prevent and reduce pollution and environmental risks in the Arctic. The action plan will cover key sources from each of the four selected case study river catchment basins in Canada, Norway, and Russia. A probabilistic modelling approach based on the Relative Risk Model will

be applied for assessing the environmental risk associated with mercury exposure in these sites. The risk-based approach, including a review of sources and identification of key measures in the four localities, aims to recommend concrete reduction measures with high relevance for a larger part of the Arctic. The project aims not only to identify and assess the current risks, but also to assess the risks associated with different plausible future environmental (e.g. climatic), societal and economic development scenarios.

2.11.11
The Antarctic Fairy Shrimp Branchinecta Gaini As Promising Model Species to Assess the Impact of Nanoplastics in Antarctic Freshwater Lakes
E. Bergami, University of Siena / Physical, Earth and Environmental Sciences; A. Krupinski Emerenciano, L. Palmeira Pinto, W. Reina Joviano, University of São Paulo / Cell and Developmental Biology, Institute of Biomedical Science; A. Font, Chilean Antarctic Institute / Scientific Department; T. Almeida de Godoy, J.M. Silva, University of São Paulo / Cell and Developmental Biology, Institute of Biomedical Science; M. González-Aravena, Chilean Antarctic Institute / Scientific Department; I. Corsi, University of Siena / Physical, Earth and Environmental Sciences
Antarctic lakes and natural pools represent isolated and delicate freshwater ecosystems, found in Maritime Antarctica and Antarctic Peninsula regions, which have been experiencing relevant environmental and anthropogenic perturbations. An example is represented by the lakes near historical open waste deposits along the Fildes Peninsula at King George island (South Shetland archipelago), which are contaminated by plastic litter materials and potentially micro- (< 1 mm) and nanoplastics (< 1 µm). Antarctic lakes are characterised by simple trophic webs, dominated by bacteria, rotifers and few microcrustaceans, such as the fairy shrimp *Branchinecta gaini* (Order Anostraca), which developed peculiar adaptations to inhabit such extreme environments. To date, studies on the biological responses of these organisms to anthropogenic contamination such as plastic debris, are lacking. Here, we explored for the first time the effects of surface charged polystyrene nanoparticles (PS NPs), as a proxy for nanoplastics, on *B. gaini*. Based on our knowledge acquired with the anostracan *Artemia franciscana*, a common model organism in ecotoxicology, we developed a short-term toxicity test (48 h) with *B. gaini* and proposed suitable endpoints to evaluate the effects of PS NPs as emerging contaminants to this freshwater organism. For the experiments, adult individuals of *B. gaini* collected from an Antarctic lake in the Fildes Peninsula in February 2018 were used. Two males and two females in each replicate were exposed to carboxyl (-COOH, 60 nm) and amino-modified (-NH_2, 50 nm) PS NPs (at 1 and 5 µg/ml). Lethal and sub-lethal (i.e., swimming, moulting, histology, gene expression) endpoints were determined. Ingestion/excretion of fluorescently labelled PS-COOH was also evaluated. Dynamic light scattering showed that both PS NPs maintained their nanoscale size in the Antarctic lake water up to 48 h. Surface charged PS NPs did not affect the survival of fairy shrimp adults over short-term exposure. However, an increase in moulting rate in the experimental groups exposed to PS NPs was observed. Significant alterations at the behavioural (ventilation rate), morphological (digestive tract epithelium) and molecular (upregulation of *Hsp70mit*, *Hsp83*, *Sod*) level were related to PS NP surface charge and associated to PS-NH_2 exposure only. Our results show that the Antarctic fairy shrimp *B. gaini* is a valuable model organism for the environmental impact assessment of emerging contaminants.

2.11.12
Modeling Nanoplastic Fate in the Polar Regions: Transfer From Saltwater to Ice
A. Pradel, Université de Rennes 1 / Géosciences Rennes; M. Gautier, C. Le Carlier, D. Bavay, Univ Rennes, CNRS / Géosciences Rennes; J. Gigault, TAKUVIK CNRS, Université Laval
The dissemination of plastic within ecosystems is a proven ecological risk. This type of waste includes nanoplastics: colloids that originate from the unintentional degradation of plastic. The colloidal nature of nanoplastics allows them to remain stable in aqueous environments during long periods. However, their detection and quantification is more complicated than that of macro- and micro-plastics since they cannot be detected by optical methods. The environmental fate of nanoplastics must be characterized in order to quantify organisms' exposure rate and *in fine* describe the ecological and health risks that are specific to this particle. One of the most fragile environmental compartments which reveals the consequences of the Anthropocene is located in the polar regions. In oceans and sediments worldwide, plastic debris belonging to all size classes (macro, micro and nanometric) have been quantified. However, in polar regions, plastic debris is only beginning to be quantified. Indeed, in sea ice only microplastics have been studied. Thus, this study aims to shed some light on the behavior of nanoplastics at the interface between seawater and sea ice. A bench-scale experiment reproduced the seawater/sea ice interface in order to determine the capacity of sea ice to capture or expel nanoplastics. To describe the thermohaline gradients occurring in this experimental setup, the system was numerically modelled using COMSOL Multiphysics. Three nanoplastic models were used, with varying sizes and shapes, and one microplastic model. The effect of natural organic matter (NOM) was also considered, in order to more closely represent environmental conditions. The results show that whilemicroplastics accumulate in sea ice, nanoplastics appear to be expelled from sea ice. The presence of NOM does not alter nanoplastic's expulsion from sea ice but stabilizes the nanoplastics, hence decreasing their sedimentation rate. Due to the technical difficulty in sampling nanoplastics in the environment, these experimental results are useful in assessing the fate of nanoplastics in polar regions. Indeed, these first experimental results suggest that, while microplastics may accumulate in sea ice, nanoplastics may accumulate in the water column and eventually sediments.

Wildlife Poisoning Incidence: Impacts on Biodiversity, Ecosystems, Human Health and Legislative Regulations

2.12.04
Temporal Persistence of Bromadiolone in Carcasses of Common Kestrel (Falco tinnunculus)
I. Valverde, Universidad de Murcia (Q-3018001-B) / Toxicology and Forensic Veterinary Service, Faculty of Veterinary, Department of Health Sciences.; S. Espin, University of Murcia / Department of Health Sciences, Area of Toxicology; P. Gomez-Ramirez, University of Murcia (Q-3018001-B) / Sociosanitary Sciences; I. Navas, University of Murcia / Toxicology and Forensic Veterinary Service, Faculty of Veterinary, Department of Health Sciences.; P. Sánchez-Virosta, University of Murcia / Department of Health Sciences, Area of Toxicology; M. Torres, Universidad de Murcia / Sociosanitary Sciences; P. Jiménez, University of Murcia / Department of Health Sciences, Area of Toxicology; P. María-Mojica, University of Murcia / Wildlife Rehabilitation Center of Alicante, Generalitat Valenciana, Alicante, Spain; A. Garcia-Fernandez, University of Murcia / Health Sciences
Bromadiolone is a second generation anticoagulant rodenticide (SGAR) used to control pest rodents worldwide. SGARs are frequently involved in secondary and tertiary poisoning in predators due to their persistence and toxicity. This study aims to evaluate the persistence of bromadiolone in liver at different stages of carcass decomposition using experimentally-dosed common kestrels (Falco tinnunculus) to understand the possibility of detecting bromadiolone in cases of secondary wildlife poisoning in raptors and the potential risk of tertiary poisoning in the field for scavengers. For this purpose, 6 individuals were assigned to the bromadiolone-dose group (dosed with 55 mg/kg b.w) and 6 individuals to the control group. These kestrels were admitted in the "Santa Faz" Wildlife Recovery Centre (Alicante, south-eastern Spain) and were non-releasable and destined to be euthanized due to traumatic wing injuries. Hepatic bromadiolone concentrations found in each stage of decomposition in the dose group were: 3.00, 2.89, 4.80, 4.24, 8.84, and 0.76 µg/g dry weight at 1–2 h (fresh carcass), 24 h (moderate decomposition), 72 h, 96 h (advanced decomposition), seven days (very advanced decomposition), and 15 days (initial skeletal reduction) after death, respectively. Liver bromadiolone concentrations in carcasses remained relatively stable over the first four days and raised on day 7 of decomposition under the specific conditions of this experiment, presenting a risk of causing tertiary poisoning. However, at the initial skeletal reduction stage, liver bromadiolone concentration declined. This experiment provides for the first time some light to better understand the degradation of SGARs in carcasses in the wild. However, more studies should be performed to interpret toxicological analyses and for proper diagnosis in different scenarios. All procedures performed complied with the ethical standards of the Comité Ético de Experimentación Animal (CEEA)—University of Murcia (code: 549/2019) as well as applicable institutional, local, and national guidelines and laws. Acknowledgments: This study was funded by the Seneca Foundation (MASCA 2014, 19481/PI/14 and MASCA 2018, 20945/PI/18). Silvia Espín was supported by the MICINN (IJCI-2017-34653). This communication is dedicated to the memory of Prof Richard F. Shore. Keywords: anticoagulant

rodenticides, carcass decomposition, wildlife poisoning, bromadiolone degradation

2.12.05
Anticoagulant Rodenticides Exposure in Non-Target Predators and Scavengers
P. Gomez-Ramirez, University of Murcia (Q-3018001-B) / Sociosanitary Sciences; S. Castán, University of Murcia / Toxicology and Veterinary Forensic Service, Faculty of Veterinary, Campus de Espinardo, 30100, Murcia, Spain; I. Valverde, Universidad de Murcia (Q-3018001-B) / Toxicology and Forensic Veterinary Service, Faculty of Veterinary, Department of Health Sciences.; I. Navas, S. Espín, P. Sánchez-Virosta, P. Jiménez, University of Murcia / Department of Health Sciences, Area of Toxicology; P. María-Mojica, University of Murcia / Wildlife Rehabilitation Center of Alicante, Generalitat Valenciana, Alicante, Spain; A. Garcia-Fernandez, University of Murcia / Health Sciences
Despite legal restrictions in EU to the use of anticoagulant rodenticides (ARs), they are commonly used in urban, semi-urban, farming and even forest environments. This may pose a risk for wildlife by ingestion of commercial baits (primary intoxication) or predation of both target/non-target species (secondary/tertiary intoxication). The prevalence and risk of secondary exposure to ARs in mesocarnivores and birds from Spain was studied analysing carcasses from different wildlife rehabilitation centres from Eastern (autonomous communities of Valencia and Murcia) and Central (Autonomous Community of Castilla-León) Spain. Liver was collected for simultaneous analysis of 8 ARs (warfarin, coumatetralyl, chlorophacinone, bromadiolone, brodifacoum, diphacinone, difenacoum and flocoumafen) with a HPLC connected to a Q-TOF MS operated in negative mode, after an extraction using a modified QuEChERs method. A total of 60 samples were obtained from 22 birds of 5 species [3 scavengers (9 Griffon vultures-*Gyps fulvus*, 1 Golden eagle-*Aquila chrysaetos*, 1 Common raven-*Corvus corax*) and 2 non-scavengers (9 Eurasian eagle owls-*Bubo bubo*, 2 Common buzzards-*Buteo buteo*)] and 38 mammals of 7 species [1 dog-*Canis lupus familiaris*, 2 cats-*Felis silvestris catus*, 7 Red fox-*Vulpes vulpes*, 4 Common genets-*Genetta genetta*, 10 Stone martens-*Martes foina*, 11 Badgers-*Meles meles* and 3 Eurasian otters-*Lutra lutra*)]. Residues of one or more ARs were found in 37 samples (62%). Coinciding with previous studies, the most common ARs detected were brodifacoum (n=23, 38%) and difenacoum (n=19, 32%), followed by flocoumafen (n=15, 25%), bromadiolone (n=7, 12%), and coumatetralyl (n=6, 10%). The other ARs were not detected. Twenty-three samples (38%) contained more than one AR. Brodifacoum was also found at the highest level (max = 1088.46 µg/kg), followed by bromadiolone (max = 382.99 µg/kg) and flocoumafen (max = 167.13 µg/kg). Total ARs were significantly different between owls/vultures, badgers/genets, badgers/foxes, and genets/stone martens ($p< 0.05$). Our results indicate that there is a higher risk for exposure to ARs in mesocarnivores in areas with higher density of human population and abundant crops. Also, as scavenger birds do not feed on rodents, they show lower risk of exposure than mesocarnivores. *Acknowledgements*: Funded by Seneca Foundation (20945/PI/18). SE supported by IJCI-2017-34653. This communication is dedicated to Prof Richard F. Shore.

2.12.09
Poisoning Incidence in Wild Birds Patients of Wildlife Rehabilitation Centre As Factors for Disabilities and Mortality
A. Lazarova, G.N. Balieva, Trakia University, Faculty of Veterinary Medicine / Hygiene, technology and control of foodstuffs from animal origin, veterinary legislation and management; S.B. Dimitrova, Wildlife Rehabilitation and Breeding Centre, Green Balkans, Stara Zagora
Wildlife rehabilitation centers could provide valuable information regarding the nature of causes resulting in wildlife incidents. Among these causes fall various activities from anthropogenic character leading to injuries and mortality of wild animals. Regarding the database of the Wildlife Rehabilitation and Breeding Centre at Stara Zagora, Bulgaria (WRBC), a particular share of the human-related injuries in the hospitalized patients appeared to be due to poisoning. We analyzed data for a number of patients admitted with suspicion of poisoning by the history taken and/or clinical symptoms of intoxication. For some of the patients – injured wild birds, samples were sent for laboratory testing after which the results confirmed presence of anticholinesterase's inhibitors. These findings indirectly indicated pesticides poisoning. For another range of injured birds, we tried to identify if the primary reason for the incidents was related to intoxication with sub-lethal doses which led to subsequent exhaustion or body traumas. The laboratory tests didn`t detect any pesticides residues in this group of patients, admitted to the WRBC with different symptoms and history. In order to be able to come to a conclusion if the primary cause for body injuries in some of the cases was related to sub-clinical intoxication, it is necessary further detailed analyses to be conducted.

2.12.10
Protocol to Classify the Stages of Carcass Decomposition and Estimate the Time of Death in Small-Size Raptors
I. Valverde, Universidad de Murcia (Q-3018001-B) / Toxicology and Forensic Veterinary Service, Faculty of Veterinary, Department of Health Sciences.; S. Espin, University of Murcia / Department of Health Sciences, Area of Toxicology; P. María-Mojica, University of Murcia / Wildlife Rehabilitation Center of Alicante, Generalitat Valenciana, Alicante, Spain; A. Garcia-Fernandez, University of Murcia / Health Sciences
Malicious poisoning involving birds is one of the most common wildlife crimes in the world. Post-mortem examination and toxicological analysis are essential for a proper diagnosis in poisoning cases. Moreover, an estimate of the time of death is often required. The aim of this poster is to present a scoring method to classify the stages of carcass decomposition and to provide an estimation of the time of death in small-size raptors. For this purpose, 12 carcasses of Common kestrel (*Falco tinnunculus*) were exposed to external weather conditions in the summer of 2019 in Southeastern Spain. Several parameters were measured at intervals over the study period: ambient temperature, relative humidity, body core temperatures and carcass weights. Necropsies were performed (2 birds at each interval) at 1–2 h, 24 h, 72 h, 96 h, 7 days and 15 days after death. The necropsy of a previously frozen bird was performed to act as a comparison with non-frozen fresh individuals. Six stages of the post-mortem autolytic process were selected: fresh carcass, moderate decomposition, advanced decomposition, very advanced decomposition, initial skeletal reduction and complete skeletal reduction. To classify the carcasses according to these categories, a scoring method is proposed considering 5 parameters: state of the eyeballs, tongue/oral cavity, pectoral muscle, internal organs and other features. Several parameters affecting the process of the decomposition are discussed. This protocol can be used by practitioners, forensic veterinarians, researchers, authorities and personnel collecting carcasses in order to standardize methods and minimize subjectivity. *Acknowledgments*: This study was funded by the Seneca Foundation (MASCA 2014, 19481/PI/14 and MASCA 2018, 20945/PI/18). Silvia Espín was supported by the MICINN (IJCI-2017-34653). This communication is dedicated to the memory of Prof Richard F. Shore. ***Keywords***: autolysis, decomposition, forensic, necropsy

2.12.11
Use of Toxicants Baiting for Reduction of Wild Boar Populations in Contagious Animal Disease Management - Implications for Biodiversity and Legislation
G.N. Balieva, Trakia University, Faculty of Veterinary Medicine / Hygiene, technology and control of foodstuffs from animal origin, veterinary legislation and management; D. Tanchev, Bulgarian Food Safety Agency / Regional Food Safety Directorate - Yambol
Wildlife is an essential natural resource which is in tight interconnection with all other units of the ecosystem in a particular area, including domestic animals and human population. At the same time wild animals appear to be a key factor in the occurrence, transmission and prevalence of a myriad of contagious animal diseases, being natural reservoirs, vectors or both. This is the case for the wild boar populations and their participation in the African swine fever (ASF) transmission to domestic pigs. ASF entered the European Union in 2014 and since then a lot of measures have been implemented by the member states to bring the disease under control. Bulgaria falls among the countries which have tried to introduce new measures for reduction of wild boar populations with regard to ASF through legislative amendments. Legislative proposals in the Bulgarian Law on hunting and protection of game from the autumn of 2020 tried to make legitimate the culling of wild boars with some unselective approaches like the use of chemical poisonous or intoxicating substances, as well as baits with such substances. The proposal did not pass and received wide negative public reaction. This provoked the authors to study the experience of other countries which have made research and field studies on particular toxicants used for baiting for control of wild boar populations. We investigated the available scientific literature on the topic with the focus on the selective character of chemical baiting, the effect on non-target species and the success of combination of baiting with other measures for population reduction. Moreover, the participation of different stakeholders in the process of development and implementation of the mentioned measures was discussed. Thus,

proposals were made for better preparatory, scientific and efficiency investigation at the preliminary stage of animal disease control measures development.

2.12.12

Potential Risks of Certain Key Classes of Pharmaceuticals to Avian Scavengers

M. Herrero Villar, University of Castilla La Mancha / Ecotoxicology; R. Shore, Centre for Ecology & Hydrology / Lancaster; R. Mateo, Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM); M. Taggart, Environmental Research Institute / Environmental Contamination and Ecological Health

Pharmaceuticals are considered as emerging toxicants in the environment, and one of the sentinel groups thatcan act as environmental indicators of their potential impact are top predators. For instance, dramaticecological effects have been observed due to the non-steroidal anti-inflamatory drug (NSAID) diclofenac whichcaused the collapse of asiatic vulture populations in recent decades. Moreover, there are other NSAIDs anddrug groups, such as antibiotics, antiparasitics, barbiturates, etc., that can cause adverse effects in wildlife.Within the European Raptor Biomonitoring Facility (ERBF) COST Action(https://www.cost.eu/actions/CA16224), we performed a review to compile published data reporting on thetoxicity of key pharmaceutical groups in avian scavengers worldwide, and especially in Europe. One of theobjectives was to identify the most commonly used and prescribed pharmaceuticals in Europe in order to pointto potential drugs widely used in veterinary and human medicine. Further, data was compiled includingresidues and concentrations detected in carcasses available to avian scavengers, toxicokineticstoxicodynamics(based on data from the European Medicines Agency (EMEA) and other publised dataregarding domestic animals and raptors. In addition, we collected data concerning metabolic pathways,accumulation, clinical pathology parameters and histopathology. Also, we noted the main analytical and riskassessment techniques used, as well as the results obtained and the regulation and effectiveness of measuresimplemented to mitigate the potential adverse effects of pharmaceuticals in the environment. All thisinformation pointed to gaps in knowledge that may help to determine further steps needed in order to avoiddramatic ecological scenarios similar to those seen in Asia. We found that pharmaceutical residues detectedin avian scavengers were mainly of veterinary use, especially NSAIDs such as diclofenac, ketoprofen, flunixinor meloxicam. Antibiotic residues are also reported in avian scavengers (mainly quinolones; ciprofloxacin,enrofloxacin and marbofloxacin), as well as observed mortality linked to consumption of barbiturateeuthanaized animal carcasses. Antiparasitic residues of diazinon and permethrin in bearded vulture (Gypaetusbarbatus), and other pharmaceuticals present in water systems were detected in osprey (Pandion haliaetus).Lastly, we created a template to record visceral gout postmortem examination findings - including general tips,precautions and useful information for vets working in Europe.

2.12.14

Non-Invasive Method to Measure the Dermal Exposure of Amphibians to Pesticides

D. Schenke, Julius Kühn-Institut - Federal Research Centre for Cultivated Plants / Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection; J. Sadowski, A. Esther, Julius Kühn-Institut - Federal Research Centre for Cultivated Plants / Institute for Plant Protection in Horticulture and Forests - Vertebrate Research, Toppheideweg 88, D-48161 Münster

Pesticide exposure of amphibians is difficult to assess due to the various possible exposure routes and the movement behavior between aquatic and terrestrial habitats (EFSA, 2018). In particular, little is known about the dermal uptake of pesticides in field, which appears to be very important for amphibian's exposure risk and survival (Llewelyn et al., 2019). We have used swab samples to measure pesticides on the skin of amphibians which possibly originate from contact to contaminated soil, sediment, water, plants or air. We investigated adult amphibians, nine common toads (*Bufo bufo*) and one common frog (*Rana temporaria*), which were trapped in and around maize fields in North Rhine-Westphalia (Germany) in summer 2018. About 10 cm² of the ventral and dorsal side of each animal were swabbed separately using individual sterile Dryswab™ with fine tip rayon buds. The selection of pesticides based on a proposal for a representative monitoring in the framework of the "Implementation of the National Action Plan on the Sustainable Use of Pesticides" (UBA, 2019). The substances were extracted with a solution of water/methanol (1:1, 1% formic acid) from the swabs. The amounts were measured with liquid and gas chromatography-mass spectrometry. We detected terbuthylazine in samples of three animals. Plant protection products containing terbuthylazine may be used in the pre- and post-emergence (BBCH 10-17) of maize with maximal 750 g/ha (BVL, 2018). The highest amount of 75 pg/cm² was found on the ventral side of a toad. This amount corresponds to 0.001% of the maximum application rate permitted for maize fields. The detection of terbuthylazine in swab samples from animals in and around maize fields is an expression of the spatio-temporal behaviour of amphibians. DOI 10.5073/20201029-143153**References** EFSA, 2018: Scientific Opinion on the state of the science on pesticide risk assessment for amphibians and reptiles. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2018.5125. Llewelyn et al., 2019: Effects of skin region and relative lipophilicity on percutaneous absorption in the toad *Rhinella marina*. Environmental Toxicology and Chemistry 38 (2) 361-367. UBA, 2019: Umsetzung des Nationalen Aktionsplans zur nachhaltigen Anwendung von Pestiziden - Teil 2. Texte 08/2019, ISSN 1862-4804. BVL, 2018: Pflanzenschutzmittel- Verzeichnis 2018, Teil 1 Ackerbau – Wiesen und Weiden Hopfenbau – Nichtkulturland, 66. Auflage 2018, ISSN 0178-059X.

2.12.15

Has the Risk Profile of Authorized Plant Protection Products for Birds and Mammals Changed Over the Last Years?

R. Gauch, A. Aldrich, O. Daniel, Agroscope

In Switzerland, many authorized plant protection products and their uses have been re-evaluated, and authorization and risk management has changed accordingly since 2010. To this end, more than 2000 uses from plant protection products with about 100 active ingredients have been assessed and the authorization of several active ingredients have been withdrawn. Plant protection products from active ingredients with high sales volumes in Switzerland were usually assessed first. Furthermore, results from Swiss monitoring programs on concentrations of plant protection products in surface water and groundwater, as well as residues in harvested crops are included in this prioritization. For terrestrial vertebrates, however, no plant protection product-related monitoring data exist for Switzerland, even if some postulate from information of conservational programs that a risk might occur. Suitable, precautionary management options are not easy to implement. The question remains, whether the situation with terrestrial vertebrates has changed. This poster investigates whether changes in authorisations in the last years, almost entirely due to non-vertebrate assessments, also had an effect on risks to birds & mammals.

2.12.18

Histopathology in the Liver of the Scalloped Hammerhead, Sphyrna Lewini, From the Coast of KwaZulu-Natal, South Africa

H.J. Coetzee, I. Wagenaar, University of Johannesburg / Zoology

The presence of xenobiotic compounds in the marine ecosystems is anemerging concern. It is known that xenobiotics such as polychlorinatedbiphenyls (PCB's) used in electronics, paints and oils have a toxicityeffect in the environment and animals. PCB's are also carcinogenic andhave caused liver damage in animals and humans. The aim of the studywas to determine the health status of the hammerhead shark (*Sphyrnalewini*) by evaluating the histology and bioaccumulation of PCBs in theliver of *Sphyrna lewini*. Six mature *S. lewini* males were caught as by-catch by the KwaZulu-Natal Sharks Board netting program of the coastof KwaZulu-Natal South Africa. Standard necropsies were performed oneach specimen and 20 representative samples were collected from eachshark's liver. For bioaccumulation, 10 grams of the liver was frozen andanalyzed for the presence of PCB's. Representative samples werefixated and standard histological procedures followed for the microscopyassessment. Upon investigation histopathological changes wereidentified such as lymphocyte infiltration, steatosis, hypertrophic tissueand necrosis. PCB's were present in the shark livers and differentisomers were found such as 153, 138 and 180 with PCB 153 the highest(114 µg/kg) and PCB 138 the lowest (30.4 µg/kg). The PCB's thatbioaccumulated in the livers could have caused the histopathologicalchanges which could lead to liver damage. In conclusion, the pathologyof the liver tissue could affect the health status of the scallopedhammerhead. Better legislation on pollutants in the marine ecosystemsshould be implemented to protect the hammerhead shark which hasbecome critical endangered

Extended submission 2 - Ecotoxicology becomes stress ecology: from populations to ecosystems and landscapes

2.13.03

Antagonistic Action of Selenium Against "Chemical Cocktails" and the Role of Gut Microbiota

A. Arias Borrego, Universidad de Huelva / Department of Chemistry; M.P. Selma-Royo, Institute of Agrochemistry and Food Technology (IATA-CSIC), Food Biotechnology, Agustin Escardino 7. Paterna (Valencia) / Food Biotechnology / Chemistry. Faculty of Experimental Sciences; M.P. Collado, Institute of Agrochemistry and Food Technology (IATA-CSIC), Food Biotechnology, Agustin Escardino 7. Paterna (Valencia) / Food Biotechnology; N.P. Abril, University of Córdoba, Campus de Rabanales, Edificio Severo Ochoa / Department of Biochemistry and Molecular Biology; T. García-Barrera, University of Huelva / Chemistry

Controlled exposure experiments to "chemical cocktails" that integrate several environmental pollutants of different chemical groups allows evaluating the real effects of contaminants in the environment [1], since they usually interact though antagonistic or synergistic mechanisms [2]. Selenium (Se) is one of the most known antagonistic element against numerous contaminants (*i.e.* metals and pesticides). The aim of this study was the evaluation of the impact of a mixture including arsenic (As), cadmium (Cd) and pharmacologically active compounds on mice gut microbiota and the role of Se as an antagonist. To this end, 50 *Mus musculus* mice were divided in 5 groups: Group C (control), Group CC (mice fed regular diet exposed to the chemical cocktail), Group CC-Se (mice fed Se-supplemented diet exposed to the chemical cocktail), Group CC-Abx (mice fed regular diet with microbiota depleted by antibiotics exposed to the chemical cocktail) and group CC-Abx-Se (mice fed Se-supplemented diet with microbiota depleted by antibiotics exposed to the chemical cocktail). Selenoproteins were determined by heteroatom-tagged proteomics using an atomic detector such as inductively coupled plasma mass spectrometer (ICP-MS) for the sensitive detection of Se. The absolute quantification of the major selenoproteins in plasma was accomplished by the chromatographic separation using a column switching method, which combines size exclusion and affinity chromatographies (2D-SEC/AFC) coupled to ICP-MS. Gut microbiota profile was analyzed by 16S rRNA gene sequencing. As speciation was carried out in mice plasma by anionic exchange chromatography coupled to ICP-MS. Interesting and new correlations were obtained between gut microbiota, selenoproteins and arsenic species in mice plasma. Moreover, the impact of the chemical cocktail on gut microbiota and the action of Se has been eveluated.

2.13.07

Toxicity and Risk of Plant-Produced Alkaloids to Daphnia magna

M. Griffiths, Ricardo Energy & Environment / NCEC; B. Strobel, J. Hama, N. Cedergreen, University of Copenhagen / PLEN - Department of Plant and Environmental Sciences

Many plants contain phytotoxic alkaloids to deter herbivorous pests and grazing animals. Alkaloids include quinolizidine and indole alkaloids found in the lupin (*Lupinus* spp.), an ornamental flower and emerging protein crop, as well as pyrrolizidine alkaloids in the ragwort (*Senecio jacobaea*), an invasive, weed-like flower. When lupins and ragworts are present in large densities in fields, there is a concern that alkaloids may leach into freshwater environments in amounts that may affect non-target organisms, such as *Daphnia magna*. This study aimed to investigate (i) the acute toxicity of alkaloids (gramine, heliotrine, lupanine, lupinine, monocrotaline, monocrotaline N-oxide, senecionine and sparteine) in *D. magna*, (ii) the contribution of these individual alkaloids to lupin plant extract toxicity, (iii) the longer term reproductive effects of a representative alkaloid, sparteine, and conclude with (iv) a tentative risk assessment for the sum of alkaloids measured in soil and surface waters. The alkaloids exhibited toxicity, with 48 h EC_{50} values in the range of 5.6 to > 100 mg/L. The 48 h EC_{50} of the *Lupinus angustifolius* plant extract was 1.38 mg/L, which was far more toxic than the simulated extract where lethality was 2.5 mg/L sparteine produced significantly fewer and smaller offspring during the 21-day exposure, making chronic effects occur at concentrations approximately 10-fold lower than the 48 h EC_{50} for sparteine. The risk assessment of cumulated alkaloids measured in drain, running and pond waters showed a potential risk, particularly for stagnant pond water, where concentrations were severalfold higher than in the drain and running waters. The results highlight that natural toxins may contribute to poor chemical quality of natural waters, and that natural toxins from upcoming crops or invasive weeds should be considered in aquatic risk assessments.

2.13.08

Impact of Bare and Peg-Functionalised Up-Converting Srf2: Yb3+,Er3+ Nanoparticles on Lifespan, Moulting, Metabolism and Bioaccumulation of the Beetle Tenebrio Molitor

A. Ekner-Grzyb, Adam Mickiewicz University in Poznan / Department of Plant Ecophysiology; S. Chowanski, Adam Mickiewicz University in Poznan / Animal Physiology and Development; D. Mańczak, Adam Mickiewicz University in Poznan / Department of Plant Ecophysiology; T. Grzyb, N. Jurga, Adam Mickiewicz University in Poznan / Department of Rare Earths

Nanoparticles (NPs) are characterised by unique properties, such as their high surface area to volume ratio and higher surface reactivity, comparing to bulk counterparts. Therefore they are used in many fields of industry and science. Lately, intensively investigated are lanthanide-doped up-converting NPs, because of their uncommon physicochemical properties and luminescence under near-infrared excitations. However, along with their benefits, the NPs may also cause a threat to animals, including human. The effect depends on many factors, such as composition, size, shape or surface functionalisation. The NPs are widely functionalised with polyethylene glycol (PEG) to improve their properties. Despite that, the research about the influence of the PEG coating on the toxicity of the NPs on invertebrate is insufficient. Therefore, in the presented study, the impact of SrF_2: Yb^{3+}, Er^{3+} and SrF_2: Yb^{3+}, Er^{3+}@ PEG on *Tenebrio molitor* larvae was evaluate. This species is commonly used in ecotoxicological tests. A lifespan and time between moulting were checked. Moreover, the metabolism of the beetle was investigated by analysis of the level of the following parameters: glucose, glycogen, triglycerides, free glycerol and proteins in a fat body, as well as trehalose, glycogen, triglycerides, free glycerol in the hemolymph. Also, bioaccumulation of the up-converting NPs was assessed. Funding for this research was provided by the National Science Centre (grant no. UMO-2016/23/D/NZ8/01112)

2.13.09

Current Challenges Associated With Advancing the Field of Terrestrial Ecotoxicology

C.d. e Silva, Wageningen Environmental Research / Alterra - ERA; J. Kayode, Wageningen Environmental Research (Alterra) / Environment and Risk Assessment (ERA); G. Arts, Wageningen Environmental Research - WUR / Environmental Risk Assessment

Introduction: In recent years, results of different research are showing an increasing loss of species of insects and other taxa due to the use of pesticides among other factors. These losses will certainly have different impacts on different ecological levels, and eventually, lead to a negative effect on biodiversity. However, when faced with the quest of protecting it under the viewpoint of Environment Risk Assessment (ERA), one is confronted with basic questions: which species are endangered? which is the scale of the protection needed? and how to measure the impact, e.g. of plant protection products to biodiversity? **Discussion:** In the field of Terrestrial Ecotoxicology (TEx), biodiversity has been deeply affected by the use of chemicals, such as pesticides. When compared to the aquatic toxicity testing, the number of species used in soil is limited, and so are the number of methodologies available. The technical suite of guidelines can, sometimes, be restrictive concerning the questions asked, such as: Which ecosystem services (ES) are present and which is/are important to protect? What are the toxic effects on local species of soil invertebrates and their population? Normally, the answers to some of these questions are developed by extrapolating results obtained in laboratory to the field, which can bring a great amount of uncertainty when performing a risk assessment of a chemical. In order to advance the field of TEx, researchers are pointing towards improving the current methodology suite in order to better deal with present and potential threats. However, there are challenges associated with this quest, and some of the specific ones are related to: (i) the necessity to use different model species with different traits/characteristics, such as *Sinella curviseta* (Collembola) or *Aporrectodea caliginosa* (Annelida); (ii) how to use newly standardized test species e.g. *Oppia nitens* (Acari) for more toxicity tests; (iii) absence of standardized intermediate tier, such as multigeneration testing representing long term effects or mesocosm tests which can represent community testing; (iv) identification and measurement of ES in order to ascertain quality of the soil, such as decomposition or competition and to protect different species; (v) how a cocktail of similar or different chemicals/stressors impact soil invertebrates? And finally (vi) how to integrate spatial and temporal differences of landscape in terrestrial ecotoxicology.

Advances in Methods to Evaluate Environmental Degradation and Persistence

3.01.02

Biodegradation Kinetics of Industrial Chemicals in the Presence or Absence of Substrate Mixture

H. Birch, DTU Environment / DTU Environment; P. Mayer, Technical University of Denmark / DTU Environment

In the environment, biodegradation of xenobiotic chemicals occurs in the presence of other chemicals, whereas laboratory biodegradation tests are often conducted with one substance at a time. The aim of this study was to investigate the effect of presence or absence of an additional test mixture on biodegradation kinetics of individual chemicals. One experiment was conducted in June, and one in August; the first consisting of four parallel biodegradation tests containing one chemical each and one test containing a mixture of 18 chemicals, the second experiment containing five single chemical tests and a mixture test. A large number of test systems were prepared in 20 mL headspace vials using water from a Danish stream as inoculum for biotic test systems and ultrapure water for abiotic test systems. Stock solutions in ultrapure water was prepared directly for the more water soluble chemicals and by equilibrium passive dosing from a silicone rod for hydrophobic chemicals. These were used to spike test systems at concentrations from 0.002-7.4 mg/L for the different test chemicals. Test systems were then incubated at 12°C for 1-30 days. At seven time point, triplicate biotic and abiotic test systems were analyzed using direct immersion SPME coupled to GCMS. The ratio between abiotic and biotic signal response was used to fit a first order degradation with lag phase (substrate depletion). The two inoculum samples were comparable in sampling temperature (20 and 24°C), pH (6.5 and 7.4), oxygen content (9.0 and 7.4 mg/L), dissolved non-volatile organic carbon (4.4 and 5.7 mg/L) and heterotrophic plate count ($1.3 \cdot 10^3$ and $2.1 \cdot 10^3$ CFU/mL after 72 h at 20°C). Oxacycloheptadec-10-en-2-one, decan-5-olide, phenethyl benzoate and α-ionone degraded similarly in all three tests (single and two mixture tests) or slightly faster in the single chemical test. Octan-2-one had a similar lag-phase in the two mixture tests, but a shorter lag-phase in the single chemical test. Methyl 2-naphthyl ether did not degrade in the mixture test, but degraded rapidly between day 25 and 30 in the single chemical test. (+)-Menthone was also only degraded at the last two time-points in the single chemical test. However, methyl N-methylanthranilate was only degraded in the two mixture tests. The mixture effect on biodegradation of individual chemicals was therefore chemical specific included positive, negative, or no effects.

3.01.03

Pesticide Mixtures and Their Environmental Fate in Soil

C. Wijntjes, RWTH Aachen University / RWTH Aachen - Institute for Environmental Research; S. Hoger, Y. Weber, W. Völkel, D. Adam, Innovative Environmental Services (IES) Ltd; A. Schäffer, RWTH Aachen University / Institute for Environmental Research

Soil microbial communities which are essential in preserving productivity in soil ecosystems can be disturbed by a variety of environmental variables, including pesticides. Although fungicides are reckoned as effective crop protection chemicals in modern agriculture, as a side-effect due to their wide assassination and inhibitory capabilities, broad-spectrum fungicides play a dominant role in alteration of soil microflora. In the production of agricultural crops, the successive application of distinct pesticides, or mixtures of them, is a frequent practice and tank mixtures including two or more individual chemical compounds are preferred by farmers as they save fuel and labor hours, while causing less soil compaction and obtaining a larger pest control range and efficacy when compared to single chemical compound application. Consequently, when the soil or aquatic microbial composition is harmed or altered due to the presence of *e.g.* fungicides, the environmental fate of co-existent chemicals with degradation behavior dependent on biotic abundance might also be affected and hence, the degradation rate of such compounds might be inhibited and its dissipation half-life in the environment could be hazardously enlarged. However, currently installed international Guidelines for testing of chemicals are designed for examination and risk assessment of a single chemical compound per study and respective test system. While mixtures have been thoroughly studied concerning their agronomic efficacy, possible cumulative or synergistic effects and interactions between individual mixture compounds in the environment, as well as indirect side-effects such as alteration of soil, water and sediment microbial communities have only very rarely been investigated. As of 2019, in conjunction with RWTH Aachen University, Germany, Innovative Environmental Services (IES) Ltd, Switzerland, has been investigating the potential environmental impact of tank mixtures applied to soil and aquatic sediment. For this presentation, the effect of broad spectrum fungicides on herbicidal degradation in various US American and German soils has been studied under controlled laboratory conditions in accordance with the OECD 307 Guideline.

3.01.05

Comparison of Approaches for Volatilisation Correction in Degradation Simulation Tests

E. Uotila, M. Vega, ERA-Consult

The evaluation of degradation and persistence in the environment is an important aspect of the hazard and risk assessment of chemicals and an effective chemicals management. Higher tier degradation data will be needed for more substances as the European Commission plans to introduce persistent, mobile and toxic substances as a category of SVHC in REACH. This means that more substances with difficult to test properties, e.g. volatility, will be tested in degradation simulation studies, which may difficult the interpretation of the results. In simulation tests it is crucial to differentiate losses from the test system due to degradation and dissipation. . FOCUS Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (2014) includes correction procedures to account for volatilisation in the kinetic analysis. According to this guidance, volatilisation correction is most straightforwardly derived by considering a parent compound that is subject to an overall rate of loss from the water-sediment system by degradation and volatilisation, and that each loss process is described by SFO kinetics. In this case, kTOT, kVOL and kDEG are the SFO rate constants for the total overall loss from the water-sediment system, and the losses by volatilisation and by degradation, respectively. Assuming that: $k_{TOT} = k_{VOL} + k_{DEG}$, then k_{DEG} can be estimated simply from the difference between these two parameters by re-arranging the above equation. In this work different volatilisation correction approaches used in simulation studies will be searched for, and the following approaches will be compared by applying them in the calculation of DegT50 for two substances, with high and medium volatility: A. a) Fitting volatilisation and whole system data separately with CAKE in OECD 308 (FOCUS, 2014), B. b) Simultaneous fitting of whole system and volatilisation data in OECD 308 using Model Maker (Jene B. 2007b as cited in Annex XV CLH report, 2018) , and C. c) Simultaneous fitting of soil and volatilisation data in CAKE using a model by Shrestha et al (2019) for OECD 307. Annex XV CLH report, 2018: Proposal for Harmonised Classification and Labelling based on CLP Regulation. Pendimethalin. Submitted by the Netherlands, Feb 2018. FOCUS Guidance, 2014. Shrestha et al, 2019. Biodegradation of Volatile Chemicals in Soil: Separating Volatilization and Degradation in an Improved Test Setup (OECD 307). Environ Sci Tech, 2019 53(1)

3.01.08

Improving Available Guidance for Persistence Assessment of Substances (CEFIC-LRI ECO52) - Complex and Difficult Test Substances

C. Hughes, Ricardo Energy & Environment / Chemical Risk; D. Brown, M. Griffiths, E. Delouvrier, Ricardo Energy & Environment / NCEC; S. Hahn, Fraunhofer ITEM / Chemical Safety and Toxicology; D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry; G. Whale, Whale Environmental Consultancy Limited

Chemical persistence plays a key role in the determination of environmental exposure making it an important component in risk assessment and regulation. However, existing frameworks under which persistence is assessed have shown some limitations. Chemical degradation rates are prone to wide variability depending on environmental conditions, which is difficult to address in a weight of evidence approach. In addition, many substances are problematic, or fall outside the applicability domain of existing frameworks due to their specific characteristics. These include substances that are difficult to test, substances of unknown composition, complex reaction products or biological materials (UVCBs), and polymeric substances, including microplastics. Finally, evaluating degradation half-lives using a compartment-by-compartment approach is overly simplistic, and neglects dynamic multimedia exchanges and degradation processes that may have an important bearing on the overall persistence of a substance in the environment. This European Chemical Industry Council Long-Range Research Initiative (CEFIC-LRI) project aims to address these issues by improving available guidance for persistence assessment of substances, including those considered problematic to evaluate under the current frameworks. This poster will present an update on the development of guidance for difficult test substances and substances of unknown composition, complex reaction products or biological materials (UVCBs).

Difficult test substances present challenges when applying standard testing protocols due to their physicochemical properties, while UVCBs are complex and variable in nature, presenting significant challenges in their characterisation, testing and regulatory evaluation. The guidance will consolidate recent improvements to test protocols that have increased the success of testing substances with some of these challenging characteristics. A guiding principle for both types of substance will be test substance bioavailability, a confounding factor when assessing biodegradation and persistence. Furthermore, recent developments in the approaches for assessing UVCBs will be incorporated. It is anticipated that this guidance will improve future assessment of substances and increase the applicability of persistence frameworks to encompass a broader range of substance types.

3.01.09

Application of Inoculum Preconditioning and Metagenomics Analysis to Address Variability Challenges and Improve Understanding of Microbial Dynamics During OECD 301F Ready Biodegradation Testing of Petrochemical Substances

L. Camenzuli, ExxonMobil; B. Hedgpeth, ExxonMobil Biomedical Sciences, Inc. / Toxicology and Environment Science Division; J. Arey, EMBSI; K.M. McFarlin, J. Zukowski, B. Kelley, ExxonMobil Biomedical Sciences Inc

The OECD 301 Ready Biodegradation test is used to meet data requirements for environmental hazard classification, including persistence assessment. It is designed to assess the extent of chemical mineralization under aerobic conditions within 28 days. Although the test is designed to provide a conservative estimate of biodegradation, it sometimes exhibits high intra-replicate and test to test variability, which suggests that the method may be poorly optimized. For these tests, an inoculum preconditioning method was used to determine the impact to ready biodegradation test results for a range of petrochemical substances. Preconditioning consisted of aerating the inoculum in mineral medium for 5-7 days prior to test initiation. The OECD 301F test was conducted with and without preconditioning to 13 different petrochemical substances spanning six chemical categories. Genomics analysis was conducted on inocula at test initiation and termination for one of eight chemicals which demonstrated improved biodegradation (C20-30 branched alkane). Results indicate inoculum preconditioning improved biodegradation in 8/13 tests, elicited no change in biodegradation in 3/13 tests, and decreased biodegradation in 2/13 tests. Preconditioning improved intra-replicate variability by reducing the range of replicate values in 10/13 tests. From genomics data, a principal component analysis indicated that preconditioning produced a microbial community distinct from non-preconditioned samples and reduced relative abundance variability within the microbial community. Phylogenetic diversity measurements indicated no statistically significant difference in diversity when comparing preconditioned to non-preconditioned inocula, at either test initiation or test termination. A known alkane degrader (Gordonaceae) was identified along with an increase in relative abundance amongst samples which demonstrated biodegradation, regardless of preconditioning status. This study indicates preconditioning positively impacted biodegradation and replicate variability for most of the substances tested. Genomics analysis indicated decreased heterogeneity in microbial abundance, though this occurred in the absence of a significant shift in the phylogenetic diversity index. Preconditioning did not impair hydrocarbon degradation, which may evidence the ubiquity of populations capable of using hydrocarbons as their sole or major carbon source.

3.01.12

Marine Enrichments Using Several Novel Plastics Result in Different Plastic-Degrading Assemblages

V.C. Mattelin, Ghent University (UGent); K. Kundu, N. Boon, Ghent University - LabMET

Plastic pollution is a notorious environmental problem for which no robust solution is found so far. Recent microbial research explores the microbiomes present in plastic-rich natural environments. Therefore, most attention goes to microorganisms associated with synthetic plastics. Synthetic plastics are not made to be biodegradable and are likely to persist in nature, nonetheless microbial colonization is observed. Even commercially available biodegradable plastics are only degraded efficiently in some habitats. Understanding the polymer structure – degradation relationship in different habitats and developing novel biodegradable plastics is of utmost importance. We hypothesize that designing novel plastics to be prone to the metabolisms of plastisphere organisms will eventually allow us to reduce plastic pollution. A crucial step in this designing process is to understand how specific microbial species and polymers interact with each other. To study these interactions, we performed an enrichment experiment with a variety of novel and unique plastics (as sole carbon source) in seawater with different nutrient and temperature conditions. We measured gas composition and cell growth throughout time. Both measurements were dependent on nutrient concentration and plastic type. The degradation potential of the enriched microbial communities was reflected by cell growth. Besides phenotypic fingerprinting by flow cytometry, we performed a 16S rRNA gene sequencing on the stable enriched communities to understand how nutrient concentration and plastic type influenced the microbial community composition. The taxonomic composition of microbial communities in the enrichments confirmed the dependency on nutrient addition and plastic type. This suggests a “specialist” microbial population for specific plastic-type. Further steps are to elucidate the degradation enzymes, and pathways to break down different types of plastic material in the marine environment.

3.01.16

New Formulations to Reduce the Pesticide Residues in Tomatoes

M. Yalcın, Adnan Menderes University / Faculty of Agriculture; C. Turgut, Adnan Menderes University / Environmental Toxicology and Biotechnology

Tomato (*Solanum lycopersicum* L.) is one of the most popular and widely grown vegetables in the world. Many pests are fed on tomatoes and cause crop losses very intensively during the production. Although many different methods are used to control pests in tomatoes, pesticide usage is preferred mostly. That’s why it is vital to evaluate the pesticide residue removal rate. The present study aimed to determine different formulations' possible role in the degradation of acetamiprid and boscalid. Three enzyme-based formulations which are FR1, FR2, and FR3 used in the tomato greenhouse experiment. Experiments were conducted in Aydın province according to the random blocks trial design which is refining by the Republic of Turkey Ministry of Agriculture and Forestry. Each experiment was replicated three times. Insecticides were applied as recommended doses with a knapsack sprayer. Two hours after pesticide application formulations were applied. After 3 days passed 1 kg sample was taken from each plot. In the next step, the samples were transferred to the laboratory in the steril bags. QuEChERS multi-residue extraction method was used to prepare the samples for the analyses and analyses were utilized by LC/MS-MS instrument. According to the results of the acetamiprid experiment, there was not any significant difference between formulation effectiveness for pesticide removal. But in contrast to the acetamiprid results boscalid removal was observed by using FR2 formulation. It thus seems that the active applicability of the FR2 formulation can be recommended to be used as a commercial product for pesticide removal.

3.01.18

Identifying Chemicals of Global Concern in 2021 and Beyond: A Canadian Perspective

M.A. Bonnell, Environment and Climate Change Canada / Ecological Assessment Division

While efforts continue to identify new PBT and POP-like substances in chemical inventories and the environment, there are few studies or regulatory paradigms that trend away from standard “hazard” criteria and use or suggest other metrics or descriptors, such as persistent, mobile and toxic substances (PMTs), to identify chemicals of concern. In Canada, prioritization of organic substances for further evaluation has used a multi-descriptor weight of evidence approach known as the Ecological Risk Classification (ERC) system since 2016 (ECCC 2016). The second version of the system (ERC2) was developed in 2018 to incorporate confidence and severity scoring (Bonnell 2018). One of the benefits of both the ERC approaches is that they are risk-based allowing both hazard and exposure potential to be evaluated. The ERC approaches incorporate descriptors to identify highly potent chemicals at different spatial and temporal scales of exposure with one of its goals to target substances that have the potential to affect the *planetary boundary*. The planetary boundary concept was first described by Rockstrom et al. (2009) and later extended to identifying chemicals that are threats to the planetary boundary by MacLeod et al. (2014). This work then lead to recommendations for technological and societal changes to avoid global chemical pollution by Diamond et al. (2015) and an approach for prioritizing chemicals based on this concept by Reppas-Chrysovitsinos et al. (2019). ERC2 examines a specific aspect of the planetary boundary chemical threats described by situation C2-2 and C3-2 in MacLeod et al. (2014). This scenario describes a threat from persistent and mobile substances (and their transformation products) that have a hazard potential capable of causing permanent genetic damage in exposed organisms the effects of

which may also be expressed in subsequent generations (i.e., epigenetic inheritance). This type of effect is known to be "irreversible or poorly reversible" and result in a "regime shift" in populations (MacLeod et al. 2014). The impact of this type of chemical profile is that local and global effects continue after emissions have ceased due to regulatory or non-regulatory actions. In other words, as cited in MacLeod et al. (2014) *effects are distributed widely across populations and are independent of global or local concentrations* (MacLeod et al. 2014). Concentrations in the far field, normally quite diluted compared to near field or local concentrations, become highly relevant because exposure at very low concentrations can result in adverse effects (i.e., very narrow margin of exposure). ERC2 prioritizes substances of this type as "chemicals of global concern" (CGC) where the risk can scale up to the far field. A case study with ~200 substances will be presented to show how ERC2's rule-based logic classifies substances as a CGCs. Rules for classifying hazard (e.g., chemical reactivity and genotoxicity) as well as exposure (e.g., response time, mobility, foodweb exposure) and risk as well as the confidence associated with these outcomes will included.

3.01.19
Improving Available Guidance for Persistence Assessment of Substances (CEFIC-LRI ECO52) - Sensitivity Analysis to Identify Key Modelling Input Parameters for Overall Persistency

J. Klein, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecotoxicology; M. Klein, Fraunhofer IME / Ecological chemistry; S. Hahn, Fraunhofer ITEM / Chemical Safety and Toxicology; D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry; C. Hughes, Ricardo Energy & Environment / Chemical Risk; G. Whale, Whale Environmental Consultancy Limited

Chemical persistence plays a key role in the determination of environmental exposure making it an important component in risk assessment and regulation. However, existing frameworks under which persistence is assessed have shown some limitations. Chemical degradation rates are prone to wide variability depending on environmental conditions, which is difficult to address in a weight of evidence approach. In addition, many substances are problematic, or fall outside the applicability domain of existing frameworks due to their specific characteristics. Finally, evaluating degradation half-lives using a compartment-by-compartment approach is overly simplistic, and neglects dynamic multimedia exchanges and degradation processes that may have an important bearing on the overall persistence of a substance in the environment. This CEFIC-LRI project aims to address these issues by improving available guidance for persistence assessment of substances, including those considered problematic to evaluate under the current frameworks. This poster will present how fate modelling can support the guidance on persistence assessment. Multimedia fugacity box models (Level IV) and the overall persistency approach is used to get a better understanding on persistence assessment methodologies. A one-at-a-time (OAT) sensitivity analysis is performed for both, environmental and substance specific model parameters, to identify important model parameter influencing overall persistency of the substance and other reasonable endpoints such as recovery time. This includes the consideration of several different emission scenarios. The aim is to evaluate how multimedia modelling and overall fate can be incorporated to improve the robustness of current persistence assessment methodologies.

3.01.20
Developing Guidance and Recommendations for Using Overall Fate and Monitoring Data in a Weight-Of-Evidence for Assessing Petroleum Hydrocarbon Persistence Under REACH

T.N. Brown, TNB Research; A. Sangion, University of Toronto Scarborough / Department of Physical and Environmental Sciences; L. Li, University of Nevada, Reno / School of Community Health Sciences; J.M. Armitage, AES Armitage Environmental Sciences, Inc / Physical and Environmental Sciences; L. Toose, ARC Arnot Research and Consulting. Inc; T. Gouin, TG Environmental Research; X. Zhang, Ontario Ministry of the Environment, Conservation and Parks; F. Wania, University of Toronto, Scarborough / Department of Physical and Environmental Sciences; J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology

Typical methods for assessing the indication of persistent (P) and very persistent (vP) are the comparison of degradation half-lives against pass/fail criteria. It has been suggested that evaluating "P" is only relevant for a medium if the fraction of chemical mass distributed in the environment exceeds 0.05 of the total. The overall persistence (P_{OV}) integrates the effects of chemical distribution and different "P" criteria in multiple media and has been advocated by organizations such as the SETAC and the OECD. According to REACH guidance, chemical fate information can be used to identify specific compartment(s) of concern, and as such, chemical fate information may be used as supporting information in a weight of evidence (WoE) approach for "P" assessment. Petroleum-derived substances include a wide number of individual hydrocarbon constituents (~15,000 possibly present structures). To help address the structural complexity of petroleum substances, various modelling approaches have been developed adopting the hydrocarbon block (HCB) approach. This research project develops guidance and recommendations for combining environmental fate modelling, experimental and predicted degradation data, and monitoring data to infer P_{OV}, which can used in a WoE approach for "P" assessment and for improving "P" assessment of hydrocarbons. The guidance and recommendations arise from: (i) collecting monitoring data, physicochemical properties and degradation half-life data for hydrocarbons; (ii) applying a multimedia environmental model to calculate the fate and P_{OV} of individual hydrocarbons and hydrocarbon blocks (HCBs); (iii) examining the key factors affecting chemical fluxes of these substances in the environment including emission estimates; and (iv) comparing monitoring data and model output to determine whether monitoring data could provide additional information on environmental fate processes and "P" assessments. The results were analysed by conducting case studies of specific regions with detailed monitoring data for individual hydrocarbons, and by Europe-wide regional modelling combined with all available monitoring data. The data were also processed using a chemical space approach to relate P_{OV} to other chemical properties.

3.01.21
Eas-E Suite: An Integrated Platform to Facilitate Hazard, Exposure, and Risk Assessment

A. Sangion, University of Toronto Scarborough and ARC Arnot Research and Consulting Inc. / Department of Physical and Environmental Sciences; L. Li, University of Nevada, Reno / School of Community Health Sciences; J.M. Armitage, AES Armitage Environmental Sciences, Inc / Physical and Environmental Sciences; L. Toose, ARC Arnot Research and Consulting. Inc; T.N. Brown, TNB Research; J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology

For most chemicals requiring hazard, exposure and risk assessment, information is limited, unstandardized and documented across multiple databases (e.g. CompTox, ChEMBL, PHYSPROP database). Environmental persistence is a key property for hazard classification ("P or not P") and for fate and exposure modelling. The persistence of organic compounds is driven by the rates at which they are removed by biological and chemical processes, such as biodegradation, hydrolysis and atmospheric oxidation. Few publicly available databases and tools (e.g. BIOWIN) exist to estimate degradation processes, rates and half-lives, however, these are not user-friendly or easily-usable. Most importantly they are not integrated, meaning that scientists and regulators need to gather information from many diverse sources before being able to perform a comprehensive environmental risk assessment.Here we introduce the Exposure And Safety Estimation (EAS-E) Suite platform to bridge the gap between evolving scientific research and regulatory assessment challenges. EAS-E Suite is comprised of databases, quantitative structure activity relationships (QSARs) and tools to aid chemical assessment decision-making. EAS-E Suite includes curated, measured physical-chemical properties, environmental biodegradation and biotransformation half-lives for thousands of chemicals. EAS-E Suite also houses multi-media mass-balance environmental fate, bioaccumulation and exposure models as well as the Chemicals in Products - Comprehensive Anthropospheric Fate Estimation (CiP-CAFÉ) model for estimating life cycle chemical mode-of-entry and emission rates. EAS-E Suite currently includes the Risk Assessment IDentification And Ranking (RAIDAR), and RAIDAR-Indoor and Consumer Exposure (RAIDAR-ICE) models for simulating chemical fate in natural and indoor environments and aggregate exposures to representative ecological receptors and humans. With entry of only chemical structure as an input parameter, a weight of evidence approach considering existing QSAR predictions and extrapolation methods is used to provide environmental degradation half-lives in air, water, soil and sediment, overall persistence (POV) and provides screening-level uncertainty estimates for biodegradation half-lives in water. An overview of existing and emerging databases,

models and tools in EAS-E Suite is presented with a focus on addressing data gaps and uncertainty for chemical persistence.

Air Pollution, Exposure Assessment, and Health Effects

3.02.04
Endocrine Activity of Semivolatile Organic Chemicals in Indoor Air
A.K. Halse, E. Mariussen, NILU - Norwegian Institute for Air Research; P. Bohlin-Nizzetto, NILU - Norwegian Institute for Air Research / MILK - Environmental Chemistry Department
Air may act as a source for human exposure to organic pollutants, and we spend as much as up to 90 % of our time in indoor environment making the indoor air of special importance. The indoor environment contains various materials and products which may contain organic pollutants, e.g. halogenated flame retardants, bisphenols, chlorinated paraffins. These compounds may, over time, be emitted to indoor air. These compounds may be toxic, persistent, bio-accumulative and inherent endocrine disruptive potential. In this study we aimed at identifying sites (i.e. residential/public buildings) with elevated levels of organic pollutants and to further elucidate whether their presence can pose endocrine and/or genotoxic disrupting potential. A selection of air sampling devices was co-deployed at five indoor locations in Tromsø (69.6437°N, 18.9474°E). Three locations were located inside the high north Research Centre for Climate and the Environment (Fram Centre), and two locations were private households. At the locations in Fram Centre, two types of commonly used passive air samplers (PAS) were deployed: i) XAD-PAS and ii) polyurethane foam (PUF)-PAS, together with a low volume air sampler using ABN as adsorbent. In the private households, only the two PAS samplers (XAD-PAS and PUF-PAS) were deployed. Duplicate samplers were deployed side by side at all locations, to enable chemical analysis and toxicity testing on the same type of air (same exposure) in parallel. The PAS samplers were deployed for 1 month, while the low volume sampler collected air for approximately 24 hours. One of the duplicates were used for targeted chemical analysis of chlorinated paraffins (SCCP and MCCP), novel flame retardants, polycyclic aromatic hydrocarbons (PAHs) and bisphenols. The other duplicate was used for toxicity assay to assess the estrogenic activity with U2-OS CALUX (BDS-company) assay, which is a cell line expressing the endogenous estrogen receptors. Based on the results of the chemical analysis and the CALUX toxicity assay, some samples were also subjected to DNA-damage assay (genotoxic activity), with use of the comet assay. We link the estrogenic activity with the chemical analysis.

3.02.07
The Influence of Aqueous Phase Processes and Their Seasonality on Oxidative Potential of Fine Aerosols and Formation of Reactive Oxygen Species in the Lung Lining Fluid
P. Shahpoury, Environment Canada / Air Quality Processes Research Section; A. Arangio, Ecole Polytechnique Fédérale de Lausanne (EPFL) / School of Architecture, Civil and Environmental Engineering; V. Celo, Environment and Climate Change Canada / Analysis and Air Quality Section; E. Dabek, Environment and Climate Change Canada / Air Quality Research Division; L. Greco, T. Harner, Environment and Climate Change Canada / Air Quality Processes Research Section; A. Nenes, Ecole Polytechnique Fédérale de Lausanne (EPFL) / School of Architecture, Civil, and Environmental Engineering; Z. Zhang, Environment and Climate Change Canada / Air Quality Processes Research Section
Air pollution is a global health risk. The inhalation of particulate matter with aerodynamic diameter ≤2.5 μm ($PM_{2.5}$) has been associated with respiratory-cardiovascular diseases and increased mortality. Oxidative potential is defined as the ability of PM-bound chemicals to oxidize the lung antioxidants directly or through catalytic generation of reactive oxygen species (in particular OH radical, which is a strong oxidizing agent). This process may result in oxidative stress, inflammation of the epithelial tissue, and chronic diseases. Among PM components, transition metals Fe and Cu are well known for their contribution to OH radical formation through Fenton reactions; the oxidation state of these metals and their water-solubility play important roles in this process. The solubility of PM-bound metals is affected by the aerosol aqueous phase processes which influence aerosol acidity and metal complexation with organic ligands. In this work, we investigated the influence of aerosol aqueous phase processes and their seasonality on the ability of $PM_{2.5}$ to generate OH radical in a simulated lung lining fluid (SELF). The fluid consists of an aqueous solution containing lung antioxidants, inorganic salts, and a molecular probe that acts as scavenger of OH radical. The $PM_{2.5}$ samples were collected from the Canadian National Air Pollution Surveillance sites in Toronto and Hamilton. The two sites are characterized by intense traffic and industrial (steel manufacturing) emissions, respectively. The samples were subject to extensive chemical speciation including the near-total and water-soluble transition metals, ionic species, organic and black carbon, biomass burning markers, and acidic and basic gases. The $PM_{2.5}$ oxidative potential and OH radical formation were quantified in SELF using a new bioanalytical method. In addition, the aerosol pH and liquid water content were calculated using ISORROPIA II model. The results showed a clear seasonal pattern in water solubility of transition metals, especially Fe, which corresponded with an increase in the concentrations of organic ligands and reduction in aerosol acidity in the spring-summer period. These features were influenced also by emission sources, with the industrial site showing noticeably higher Fe solubility and less dependency on aerosol acidity, likely due to pyrogenic Fe emissions. Preliminary results showed that OH radical formation was enhanced by increase in Fe solubility related to the aqueous phase processes.

3.02.08
Importance of Local and Background Aerosol on Indoor Air Quality: A Study of the Office Air
J. Pauraite, A. Minderyte, T. Gill, K. Plauškaitė, I. Garbarienė, V. Dudoitis, State research institute Center for Physical Sciences and Technology / Department of Environmental Research; J. Ovadnevaitė, National University of Ireland; S. Kecorius, Leibniz Institute for Tropospheric Research; G. Mainelis, RUTGERS UNIVERSITY; S. Byčenkienė, State research institute Center for Physical Sciences and Technology / Department of Environmental Research
High levels of air pollutants have a negative impact on environmental and human health. Outdoor air quality largely depends on aerosol sources in both local and distant areas. In order to take actions in reducing personal exposure, measurement of outdoor and indoor aerosol concentrations and the assessment of their sources are essential. 7-wavelenght Aethalometer (AE31) (Magee Scientific) and Aerodynamic Particle Sizer (APS) (TSI 3321) were deployed in the building of FTMC main campus (urban background environment) office room between 30th September and 10th November, 2020. Instruments were operating for both outdoor and indoor measurements. Room air was treated with G4 pre-filters and F7 filters, which are typical for office air supply. 'Aethalometer model' was used to separate black carbon (BC) released from fossil fuel combustion (BC_{ff}) and biomass burning (BC_{bb}). During measuring campaign a significant increase of BC_{bb} was registered due to air mass transfer from wild fires in Ukraine. Furthermore, the same source was linked with an increased particle number concentration of 1-4 μm size particles. Observed ratios between BC_{bb} and BC_{ff} in indoor and outdoor air were the highly similar. It was estimated that indoor air was filtered insufficiently (< 44.1%) for PM_1 fraction particles. Wild fire related particles due to larger particles size showed a lower impact on indoor air quality than local. Thus, due to poor filtering of local emissions improved office air filtering standards could be implemented.Acknowledgments. This research was funded by a grant (No. S-MIP-20-28) from the Research Council of Lithuania.

3.02.09
Identification of Smoke From Fire Event in the Indoor Air
J. Pauraite, I. Garbarienė, T. Gill, K. Plauškaitė, S. Byčenkienė, State research institute Center for Physical Sciences and Technology / Department of Environmental Research
To this day importance of outdoor PM levels to the indoor air data is poorly investigated. While widely used office filtering systems help to dramatically remove coarse particles, the fraction of submicron particles often remains not monitored. Outdoor and indoor carbonaceous particles were measured by the Aerosol Chemical Speciation Monitor ACSM (Aerodyne Research, Inc., Billerica, MA, USA) and Aethalometer TM, Model AE31 Spectrum, manufactured by Aerosol d.o.o., Slovenia. Time resolution of instruments were ~30 and 2 minutes, respectively. While ACSM measures main chemical composition of PM_1, Aethalometer based on optical transmission of carbonaceous aerosols provides data of black carbon (BC) mass concentration. Measurements were deployed in the capital city Vilnius (Lithuania) during April 25-26 and April 26-29 (2019) in outdoor and indoor data, respectively. For indoor measurements FTMC campus room was selected which could be characterized as typical office. Air supply and filtering system consisted of G4 pre-filters, F7 and F9 filters. During measurement campaign several fire events occurred in the neighborhood of Vilnius. The aim of the study was to identified smoke particles in the office air and compare it to

smoke from the outdoor data. Aerosol mass spectra was separated to more oxidized aerosol and biomass burning aerosol which showed a great similarities between those from outdoor and indoor measurements (θ=10.85 and θ=16.30, respectively). PM_1 in both outdoor and indoor air was dominated by organic matter (83% and 71%, respectively). BC source apportionment showed that BC from biomass burning (BC_{bb}) contribution to BC was the highest for both outdoor and indoor environments (68% and 67%, respectively). Thus, the impact of neighborhood fire event were significant in indoor air.Acknowledgments. This research was funded by a grant (No. S-MIP-20-28) from the Research Council of Lithuania.

3.02.11

Aerosol Formation and Semi-Volatile Organic Compound Concentrations in Car Cabins - Influence of Temperature and Ventilation

J.K. Lexén, Volvo Car Corporation / Sustainability Centre; C. Gallampois, Umea University / Department of Chemistry; M. Bernander, Volvo Car Corporation / Sustainability Center; J. Vanhanen, Airmodus; P. Haglund, Umea University / Department of Chemistry; A. Sebastian, Volvo Car Corporation / Sustainability Center; P.L. Andersson, Umea University / Department of Chemistry

Car cabins are complex microenvironments containing several different materials such as polymers, textiles and leather. Semi-volatile organic compounds (SVOCs) are used as additives in these materials to enhance material performance and are mixed into the materials without forming chemical bonds to the material as such. They can thus be emitted from the materials at a slow release rate to the surrounding and can form particles or adsorb to airborne particles, settled dust and surfaces in the car cabin. Along with that, humans using cars are exposed to these SVOCs. Exposure to some SVOCs can cause adverse human health effects, such as endocrine disruption, cancer, neurotoxicity and developmental effects. In this study, we measured concentrations of 13 endocrine disrupting SVOCs, by collecting air samples covering gas phase and airborne particles. Three different cars were sampled using low volume active samplers consisting of a glass fiber filter followed by two polyurethane foam plugs (PUFs). During sampling, the cars were placed indoors in a test chamber equipped with UV-lamps where it is possible to control temperature and humidity. SVOC concentrations where measured at four different interior temperatures (25, 40, 60 and 80 °C). At 25 °C, samples were collected with ventilation off and on, to simulate conditions in a parked car versus during driving. Samples, both filters and PUFs, were extracted using three rounds of sonication and dichloromethane (20 ml), extracts were evaporated and analyzed with GC-MS and LC-MS. During the entire sampling campaign, particle concentrations (#/cm^3) were recorded every minute for particles in the size ranges 1.3 – 1000 nm and 10 nm – 35 µm using an Airmodus A11 Nano Condensation Nucleus Counter (nCNC) and a Grimm Mini-Wras 1371 respectively. Nine of the 13 SVOCs were detected in both gas phase and airborne particles, while two SVOCs were only detected in airborne particles. Concentrations increase with temperature, and some of the SVOCs were only detected at high temperatures. The particle instruments used provide high time resolution of data compared to the active sampling, allowing us to see what we believe is secondary aerosol formation from gas phase SVOCs to particulate phase. Also, growth from sub 2 nm cluster size to >4 nm particles was detected. When turning on the ventilation, a rapid decrease in particle concentration was observed, but no uniform trend was observed for the SVOC concentrations.

3.02.12

Using Isotope Fingerprints to Track Sources of PM2.5 in Air Pollution

M. Tuthorn, Thermo Fisher Scientific; C. Brodie, Thermo Fisher Scientific GmbH; J. Renpenning, Thermo Fisher Scientific / CMD

The natural and synthetic materials in our air, water and on our land have a fingerprint, a unique chemical signature that allows them to be identified and differentiated from one another. Consequently, we can interrogate samples, chemically, to determine where they came from, allowing measures to be developed and executed to reduce and remove environmental pollution. In this work, we focus on air pollution caused by sub-micron particles (PM2.5) in air in the form of sulfate aerosols and nitrous oxides derived from fossil fuel burning, ozone and volatile organic compounds. PM2.5 particles were collected on quartz microfiber filters in Seoul, South Korea and analysed by Isotope Ratio Mass Spectrometry. We explain the analytical technique and how acquired stable isotope fingerprints of carbon, nitrogen and sulfur are used to identify the origin of air pollution.

3.02.13

Modeling and Transport of Volatile Organic Compounds in the Atmosphere From the Chimneys of the Matosinhos Refinery - Risk Analysis for Nearby Hospital Populations

M. Vila, Faculty of Engineering - University of Porto; F. Mahú, University of Porto - Faculty of Engineering

In this work it is made an evaluation of the dispersion of volatile organic compounds (VOC) from the chimneys in a refinery, in Matosinhos, at two hospitals in different relative positions to the chimneys. Two different methods were used to estimate the VOCs (BTEX) concentration in the air, which were then programmed in MatLab. The first program based on the box type model allows the study of the VOC concentration either in the air and other sub-compartments. Based on this information, it is also possible to study the inhalation dose and the risk (based on toxicological and carcinogenic properties) to which the permanent (such as hospital staff) and temporary (such as users of these hospitals) population is exposed. The second method account with the Pasquill equation modified by Gifford in the transient state. The results obtained allows a detailed understanding of the evolution of the released plume. The wind is a decisive factor in the dispersion of pollutants, but the importance of other factors such as its distance to the point of emission, and the stability of the meteorological conditions of the site, is also evident.

Assessment of the Exposure and Effects of Contaminants of Emerging Concern in Drinking and Wastewater Systems and Assays to Evaluate their Removal

3.03.03

Lanthanides in Croatian Wastewaters

. Fiket, N. Udikoviń-KoliDŽ, RuDŽer BoxkoviD Institute

The distribution of lanthanides in wastewaters collected at the outlet of the municipal wastewater treatment plants receiving the wastewater from different Croatian cities was investigated. Seven cities of different population sizes, from 15 000 to 800 000 inhabitants, were chosen for this study. The total concentration of 14 lanthanides (Ce, La, Nd, Pm, Dy, Er, Eu, Gd, Ho, Lu, Sm, Tb, Tm, Y, and Yb), including Y, (REY) were analyzed in collected wastewater samples by High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS). Obtained REY levels in samples were in accordance with previously published data for other major cities in Europe. Additionally, the intensity of positive Gd anomalies observed in wastewaters indicates an influence of pharmaceuticals on the urban wastewater load comparable to larger European cities. The obtained data also confirm the inability of their efficient removal during wastewater treatment and purification processes.

3.03.04

Anticoagulant Rodenticides in the Aquatic Environment

J. Regnery, M. Brinke, S. Schaefer, G. Reifferscheid, Federal Institute of Hydrology / Biochemistry and Ecotoxicology; A. Friesen, German Environment Agency UBA / Section IV 1.2 Biocides

Anticoagulant rodenticides (AR) are used worldwide to control commensal rodents for hygienic and public health reasons. Despite the identified environmental risks within the biocidal active substance approval process, AR were authorized as biocidal products imposing strict conditions (risk mitigation measures) due to the lack of safe alternatives to control rodents. Although possible risks for the aquatic environment resulted from risk assessment for fish-eating birds and mammals via the aquatic food chain, no other information or experimental data were available to assess the occurrence of AR in aquatic ecosystems. According to exposure assessments, the predicted concentration of AR in surface water as a consequence of rat control in sewer systems was well below the corresponding ecotoxicological threshold values derived for aquatic organisms. The main aim of this study was to test the research hypothesis that rodent control in municipal sewer systems using AR can lead to emissions of active ingredients into receiving surface waters and thus contamination of aquatic organisms. The occurrence and fate of eight AR was monitored in samples collected from two different German wastewater treatment plants (WWTP) and adjacent receiving surface waters during and after rodent control measures with AR-containing bait in connected municipal sewer systems. Furthermore, a retrospective biological monitoring was carried out by analyzing fish tissue samples from different WWTP fish monitoring ponds in Germany that are exclusively fed by municipal effluents. Study results showed incomplete removal of AR during conventional wastewater treatment and confirmed the hypothesis regarding the

exposure of aquatic organisms via municipal WWTP effluents. It was demonstrated that the common sewer baiting practice, i.e., deployment of AR-containing bait by wire in manholes, contributes to the release of AR into wastewater and consequently into the aquatic environment. Findings also showed that second-generation AR active ingredients bioaccumulate in fish liver under environmentally realistic conditions and exposure scenarios. Thus, ecotoxicological consequences of chronic AR exposure to indigenous freshwater fish at concentrations relevant for surface water bodies need to be identified. As for most terrestrial species, a link between hepatic AR residue levels in fish and species-specific lethal or sub-lethal effect concentrations and their population relevance is still missing.

3.03.05

Relevance of Disinfection Byproducts (DBPs) for the ENvironmental Risk Assessement of Biocides

M. Huben, Fraunhofer IME / Ecological chemistry; M. Diehle, Umweltbundesamt; M. Herrchen, Fraunhofer IME / Ecological chemistry; M. Usman, Institute of Environmental Engineering, RWTH Aachen University; V. Linnemann, Institute of Environmental Enginnering RWTH Aachen University; M. Klein, Fraunhofer IME / Ecological chemistry; S. Hahn, Fraunhofer ITEM / Chemical Safety and Toxicology

Relevance of disinfection byproducts (DBPs) for the environmental risk assessment of biocides *M. Hüben[1], M. Diehle[4], M. Herrchen[1], M. Usman[2], V. Linnemann[2], M. Klein[1], S. Hahn[3] [1]Fraunhofer IME, Auf dem Aberg 1, 57392 Schmallenberg, Germany [2]Institute of Environmental Engineering, RWTH Aachen University, Mies-van-der-Rohe-Str. 1, 52074 Aachen, Germany [3]Fraunhofer ITEM, Nikolai-Fuchs-Straße 1, 30625 Hannover, Germany [4]Umweltbundesamt, Wörlitzer Platz 1, 06844 Dessau-Roßlau, Germany* Keywords: disinfection byproducts, biocides, regulation

Disinfectants are widely used biocidal products which are regulated by the EU Biocides Regulation 528/2012. Their scopes of applications are quite broad and include different fields such 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 byproducts (DBPs) are formed, especially if organic matter is present. The specific uses and the reaction conditions have great influence on the formation of DBPs. The intended use also determines possible releases of the formed DBPs into the environment. This complex situation is not adequately considered in the guidance within the European Biocides Regulation at the moment. 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. In order 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). 1. As a first step in this project, a list of those active substances has been generated which are potentially competent to form DBPs. 2. Based on a systematic literature search, already identified DBPs have been assigned to these active substances together with the conditions of use in the respective experiments. 3. For these combinations of active substances and their DBPs, the entry pathways into the environment have been investigated to decide on the relevance of DBPs for the ERA. At the moment, this list is reviewed by an experimental validation via chemical analysis of simulated product applications. The experimental setup and the analytical results of the validation experiments will be presented. *Characters text: 2133 (allowed: max. 2500)*

3.03.06

Laboratory Scale Simulation of Ozonation With the Example of Methyl-Desphenyl-Chloridazon

M. Floers, W. Seitz, W. Schulz, Zweckverband Landeswasserversorgung, Am Spitzigen Berg 1, D-89129 Langenau / Laboratory for Operation Control and Research; A. Michel, P. Cirpus, BASF SE / Environmental Fate

Oxidative treatment methods, like ozonation or chlorination, are valuable tools to the water industry to meet high quality standards for our drinking water. These methods contribute to the elimination of pathogens as well as chemical contaminants. The reaction of the oxidant with natural substances or anthropogenic contaminants present in the raw water can lead to the formation of transformation products (TPs), of unknown structure and toxicity. Concerning plant protection products (PPP), the Regulation 1107/2009 includes as a condition for approval that "the residues of PPP […] shall have no immediate or delayed harmful effects on human health […], directly or through drinking water (taking into account substances resulting from water treatment)." Up to now, no agreed guidance or testing procedure has been available to address the nature of the residues formed during water treatment. Recognizing this situation, the European Commission has initiated the development of a new Guidance. To reduce the need for unnecessary testing, especially vertebrate testing, and prioritize the efforts on possible real concerns, a tiered approach will be necessary. Exposure calculations and the known reactivity of certain chemical structures during ozonation/chlorination should be considered. Experiments should be conducted only if possible problematic TPs cannot be ruled out. As the TPs formed during ozonation depend on the conditions (water matrix, reaction time, oxidant concentration, …), the experiments should stay as close as possible to the conditions prevalent in waterworks. The ozonation of methyl-desphenyl-chloridazon, a metabolite of the herbicide chloridazon, was investigated in a lab scale simulation combining ozonation with biofiltration (COMBI unit). To confirm the pertinence for waterworks of the TPs observed in the COMBI unit, the ozonation of a pharmaceutical present in the Danube river in the µg L^{-1} range was investigated in the COMBI unit and the outcomes were compared to the situation in a waterworks. One TP detected in the COMBI unit could be detected also in the waterworks, confirming the COMBI unit as a realistic lab simulation of ozonation. The concentration of the TP was evaluated by semi-quantitative analysis at 0.5 ng L^{-1} and 2 ng L^{-1} in the waterworks after ozonation and in the COMBI unit, respectively.

3.03.07

Progress on Disinfection of Emerging Pathogenic Microorganisms With the AID of Light Emitting Diodes (LEDs)

N. Salve, BITS Pilani K. K. Birla Goa Campus / Chemistry; J. Gandhi, L. Raikar, H. Prakash, BITS-Pilani K K Birla Goa Campus / Department of Chemistry

Here, we present the progress on the application of LEDs for the disinfection of drinking water contaminated with pathogenic microorganisms. Fecal contamination of water leads to a serious outbreak of diseases. Frequently, there is a risk of wastewater treatment system failure, inconsistent effectiveness, and by-pass during high flow. Particularly in economically tested places, proper water treatment is a major challenge and people live with almost no access to safe water. Disinfection of drinking water to remove emerging pathogens is an important step in the prevention of water-borne diseases. LEDs have the power to emit light with various wavelengths (UV and visible range). Light from LEDs was reported to promote the inactivation of microorganisms. LEDs were used to promote photo-Fenton, photocatalytic, and direct UV light exposure for inactivation of pathogenic microorganisms such as bacteria, viruses, protozoa, and unknown emerging species. Importantly, mercury has adverse effects on human and environmental health. For this reason, the Minamata Convention (2013) has called for a ban on mercury-based products, including the light sources made up of mercury. LED light sources are considered as important alternative light sources (free of mercury) that would positively impact the water treatment sector. The effectiveness of the wavelength required for inactivation varies among microorganisms. The advantages of LEDs over the conventional lamps (Mercury, Xenon, etc. which require a high warm-up time, electricity cost, large space) will be discussed. Technological progress on the generation of pulsed LEDs, and compact size high power LEDs will be discussed in the presentation, and highlight the progress of lighting technologies for removal of pathogenic microbial emerging contaminants in water.

3.03.13

Fate and Distribution of Micropollutants During Long-Term Sub-Surface Irrigation With Sewage Effluent

D.M. Narain-Ford, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam; R.P. Bartholomeus, KWR Water Research Institute; R. Helmus, University of Amsterdam / IBED; S.C. Dekker, Copernicus Institute of Sustainable Development Utrecht University / Department of Environmental Sciences; A. van Wezel, University of Amsterdam/IBED Institute

Alternative water resources such as the reuse of sewage treatment plant (STP) effluent through sub-surface irrigation (SSI) offer the possibility of making optimal use of soil processes while irrigation water is being provided to crops. Indeed, studies done in river bank filtration, managed aquifer recharge and constructed wetlands have proven that the soil may have the ability to act as a filter and buffer zone. However, these systems were constructed for purification functions, while SSI also serves as

an irrigation system which may have shorter residence times with regards to crop uptake and longer residence times for groundwater flow. In this study we investigate the fate of contaminants of emerging concern (CoECs) for three consecutive years in a cropland currently sub-surfacely irrigated with STP effluent from the STP Haaksbergen, The Netherlands. STP effluent, surface water, soil moisture and groundwater samples are collected at different monitoring points in depth and time. Both the Cl:Br ratio as well as the total concentration of the negative modus hint that STP effluent seeps towards deeper groundwater, but may also reach the rootzone. So far the CL:Br and the total micropollutant concentration is higher in the groundwater than in the rhizosphere.The aim of this study is to detect major distribution trends across the sub-surface irrigation system transect and characterize the identities and fate of organic micro-pollutants by applying state-of-the art.

3.03.14

Water Treatment and Disinfection By-Products: A Comparison of Approaches for Crop Protection and Biocidal Products in the EU

J. Achtenhagen, S. Dorn, F. Schnitzler, knoell Germany GmbH

Regulation (EC) No 1107/2009 lays down the rules for the authorisation of crop protection products in Europe with the aim of protecting humans and the environment. During primary disinfection processes for central water treatment (e.g. ozonation, chlorination) certain active substances included in crop protection products and their metabolites have the potential to form unwanted by-products with e.g. toxic, carcinogenic and genotoxic characteristics. Notwithstanding that the EFSA (European Food Safety Authority), the scientific advisory body of the European Commission, has recently identified data gaps during the approval process of active substances, water treatment processes have not (yet) been implemented in the European data requirements (Reg. 283/2013 or 284/2013) relevant for the authorisation of crop protection products. Further, no guidance document for experimental testing is available. With this information pending, addressing water treatment processes successfully becomes a challenge for applicants of crop protection products. The Biocidal Product Regulation (BPR) (EU) 528/2012 regulates the application of disinfectants for water treatment. The disinfection by-products (DBPs) are formed during the disinfection process using oxidizing, halogen-containing biocidal products. According BPR, the effect of residues, which includes per definition also reaction products like DBPs, should be further evaluated in the risk assessment, as known DBPs can be biologically active and stable in the environment. A recently published guidance document (Guidance on the Biocidal Products Regulation, Volume V, Guidance on Disinfection By-Products, 2017) defines a stepwise approach for an environmental risk assessment of DBPs. Besides major DBP groups trihalomethanes, and the haloacetic acids, halogenated acetonitriles and bromate should be included in the assessment. Following most recent developments in the area, the poster will provide a comparison between crop protection and biocides regulations highlighting recent activities and challenges around water treatment and disinfection by-products.

3.03.15

Landscape Level Exposure Assessment of Pesticide Concentration at Drinking Water Abstraction Locations - a Surface Water Perspective

S. Gebler, BASF SE / Agricultural Solutions - Global Environmental Fate Modelling; S. Li, Environmental Modelling Consultant; T. Schröder, BASF SE / Agricultural Solutions - Environmental Fate Modelling

The exposure assessment of plant protection products (PPP) at drinking water abstraction points is an intrinsic part in the EU regulatory framework (regulation 1107/2009) and therefore of growing interest for authorities, water suppliers, industry, and other stake holders. Guidance development has recently been initiated by the EU commission and foresees a joined cooperation between EFSA and ECHA. From a landscape level exposure perspective, the target is amongst others to derive dilution factors from edge-of-field surface water (SW) concentrations (PEC_{sw}) to a potential drinking water abstraction location at the catchment outlet. This dilution factor depends on various variables, e.g. the agricultural use area, the hydrology and other processes that determine the fate of a chemical substance in a catchment. Based on a concept that was developed on national level in the Netherlands considering the above-mentioned variables and processes, an advanced concept was presented at SETAC 2020 by Gebler *et al.* presenting a workflow established to identify vulnerable surface water catchments that potentially can be used for drinking water assessment in the EU, including high-resolution EU-wide surface water catchment map of dilution factors by upstream land use. In order to identify vulnerable catchments advanced modelling techniques can be adopted, e.g. SWAT tracer experiments, which considers all these different processes that determine the dilution factors holistically. These modelling experiments, however, are computationally very intensive. Therefore, approximations are required to derive the key factors that drive the vulnerability, i.e. determine the dilution factors, of a SW catchment. The main factors considered are hydrology, resp., the surface water distribution within a catchment, field connectivity, and application timing. Based on approximation of these factors and validation by means of modelling for individual catchments, a vulnerability ranking could be established and generic dilution factors for agricultural surface water catchments be derived, which is the key for any further higher Tier refinement on catchment level.

3.03.18

Application of Online Biomonitoring Systems in Wastewater: Potential to Monitor Changing Wastewater Compositions

D. Schmidt, Ecotox center EAWAG-EPFL; A. Kizgin, C. Kienle, Ecotox Centre Eawag-EPFL; M. Langer, Oekotoxzentrum Eawag-EPFL / Aquatic Ecotoxicology

Wastewater treatment plants (WWTP) are a major source of micropollutants to surface waters. To generate knowledge about the presence, potential effects and removal of micropollutants it is a valuable approach to monitor treated wastewater effluent of WWTP. Currently, this is realised by using grab samples or mixture samples of the wastewater. This means, that the samples only have a temporary value, as they only contain a snapshot of the current wastewater composition. However, especially in WWTP with industrial input, the wastewater composition can be highly variable and a continuous testing would be advantageous, but very labour and cost intensive. A promising concept is to monitor the wastewater by automated online-biomonitoring systems. Generally, they are applying living organisms to monitor constantly water quality and are designed to raise an alarm if the organism's responses exceed a harmful threshold. Currently online-biomonitoring systems are established for drinking water and surface water but only few experiences exist where wastewater comes in the focus. In this project, two biomonitors were selected to address this research gap. The DaphToxII, which is based on video tracking of the movement pattern of Daphnia magna and the SensaGuard, which is based on changes in the electromagnetic field with Gammarus pulex as a test species. The aim of this study is to see if the selected systems can be applied successfully in wastewater and to understand the behavioural changes of the test organisms exposed to changing wastewater compositions. To have a better understanding of a change in behaviour, the biomonitors were tested with a flow through of wastewater and spike concentrations of different micropollutants under controlled conditions. It could be shown that, within the wastewater matrix, not every change in behaviour, which could provide relevant information about toxic compounds within the wastewater, is triggering an alarm. This indicates that more information can be extracted from the generated behaviour data. With this background, the systems are currently installed at a WWTP with varying wastewater composition for the first time. During a six-week period, their applicability in the field will be investigated and the potential of continuous biological monitoring additionally to chemical monitoring will be evaluated. Results of this experiment will be presented. If successful, the online-biomonitoring systems could open up new possibilities to check the effectiveness of wastewater treatment.

3.03.19

Ecotoxicological Testing With Algae for Toxicity Assessment in a Tertiary Treatment by Adsorption on Powdered Activated Carbon (PAC) in a Municipal Wastewater Treatment Plant

F. Cappelli, IRSA-CNR / University of Insubria, Department of Science and High Technology; M. Palumbo, Water Research Institute, CNR; S. Pascariello, Water Research Institute - Italian National Research Council, IRSA-CNR; S. Polesello, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute; S. Russo, Water Research Institute - Italian National Research Council, IRSA-CNR; S. Valsecchi, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute; S. Villa, University of Milano Bicocca / Department of Earth & Environmental Sciences; G. Bellotti, S. Culatina, M. Stante, Veolia Water Technologies Italia S.p.A.; A. Turolla, M. Antonelli, F. Malpei, Politecnico di Milano / Department of Civil and Environmental Engineering

The addition of powdered activated carbon (PAC) is currently considered one of the most appropriate technology to upgrade conventional

wastewater treatment plants (WWTPs) in order to achieve a significant reduction of refractory organic contaminants in receiving waters. The chemical monitoring of the adsorption process in WWTPs at pilot and full-scale plants showed that the activated carbon treatment is efficient in reducing the load of a broad range of micropollutants by over 80%. However, lower removal efficiencies are reported for several pollutants. Additionally, some pollutants could be ignored by the chemical analyses and some toxicants might be overlooked or accidently ignored. Ecotoxicological assays are capable of integrating the joint effects of all residual chemicals that remnant after the PAC-treatment of the effluents. Thus, they are essential to evaluate the actual improvement of the effluent quality by verifying the toxicity removal following the adsorption process.In this work the algal growth inhibition test was selected as a sensitive chronic bioassay suitable to detect low levels of contamination in wastewaters after a pilot-scale PAC treatment of wastewaters in a municipal WWTP. The PAC was dosed in an Actiflo® Carb system; this patented Veolia technology consists in a pre-contact tank where PAC is dosed followed by coagulation, flocculation and lamellar sedimentation steps; the solution is compact thanks to microsand recirculation that allows the ballasting of the particles (TSS with PAC) to be settled. The bioassays and chemical analyses were repeatedly performed using the inlet and the outlet samples taken from the PAC pilot system in different operating conditions.Following the OECD Guidelines, all samples were 0.45µm filtered and tested using the green alga *P. subcapitata* as test organism. The experimental strategy additionally included the parallel use of the outlet unfiltered samples with the specific aim to evaluate the possible role of residual micropollutant-loaded carbon particles as affecting the toxicity changes. After 72h exposure the toxicity effect was calculated as % growth inhibition of the samples relative to the control cultures. The bioassay revealed capable of measuring the changes of toxicity effects following the PAC treatment, both in the soluble as well as in the particulate fraction.

3.03.20
Ecotoxicological Evaluation of Antiscalants From Reverse Osmosis/Nanofiltration Plant Discharges and the Effects of Post-Treatment With Ozon and PerfluorAd

S. Johann, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology; G. Schertzinger, IWW Water Centre / Aquatic Ecology / Biology; O. Happel, DVGW Water Technology Center / Analysis and Water Quality; D. Armbruster, B. Schmutz, DVGW Water Technology Center; V. Hartmann, Y. König, M. Nagler, Goethe University Frankfurt; S. Schiwy, Goethe University Frankfurt am Main / Department Evolutionary Ecology and Environmental Toxicology, Faculty Biological Sciences; H. Hollert, Goethe University Frankfurt / Department Evolutionary Ecology and Environmental Toxicology, Faculty Biological Sciences

Membrane-based technologies such as nanofiltration (NF) or reverse osmosis (RO) for water reuse, and drinking water purification are increasingly applied in Europe due to high water quality requirements for human consumption. The efficiency of such membrane technologies is limited by inorganic scaling, which refers to membrane blocking caused by the precipitation of insoluble compounds. Hence, additives, namely antiscalants (AS), are applied in the water flow, which inhibits scale formation and elongating the lifetime of membranes. NF/RO filtration produces the permeate (deionized water) and a concentrate (wastewater), the latter of which is enriched with all inorganic constituents and micropollutants, including the dosed AS. Direct or indirect disposal of the concentrate into the water cycle poses a high challenge for practical application. In this context, the project *KonTriSol*, funded by the Federal Ministry of Education and Research, focuses on improving technical solutions and regulation for RO/NF membrane technologies. Furthermore, the ecotoxicological characterization of concentrates, permeates, and, in particular, relevant for the present work, AS solutions are included to complement a comprehensive risk assessment. AS solutions are important for the ecotoxicological assessment since in particular phosphonate-based AS are poorly biodegradable and highly active due to their complexation activity. Furthermore, though an overall efficient removal during wastewater treatment processes (> 80 %) has been reported, they can be found in a low µg/L range in rivers due to a broad field of technical and industrial application (e.g., detergents). Hence, the present work investigates the acute and mechanism-specific toxicity of selected AS reference compounds and commercially available AS products using representative freshwater model species, such as zebrafish (*D. rerio*), daphnids (*D. magna*), and algae (*R. subcapitata*). A direct comparison of reference and products allows evaluating potential adverse effects caused by unknown additives from the products, which composition is typically a trade secret. Furthermore, AS solution and -enriched concentrates treated with ozone and PerfluorAd will be investigated within the effect-based methods. Ozonation was selected due to detected side products of AS in previous studies. PerfluorAd is a treatment technology for reducing contamination and is hence expected to reduce the AS content.

3.03.21
BPA Occurrence and Cumulative Genotoxic or Estrogenic Potential in Samples From a Drinking Water Treatment Plant

M. Profita, Alma Mater Studiorum - University of Bologna / Department of Biological, Geological and Environmental Science (BiGeA); E. Fabbri, University of Bologna / Department of Biological Geological and Environmental Sciences; I. Vasumini, Romagna Acque - Società delle Fonti; P. Valbonesi, Alma Mater Studiorum University of Bologna / Departmento of Biological, Geological and Environmental Science (BiGeA)

Drinking water quality is a priority issue of the environmental policy agenda; however, the presence of many Contaminants of Emerging Concern (CECs), such as Bisphenol A (BPA), in drinking water sources has drawn the attention of international environmental and health agencies. BPA is a commonly used additive in plastic products. As an endocrine disruptive chemical, BPA can modulate the endocrine system and represent a hazard to human health even at extremely low concentrations. The recent revision of the Drinking Water Directive (EU Council, 2020) represents the first regulation concerning BPA occurrence in water for human consumption, with the definition of the upper limit of 2.5 µg/L. The aim of this study was to evaluate the occurrence of BPA both in surface water entering and in treated water exiting a recently built drinking water treatment plant (DWTP) serving the Romagna area (Italy). Samples were analyzed by liquid chromatography coupled to mass spectrometry (HPLC-MS/MS). A total of 10 sampling campaigns were conducted starting from July 2016. BPA was detected in all samples at DWTP entrance, with concentrations ranging between 3 and 18 ng/L, while it was always partially or completely removed by DWTP (< LOQ – 6.35 ng/L). The 2020 sampling campaigns also addressed the BPA fate along the waterwork by analyzing its occurrence within the DWTP, in water samples collected after three treatment stages (preoxidation, flocculation, ultrafiltration) as well as after the activated carbon filtration, the last step before exiting the plant. BPA was removed during the water treatment, with an important contribution given by activated carbon filtration; final BPA concentrations were always detected well below the recent regulatory limit. In addition, biological analyses were performed to ascertain the treated water cumulative estrogenic and/or genotoxic potential. The water analyzed met the criteria of good quality. It has been suggested that drinking water may not represent a significant source of human exposure to CECs; however, the co-occurrence of different compounds may lead to additive/synergistic interactions causing unexpected effects on human health and the environment, and deserve implementation of strategies for detection and removal.

Biodegradation of Organic Trace Pollutants in the Environment

3.04.08
Trace Organic Chemicals Transformation by Microbial Communities Preadapted to Aniline and Sodium Acetate

E. Chingate, G. Tessaro, Technical University of Munich / Chair of Urban Water Systems Engineering; M. Farré, Catalan Institute for Water Research (ICRA); C. Wurzbacher, Technical University of Munich / Chair of Urban Water Systems Engineering; J. Drewes, Technische Universität München / Chair of Urban Water Systems Engineering; U. Hübner, Technical University of Munich / Chair of Urban Water Systems Engineering

@font-face font-family:"Times New Roman"; @font-face font-family:"宋体"; @font-face font-family:"Calibri"; @font-face font-family:"SimSun"; p.MsoNormal mso-style-name:Normal; mso-style-parent:""; margin-bottom:8,0000pt; line-height:107%; font-family:Calibri; mso-fareast-font-family:SimSun; mso-bidi-font-family:'Times New Roman'; span.msoIns mso-style-type:export-only; mso-style-name:""; text-decoration:underline; text-underline:single; color:blue; span.msoDel mso-style-type:export-only; mso-style-name:""; text-decoration:line-through; color:red; @pagemso-page-border-surround-header:no; mso-page-border-surround-footer:no;@page Section0 div.Section0page:Section0;Managed aquifer recharge systems and biofilters have shown high potential for the removal of trace organic chemicals (TOrCs). Previous studies indicate that microbial degradation of many TOrCs is favored under oxic and oligotrophic conditions. However, a fundamental

understanding of TOrCs bacterial transformation is still lacking. The objective of this study was to preadapt bacterial communities to individual substrates and get insights into metabolic pathways active in the transformation of different TOrCs based on chemical structure similarities. To avoid biomass and concentration gradients from column systems, chemostats with well-defined conditions were operated under oxic and oligotrophic conditions.A series of chemostats with aeration and a dilution rate of 0.09 h^{-1} were operated for microbial adaptation to a unique carbon source, either an easily degradable compound (sodium acetate) or a xenobiotic (aniline). Microbial medium also included essential elements for xenobiotics degradation and phosphate buffer to keep the pH at 6.5. Experiments were run in triplicates with daily sampling for chemical analysis and cell counting, as well as temperature and dissolved oxygen monitoring inside each reactor. Microbial adaptation to aniline and sodium acetate was evaluated by comparing the structural composition via 16S rRNA sequencing for the inoculum and samples from the reactors at steady-state. After steady-state was reached, adapted microbial communities were exposed to a mix of TOrCs in preliminary 4 hours batch experiments in order to evaluate co-metabolic transformation.Aniline reactors were operated successfully for 37 days with the removal of > 99 % of the primary substrate. Sodium acetate reactors were stopped after 14 days of inoculation and 5 days of steady-state operation. Low reproducibility in cell counting with both substrates suggests the establishment of complex microbial communities with different members capable of substrate degradation, but also the potential presence of bacteria predators such as protozoa. Results from TOrCs experiments revealed different degrees of removal by aniline and acetate degraders. Additional experiments with different dilution rates in retentostats are planned to determine survival energy thresholds and investigate multiple substrate growth strategy.

3.04.11
A New Database and Preliminary QSARs for Environmentally Relevant Biodegradation Half-Lives
T.N. Brown, TNB Research; A. Sangion, University of Toronto Scarborough / Department of Physical and Environmental Sciences; J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology
Environmental biodegradation half-lives are important parameters for performing chemical hazard and risk assessments, but there is a paucity of environmental biodegradation half-lives for relevant chemicals in commerce. To help address this challenge we are developing a new database of environmental biodegradation half-lives, focusing on primary aerobic biodegradation in water (HL_{pawb}). An initial dataset of 2326 HL_{pawb} values for 1114 organic chemicals were collected from publicly available datasets. The dataset spans a range of nearly five orders of magnitude, with a good separation of labile and highly persistent chemicals. QSARs for environmental biodegradation are being developed using the new database as a training set. The IFS-based QSARs were developed and evaluated following OECD QSAR guidance, using the algorithm described in previous publications. The QSARs include a fully defined applicability domain (AD). In an external validation dataset, chemicals which are within the model AD show adequate predictions, with a q^2 of 0.65 (correlation of predicted values regressed vs. expected values for the external validation set). Predictions for chemicals further outside the AD are less reliable. The relatively narrow AD of the model suggests that the QSAR is limited by data availability, even with the large training dataset (n=743). Future work will aim to further expand and curate the dataset to address these shortcomings and make recommendations for further experimental work.

Environmental Chemistry and Exposure Assessment: Analysis, Monitoring, Fate and Modeling

3.05.05
Quantitative Analysis of Preservatives and Sweeteners by HPLC in Commercial Fruit Juice Available in Bangladesh
M. Shoeb, M. Hossain, M. Islam, M. Reza, N. Nahar, University of Dhaka / Department of Chemistry
Two methods of reversed phase-HPLC equipped with photodiode array (PDA) detector were used for the determination of preservatives (Sodium benzoate and Sorbic acid) and artificial sweeteners (Aspartame, Saccharin, and Acesulfame-K) in 22 fruit juice samples. Separations of preservatives were affected by initial the mobile phase of methanol–acetate (40:50) buffer (pH 4.5-4.6). Separations of sweeteners were affected by acetonitrile-phosphate (15:85) buffer (pH 3.5). The correlation coefficients (r^2) of the calibration curves were found to be 0.998, 1.000, 0.998, 0.999 and 0.999, LOD were 14.77, 2.08, 0.50, 0.42 and 0.38 mg/L, LOQ were 49.23, 6.94, 1.66, 1.41, and 1.27 mg/L and the recoveries were 91, 110, 92, 93, and 110%, respectively, for Sodium benzoate, Sorbic acid, Aspartame, Saccharin and Acesulfame-K, respectively. Out of 22 samples, 12 contained Sodium benzoate in the range of 6.73-134.78 mg/L and 9 contained Sorbic acid in the range of 21.71- 370.93 mg/L. Only 2 samples, pran ping pong and pran rocket drinks contained Aspartame 12.45 mg/L and 34.47 mg/L, respectively. Saccharin was found in below detection level in all analyzed samples. Out of 22 analyzed juices, only 4 contained Acesulfame-K in the range of 5.91-12.60 mg/L.

3.05.06
Evaluation of Supercritical Fluid Chromatography Detection of Highly Polar Pesticides
L. Pérez-Mayán, Universidade de Santiago de Compostela / IAQBUS - Institute of Research on Chemical and Biological Analysis; A. González-Baizán, Universidad de Santiago de Compostela / IAQBUS- Institute of Research on Chemical and Biological Analysis; M. Ramil, University of Santiago de Compostela / IAQBUS- Institute of Research on Chemical and Biological Analysis; R. Cela, Universidade de Santiago de Compostela / Department of Analytical Chemistry- Institute of Research on Chemical and Biological Analysis; I. Rodríguez, University of Santiago de Compostela / Department of Analytical Chemistry - Institute of Research on Chemical and Biological Analysis
Nowadays, agricultural production depends on the use of phytosanitary compounds to ensure the productivity of crops and to cover the demand of the current society. To this end, different organic fungicides have been commercialized to control pests such as *mildew* and *oidium* in viticulture . Wine production requires the use of different pesticides depending on the weather and soil conditions. Fungicides and insecticides used in viticulture can remain in the application crop , migrate to other environmental compartments or even be tranferred to manufactured products. Additionally, applied compounds could suffer degradation, through different pathways, leading to transformation products with different chemical and toxicological characteristics. In this vein, dithiocarbamate compounds, as mancozeb, maneb, metiram and propineb, have been extensively used due to their low toxicity and efficiency, combined with organic fungicides, in the treatment of fungal infections afecting vines. However, hidrolysis and photodegradation of these compounds produce ethylenethiourea (ETU) or propylenethiourea (PTU), as major metabolites, which are more toxic than precursor ones. Another example are triazolic fungices, such as penconazole, tebuconazole and myclobutanil, that are degradated leading to 1,2,4-Triazole. Aminobenzimidazole derived from carbendazim, that is, in turn, a metabolite of methyl thiophanate, a systemic fungicide authorized until year 2020 in some European countries.The above pesticide transformation products are highly polar species, hard to be introduced in routine multiresidue methodologies, either using reversed-phase liquid chromatography (LC) for pesticides detemination. Most common methods are centered in hydrophilic interaction liquid chromatography (HILIC), resulting in high consumption of solvents and long run times. In this work, we evaluate the use of supercritical fluid chromatography (SFC) coupled to an hybrid quadrupole time-of flight detector, to assess the suitability of this enviornmentally-friendly technique for the determination of highly polar pesticides. Optimization of the analytical methodology addresses the study of the type of stationary phase, CO2 additives and make-up solution to enhance compounds separation and detectability by mass spectrometry after electrospray ionization. The developed methodology is applied to investigate the presence of above compounds in viticulture related samples, such as vineyard soils, grapes and wine.

3.05.07
Improved Voltammetric Detection of Cadmium and Lead in Wastewater Samples
C. Van der Horst, Cape Peninsula University of Technology (CPUT) / Chemistry; M. Makombe, CPUT / Department of Chemistry; V.S. Somerset, CPUT / Chemistry
Heavy metals are the most toxic chemical pollutant present in wastewaters and enter our ecosystems through anthropogenic activities. Anthropogenic activities such as pesticide development, metal industries, nickel-cadmium batteries, plastic manufacturing, polymer production, pigment industries, electroplating, erosion, volcanic activity, electronics, photography, alloys, fertilizers, refining, and nuclear plants are the major sources of heavy metals in the environment. Exposure to toxic heavy metals such as cadmium and lead can

produce a wide variety of acute and chronic effects in humans such as kidney damage, psychological disorders, damage to the central nervous system, lung cancer, and prostate cancer. In the determination of heavy metals, researchers have used several analytical procedures such as inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS). The search for new advanced materials with a high absorption coefficient, high surface area, and high sensitivity for the determination of heavy metals has increased. Due to the electrical, mechanical, thermal, catalytic, and optical properties, bimetallic nanocomposites are excellent electroactive material for heavy metal detection. However, iron-silver bimetallic nanocomposites are not extensively applied as a chemical sensing material for heavy metal detection. In this study, we drop-cast chitosan-iron/silver nanocomposite on a glassy carbon electrode and the electrochemical characterization was done by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Differential pulse adsorptive stripping voltammetry was used to detect these heavy metals in wastewater samples. The differential pulse adsorptive stripping peak current signal was linear from 0.2 to 1.2 µg/L (n = 3) range, with limit of detections for Cd(II) (0.10 µg/L) and Pb(II) (0.09 ng/L), respectively. The results reported in this study demonstrate that the synthesized chitosan-iron/silver nanocomposite can be used for sensor construction and the determination of heavy metals in environmental samples.

3.05.08
Analysis of VOCs According to EPA Method 8260
A. Ladak, J. Renpenning, D. Lee, T. Jeffers, D. Cardona, Thermo Fisher Scientific / CMD; A. Nutter, Teledyne Tekmar
Volatile organic compounds or VOCs are common in modern life and come from both human-made and natural sources, but the human-made sources of VOCs in populated and industrialized areas are the main contributors to environmental pollution. Many of these compounds contaminate our environment today and cause negative health effects to humans when they are exposed to elevated levels. Analytical testing laboratories must monitor the environment to ensure the public is not exposed to elevated levels of VOCs. The latest version of US EPA Method 8260—EPA Method 8260D—is used to monitor a variety of matrices for the presence of VOCs. In this work an analytical method that meets the requirements outlined in U.S. EPA Method 8260D utilizing U.S. EPA Methods 5030 and 5035 preparation methods for the quantitation of purgeable organic compounds (POCs) in wastewater and soil, using a purge and trap system along with a ISQ 7000 single-quad MS system coupled with a TRACE 1310 GC and a Chromeleon Chromatography Data System (CDS) is demonstrated. Method linearity, method detection limit (MDL), Initial Demonstration of Capability (IDC) and method robustness were assessed to evaluate method performance.

3.05.09
Consolidated GC-MS/MS Analysis of Pesticides, PAH, OCB and PCB in Water Samples Through Automated In-Vial Liquid-Liquid Extraction (LLE) and Large Volume Injection (LVI)
G. Pintonello, Veritas S.p.A.; J. Renpenning, D. Cavagnino, Thermo Fisher Scientific / CMD; I. De Dobbeleer, Interscience
Environmental contaminants remain a constant cause of concern for human health and there is the need for many laboratories to analyze samples in a fast and cost-effective way. Environmental testing laboratories are facing a growing demand for target contaminants determination in water samples and look for options to save on solvent costs, minimize sample preparation time and consolidate different methods in a single workflow. However, time-saving and cost driven measurements should not compromise the analytical results in terms of sensitivity, robustness, or quality controls. This poster shows how the capability of automated sample preparation combined with the flexibility of injection mode (such as large volume injection), and sensitivity at ultra-low levels, play together to minimize costs and efforts from the laboratory, whilst maintaining high quality standards and compliance with the official regulations. A full automated workflow for in-vial liquid-liquid extraction on-line with LVI and subsequent detection of semi-volatile contaminants at ultra-low levels in water samples is presented, demonstrating how automation improves the quality of the analytical data, removing the variance due to manual operations and increasing the precision of each step of the workflow. Additionally, the presented workflow demonstrates a consistent resources optimization by reducing solvent consumption with consequent less wastes and less exposure for the user. Water sample amount is also significantly reduced, with important improvement of the logistic by limiting storage and transportation costs.

3.05.10
Determination of Glyphosate in Different ENVIRONMENTAL Samples
Y. Soriano, University of Valencia / Desertification Research Centre (CIDE); R. Alvarez-Ruiz, University of Valencia / Food and Environmental Safety Research Group (SAMA-UV), Desertification Research Centre (CIDE, UV-CSIC-GV),; Y. Pico, University of Valencia / Environmental Quality and Soil
Currently, Glyphosate —a broad spectrum herbicide linked to both agricultural and urban uses — is one of the most applied pesticides, so the need to evaluate its impact on the environment is urgent. One of the drawbacks that makes its determination complicated is the difficulty to incorporate glyphosate in the multiresidue schemes due to its high polarity and solubility in water, low volatility and molecular weight, amphoteric behavior and lack of chromophores. Based on existing methods, we developed a simplified procedure for the determination of glyphosate and its main metabolites, aminomethylphosphonic acid (AMPA) and glufosinate (GLU) in different matrices, such as water, sediment, honey and honeybee samples. The analytical method is based in the derivatization with fluorenylmethyl chloroformate (FMOC-Cl) to achieve sufficient retention on reversed phase chromatographic columns and liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QqTOF-MS) for its detection. Sensitive and selective liquid chromatography – mass spectrometry (LC – MS) analysis is a powerful and essential tool for contaminant identification in environmental studies. The derivatization step will be modified by changing parameters such as volume and / or concentration of the sample and reagents to lower the limits of quantification (LOQ). As mentioned above, pre-column procedures are primarily based on derivatization to form fluorescent derivatives (improve detection) and / or reduce the polarity of analytes facilitating chromatographic retention. The method was widely tested in water samples from L´Albufera Natural Park (Valencia, Spain) and in different honeys and honeybee samples provided by the beekeepers of the area [1]. The results of this study demonstrate the interest of high resolution mass spectrometry to draw the profile of the contaminant in matrices of water and honey. Further research is needed to assess the real environmental risk related to this pesticide and its possible effects on the surrounding ecosystems. Reference: [1] L. D. Demonte, N. Michlig, M. Gaggiotti, C. G. Adam, H. R. Beldoménico, y M. R. Repetti, «Determination of glyphosate, AMPA and glufosinate in dairy farm water from Argentina using a simplified UHPLC-MS/MS method», *Sci. Total Environ.*, vol. 645, pp. 34-43, 2018, doi: 10.1016/j.scitotenv.2018.06.340.Acknowledgments This work has been supported by the Spanish Ministry of Science, Innovation and Universities and the ERDF (European Regional Development Fund) through the project CICLIC—subproject WETANPACK (RTI2018-097158-B-C31) and by the Generalitat Valenciana through the project ANTROPOCEN@ (PROMETEO/2018/155). R. Álvarez-Ruiz and Y. Soriano acknowledge the Spanish Ministry of Science, Innovation and Universities and the ERDF (European Regional Development Fund) for their grants BES-2016-078612 and PRE2019-089042, respectively.

3.05.11
Development and Optimisation of a GC-MS/MS Protocol for Analysis of Selected Organophosphorous Pesticides in Juice Samples
K. Malatji, CPUT / Department of Chemistry; M. Louw, Hearshaw and Kinnes Analytical Laboratory, / HKAL, Westlake, Cape Town; V.S. Somerset, CPUT / Chemistry
Excessive use of organophosphate pesticides on fruits and vegetables has severe effects on the environment and poses harm to humans due to their high acute toxicity. Food safety is ensured by strict monitoring of residue levels because organophosphate pesticides and carbamates inhibit the enzyme acetylcholinesterase (AChE) that results in accumulation of acetylcholine (ACh) at chlinergic receptor sites, leading to paralysis. Various standard methods including liquid chromatography (LC) and gas chromatography (GC) are used for the detection of multiple classes of pesticides in various sample matrices including juice samples. The study discusses a developed and optimised large-volume injection method for the analysis of malation, chlorpyrifos, bromophos-methyl, methidathion and profenofos in fruit juices on a gas chromatographic instrument coupled to a triple quadrupole mass spectrometer (GC-MS/MS). The developed large-volume-injection method and a currently used hot-splitless-injection method are compared by means of evaluating percentage relative standard deviation (%RSD), limit of detection (LOD), limit of quantification (LOQ) and percentage measurement of uncertainty (%MU). A simple and cost-effective QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction

method is used in the study for accurate and reliable analytical results.

3.05.12
Chromatographic Determination of Selected Carbamate Pesticides in Soil Samples
L.L. Luzi-Thafeni, Department for Environmental Food & Rural Affairs / Department of Agriculture; V.S. Somerset, CPUT / Chemistry
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 pre-concentrated 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 how much of the applied carbamates are found in the fruit, compared to the soil in the same geographical location. The study also aims to examine if a sonicated SPE procedure promotes the release of the carbamates in the soil, before samples are subjected to a GC-MS/MS analysis procedure.

3.05.13
Evaluation of Two Extraction Methods for Multiresidue Analysis of Pesticide From Sediment Samples by LC-MS/MS
L.V. Herrera, D. Sadutto, Environmental and Food Safety Research Group of the University of Valencia (SAMA-UV) / Desertification Research Centre (CIDE); Y. Pico, University of Valencia / Environmental Quality and Soil
Pesticides are compounds widely used in croplands to control pests and weeds and to repair the damages they cause, providing important benefits for humanity and agriculture. However, at the same time they represent a potential risk to human health and the environment due to their high environmental persistence, stability and toxicity. The development and optimization of an extraction method is the first essential step for the chemical analysis of these compounds, as it helps to achieve high recovery rates in the selected study matrices. The present study describes the evaluation and comparison between Mcllvaine-EDTA buffer solution (pH 4.0) extraction followed by solid phase extraction (SPE) with QuEChERS method for the determination of pesticides in sediment samples. For the SPE Strata-X-33u Polymeric Reversed Phase and Strata-X-CW-33u polymeric weak cation exchange cartridges (Phenomenex, Torrance, California, USA) were tested. The QuEChERS method used acetonitrile as extraction solvent and required a clean-up step with a salt mixture prior to analysis. Both extraction methods used high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) for analysis. The methods were applied to determine pesticides in sediment samples, collected in the Natural Park of the Albufera (Valencia, Spain). More than 60 target pesticides have been identified and quantified. As the main result, the QuEChERS method has proven to be more accurate with an average recovery of 60% and relative standard deviation less than 20% for 86% of the cases. The recoveries obtained with the two SPE extractions tested were much lower, with a maximum recovery of 57% for the Strata-X cartridges and 32% for the Strata-X-CW cartridges. Based on the instrument and method performance validation results, QuEChERS seems to be the most recommended method for the extraction of pesticides in the sediment matrix. The results obtained can contribute to the optimization of pesticide detection methods in environmental pollution studies of complex natural environments, such as Mediterranean coastal wetlands, which suffer significant impacts from agricultural activity carried out in their vicinity.

3.05.14
Small & Polar Organic Trace Compounds: Future Environmental Monitoring Via Supercritical Fluid Chromatography-Mass Spectrometry?
N. Günther, M. Schulz, M.P. Schluesener, J.S. Kirchner, T.A. Ternes, Federal Institute of Hydrology; A. Wick, Federal Institute of Hydrology / G2 -Water Chemistry
Because of the diverse nature of organic water contaminants, many substances are not captured using current monitoring routines. Especially analytical methods for small and polar organic compounds as EOCPs (emerging organic chemical pollutants) are scarce. Bieber et al. (2017) showed, that more than three-quarter of organic compounds, originating from a wastewater treatment plant, have a negative $\log D_{\mathrm{pH\,7}}$ value. Thus, novel multi-methods are required to add such compounds to the current analytical window. Supercritical fluid chromatography coupled with mass spectrometry (SFC-MS) can be used to simultaneously analyse very polar and non-polar analytes, using a gradient, starting under supercritical and ending under liquid chromatographic conditions. Recent studies already showed its applicability for $\log D_{\mathrm{pH\,7}}$ values down to -7,71 (Bieber et al., 2017; Schulze et al., 2019). We studied a further extension of the analytical window and the general suitability of SFC for future environmental monitoring. Therefore, about 100 target compounds were selected, including scarcely investigated compounds, their main transformation products, and human metabolites. Despite the challenging chemical characteristics, a simple as well as non-discriminatory sample preparation is used. SFC-specific and common LC parameters are optimised and compared considering their detectable analytes and their effects on peak shape. Furthermore, we address typical SFC-associated problems and provide solutions, e.g. an adaptation of the SFC-MS-interface. Matrix effects are studied as well as the benefits and limitations of SFC-MS, as a sensitive but robust and polarity-independent monitoring method. The method will be applied to aqueous environmental samples of various origins in order to investigate the occurrence, distribution and sources of the numerous selected polar trace compounds. References: Bieber, S., Greco, G., Grosse, S., & Letzel, T. (2017). RPLC-HILIC and SFC with mass spectrometry: polarity-extended organic molecule screening in environmental (water) samples. Analytical chemistry, 89 (15), 7907-7914. Schulze, S., Zahn, D., Montes, R., Rodil, R., Quintana, J. B., Knepper, T. P., Reemtsma, T. & Berger, U. (2019). Occurrence of emerging persistent and mobile organic contaminants in European water samples. Water research, 153, 80-90.

3.05.15
Silylation: A Reproducible and Readily Applicable Method for Characterization of Non Extractable Residues (NER) of Chemicals and Pesticides in Soil and Sediment
D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry; A. Schäffer, RWTH Aachen University / Institute for Environmental Research; D. Classen, German Environment Agency / Institute for Environmental Research; C. Gaertner, Fraunhofer Institute for Molecular Biology and Applied Ecology IME
All chemicals produce besides extractable and volatile degradation products also non-extractable residues (NER) in solid environmental media like soil and sediment, however to various extents from less than 10 to more than 90% of the applied amounts. Since it has been found that parent substances and relevant metabolites can be contained and stabilized in these immobilized residues there is currently much debate on how to include NER in the environmental persistence assessment. Using test substances labelled with radioactive, e.g. ^{14}C, or stable, e.g. ^{13}C, isotopes, three types of NER can be experimentally discriminated[1]: sequestered and entrapped residues (type I) having the potential to be released, and type II NER, i.e., residues covalently bound to organic matter in soils or sediments, the latter being considered to have very low remobilisation potential. Type III NER (bioNER) are formed after degradation of the xenobiotic chemical to residues which are anabolically used to form biomolecules like amino acids and other biomass compounds, and are, thus, of no environmental relevance. Silylation has been suggested as a methodology to differentiate types I and type II NER but concern has been addressed that this procedure is not suitable or manageable for routine analysis, e.g., in the frame of registration studies. We here describe a readily applicable and reproducible experimental procedure to use this method for analysis of NER, i.e., the distinction of sequestered and covalently bound residues of chemicals, biocides, pharmaceuticals and pesticides in soils and sediments, including subsequent structural identification of residues entrapped in type I NER. [1] Schaeffer, A., Kaestner, M., and Trapp, S. (2018) A unified approach for including non-extractable residues (NER) of chemicals and pesticides in the assessment of persistence. Environmental Sciences Europe 30:51

3.05.16
Rapid & Sensitive Analysis of Underivatized Polar Pesticides in Water by LC/MS/MS
T. Anumol, J. Roy, Agilent Technologies, Inc.; J. Grossman, Agilent Technologies
Glyphosate is a synthetic, broad-spectrum herbicide widely used in both agricultural and residential sectors. Glufosinate is naturally produced by plants but is also produced

synthetically on an industrial scale. Both are degraded by bacteria in plants, soil and water, to Aminomethylphosphonic acid (AMPA) and 3-(methylphosphinico)propionic acid (MPPA), respectively. The accurate quantitation of these compounds and other polar pesticides (2-hydroxyethyphosphonic acid (HEPA), N-acetylglufosinate, Ethephon, Fosetyl) at low ng/L levels in surface water, and low-ug/L levels in other matrices, have proven to be challenging, given their very polar nature. A simple yet effective methodology was developed that includes quick sample preparation, very robust reversed-phase chromatography and sensitive mass spectrometry detection for routine analysis. An innovative method using a new superficially porous reversed phase LC column, which excels at retaining difficult polar molecules was used for robust analysis and good seperation. In contrast to HILIC approach, this method allows for a larger injection volumes of the 100% aqueous extracts allowing no need for organic solvent exchange for water samples, resulting in lower detection limits without sacrificing peak shape. The peak shape of all studied compounds remained stable throughout multiple matrices, even though a large injection volume is used and the pesticides are not derivatized. The retention times and peak areas are very reproducible across all studied matrices including drinknig water, surface water, wine, and honey , even in the ng/L range, indicating that the overall strategy (very simple and short sample clean-up followed by aqueous injection on reversed-phase column) is insensitive to matrix. The use of a labelled internal standard can help to counter matrix effect in some cases, especially for the two first eluters (AMPA and glufosinate). Method reporting levels of 10-100 ng/L in water were noticed for the polar pesticides.

3.05.17
Use of Ex Situ Passive Samplers to Measure Bioavailable Polycyclic Aromatic Hydrocarbons in Sediment
S.B. Kane Driscoll, Exponent / EcoSciences; S. Ahn, Exponent / Environmental and Earth Science; M.W. Kierski, Exponent / Ecological and Biological Services Practice; P. Schierz, Helmholtz-Zentrum Dresden-Rossendorf e.V.; J. Clock, EPRI
The concentration of freely dissolved polycyclic aromatic hydrocarbons (PAHs) in sediment porewater has been shown to be closely related to the concentration of bioavailable PAHs. The purpose of this study was to evaluate the effectiveness of *ex situ* polyethylene (PE) passive samplers for measuring the concentration of freely dissolved PAHs in porewater from PAH-contaminated sediments. *Ex situ* passive sampling in the laboratory is easier and simpler than *in situ* deployment of passive samplers in the field, which is logistically more difficult and expensive. Nine sediment samples were collected from a river adjacent to a former Manufactured Gas Plant (MGP) site and analyzed for PAHs in bulk sediment. Subsamples of the same sediment samples were exposed *ex situ* in the laboratory to PE passive samplers. A pre-determined amount of PE was exposed to known amounts of sediment for six weeks, which is expected to be long enough for the PE to reach equilibrium with the sediment samples. Retrieved passive samplers were extracted for analysis of PAHs, and concentrations of PAHs in porewater were calculated using PE-water partitioning coefficients. PE passive samplers sorbed enough PAHs so that all PAHs were detected above method detection limits (MDLs) despite low PAH concentrations in some bulk sediment samples. To evaluate the feasibility and effectiveness of using passive samplers to measure porewater concentrations, the porewater concentrations calculated from PE sampler concentrations were compared to porewater concentrations predicted using non-site-specific equilibrium partitioning-based models, including the one-carbon model, which accounts for partitioning of PAHs to natural organic carbon (OC), and the two-carbon model, which accounts for partitioning of both natural OC and "black carbon" (BC) in sediment. This study demonstrated that *ex situ* PE passive samplers can be used to measure freely dissolved PAHs in porewater from bulk sediment samples and potentially to identify or "screen out" samples or areas of a site that do not require further analysis of risk.

Environmental Forensics - State of the Science and Global Applications

3.06.06
Source Apportionment of Perflouro Alkyl Substances in Great Lakes Fish
L.A. Rodenburg, RUTGERS UNIVERSITY / Environmental Sciences; Y. Lin, Xiamen University / Collage f the Environment & Ecology; S.L. Capozzi, Geosyntec Consultants, Inc. / Groundwater Remediation; K.R. Cooper, RUTGERS UNIVERSITY / Environmental and Occupational Health Science Institute
A data set of measurements of perfluoro alkyl substances (PFAS) in fish caught in near shore areas of the Great Lakes was analyzed using Positive Matrix Factorization and stepwise regression to investigate the primary sources of PFAS as well as the role of metabolism of PFAS compounds and their precursors. PMF analysis produced five fingerprints that are thought to be related to sources including textiles and aqueous film-forming foams (AFFFs) used in fighting fires, especially in aviation. The dominant measured PFAS compound in the fish, PFOS, increased in abundance as the water moves downstream from Lakes Superior and Michigan to Huron and thence Erie and Ontario. This may suggest that PFOS accumulates in the water column and that processes that remove it (biodegradation, volatilization, etc.) are not fast enough to prevent the accumulation of PFOS. This spatial trend may also indicate that a significant fraction of the PFOS arises from precursors that are transformed during atmospheric transport, since the prevailing winds in this region blow from west to east, mimicking the pattern of concentration increase, and large urban areas that could be sources of precursors, such as Chicago and Minneapolis lie to the west of the Great Lakes. Textiles and AFFFs also appear to be important sources of PFAS to Great Lakes fish. Stepwise regression was used to determine whether the PFAS and PMF derived factors were correlated with other chemicals measured in the fish including polychlorinated biphenyls (PCBs), brominated diphenyl ethers (BDEs), and Hg, as well as ratios of metabolized to unmetabolized PCBs and BDEs. Several lines of evidence, including the PMF-derived fingerprints and the correlations with metabolism ratios indicate that ADME (Absorption, Distribution, Metabolism, and Elimination) processes affect the levels of PFOS and PFOSA in the fish.

3.06.08
Bioaccumulation of Organic Pollutants in Mussel and Influence of Microplastics During the Process
R. Alvarez Ruiz, Centro de Investigación sobre Desertificación (CIDE) / SAMA-UV; J. Campo, CIDE-CSIC / Degradación y Conservación de Suelos; Y. Pico, University of Valencia / Environmental Quality and Soil
Coastal environments are heavily influenced by human activities. Organic pollutants are one of the most important indicators of the anthropic influence on the environment, furthermore, recent studies show interactions between the presence of microplastics and organic pollutants intake [1]. Mussels are suitable for in-lab aquarium experiments, such as bioacumulation studies, which provide insigth about the occurrence and fate of pollutants in the organisms. In the present work, bioacummulation in *Mytilus galloprovincialis* of 23 pollutants, including pharmaceuticals, pesticides, PFASs and illicit drugs and the influence of microplastics assesssed. Mussels (*Mytilus galloprovincialis*) were purchased from a local market (Valencia, Spain) and distributed randomly in three groups: control group (B), the group exposed to the organic pollutants mix (P) and the group exposed to organic pollutants and microplastics (P+M). The study was carried out during 58 days separated in two stages. Exposition stage during days 0-28 and depuration stage during days 28-58. Mussel were pooled, homogenized and extracted with QuEChERS combined with dispersive solid phase extraction (dSPE) Enhanced Matrix Removal (EMR-Lipid) clean-up. Analysis was performed via UHPLC-MS/MS. Results showed concentrations of several PFASs, pharmaceuticals and pesticides during the exposition stage. However, just 2 PFASs and 4 pesticides showed concentrations during depuration stage, suggesting effective bioaccumulation. Just terbuthylazine, chlorhenvinphos and chlorpyrifos showed differences between P and P+M with higher concentrations in P+M, especially during the depuration stage. The results of the present study suggest the effective bioaccumulation of several PFASs and pesticides in the visceral mass of mediterranean mussels. Furthermore, the differences between P and P+M also suggest that the presence of microplastics can influence the depuration capability of mussels for several pesticides. However, further research ins need to elucidate the particularities of this process.
References [1] Vieira Y, Lima EC, Foletto EL, Dotto GL. 2021. Microplastics physicochemical properties, specific adsorption modeling and their interaction with pharmaceuticals and other emerging contaminants. Sci. Total Env. 91: 1103-1113.

3.06.09
Detecting the Sources of Nitrate Pollution by Using Isotope Fingerprints
M. Tuthorn, Thermo Fisher Scientific; A.C. Smith, British Geological Survey; O. Kracht, C. Brodie, Thermo Fisher Scientific GmbH; J. Renpenning, Thermo Fisher Scientific / CMD
Nitrates are a key nutrient for plants and are largely applied in agriculture to enhance crop productivity. Their excessive use pollutes our environment on local levels, but also spreads

globally through oceanic and atmospheric systems. Consequentially, contamination of the hydrosphere affects human health through drinking water quality and environmental health. Whilst for surface water, soils and to some extent the atmosphere environmental clean-up can be undertaken in a matter of years, groundwater is far less accessible and more complex to clear of contaminants. For this reason, it is essential that we understand the sources and flow pathways for key contaminants in venerable groundwater aquifers. Stable isotopes are one key tracer for fingerprinting pollutants within the subsurface. Detailed studies of groundwater nitrate isotopes (N and O) can assist in identifying anthropogenic sources of nitrogen, which are responsible for the contamination of aquifer water. This work will give an overview of these stable isotope systems and some examples of how isotope fingerprints support the Nitrate Directive by offering a tool for nitrate source identification and tracking changes in our environment

3.06.10
Studying Multi-Residue Extraction Procedures in Mussels
R. Alvarez Ruiz, Centro de Investigación sobre Desertificación (CIDE) / SAMA-UV; D. Sadutto, Environmental and Food Safety Research Group of the University of Valencia (SAMA-UV) / Desertification Research Centre (CIDE); Y. Pico, University of Valencia / Environmental Quality and Soil; J. Campo, CIDE-CSIC / Degradación y Conservación de Suelos
The development of multi-residue methods aims to create procedures capable to extract a wide range of compounds at once, saving resources and time. In the present study, 10 pharmaceuticals, 5 pesticides, 5 perfluoroalkyl substances and 2 illicit drugs were chosen as target compounds for their extraction from mussel (*Mytilus galloprovincialis*). Different combinations of 4 different quick, easy, cheap, effective, rugged, and safe (QuEChERS) procedures and 12 clean-ups were tested. Finally, three methods were selected for validation according to the quality of the results. One based on the QuEChERS by the European Standards CSN EN 15662 [1], combined with a dispersive solid phase extraction (dSPE) specific for lipid elimination: the Enhanced Lipid Removal (EMR-lipid) dSPE by Agilent Technologies. The other two employed the official AOAC QuEChERS method [2] combined with two different dSPE clean-ups. One based on zirconium (Z-sep+ bulk) and other based on graphitized carbon black (GCB), both along with other sorbents. These clean-ups were selected in order to eliminate complex matrix interferences, such as lipids, proteins and pigments. Samples were analysed using high performance liquid chromatography-mass spectrometry (HPLC-MS/MS) with an Exion LC AD coupled to a Sciex QTRAP 6500+ mass spectrometer. Results showed limits of detection < 10 ng/g for most of the compounds. Strong matrix effect (>30%) was observed for more than the 40% of the compounds in the three methods. All the validated methods achieved satisfactory recoveries (70-120%) for most of the compounds. EMR-lipid showed recoveries ranging 53.9-124.0%, zirconium 58.5-124.0%, and GCB 60.3-127.0%. As an exception metformin, showed recoveries < 10% for the three methods. On the other hand, the illicit drug 4-MeO-PCP was satisfactorily recovered just when the carbon clean-up was employed. In conclusion, simple changes made to the classic QuEChERS and clean-ups can improve the overall recoveries for many types of different contaminants without sacrificing the performance of a multi-residue method.References: 1. EUROPEAN STANDARDS CSN EN 15662. “Foods of plant origin - Determination of pesticide residues using GC-MS and/ or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method”. 2. AOAC-INTERNATIONAL, Pesticides Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesion Sulfate. 2007.

Fate and Metabolism of Wastewater-Derived Pollutants

3.07.02
Rapid River and Tributary Catchment Profiling of Emerging Contaminants Using Direct Injection Liquid Chromatography Mass-Spectrometry (LC-MS/MS)
M. Egli, Imperial College London / School of Public Health (Environmental Research Group); H. Rapp-Wright, Dublin City University / School of Chemical Sciences; K. Ng, Imperial College London / School of Public Health; L. Barron, Kings College London / School of Public Health
There are currently an estimated 350,000 chemicals approved for manufacture and use globally. Population density in fast-growing cities such as London (UK) can result in more water pollution, such as combined sewer overflows, where untreated sewage directly enters a river [1]. Pinpointing impacted catchments depends on high frequency monitoring campaigns for hundreds of chemicals. This presents a major analytical challenge. The aim of this work was to assess a new rapid direct injection liquid chromatography tandem mass spectrometry method [2] for quantitative determination of 135 emerging chemicals in < 6.5 min and across an 80 km portion of the River Thames (Reading to Southend) as well as five tributaries (Rivers Brent, Grand Union Canal, Hogsmill, Lee and Wandle) at high spatial resolution. Water samples were collected in Dec 2019 and Nov/Dec 2020. For quantification, matrix-matched calibration (N=12, ranging from 5 – 5000 ng L^{-1}) showed excellent linearity (mostly R^2>0.99) and range for practical environmental application with a mean limit of detection at 4 ng/L for the vast majority of compounds with only 10 µL of filtered sample. Upon application, differences in contamination are presented for several analytes and selected sites were identified as being impacted more than others across both campaigns, e.g., Kew in East London. Interestingly, of over 200 samples collected and analysed in triplicate, together with quality control standards, blanks and multiple matrix-matched calibration lines prepared in marine, brackish and freshwater, this represented a total instrumental analysis time of only five days. For selected lifestyle markers, up to four-fold lower nicotine and 10-fold lower benzoylecgonine concentrations were observed in some Thames river stretches, which might be linked to impact of the COVID-19 pandemic. Moreover, this project provides new insights into potentially localised contamination of tributary rivers and their impact on the main river Thames using a streamlined LC-MS/MS analysis approach to help enable near real-time monitoring programmes at high resolution.

3.07.04
Exposure Assessment of Pharmaceuticals in the Lagoon of Venice: From the Emission Inventory to the Application and Evaluation of a Level III Fugacity Model
L. Calgaro, University Ca Foscari of Venice / Department of Environmental Sciences Informatics and Statistics; E. Giubilato, University Cà Foscari Venice / Department of Environmental Sciences Informatics and Statistics; L. Lamon, University Cà Foscari Venice; E. Marchese, University Cà Foscari Venice / Department of Environmental Sciences Informatics and Statistics; F. Calore, University Ca'Foscari Venice / Department of Environmental Sciences Informatics and Statistics; E. Semenzin, University Cà Foscari Venice / Department of Environmental Sciences Informatics and Statistics; A. Marcomini, University of Venice / Department of Environmental Sciences Informatics and Statistics
Pharmaceuticals are fundamental to effectively treat a multitude of diseases both in humans and animals but, at the same time, there is evidence of risks to the environment and, particularly in relation to antimicrobial resistance, to human health caused by the environmental release of these chemicals during their manufacture, use and disposal. The monitoring of pharmaceuticals in the environment is still limited, but the use of predictive exposure models has been identified as a complementary, low-cost tool to support the investigation of the behaviour and environmental fate of these contaminants and the assessment of associated risks.The lagoon of Venice, a highly valuable coastal transition environment surrounded by a densely populated area, is potentially subject to pharmaceutical contamination through several sources such as freshwater inputs from its drainage basin, discharges from wastewater treatment plants and Venice historical city centre. Despite various water quality monitoring programs conducted in accordance with the Water Framework Directive and other regional and national regulations, there is little information on the presence of pharmaceuticals in both water and sediment compartments. The aims of this work, developed within the Venezia2021 project, are: (i) to provide an emission inventory of several pharmaceuticals in the study area, (ii) to investigate the environmental distribution and fate of these contaminants in the lagoon of Venice, with particular attention to the processes involved in their natural attenuation, and (iii) to define a risk-based priority list of contaminants specific to the case study area, taking into consideration the overall mass balance of the pollutants under consideration. The emission inventory was developed by integrating pharmaceutical sales data for human consumption obtained from the Veneto Region Authority with census and geographical information through statistical data treatment and GIS-based spatial analysis. In addition, an exposure assessment of these chemicals for the lagoon of Venice was carried out through the application and evaluation of a multimedia level III fugacity model. Model evaluation was carried out by performing uncertainty and sensitivity analysis on the predicted results, as well as by comparing concentrations calculated by the model after

calibration with the concentrations measured in lagoon water and sediments samples taken during two recent sampling campaigns (2019).

3.07.06
In Vitro Toxicity and Metabolism of a Potentially Sustainable, Fungal Colourant Dermocybin
J. Yli-Öyrä, M. Herrala, R. Juvonen, M. Lehtonen, University of Eastern Finland / School of Pharmacy; G. Umbuzeiro, School of Technology- UNICAMP / LAEG Laboratory of Ecotoxicology and Genotoxicity; R. Raisanen, University of Helsinki / HELSUS Helsinki Institute of Sustainability Science; J. Rysä, University of Eastern Finland / School of Pharmacy
The current industrially used colourants pose a significant occupational health risk and cause environmental complications upon their release within industrial effluents. In the future, colourants obtained from biological matter may serve as a sustainable colourant source. However, their toxicity is scarcely studied although natural origin is not a safety guarantee. Dermocybin is an orange anthraquinone colourant obtained from a fungal species *Cortinarius Sanguineus*, also known as bloodred webcap. We studied the toxicity of dermocybin in two human cell lines: human breast cancer line MCF-7 and a hepatic cell line HepG2. We determined the effect of dermocybin on cell viability with MTT and propidium iodide (PI)-digitonin assays, cytotoxicity with lactate dehydrogenase test, production of reactive oxygen species (ROS) with dihydrodichlorofluorescein diacetate (DCF) assay, and superoxide formation both in cytosol with dihydroethidium assay and mitochondria with mitochondrial superoxide assay. We used six dermocybin concentrations from 0.035 to 7 µg/ml accompanied by positive and negative controls. The experiments were repeated three times and each concentration had four replicates. Cells were seeded onto 48-well plates for 24 h prior to exposure and then exposed to dermocybin for another 24 h. We observed a 2.2-fold increase in mitochondrial superoxide production with MCF-7 cells at 7 µg/ml compared to control (DMSO 0.1 %) ($p< 0.01$). MTT, PI-Digitonin and DCF tests were conducted also in HepG2 cell line. MTT test showed a 23.4 % decrease compared to control in cell viability at 7 µg/ml ($p< 0.05$), and the rest of the experiments are to be completed. Lower dermocybin concentrations did not show any statistically significant effects compared to controls in either cell line. In addition, dermocybin was not mutagenic in a miniaturized Ames test. Further, the metabolism of dermocybin was studied in human liver microsomes and cytosol which contain the metabolic enzymes UDP-glucuronosyltransferase, sulfotransferase, cytochrome P450 enzymes, and catechol O-methyltransferase. We observed metabolism through glucuronidation, sulfonation, and oxidation reactions but further studies in single-enzyme assays are needed. Completion of the studies is needed to evaluate if dermocybin can be considered safe. This study is a part of the BioColour project that aims to identify biodegradable and environmentally friendly set of colourants that are safe for humans as well.

3.07.08
Treated Wastewater Significantly Affected the Community Composition of Beneficial Bacteria in Lettuce Roots
Y. Bigott, Helmholtz Zentrum Munchen / Comparative Microbiome Analyses; S. Gallego, M. Breuil, AgroSup Dijon, INRAE Agroécologie, Univ. Bourgogne, Univ. Bourgogne Franche-Comté; C. Cruzeiro, Helmholtz Zentrum Muenchen / Research Unit for Comparative Microbiome Analysis (COMI); N. Montemurro, Jordi Girona 18-26, null / Dipartimento di scienze agro-ambientali e territoriali; S. Perez, IDAEA-CSIC / Department of Environmental and Food Chemistry (ENFOCHEM); F. Laurent-Martin, AgroSup Dijon, INRAE Agroécologie, Univ. Bourgogne, Univ. Bourgogne Franche-Comté; P. Schroeder, Helmholtz Zentrum Muenchen / Microbe Plant Interactions
The reuse of soil and water for agricultural practices becomes progressively more important due to the increasing demand for a transition to a circular economy. Therefore, treated wastewater can be an alternative option for the irrigation of crops especially in arid and semiarid regions. Besides this considerable benefit of treated wastewater, it might pose potential risks by pharmaceuticals and personal care products (PPCP) disposed to the sewage system. Those wastewater-borne contaminants and their metabolites can be taken up by edible plants and therefore enter the human food chain. Thus, this study compares the uptake and metabolisation of PPCPs derived from freshwater and treated wastewater into lettuce as well as their impact on the plant-associated microbiome. Moreover, the reuse of the same soil was evaluated during a consecutive campaign. For the experimental setup under greenhouse conditions, four-week-old lettuce was transferred to pots filled with soil and irrigated with freshwater or treated wastewater spiked with either none, 10 µg/L or 100 µg/L of 14 different PPCPs. After seven weeks, the plants were harvested and the same soil was reused for a second cultivation campaign of lettuce. First results showed that the concentration of several PPCPs was multiple times higher when irrigated with spiked treated wastewater compared to freshwater in lettuce roots but not in leaves or soil in both campaigns. The bacterial diversity and community structure was examined by 16S rRNA sequencing. A PERMANOVA analysis revealed a significant different bacterial distribution in both campaigns affected by the treatments. Moreover, the type of irrigation water (freshwater or treated wastewater) significantly affected five bacterial families in the first campaign. Four out of these five families belong to the most abundant ones (> 2% relative abundance) and furthermore all of them had been shown to possess plant growth-promoting activities in previous studies. In the second campaign of lettuce cultivation in only minimal disturbed soil, eighteen bacterial genera were significantly affected by the type of irrigation water and seven by the concentration of the PPCPs. Only members belonging to the Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium clade were increased by the PPCP concentration. Results are critically discussed with view to agricultural practice within a circular economy focused on the direct reuse of soils and irrigation with treated wastewater.

3.07.10
Uptake and Translocation of the Opioid Painkiller Tramadol in Plants
D.M. Kamel, Helmholtz Zentrum Munchen / Research Unit Comparative Microbiome Analysis (COMI); C. Cruzeiro, Helmholtz Zentrum Muenchen / Research Unit for Comparative Microbiome Analysis (COMI); P. Schroeder, Helmholtz Zentrum Muenchen / Microbe Plant Interactions
Pollution of the aquatic environments, as well as water scarcity, are threatening human health and the sustainability of our ecosystems. Several studies revealed that contaminants of emerging concern (CECs) not only might pose risk to< w:sdt sdttag="goog_rdk_0" id="165785446"> aquatic life but also to humans due to the pervasive use and frequent disposal into the environment, comprising pharmaceuticals and personal care products, endocrine-disrupting compounds, flame-retardants, disinfection byproducts, pesticides and artificial sweeteners. From these CECs, pharmaceuticals ranked among the 40 most important issues in the US, and in Europe, the EU Parliament added several drugs to their Watch List of water pollutants. Among critical compounds contaminating our water systems, the opioid, tramadol (TRD) that is widely used to treat moderate to severe pain was of interest. TRD has been detected in various water resources, such as surface water (7.7 µg L^{-1}), wastewater treatment plant (WWTP) effluent (55 µg L^{-1}) and WWTP influent (87 µg L^{-1}) as a consequence of misuse and/or inefficient wastewater treatment. Phytoremediation via constructed wetlands is considered as a promising technique for CECs and pharmaceuticals removal from wastewater. However, little information is available regarding TRD uptake by plants. In order to evaluate phytotechniques for the removal and detoxification of TRD, we investigated root uptake and translocation in seedlings to scrutinize the fate of TRD during the process of phytoremediation. The first technique was used to explore the short-term uptake of TRD for 24h using a Pitman's chamber setup. The results were then strengthened with the second technique using barley seedlings for 15 days' time exposure. The short-term root uptake experiment showed a transport rate of TRD up to 5 µg $L^{-1}day^{-1}$ through barley and cattail roots. As a complement, the barley seedlings revealed, at the end of the experiment, the removal of approx. 90% of the compound from the residual growth medium. We succeeded to detect and quantify TRD inside the root and shoot tissues of the barley seedlings. The results showed values of bioconcentration and translocation factors close to 9 and 1, respectively. In conclusion, the combination of techniques provides a rapid-robust dataset that can be used to estimate the uptake and translocation of any new emerging contaminants to successfully implement phytoremediation.

Harmonized Data Reporting and Analyses in Micro- and Nanoplastics Research

3.08.06
EUROqCHARM - Assuring Reproducible, Harmonised and Quality-Controlled Assessments of Plastic Pollution
B. Van Bavel, NIVA / Environmental Pollutants; A. Lusher, NIVA Norwegian Institute of Water Research / Environmental Contaminants; S. Aliani, Italian National Research Council / Institute of Marine Sciences; J. de Boer, VU University / Environment & Health; F. Galgani, IFREMER; E. Stoica,

RMRI; J. Fabres, SALT; M. Farre, IDAEA-CSIC / Department of Environmental Chemistry; D. Herzke, NILU - Norwegian Institute for Air Research / FRAM Centre Tromso; B. De Witte, ILVO Institute for Agriculture and Fisheries Research; S. Primpke, Alfred Wegener Institute / Shelf Sea System Ecology; J. Strand, Aarhus University / Department of Bioscience; J. Johansen, Chiron AS / Administration; R. Kaegi, Eawag - Swiss federal Institute of Aquatic Science and Technology / Process Engineering; A. Giorgetti, OGS; C. del Cerro, AFNOR

Plastic pollution (macro to nano) has become a global environmental and societal concern. Numerous protocols have been developed to monitor plastic debris, but these are rarely comparable. This has hindered gathering of knowledge regarding pollution sources, development of monitoring programmes, risk assessments and the implementation of mitigation measures. To develop long-term solutions to reduce plastic pollution, it is essential to establish harmonised methodologies. EUROqCHARM, a recently funded H2020 project, will address this by critically reviewing state-of-the-art analytical methods and validate them through an interlaboratory comparison (ILC) study. The EUROqCHARM consortium consists of 15 partners, a scientific advisory board composed of international experts and a network of more than 25 associated laboratories.

EUROqCHARM recognises that harmonisation for large scale monitoring requires flexibility, comparability and reliability. As this is a Coordination and Support Action (CSA), our aim is to provide a cross-Europe and international platform validate several methods for monitoring plastic in the environment and put forward recommendations and standards for monitoring. In more detail, this project aims to identify, test and optimise monitoring approaches through quality assured and rigorously validated methods based on current state-of-the-art techniques which cover all relevant environmental matrices.

EUROqCHARM will focus on harmonizing and possibly standardisation of methods and reporting formats to facilitate data comparability and meta-level analysis on regional, national and international scales. By including multiple national and international organisations and working groups currently participating in the proposal of harmonisation and standardized methods for research and monitoring, a coordinated and strategic action to bring these key players together, merge working group ideas and facilitate a framework for urgently required procedures for monitoring and assessment of plastics in our environment.

3.08.07

Microplastic Sampling, Source Identification and Data Management System for Researchers, Policy Makers, and Community Groups

A. Abeynayaka, Tokyo City University / Graduate School of Environmental and Information Studies; Y. Fukunaga, Pirika Inc.; N. Itsubo, Tokyo City University / Graduate School of Environmental and Information Studies; F. Kojima, Pirika Inc.

Microplastic associated pollution is a global environmental concern. Microplastic contaminants in freshwater itself a threat to the ecosystem and a potential health hazard to humans. Apart from that, freshwater resources such as rivers carry plastic litter into marine environments, where local and regional pollution becomes a global concern. Rivers are considered as a main contamination pathway of microplastics to the oceans. The number of research groups focussed on microplastic sampling and analyses. The projects “the ocean and Japan project” and “Japan fund for global environment” provided support for a large number of sampling activities with a sampling method developed using the sampling device “Albatross”. More than 200 sampling locations were covered in various parts of the world. Sample analysis and data management system has been introduced to build a unified system to facilitate various stakeholders. Sampling device development for accurate flow rate measurement and larger volume sampling (3 to 20 m^3) within a short period (3 min) is described by Abeynayaka et al 2020. The microplastic identification methods were developed with the assistance of several stakeholders (researchers, plastic manufacturers, etc.) This includes common polymer identification methods (i.e. FT-IR), microscopic observations, color and shape observations, etc. The data management system is prepared to handle the accumulating data. The data management system includes spatial information, concentrations of microplastics, and other relevant information collected. Moreover, we have developed relationships of different parameters using our large number of microplastic samples. For example, correlation analyses were conducted for area, mass, thickness, and volume. Starting in 2020, the sampling process has been conducted under several streams. Apart from the Pririka, the equipment was lent for sampling by local authorities, environmental groups, universities, etc. This has diversified the geographical distribution of sampling locations and the quantity of data. Efforts to maintain the quality of data have been taken by preparing manuals, video demonstrations, training sessions, etc. An issue faced by sample collectors such as local authorities, environmental groups, universities in developing countries is the lack of skills to analyze the samples. A proper mechanism has been developed to support the steps after sample collection.

3.08.08

Development of a Prototype of Microplastics Sampler for River Waters

C. Campanale, Italian National Research Council / Water Research Institute; C. De Palma, De Palma Thermofluid s.r.l. - Industrial projects division; B. Bollino, De Palma Thermofluid srl - Divisione Progetti Industriali; C. Massarelli, Italian National Research Council / Water Research Institute; V. Uricchio, Italian National Research Council / Water Research Institute / Water Research Institute (IRSA)

Until now, microplastic studies in river waters have mainly been adopting sampling methods from marine research, as plankton or manta nets, usually with a mesh size of 300 µm or 333 µm. However, some criticalities such as the application of these systems on smaller freshwater bodies, the filtered water volume not precisely defined and the clogging of nets appear during sampling of rivers. Only a few studies have presented microplastic sampling systems based on pump samplers. In light of this, we developed a mobile sampling system using a pump and including a set of stainless steel filters that has many advantages to collect microplastics from river waters. The system is based on the technology of diversified fineness microfiltration carried out by mechanical sieving using the differential pressure created on the two sides of a selective barrier used to separate the liquid part from the suspended solids. The highly innovative prototype consists of two elements: a floating funnel positioned in the centre of the watercourse and a filtration system positioned on the shore, connected by a flexible tube. The funnel, with a rectangular mouth, is positioned on the surface of the water and, equipped with a 2cm pre-filter, has the function of sampling the water by conveying it with a pumping system, towards the filtering station, taking care to maintain the laminar regime in all phases. Reached the filtration station, equipped with a series of portable stainless filters with different mesh-sizes and a clogging warning system, the water falls by gravity, and the solid material present is sieved allowing a first classification of particles for size. The sampler allows an accurate calculation of the filtered water volumes and a continuous sampling of up to ten consecutive hours in order to monitor the daily trends in the concentration of microplastics useful for representing the quality status of inland waters.

3.08.09

A Promising Approach for Economical Microplastic Analysis in River Sediments

S. Schirrmeister, L. Kurzweg, HTW Dresden / Technical Chemistry; Y. Adomat, HTW Dresden / Department of Civil Engineering; S. Faist, HTW Dresden / Electrical Engineering; T. Grischek, HTW Dresden / Department of Civil Engineering; K. Harre, Dresden University of Applied Sciences / Agriculture, Environment, Chemistry; R. Bauer, Dresden University of Applied Sciences / Electrical Engineering; M. Socher, HTW Dresden / Department of Chemical and Environmental Process Engineering

Occurrence and impact of microplastic (MP) in environmental compartments are topics of growing concern. Efficient and harmonised monitoring is urgently required to obtain information about pathways of MP in the environment. Rivers play a major role in MP transport and access to marine ecosystems[i]. Right now, MP monitoring has limited availability because of its high costs and expertise. This project aims to reduce the costs and duration of MP analysis in sediments and to simplify the workflow to establish governmental driven MP monitoring campaigns. A promising approach for MP analysis in river sediments combining electro separation (corona roller separator, Hamos, Prenzberg, Germany) and differential scanning calorimetry (DSC, Netzsch, Germany) will be presented. Robustness against organic matter and fast preparation of samples by drying and separation in a kilogram per hour scale provide an economic potential for monitoring. In experiments we have used MP particles of ultra-high molecular weight polyethylene (UHMW-PE, size: 100 to 200 µm) which are separated from matrices such as river bed sediments and sand. Samples with MP concentrations up to 100 ppm (parts per million) PE-UHMW were investigated. Recovery rates of the polymer in the enriched fractions were determined using the thermodynamic fingerprints measured by DSC. The separation efficiency has been tested for artificial as wells as natural sediments. The grain size distribution was found to be a crucial

parameter affecting the efficiency of separation. We also determined the limit of quantification. The recovery rate of UHMW-PE shows a decrease in environmental matrices compared to artificial sediments. The processing time to complete the sequence of sample drying, sieving, electrostatic separation, DSC measurement and evaluation is crucial for future application in environmental monitoring. Following the procedure, five samples could be processed in eight hours after drying. In conclusion, the approach has a potential as a reliable and fast method for monitoring of highly polluted environmental compartments. Subsequent research aims at an optimisation of the electrostatic separation to increase the recovery rate for environmental samples as found for artificial sediments to determine MP in river sediments. [i] McCormick, A. R. & Hoellein, T. J. *Limnol. Oceanogr.* **61**, 1718–1734 (2016)., Hoellein, T.J., Shogren, A.J., Tank, J.L. *et al. Sci Rep* **9,** 3740 (2019). https://doi.org/10.1038/s41598-019-40126-3

3.08.10

Extraction of Microplastic Particles From Digested Sewage Sludge

M. Philipp, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Process Engineering; M. Zuber, Agroscope ART; T.D. Bucheli, Agroscope / Environmental Analytics; R. Kaegi, Eawag - Swiss federal Institute of Aquatic Science and Technology / Process Engineering

Wastewater treatment plants (WWTPs) are hubs controlling the retention and release of microplastic (MP) particles from wastewater streams. Several studies focused on the quantification of the MP contents in the inflow and outflow of WWTPs. However, temporal fluctuations of the MP contents, especially in the influent, make a representative sample collection challenging. In digested sewage sludge (sludge age ~30 days) these temporal fluctuations are smoothed out. Furthermore, for relevant wastewater treatment processes such as the activated sludge treatment, sedimentation and flocculation, the same behavior of MP particles as for other particulate materials is expected. The MP removal efficiency in WWTPs is therefore similar to the removal of total suspended solids generally exceeding 95%. Digested sewage sludge therefore seems well suited for monitoring MP contents in wastewater and for assessing the amounts of MP discharged from WWTPs into surface waters. We, therefore, developed an extraction protocol tailored for digested sludge by modifying existing approaches. We use a three-step protocol, consisting of an enzymatic digestion, a density separation using polytungstate and an oxidation step using Fenton reagents. The biggest challenge is the high cellulose content in the digested sludge, resulting from toilet paper in the wastewater. Cellulose is poorly digested during the enzymatic and the oxidative digestion and cannot be separated through a density separation from PET and PVC. For the detection and identification of the polymer types using for example FPA-µFT-IR, the amount of digested sewage sludge extract that can be brought onto sample carriers is, therefore, limited by the cellulose content of the wastewater. Furthermore, unknown efficiencies of MP extraction protocols often compromise the interpretation of the experimental results. We therefore spiked 10 fibers (orange, polypropylene (PP), 50µm x 600µm) and 30-50 spheres (red, PP, 50µm) to the digested sludge. The spiked fibers and spheres were not affected by our digestion protocol and allowed us to assess fibers and spheres specific recoveries using an automated optical stereomicroscope. First results indicate a quantitative recovery of both spiked particle types. We, therefore, recommend using similar spiking protocols for all matrices as this allows assessing sample specific recoveries and enables identifying and correcting for possible MP losses related to the sample preparation protocol.

3.08.12

Large-Scale Study of Microplastics in Five Bivalve Species From the Nordic Marine Environment

I. Nerland Bråte, NIVA - Norwegian Institute for Water Research; A. Lusher, Norwegian Institute for Water Research (NIVA); R. Hurley, Nowegian Institute for water research; N. Buenaventura, NIVA Norwegian Institute for Water Research / Environmental Contaminants; M.T. Hultman, Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; C. Halsband, Akvaplan-niva; N. Green, NIVA

Microplastics (MPs) are abundant in the environment, yet the ecological impact is not well understood. One important task is to identify sentinel species for monitoring to identify hot-spots where biota are at higher risk of MP interaction and possible effects. Sentinel species can also be used to help stakeholders and decisionmakers to pinpoint the most effective measures by revealing trends, specific sources, transportation routes and fate. Mussels (*Mytilus* spp.) are already highlighted as a sentinel species for MP monitoring, however mussels are not always commonly found. On behalf of the Nordic Council of Ministers, we assessed the occurrence of MP in five bivalve species including mussels, from the Nordic marine environment. One hundred sites were selected: 32 for the hard-bottom species *Mytilus* spp., 14 for the three soft-bottom species *Limecola balthica*, 31 for *Abra nitida*, 20 for *Thyasira* spp. and 3 for the hard-bottom arctic *Hiatella artica*. The sites were spread across the Baltic Sea and the coasts of Denmark, Norway (including Svalbard), Faroe Islands, Iceland and the east side of Greenland. The samples were analysed blind to remove any observer bias. Visual identification followed by point (µtrans) or semi-automated imaging (µATR) FT-IR and pyrolysis GC/MS were applied. Four of the five species were found to contain MPs above the limit of detection (LOD): *Mytilu*s spp., *L. balthica, A. nitida* and *Thyasira* spp. At eleven *Mytilus* sites MPs were above the LOD (LOD, 2.77 MPs per individual), with the inner Oslofjord mussels containing the highest concentration of microplastics (>61 MPs per individual), as was also found in 2017 and 2018. Areas of Skagerrak, Kattegat, Baltic Sea and the North Sea close to urban areas were also found to have the high levels of MPs compared to other sites. Black rubbery particles were dominant in *Mytilus* spp., *A. nitida* and *L. balthica*. These rubbery particles could stem from road-run off or other sources of rubber such as tires used in harbours. MPs between 63 and 1000µm were present in *A. nitida*, *L. balthica,* however no MPs larger than 63µm were detected in *Thyasira* spp. This study shows that urbanized areas in the Nordic marine environment are receiving high levels of MPs, and that bivalves could be used to monitor small MPs. It also highlights that bivalves from the Nordic environment are exposed to rubber, and the sources of these rubbers should be further investigated.

3.08.13

A Novel and Rapid Approach to Automatically Quantify and Classify Microplastics Based on Computer Vision and Machine Learning Algorithms

C. Campanale, C. Massarelli, Italian National Research Council / Water Research Institute; V. Uricchio, Italian National Research Council / Water Research Institute / Water Research Institute (IRSA)

Microplastics have recently been discovered as considerable pollutants due to their presence in all environmental matrices investigated. The monitoring of their presence in the environment requires laborious and time-consuming operative workflows due to the need to carry out regular campaigns and to set up always more elaborated analytical procedures to their extraction and quantification. In light of this, an innovative method for counting and classifying microplastic particles has been developed exhibiting promising results. The method makes use of both Computer Vision techniques and Machine Learning algorithms able to count and classify microplastics fastly and automatically and to discover hidden information. The system has been tested on five different samples, with a total of 2501 microplastic particles. The particles have been manually counted and sorted in different morphology and size classes by an expert and results compared to data obtained by our system. The obtained results showed very similar counting results between the two sets of data with a deviation standard ranging by 0 to 4.9% among the samples. Depending on the sample, the algorithm sometimes overestimated the real number of manually counted particles, and some other underestimated them. However, these difference falls within the acceptable margin for monitoring studies. The automatic classification of particles in different morphology and size categories achieved good results too, even if sometimes misleading classifications of particles occurred. This aspect has to be certainly improved, including other features and new rules for classifications of particles, but it is an optimum start point. Furthermore, once better the process, it will provide a standardised method to classify microplastics based on objective parameters, without leaving room for subjective evaluations.

3.08.21

Microplastic Mass Estimation: A Pyrolysis GC-MS Based Evaluation of µFTIR-imaging Estimates

J. Lykkemark, Aalborg University / Built Environment; M.L. Christensen, Aalborg University / Department of Chemistry and Bioscience; A.H. Nielsen, Aalborg Unversity / Department of the Built Environment; J. Vollertsen, Aalborg University / BUILD

The interest in microplastic (MP) and the pollution caused by it has increased over the last 10 years, and therefore a need for better analytic methods became crucial. One of the methods that have been developed and tested repeatedly for MP is Fourier-transform infrared spectroscopy (µFTIR) imaging. This method gives a 2-dimensional IR-image of the particles. To get from here to the particle volume and finally mass, the 3rd dimension can be estimated, an approach which for example is used in

software such as siMPle (www.simple-plastics.eu). So far the trustworthiness of this mass estimation approach has though not been assessed and an independent validation is hence called for. Although a few articles recently have compared the results using µFTIR imaging and a thermal degradation analysis, the mass estimation has not been evaluated for individual particles. This study investigates the goodness of the µFTIR imaging mass estimation in comparison to a pyrolysis GC-MS mass quantification. The validation is done on single well-defined polymers particles created by cryomilling. Seven polymers are investigated, firstly particles in the range from 150-180 µm are obtained for each polymer. Each particle is first measured individually using µFTIR imaging and then destructively analyzed by pyrolysis GC-MS with internal and external standard - calibration curve. The mass results obtained from the two analytical methods are then compared for each particle together with the overall mass for each polymer type.*Keywords: µFTIR imaging, Pyrolysis GC-MS, siMPle*

3.08.22
Open Specy: An Open-Source Online Tool for Raman and FTIR Spectral Analysis of Microplastics and Beyond
W. Cowger, A.B. Gray, University of California Riverside / Environmental Science
Spectroscopy is an essential tool for microplastic research. Spectral analysis is used to characterize polymer types, mixtures, and weathering, and provide an independent evaluation of particles identified through taxonomic approaches. Microplastic spectral analysis is a rapidly developing field with a diversity of methods, which makes comparability and reproducibility across studies challenging. Spectral analysis software and libraries can cost thousands of dollars, yet often do not have reference materials that are relevant to microplastic research. Several open access spectral software and reference databases have been developed that include weathered and non-virgin polymer materials absent from many commercial libraries. Open Specy is an online, open-access spectral analysis tool that includes many of these reference databases, and encourages users to submit reference spectra to keep up with the ever-growing diversity of plastic materials in the environment. Open Specy allows researchers to freely and accurately process and identify FTIR and Raman spectra of environmental plastic pollution.

3.08.23
Harmonization of Microplastics Analysis by a Manufacturer Independent Software Tool: (siMPle)
S. Primpke, Alfred Wegener Institute / Shelf Sea System Ecology; A. Vianello, Aalborg University / Civil Engineering Department - Section of Water and Environment; G. Gerdts, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research / Shelf Sea System Ecology; J. Vollertsen, Aalborg University / Department of the Built Environment
The contamination of the environment by microplastics (MP) is of emerging concerns for legislation and societies. While first monitoring programs are currently starting, the harmonization of the analysis of these particles is still in progress. For this process, various steps are involved, which are ranging from sampling, work up/purification to chemical analysis. Here, different types of challenges are connected with each of these steps, and the analysis in addition, is based on different methods using either spectroscopy or thermoanalysis. These are generating already data sets of different data quality and comparability. One broadly used spectroscopic method is based on FTIR imaging. It allows the chemical mapping and analysis of complete filter areas independent of human bias in a short amount of time. While archiving broad information about the particles present in the sample, a comparable data determination is hampered by the different manufacturer and commercial software packages available. Due to this, the development of standardized operational protocols (SOPs) is limited and will receive a broad diversity of the generated data. To allow a harmonized analysis of spectral data and therefore to overcome these limitations, we developed the software tool siMPle (www.simple-plastics.eu). At the current stage, it already allows the analysis of datasets measured on different machines from various manufacturers including Agilent, Bruker, Perkin Elmer and Thermo Fisher (further imports in development). Within this tool, every spectrum can be investigated individually or the chemical map treated by two automated analysis pipelines, the original MPhunter- and the widely applied automated analysis pipeline. Within the software, large datasets containing several million spectra can be handled with relative ease. In addition, the generated data was recently benchmarked and validated in accordance with the original automated analysis approach based on Bruker OPUS, allowing a harmonized comparison of results to previous studies. Further, it was found that the calculation time was significantly reduced from more >24 h down to 2 h for a reference data set containing 1 million spectra. The software is available as Freeware for all types of users and allows the harmonization of MP data analysis for spectroscopic data for future research.

3.08.25
"From Debris to the Lab: A New Approach on Microplastic Research"
J.I. Serrão, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research; J. Lourenco, University of Aveiro; V. Nogueira, Faculty of Sciences and CIIMAR - Porto University; R. Silva, GreenUPorto & Faculty of Sciences, University of Porto; R. Magalhães, N. Martins, H. Fernandes, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research; T. Rocha-Santos, University of Aveiro & CESAM / Department of Chemistry; S. Monteiro, CESAM & Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; J.P. da Costa, Aveiro University & CESAM / Department of Chemistry; R. Pereira, Faculdade de Ciências da Universidade do Porto / Biology
Awareness of huge quantities of plastic debris in marine environments lead the scientific community to focus their attention on the topic and the number of scientific publications has increased every year. Although much work has been done to qualify and quantify the pollution, identify sites of concern and to understand the potential impacts that plastics can cause in organisms and ecosystems, there are still many questions to answer. Among these, the achievements obtained in microplastic-related toxicity are yet limited with many authors pointing out the gap between the laboratory methodologies and subsequent results and their environmental relevance. One of the most referred limitations is the difference between the homogeneity of laboratory tested elements and the heterogeneity found in nature. In this study, an acute toxicological bioassay using gilthead seabream juveniles, *Sparus aurata* Linnaeus, 1758, was performed using as test substance microplastic particles collected from the Portuguese shore. Microplastics with a variety of forms, colours, stages of degradation, chemical composition and with sizes between 1 to 5 mm were used “as is” in a concentration of 30mg/L. Two variables were tested: the presence/absence of plastics and the presence/absence of food in the tanks. For this later variable fish were either starved during the assay, or to promote similarities with the natural environment, microalgae, *Nannochloropsis sp.*, were added to tanks and fish were feed with *Artemia*. In the end of the bioassay, after 96 hours of exposure, fish were weighted and measured, samples of blood, digestive tube, liver and gills were collected. The presence of microplastics in the digestive tube was also recorded. Results showed that a high percentage of fish ingested microplastics, both from unfed and fed treatments, being slightly higher in the last one, 67.86% and 82.14%, respectively. Furthermore, oxidative stress parameters, intestinal permeability and tissues histology are being assessed in the different organs through a battery of several analyses. Moreover, transcriptomic analyses are being performed in gill and gut samples to determine changes in the gene expression profile of these organs due to the potential physical and chemical stress induced by ingested microplastics.

Importance of Chemical Speciation and Bioavailability in Hazard Assessment, Risk Assessment and Regulation of Metals in Multi-Stressor Environmental Conditions

3.09.01
Sulfur Control on Selenium Speciation
M. Martinez, FHNW / Institute for Ecopreunership; M. Lenz, University of Applied Sciences and Arts Northwestern Switzerland FHNW / Institute for Ecopreunership
Selenium (Se) is an essential yet toxic trace element with one of the narrowest nutritional optimums of all elements. Se speciation plays a crucial role in its mobility, bioavailability, bioaccumulation, and toxicity. The current perception of Se environmental cycling encompasses a linear series of successive, bi-directional redox processes. Elemental Se is seen as a central species thermodynamically favored in redox conditions found in most environments. Most studies on Se environmental transformations focused on systems characterized by high Se concentrations. In nature though, sulfur (S) concentrations are in general orders of magnitude higher than those of Se. Sulfur can be seen as a “sister element of Se”: Se and S have many chemical similarities (for instance similar prevalent oxidation states, same geometries of the oxyanions etc). The fact that several mixed Se/S species are known to exist challenges the “selenocentric” view seeing elemental Se as central (terminal) species in sulfur dominated environments. This work

investigated sulfur and selenium cycles interconnections and shed light on novel Se/S species identified by elemental and molecular mass spectrometry (ICP-MS/MS and HPLC-ESI-qTOF-MS). The implications of the new Se/S species on Se cycling in such environments is discussed.

3.09.02
Evaluating the Potential Mobility of Cd in Contaminated Calcareous Sediments Amended With Biosolids and Biochar
K. Mitchell, St. Georges University / Public Health & Preventive Medicine; F. Avelar-González, A. Guerrero-Barrera, M. Ramos-Gómez, L. Yamamoto-Flores, Universidad Autónoma de Aguascalientes
Studies have shown that the addition of organic amendments to metal polluted soils reduces metal mobility, in many cases due to association with organic matter fractions or co-precipitation especially in substrates with low pH and carbonate content. However, variations in these physicochemical conditions may influence the prevailing mechanism involved in mobility reduction and metal – fraction associations, thus having important implications in long term metal retention. Thus, the aim of this study was to determine the prevailing mechanism(s) involved in Cd mobility reduction in contaminated calcareous sediments amended with biosolids and biochar prepared from biosolids.Sediments were obtained from the Piedras Negras River in Asientos, Aguascalientes, Mexico. After the determination of physicochemical characteristics including pH and carbonate content, and total initial metal content in sediments, the experiment was setup in triplicate as follows: 100 g sample (S), 100 g sample + 10 g biosolids (S + BS), 100 g sample + biochar (S + BC). Samples were saturated to 75% with meteoric water and was left to stand for 1 h. Then 4 g of sample was leached with 40 ml of $CaCl_2$ 0.01M pH 3.5, agitated for 2 h. The leachates were then used for pH and total Cd content determination. This was repeated every 7 days for 4 weeks, after which metals were sequentially extracted from 1 g using the Tessier batch metal fractionation procedure.Amendment resulted in a significant reduction in Cd mobility. S + BS had a 74% reduction in Cd mobility and S + BC a 91% reduction with statistically significant differences between each group when leached with $CaCl_2$ 0.01M pH 5.5. The same statistically significant tendency, though more pronounced, was observed when treatments were leached at pH 3.5, S + BS with an 83% reduction and S + BC a 96% reduction in Cd mobility. These results coincide with those observed by other research groups, where Cd mobility seems to be particularly affected by higher ferromanganese oxides content. Here higher Fe and Mn in S + BC (up to 30% more), may have played an important role in this increased mobility reduction when compared to S + BS. Metal fractionation confirmed this increased association with the Fe/Mn fraction, suggesting a shift in Cd from the potentially mobile exchangeable and carbonate fractions to the lesser mobile ferromanganese and organic matter fractions. This study highlighted the fact that though amendment of contaminated substrates with biomass, generally reduces mobility, physicochemical characteristics of contaminated substrates and amendments play an important role in specific metal-substrate fraction association. Therefore, it is important to determine prevailing mobility reduction mechanisms, due to the implications this would have in long term mobility reduction.

3.09.03
Rare Earth Elements in Soil Samples Derived From Electronic Waste: Microcosm Study
M. Makombe, CPUT / Department of Chemistry; C. Van der Horst, Cape Peninsula University of Technology (CPUT) / Chemistry; V.S. Somerset, CPUT / Chemistry
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 centers. 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. Further results of speciation are still being investigated in this ongoing study.

3.09.04
Assessing the Leaching of Metals From Ashes Through Soil Column Tests: Potential Impacts to Groundwater
I. Campos, Universidade de Aveiro / Environment; C. Junior, University of Aveiro / Department of Environment and Planning & CESAM; J. Keizer, University of Aveiro-CESAM / Department of Environment and Planning; C. Coelho, University of Aveiro / Department of Environment and Planning & CESAM; N. Abrantes, University of Aveiro-CESAM / CESAM/DAO
Wildfires have been recognized as a diffuse source of pollution to water systems. The ash deposited after a forest fire and the toxic elements associated to it, can be transported and leached after precipitation regimes, affecting both surface waters and groundwater. Nevertheless, scientific knowledge about the impacts of fires on water quality has been focused mainly on surface water and there is a gap on the processes and risks to the groundwater systems. In this sense, in order to understand the potential impact of forest fires as diffuse source of metals, such as V, Mn, Co, Ni, Cu, Zn, As, Cd and Pb) to groundwater by leaching processes, soil columns tests were carried out. Also, in order to understand the role of the soil type and fire severity on the mobilization of metals, two distinct soils [granite (SG) vs schist (SX)] and two different burnt temperatures [low (150°C) vs high (500°C)] were used. Hence, for each soil type, 5 different treatments were tested: unburnt soil (UN); top soil burnt at low temperature (BLT); top soil burnt at low temperature with ashes (BLT+A); top soil burnt at high temperature (BHT); top soil burnt at high temperature with ashes (BHT+A). The results reveal the following order of metal concentrations for both soils and treatments: Cd < Pb < Cu < Ni≈As < V < Co < Zn < Mn. When comparing the five treatments, in general, it was observed that the highest metal concentrations were recorded in the BLT+A treatments, for both soils. Concerning the soils types, SG shown to have a potential higher mobilization of metals than SX. This study emphasizes the role of forest fires as diffuse source of metals and the potential risk of exportation through leaching with potential impacts on groundwater quality.

3.09.11
Assessing the Potential Freshwater Aquatic Risks of Nickel in European Freshwaters: Data Update
I.A. Wilson, A. Peters, G. Merrington, wca; C.E. Schlekat, NiPERA Inc - test; E.T. Middleton, E.R. Garman, NiPERA Inc / Ecotoxicologist
Nickel is classified under the European Union’s Water Framework Directive as a Priority Substance. The Environmental Quality Standard (EQS) for nickel was established in 2013 with a value of 4 µg bioavailable Ni/L. Considerable efforts have been made since 2013 to support the implementation of the bioavailability based EQS. In parallel, research developments in the understanding of the behaviour, fate and ecotoxicology of nickel in freshwaters since the European EQS was set have led to a reduction in the uncertainties and increase in the ecological relevance of nickel environmental risk assessments. These developments include the collection of additional ecotoxicity data both for species already represented in the dataset and additional species, and the demonstration of the applicability of bioavailability concepts to different regions of the world, including the tropics and subtropics. Furthermore, the applicability ranges for chronic biotic ligand models have been extended through validation testing to higher pH conditions where nickel sensitivity is expected to be high. This alone has increased the applicability of the bioavailability approach to a larger proportion of national sampling sites for some countries in Europe. In addition, the development and validation of acute nickel biotic ligand models mean that for the first time the potential risks from short-term, intermittent discharges can now be readily assessed in a manner that is both consistent with the annual average EQS and ecologically relevant. As well as the models for nickel, certainly in Europe, there has been a considerable increase in the amount and availability of high-quality regulatory monitoring data that can be used for bioavailability assessments. These monitoring data include dissolved nickel, but also critically important supporting parameters that are used to account for bioavailability, such as dissolved organic carbon, pH and calcium. Additional supplementary information for nickel, beyond that from 2011, go further in reducing uncertainties and consistently show that the bioavailability based HC5 is protective of

effects thresholds.

3.09.14

Long-term Assessment of Trace Elements in a Marine Apex Predator Inhabiting a Highly Anthropized and Polluted Area, the Río de la Plata Estuary

O. Garcia-Garin, University of Barcelona / animal biology; A. Borrell, M. Vighi, A. Aguilar, University of Barcelona and Biodiversity Research Institute (IRBio); M. Valdivia, E. M. González, National Museum of Natural History (MNHN); M. Drago, University of Barcelona and Biodiversity Research Institute (IRBio)

The estuary of Río de la Plata, in the eastern coast of South America, is a highly anthropized area that brings a high load of contaminants to the surrounding waters, which may have detrimental effects on the local marine fauna. The franciscana dolphin (Pontoporia blainvillei) is a small cetacean species endemic of the southwestern Atlantic catalogued as vulnerable with a population decreasing trend by the IUCN Red List of Threatened Species. In this study, we assessed the concentration of trace elements (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr, Zn) in bone samples of 100 franciscana dolphins that stranded in Río de la Plata and adjacent coast between 1953 and 2015. The use of bone, which is available in scientific collections and museums, offers the opportunity to retrospectively examine trace elements over long periods of time and thus assess potential long-term trends in concentrations and relate them to the biologic traits of the species (size, sex), and potential environmental changes. The results obtained, in decreasing order of mean concentrations, were: Zn > Sr > Fe > Al > Mn > Cu > Pb > Cr > Ni > As > Hg > Cd > Se. The concentrations of Zn, Sr and Fe, expressed on a dry weight basis, ranged 115-518, 104-475 and 13-3712 µg g-1, respectively. The concentrations of Hg, Cd and Se ranged < Limit of Quantification (LOQ)-0.82, < LOQ-1.14 and < LOQ-1.64 µg g-1, respectively. The concentrations of As, Ni and Pb tended to significantly decrease in larger individuals (usually females). The concentrations of Al, Cr and Fe were higher in females than in males. Throughout the study period, the concentrations of Al, Cr, Cu, Fe, Mn and Ni increased significantly while the concentrations of As, Pb and Sr decreased significantly. The decrease in Pb may be due to the ban in the use of this element as an additive in gasoline and in the fabrication of car batteries. This investigation supports the validity of using bone to study long temporal trends of trace element concentrations in the environment.

3.09.15

Species Specific Sensitivity of Freshwater Organisms to Copper

T.v. Meer, Wageningen Environmental Research; K. Dirks, T. Meerwijk, T. Reijerkerk, A. Schokker, R. van Zwieten, University of Amsterdam / Institute for Biodiversity and Ecosystem Dynamics; B. van Beusekom, University of Amsterdam IBED Institute; J. van Amerongen, University of Amsterdam / Institute for Biodiversity and Ecosystem Dynamics; M. Kraak, University of Amsterdam / IBED-FAME

Copper may inhibit photosynthesis of primary producers, including algae and macrophytes. Microalgae represent a large group of primary producers in freshwater ecosystems and adverse effects of Cu on algae may therefore also affect higher trophic levels, including primary consumers like daphnids. The aim of the present study was therefore to assess if algae and daphnids are at risk at the prevailing Cu concentrations in Dutch surface waters. To this end we determined the species specific sensitivities of algae and daphnids to Cu in laboratory toxicity experiments. From the obtained concentration-response relationships EC_{50} values were derived that were plotted in an Species Sensitivity Distribution (SSD). From this SSD the HC_5 was derived which was compared with the prevailing Cu concentrations in Dutch surface waters. The *Daphnia magna* 48 h acute immobilisation test was performed according to OECD protocol 202. The selected algal test species included the green algae *Pseudokirchneriella subcapitata*, *Chlamydomonas reinhardtii* and *Monoraphidium griffithii* and the cyanobacteria *Microcystis aeruginosa* and *Anabaena variabilis*. Photosynthetic efficiency of the algae was measured after 24 h of exposure to Cu by means of Pulse Amplitude Modulation (PAM) fluorometry. Clear concentration-response relationships were obtained for the effect of Cu on algae and daphnids, from which the EC_{50} values were derived. Cu toxicity was species specific, with the daphnids being the most sensitive test species and the green alga *P. subcapitata* the least sensitive one. The calculated HC_5 of 16.8 µg/L only just exceeded the prevailing Cu concentrations in Dutch surface water, running up to 16 µg/L. Since the Cu concentrations in Dutch surface waters approach the HC_5, it is concluded that Cu poses a potential risk for freshwater ecosystems in the Netherlands.

3.09.18

Ecotoxicity of Rare Earth Elements to Freshwater Algae Using a Modified Version of the International Standard Organization Test Medium

D.A. Vignati, CNRS / LIEC UMR7360; N. Lachaux, D. Pauly, Université de Lorraine and CNRS / LIEC UMR 7360; L. Giamberini, Université de Lorraine, CNRS UMR 7360 / LIEC, CNRS; C. Cossu-Leguille, Universite de Lorraine / LIEC, CNRS UMR 7360

Rare Earth Elements (REE) are critical raw materials with a great variety of technological uses. Anthropogenic disruptions in the biogeochemical cycles of Gd, La, Nd and Sm are already occurring and characterization of REE ecotoxicological properties is receiving increasing attention. However, the chemical properties of REE, notably their tendency to form insoluble carbonate and phosphate complexes, pose significant challenges to the characterization of their effects using standardized ecotoxicological bioassays. In the case of algal ecotoxicity testing, the addition of REE to the test medium leads to the rapid formation of insoluble phosphate complexes. This phenomenon removes the essential nutrient phosphorous (P) from the test matrix, thus preventing an accurate assessment of the direct toxic effects of REE towards algae. The difficulty can be circumvented by substituting inorganic phosphate salts, which are the typical P source in ASTM, ISO and OECD standard media, with organic phosphorous sources. In a first series of experiments, the ecotoxicological effects of lanthanides toward the freshwater green alga *Raphidocelis subcapitata* were tested in standard ISO8692 medium that contains about 11.6 µM inorganic P. The EC50 values for La, Ce, Pr, Sm, Eu, Gd, Tb, Ho, Tm and Yb were between 9 and 10 µM, suggesting that the observed effects were likely caused by P losses rather than direct ecotoxicity. The other elements (Nd, Dy, Er and Lu) had EC50 between 5.1 (Nd) and 8.2 (Dy) µM, suggesting possible direct toxic effects in addition to P starvation. A modified test medium was prepared by substituting KH_2PO_4 with glycerol phosphate. Potassium chloride was also added to the modified test medium because KH_2PO_4 is the sole source of K present in the standard formulation. Algae were then exposed to Nd, Gd and Yb in both standard and modified test medium. The exposure concentrations were close to the corresponding EC50 previously determined in standard ISO medium. Algal growth after 72h of exposure was reduced by 65 % (Nd) to 50 % (Gd and Yb) in standard medium, but showed a 100% inhibition in the modified medium. Experimental work is ongoing to test the entire lanthanides series in modified test medium to provide information about the direct ecotoxicological effects for this emerging class of contaminants to primary producers.

3.09.19

Life-Cycle Toxicity Assessment of Nickel and Lead Mixtures to Chironomus riparius in Soft Waters

F. Jesus, University of Aveiro / Department of Environment and Planning & CESAM; A. Ré, Aveiro University & CESAM / Biology; I. Campos, Universidade de Aveiro / Environment; J. Pereira, University of Aveiro / Department of Biology & CESAM; F.J. Gonçalves, University of Aveiro / Department of Biology and CESAM; A.J. Nogueira, University of Aveiro / department of Biology & CESAM; N. Abrantes, University of Aveiro-CESAM / CESAM/DAO; D. Serpa, CESAM & Department of Environment and Planning, University of Aveiro

Forest fires constitute a cause of environmental pollution since ashes and soil mobilized by post-fire runoff are typically metal-enriched, especially in elements like nickel (Ni) and lead (Pb). As metals are easily adsorbed to particles they can accumulate in the sediments of watercourses located downstream from burned areas, and negatively affect the aquatic biota, in particular benthic species. So far, there is still scarce information on the effects of Ni and Pb mixtures on benthic freshwater species. Moreover, despite being widely known that hardness influences metals toxicity to the aquatic biota, little is known about the toxicity of metal mixtures in soft waters, which are highly representative of watercourses from the Northern Iberian Peninsula and Northern Europe. Thus, the aim of this work was to assess the toxicity of Ni and Pb mixtures on the life-history of the benthic species *Chironomus riparius*, in soft waters. Larvae were exposed to Ni and Pb, both individually and as mixtures, at environmentally relevant concentrations, during 28 days. The effects on growth and reproduction were assessed through the following endpoints: growth rate, time to emergence, emergence ratio, sex ratio and fecundity. This work can give insight to the potential environmental risks posed by wildfires to aquatic benthic communities.

3.09.21

Application of Modern Proteomics on

Corrosive Biofilms Accumulated on Sheet Piles Steel by Desulfovibrio Vulgaris Strains

L. Raghunatha Reddy, Federal Institute of Hydrology / Aquatic chemistry (G2); L. Helmholz, Bundesanstalt für Gewässerkunde / Aquatic Chemistry; S. Castronovo, Federal Institute of Hydrology / Aquatic Chemistry; A. Fiskal, Federal Institute of Hydrology (BfG) / Microbial Ecology; I. Herrig, Federal Institute of Hydrology (BfG) / Biochemistry/ Ecotoxicology; J. Meier, University of Koblenz / Department of Biology; R. Baier, S. Wulf, Federal Waterways Engineering and Research Institute / Department of Steel structures and corrosion protection; T.A. Ternes, Federal Institute of Hydrology; A. Wick, Federal Institute of Hydrology / G2 -Water Chemistry

Corrosion of metal structures in the environment as a result of electrochemical reactions (cathodic/anodic) induced by microbial activities in the biofilm at the metal interface is termed as microbial induced corrosion (MIC), also called biocorrosion. As a result of metal biocorrosion annual costs of loss can be accounted to around 4% of the GNP of developed countries2. The main corrosive bacterial groups are: sulfate reducing, Fe/Mn oxidising, acid producing and nitrate reducing bacteria as well as Methanogenes. To further elucidate biocorrosion functionally by sulfate reducers we have set up culture experiments with strains of Desulfovibrio vulgaris (DSM 2119). They are cultivated under anaerobic conditions with steel coupons. The steel coupons have a similar alloy composition to that of sheet piles constructed along rivers. Application of modern proteomics on the cultures identifies the up and down regulated enzymes that would potentially be expressed under biocorrosion conditions. We plan to quantify overexpressed enzymes through bicinchoninic acid assay and identify the peptides by Nano LCMS/MS and proteome discoverer software. Functional gene analysis by qPCR will be used to quantify functional genes involved in biocorrosion of the metal. These techniques would help us identify specific gene functionality and indicator enzymes/proteins expressed by Desulfovibrio vulgaris during biocorrosion. Additionally, as a part of the routine inorganic analysis of the cultures Fe2+, HS-, and SO42- and corrosion rate will be determined for all the cultures. The mineral phases of corrosion products will be analysed by FTIR and elemental analysis (C/S) and the chemical composition of the metal substrates by ICP-OES.

3.09.23

An a Priori Index to Discriminate Contaminated Wasteland of Concern From the Ones Ready to Be Upgraded to an Ecological Usage

N. Pucheux, N. Manier, D. Boulant-Cirederf, INERIS; O. Faure, Ecole des Mines Saint-Etienne / UMR CNRS EVS EMSE Géosciences et Environnement; J. Nau, F. Druez, EODD; S. Andres, INERIS / Toxicological Ecotoxicological Assessment of chemical Substances

The future of urban and industrial wasteland is at the heart of environmental, social and economic issues. They have specific characteristics (structure, composition) which are constraints for biodiversity and the biological functioning of soils. However, an abandoned wasteland (whose soil functions were not altered beyond repair) ends up hosting transitional ecosystems with ecological value. Brownfield requalification operations most often come up against difficulties linked to the presence of pollutants (in most cases metallic) at the heart of the issues linked to degraded land. The French national methodology for the management of polluted sites and soils includes carrying out a study of health and environmental risks to assess whether a site is in a state compatible with its future use. When the health aspect is not a major issue (no housing project for example), the objectives of decontamination and / or restoration of soil quality must be based on an ecological risk assessment (ERA). These are generally not or not sufficiently applied, either for financial reasons or for lack of competence issues. The Tipomo project (transfer and index of concern: tools for valuing medium-contaminated wasteland) aims to construct an index of concern that allows the classification of polluted soils hosting transitional ecosystems. The question is to differentiate the soils which are candidates for immediate upgrading to an ecological usage (biodiversity reserve) from those which need an ERA. Applicable only on plots with significant vegetation, the index will be calculated by considering the levels of soil impregnation and its ability to trap polluting substances (characterization of bioavailability fraction). The method will be tested in a case study by comparing the interpretation of the index to the result of a bioassay battery (which take into account the mixture effect). This approach follows the general thinking behind the ERA Triad (ISO 19204) by using simultaneously chemical tools, ecotoxicological information and ecological observation. For the purpose of this work, the implementation of the TRIAD is simplified, leading to an index of “concern” rather than a risk index. The Tipomo field studies have started in June 2020. They will support the definition of the principles of the method and its use in applied regulatory context.

3.09.24

Statement of the EFSA PPR Panel on a Framework for Conducting the Environmental Risk Assessment for Transition Metals When Used As Active Substances in Plant Protection Products

S. Pieper, German Federal Environment Agency (UBA) / Plant Protection Products; A. Aldrich, Agroscope; P. Berny, VETAGRO-SUP / Toxicology; A. Conrad, ANSES; C. Lythgo, EFSA European Food Safety Authority; M. Egsmose, EFSA

The European Commission asked the European Food Safety Authority (EFSA) to prepare a statement on a framework for the environmental risk assessment (ERA) of transition metals. The reason being that distinctive characteristics such as the non-degradability, essentiality and specific conditions affecting fate and behaviour as well as toxicity are not covered in current EFSA guidance for plant protection products (PPP). The poster summarises the statement, which has been in public consultation in summer 2020 and will be finalised in spring 2021.T he statement suggests to establish a strong link between the ERA, monitoring and risk regulation. Considering the non-degradability and potential long-term use of transition metals as PPP, a preliminary phase before the actual assessment is proposed, where monitoring data in relevant environmental compartments is provided in order to consider the natural background and anthropogenic residue levels in the exposure calculations. The statement then elaborates in more detail the different steps of the possible risk assessment. Following the current tiered EFSA approach, it is suggested that a first assessment step is performed assuming that residues are fully bioavailable. Should the first step fail, bioavailability issues might be considered in a refined ERA. In principle, bioavailability models as suggested by the European Chemicals Agency (ECHA) are considered appropriate to address equilibrated exposure situations. However, in the prospective ERA of PPP, non-equilibrium conditions may occur e.g. due to the repeated input via drift into surface waters. Further work is needed to judge the suitability of available models and to adapt exposure models to address the fate and behaviour of transition metals used as PPP. All model developments should follow the framework outlined in the Good Modelling Practice [1]. To adequately address the suitable timeframe of a potential product authorisation, post-registration monitoring and controlled long-term studies should also be conducted. The statement stresses the importance to consider the use of transition metals under other frameworks in a risk management decision and to align the authorisation with the goals of other overarching legislative frameworks. [1] EFSA PPR, 2014. Scientific Opinion on good modelling practice EFSA Journal 2014;12(3):3589, 92 pp.

3.09.25

Mercury Toxicity on Biomarker Gene Expressions of a Freshwater Amphipod at Two Temperatures

M. Melo, Universidade Federal de Santa Catarina / Laboratório de Reprodução e Desenvolvimento Animal; E. Gismondi, LEAE - University of Liege / Laboratory of Animal Ecology et Ecotoxicology

Mercury (Hg) is a global contaminant resulting of both natural processes and human activities. In aquatic environments, studies conducted on vertebrates highlighted changes of gene expression or activity of antioxidant defence enzymes and oxidative stress in invertebrates. However, despite the fact that Hg is a very toxic compound in aquatic environments, only few studies have evaluated the sublethal effects of inorganic Hg on *Gammarus* sp. The present study aimed to evaluate the effect of two environmentally relevant inorganic mercury $HgCl_2$ concentration (50 and 500 ng/L) on the expression of 17 genes playing crucial roles in respiration, osmoregulation, apoptosis, immune system, endocrine system, and oxidative and general stress in males of the freshwater amphipod *Gammarus pulex* exposed at 16 °C and 20 °C, for 7 and 21 days. *G. pulex* mortality was dependent on Hg concentration and temperature; the higher the concentration and temperature, the higher the mortality rate. Additionally, the Integrated Biomarker Response showed that $HgCl_2$ toxicity depended on the concentration, time and temperature of exposure. Overall, oxidative and general stress genes, together with endocrine and immune system genes were more affected by the exposure, regarding the concentration, time and temperature tested. Other genes, involved in respiration and apoptosis were also over-expressed after the exposure, suggesting an attempt to compensate the deleterious effects caused by the exposure. On the other hand,

osmoregulation was the less affected biological function. To conclude, oxidative and general stress, endocrine and immune system, and respiration gene modulations observed herein has the potential to disturb individual and population fitness at chronic exposures.

Measuring, Monitoring and Modelling of Pesticide Fate and Mitigation in a Regulatory Context

3.10.02
Sunlight-Induced Generation of Cytotoxic Reactive Oxygen Species of Parathion Suggest Photosensitization Potential
S. Amar, Us Army Engineer Research & Development Center / Environmental Lab; R.M. Styles, M.A. Chappell, K.A. Gust, U.S. Army Engineer Research and Development Center / Environmental Laboratory
Title: **Sunlight-induced generation of cytotoxic reactive oxygen species of parathion suggest photosensitization potential** *Authors:* Saroj K. Amar[1], Renee M. Styles[2], Mark A. Chappell[2], Kurt A Gust[2] *Affiliations:* [1]Oak Ridge Institute for Science and Education, Oak Ridge, TN[2]US Army, Engineer Research and Development Center, Vicksburg, MS *Abstract*: Parathion is an organophosphorus (OP) pesticide used worldwide which exhibits the characteristic OP mechanism of toxicity, the inhibition of acetylcholinesterase causing hyper-excitation of the cholinergic nervous system. Given that environmental release of parathion-based pesticides is the standard use case, we were interested in determining if sunlight transformed parathion and therefore affected toxicity. Thus, we tested the null hypothesis that parathion is not transformed by sunlight and has no change in toxicity. Ethyl-parathion dissolved in water was exposed in replicated experiments to direct sunlight for 0 (dark control), 1, 2, 3 and 4 hours where singlet oxygen species ($^1O_2$) formation and associated cell-free assessment of DNA damage through degradation of guanine nucleotides were monitored. Additionally, superoxide anion radical ($O_2^{\cdot-}$) formation was measured in conjunction with cell-free investigations of linoleic acid photoperoxidation. The generation of $^1O_2$ under aerobic conditions was measured at 440 nm which was further confirmed by decreasing parathion concentrations analyzed via high pressure liquid chromatography. Our results showed that parathion was rapidly transformed in sunlight where formation of singlet oxygen ($^1O_2$) and superoxide anion ($O_2^{\cdot-}$) radical increased through time, likely via type-II photochemical mechanisms. Genotoxic properties of sunlight-degraded parathion was suggested by increased photo-oxidative degradation of 8-hydroxy 2-deoxyguanosine (a guanine base of DNA) with increasing sunlight-exposure time which was measured at 260nm. Our results showed that up to 52% degradation of guanine at the maximum exposure to sunlight (4 hours). The generation of $O_2^{\cdot-}$ was monitored by recording the photosensitized reduction of nitro blue tetrazolium chloride (NBT) to nitroblue diformazan (NBF) spectrophotometrically at 560 nm. The generation of $O_2^{\cdot-}$ by parathion was further confirmed by specific quencher superoxide dismutase (SOD) and our result showed that 62% inhibition by 4 hours of sunlight exposure. Our results further showed the role of $O_2^{\cdot-}$ in linoleic acid photoperoxidation which was recorded photochemically at 233nm. Our results suggest potential mechanisms by which dissolved parathion in sunlight could result in photosensitization as indicated by increased readouts of the wavelength where parathion was excited to its reactive triplet state that lead to the formation of cytotoxic reactive oxygen species (ROS). These products which degrade DNA (guanine base) and increased linoleic acid photo peroxidation provide screening-level evidence for photogenotoxic and photocytotoxic properties of parathion in sunlight. *Keyword*: Parathion, Photosensitization, ROS, Sunlight, Photogenotoxicity

3.10.03
A New OECD Microcosm Wetland Test to Determine Mass Balance of a Pesticide Turnover in the Rhizosphere
Y. Jing, Helmholtz Center for Environmental Research (UFZ) Leipzig; M. Kästner, Helmholtz Centre for Environmental Research UFZ / Dept Environmental Biotechnology; A. Miltner, Helmholtz Centre for Environmental Research UFZ; K. Nowak, TU Berlin / Institute for Environmental Research (Biology V)
Various OECD tests have been suggested to determine mass balance of an organic pollutant turnover in soil, water-sediment and marine or river water. To date, however, the degradation simulation tests in the presence of plants are lacking. Therefore, we tested the applicability of a newly OECD-adapted microcosm wetland for determination of mass balance of a pesticide turnover in the rhizosphere. We chose glyphosate as a model compound for test since this herbicide is the most used globally. To investigate the fate of glyphosate in the presence of plants, microsom wetland test was accordingly adapted to OECD guidelines. The gravel which was placed into a 1 L glass jar was used as only substrate and it was planted with *Phalaris arundinacea*. After plants grew, 20 mg/L of stable isotope labelled ^{13}C2-glyphosate was applied to water.The system was semi-closed with a septum separating the sealed rhizosphere from the shoots exposed to the atmosphere. During the 30-day incubation time, the CO_2 released from mineralzed glyphosate was trapped into NaOH solution in the sealed rhizospheric segment. After incubation, plants, interstitial water and gravel were collected and analysed. Microbes from rhizosphere, interstitial water and gravel surface were collected by PBS solution extractiong and centrifugation afterwards. The extent of ^{13}C-glyphosate incorporation into microbial biomass was quantified in hydrolyzed amino acids. Remaining glyphosate and AMPA in interstitial water were determined with LC-MS/MS after derivatization with FMOC-Cl. We found that after 30 days, 3.3% of ^{13}C- glyphosate was mineralized; 2.0% was incorporated into microbial biomass and 2.8% was taken up by plants. In the interstitial water, 14% and 0.4% of the initially applied ^{13}C could be assigned accordingly to glyphosate and AMPA respectively. In unplanted microcosm, 1.6% of ^{13}C-glyphosate was mineralized and only 0.14% was incorporated into microbial biomass. However, in the interstitial water of unplanted microcosm, only 1.3% of the initial ^{13}C-glyphosate equivalents were found. This divergence could be explained by the enhanced adsorption of glyphosate on the gravel substrate in the absence of plants. Therefore, the plants enhanced not only the microbial transformation of glyphosate, but also the desorption of glyphosate from the gravel.

3.10.04
Presence of Neonicotinoids in Spanish Surface Water
A. Casillas, I. Navarro, CIEMAT; A. de la Torre, CIEMAT / Environmental; P. Sanz, M. Martínez, CIEMAT
Neonicotinoids (NNIs) are active substances, chemically similar to nicotine, that are used as insecticides mainly in plant protection products (PPPs) but also in veterinary applications such as tick control and flea collars for pets. NNIs became very important and nowadays are the most widely used group of insecticides globally, with imidacloprid being the largest selling insecticide worldwide. In Europe five NNIs were included in Annex I of Directive 91/414/EEC from 2005 to 2008 approving their use in PPP formulations. Nevertheless, the increasing evidence of affecting non-targeted organisms, particularly their implication in honey bees mortality, led the European Commission to severely restrict (Reg EU No 485/2013) or even ban outdoor uses (Reg. (EU) 2018/783-4-5). Presently, only acetamiprid (expiry date 28 February 2033; Commission Implementing Regulation (EU) 2018/113) and imidacloprid (expiry date 1 December 2020) are approved in EU, but some Member States skip these legislations granting emergency authorisations for some of the restricted neonics and therefore the presence of those regulated NNIs must be also addressed. NNIs are rather stable and not readily biodegradable and their high-water solubility has caused the proliferation and accumulation of NNIs in surface waters. For this reason, monitoring studies conducted in this matrix enable the evaluation of their current use and their influence in the ecological status of freshwater ecosystem. To do this, a study area of high representativeness, the Tagus River watershed in its Spanish section, was selected. A total of 19 river water samples were collected from June to July 2020 to determine the presence of 5 NNIs (acetamiprid, clothianidin, imidacloprid, thiamethoxam and thiacloprid). At least one target analyte was quantified in 17 of the 19 water samples, with ∑NNIs ranging from < MDL to 16.8 ng/L. As expected, imidacloprid (2.75 ng/L; mean) and acetamiprid (0.47 ng/L) were quantified in most of the samples. Quantification frequencies decreased for banned NNIs; however, their concentrations in some samples aroused high concern. In order to assess the environmental risk posed by these concentrations and the causes of those findings, a risk assessment based on the obtained data was conducted. This study was financially supported by the Spanish Ministry of Science and Innovation (project PEJ2018-002024-A) by funding the investigations of Alba Casillas Nogales. The authors thank to Confederación Hidrográfica del Tajo for providing the water samples and information related to mean daily flows.

3.10.05
Challenges of Groundwater Monitoring Well Installation in Greece
M. Tilbrook, D. Wallace, ERM Regulatory Services Limited; W. Gezahegne, Eurofins Agroscience Services GmbH
ERM and Eurofins have been involved in well installation for a groundwater monitoring study of a cotton herbicide in Greece. The installation had to overcome challenges presented by

Greece's unusual hydrogeology, and ensure that well locations allowed a comparison to Tier 1 FOCUS modelling. Extreme climatic conditions and the Covid pandemic presented further challenges. FOCUS guidance allows for a tiered approach to allow for more realistic assessments when the trigger of 0.1 µg/L is exceeded in a Tier 1 assessment. Tier 4 is centred on groundwater monitoring. The EFSA PPR panel opinion (2013) expressed reservations on whether current knowledge on groundwater hydrology is sufficient to use monitoring data to conclude a "safe use" for extensive areas of the EU. It emphasises the importance of fully characterising groundwater hydrogeology and demonstrating connectivity between application and sampling points. Therefore, well locations must be carefully chosen to demonstrate connectivity to aquifers. Some quantification of groundwater flow direction and speed is necessary to ensure the possibility of residues reaching monitoring wells within the lifetime of the study. Setting the field results into the context of Tier 1 modelling is also essential e.g. ensuring relevant FOCUS scenarios for the crop of interest are represented. Greece's hydrogeology presented a number of challenges in site selection. There are many deep aquifers confined by thick clay layers, which would not be vulnerable to agricultural activity and therefore would potentially result in false negatives. Three major cotton-producing regions of Greece were selected. GIS approaches were used to ensure that the field characteristics were suitable for groundwater monitoring and that the two FOCUS scenarios relevant for cotton (Thiva and Sevilla) were represented. Sometimes test drilling teams on the ground found unsuitable field conditions e.g. localised clay layers, which would prevent infiltration to deeper soil layers and the underlying aquifer, and so it was necessary to find need alternative locations. Greece's climate posed some challenges though extreme heat (a potential danger to exposed field workers) and severe storms including Hurricane Ianos, which caused widespread flooding. Covid-related travel restrictions presented further challenges but well installation was successfully completed.

3.10.06
Gradient of Pesticide Concentrations Along Subsurface Flow Paths From Fields to Drinking Water Sources
M. Herrmann, Rifcon GmbH / EFate; R. Sur, Bayer AG - Crop Science Division / Environmental Safety
Pesticides concentrations show gradients from on field situations after application to concentrations detectable in groundwater as drinking water source. Therefore, we determined the stepwise attenuation of solute concentrations, starting from the unsaturated zone underneath treated fields via the deeper aquifer, down to drinking water abstraction wells. This attenuation factor may contribute to make informed decisions based on decrease of concentrations from shallow leachate to raw water collection system. In this case study, we used N,N-dimethylsulfamide (DMS), a soil metabolite of the fungicides Dichlofluanid and Tolylfluanid, with no sorption and a moderate to long degradation half-life in soil. For the determination of the attenuation factors three test sites in western and southern Germany were selected. Vertical transport below the treated area via leaching was simulated using the models PEARL and HYDRUS-(1D), transport in the saturated zone was calculated with HYDRUS-(2D) and FEFLOW. The results were then validated with monitoring data from different locations across the transport pathway. The following observation points with increasing distance from the source area were considered for comparison of simulated and measured concentrations: • in the leachate in 1 m depth as common standard • at the bottom of the unsaturated zone, the aquifer's upper boundary; • at downstream monitoring wells; • at the raw water collection system. The calculated factors show for three real examples, how several influencing factors like climatic conditions, cropping, soil conditions, catchment size, depth to groundwater and dilution in the aquifer affect the pesticide concentrations (here DMS) in water in relation to distance and time. Despite the large variety in such parameters, a quite similar behaviour in terms of attenuation was demonstrated across these studies. These attenuation factors could make an appreciable contribution in a tiered or combined approach to assess and support the interpretation of groundwater monitoring studies and perform conservative and realistic higher-tier exposure assessments.

3.10.12
Modelling Effectiveness of Two Runoff Mitigation Measures in the Netherlands
W. Beltman, Wageningen Environmental Research / Environmental Risk Assessment; P. Dik, P. Groenendijk, M. Heinen, Wageningen Environmental Research; H. Massop, Wageningen University and Research / Alterra; M. Mulder, A. Veldhuizen, Wageningen Environmental Research; R. Sur, Bayer AG - Crop Science Division / Environmental Safety; t. smit, Bayer CropScience
Run-off from agricultural fields may affect surface water quality because run-off water may contain nutrients and pesticides. In areas densely populated with water courses, common in the Netherlands, farmers need support to mitigate runoff events. Emissions of pesticides to surface water are likely to be reduced by reducing the number and size of the runoff events. The aim of the study was to develop methods to quantify the effect of runoff mitigation measures on runoff, parameterized for typical Dutch landscapes and weather conditions. Two measures were considered: micro-dams in ridged fields and an infiltration trench at the edge of the field. With the Soil-Water-Atmosphere-Plant (SWAP) simulation model 30 years of rainfall events were simulated for different soil-crop combinations with and without these mitigation measures. Standard situations for arable fields were described to cover the variation of situations relevant for runoff. All arable fields in the Netherlands were attributed to one of the 57 described standard situations.The SWAP-model describes physically the hydrological processes leading to runoff. The simulated runoff volume was plotted against precipitation volume for each unique SWAP simulation run for the 30-year period (1989-2018). The plotted data were then used to determine two parameters in the rainfall-runoff relationship. This was done both for the reference and the mitigation situations considered. The ratio of both optimized rainfall-runoff relationships was then used to determine the effectiveness as a function of the precipitation. In this way the effectiveness can be calculated for any given precipitation event. Using the equations and set of 57 standard situations the effect of two mitigation measures for reduction of runoff for all arable fields present in the Netherlands can be determined. These can be used to advise farmers for which crops and for which fields the mitigation measures help to reduce the runoff from their fields.

3.10.13
Spatiotemporal Explicit Modelling of Pesticide Concentrations in Groundwater (FOCUS Tier 3b) With a New Model Package - a Case Study
S. Multsch, F. Krebs, knoell Germany GmbH / Environmental Safety; S. Reichenberger, Knoell France SAS / Environmental Fate / Modelling / GIS; L. Pape, knoell Germnay GmbH / Environmental Safety
The FOCUS groundwater guidance document (FOCUS, 2014, Sanco/13144/2010) proposes several strategies beyond the standard FOCUS Tier 1 assessment to evaluate the environmental risk of plant protection products (PPP) to groundwater bodies. Advanced spatial modelling (Tier 3b) is proposed as one of various refinement options. Tier 1 assessment focuses on the regulatory zones of the EU, which are represented by nine local scenarios covering the agro-environmental conditions of the member states. Tier 3b allows for the application on the EU scale, individual member states or regions. The risk for the region of interest is then assessed on the basis of a certain percentile of all Predicted Environmental Concentration in ground water (PECgw). So far, no official and agreed dataset, nor models are available through EFSA yet, which are under version control and ready to use within the authorisation process of crop protection products. A model package was developed which enables the spatiotemporal explicit calculation of PECgw at FOCUS Tier 3b. Public accessible geospatial data was collected on land use, climate and soils on a pan-European scale. The spatial extent and location of crops were taken from the CAPRI dataset. Climate data was received from the MARS dataset. The spatial extent of soil profiles was received from the soil geographical database (SGDBE) and soil horizon specific properties from the Soil Profile Analytical Database (SPADE). Soil hydraulic parameters were derived by using the Hydraulic Properties of European soils (HYPRES) database. The environmental fate of substances was simulated using FOCUS PEARL 444. Data processing, simulations and evaluations were automated using the Python programming language. A case study was performed for a region in northern Italy which is represented in the FOCUS Tier 1 assessment by the Piacenza GW scenario. FOCUS test compounds (herbicide) were used and the application in cereals and oilseed rape was simulated. The resulting spatiotemporal explicit PECgw were further analysed to conclude on the overall risk of groundwater contamination in the region of interest. The model package and results may help decision makers to refine the risk assessment where the region of interest is poorly covered by the Tier 1 FOCUS scenarios and/or provide information on likely vulnerable sites which could be further investigated by field leaching studies (FOCUS Tier 3c) or groundwater monitoring studies as defined by FOCUS Tier 4.

3.10.14

Modelling Landscape-Level Pesticide Concentrations With SWAT+ - an Uncertainty Assessment of Application Timing

M. Fuchs, BASF SE; S. Gebler, BASF SE / Agricultural Solutions - Global Environmental Fate Modelling; A. Lorke, University of Koblenz-Landau / Institute for Environmental Sciences

Modelling environmental concentrations of plant protection products at landscape-level is of growing interest for pesticide registration and product stewardship, including higher-tier studies in risk assessment, mitigation measures, monitoring support and decision making. However, landscape-level modelling is challenging due to uncertainties by modelling concepts and scaling as well as the extensive (geo)data demand for model parametrization and validation. This includes also limited information about application timing of pesticide products having strong impact on the model performance predicting pesticide concentrations in water bodies. Our work explores the impact of pesticide application timing using the eco-hydrological model SWAT+ (revised version of Soil and Water Assessment Tool) to explore uncertainty effects of application timing and the underlying mechanisms for the surface water exposure pattern in a small-scale catchment. Specific focus thereby was on method development to mimic realistic application timing considering plant stage, hydrology and weather conditions. On this account, we setup a SWAT+ model of the Funne catchment (54.6 km²) in the North-West of Germany. The simulated daily streamflow was calibrated using publicly available gauge data (Selm-Ondrup) showing a very good hydrological performance of the model (NSE: 0.746). The impact of application timing was subsequently explored by different synthetic application scenarios for three pesticides with varying physio-chemical properties, in combination with static and rule-based timing options. First results taking runoff and drainage into account indicated that a simple forward oriented ruleset (i.e. using weather forecast) could significantly decrease pesticide loads at the catchment outlet on average by 16 to 46%. For individual years and substances, channel loads decreased by up to 92%, which could be attributed to the interaction of rainfall and wash-off timing as the main driver of concentration variation during runoff events. We will further explore the impact of drift entry and other processes (e.g. channel dissipation) as well as different application schemes. These findings underpin the importance of realistic application timing in landscape-level simulations of pesticide concentration in surface water bodies. It is hence expected that landscape-level tools will play an important role in the future, e.g., for the development and operation of smart decision tools in agriculture.

3.10.23

Collation and Analysis of Regulatory Field Trial Data for the Validation and Refinement of Crop Development Dates Used in Pesticide Exposure Modelling and Risk Assessment

G. Hughes, Cambridge Environmental Assessments; A. Eatherall, Corteva agriscience; A. Blakey, Syngenta Ltd / Environmental Safety; A. Bolekhan, Bayer AG Crop Science Division / Environmental Safety; P. Branford, ADAMA Agricultural Solutions; S. Gebler, BASF SE / Agricultural Solutions - Global Environmental Fate Modelling; A. Lozano, Sumitomo Chemical Agro Europe SAS; P. Massey, FMC Agricultural Solutions

Determining appropriate pesticide application windows is an important component of surface water (SW) and groundwater (GW) pesticide exposure modelling; increasingly, regulators are constraining these application windows using estimates of the timing of BBCH crop growth stages from AppDate. The “repaired” version of the FOCUS SW models will have AppDate integrated directly into the software shell to remove “subjectivity” from the selection of application windows. The AppDate (Klein, 2012) software calculates consistent application dates for use in FOCUS SW and GW modelling with the purpose to align the product GAP being assessed with the fixed model crops described by the FOCUS models. The dates for major crop development stages, *e.g.* BBCH 10, captured in AppDate are based on various sources, differentiated for GW and SW, with linear interpolation used for BBCH growth stages between these. Given exposure assessments are a function of the application dates this software generates, it is important to understand how realistic and representative they are; however, there is currently no comprehensive, harmonised, quality-controlled, readily available, pan-European crop phenology dataset available to undertake such an assessment or for use in regulatory risk assessment refinement. The crop protection industry hold large quality-controlled datasets of BBCH crop growth stages within their efficacy and residue trials datasets that span the required crop types as well as the agronomic and pedoclimatic diversity of Europe, collected over several decades. Extraction and collation of a harmonised crop development dataset from such trials, held by individual companies, would allow for a robust assessment of AppDate and allow for the justification of more realistic and location specific dates in regulatory risk assessment refinements. It will also hold significant opportunities for future development of crop models to improve estimates and timing of exposure, not only in the context of exposure modelling, but also, for example, to help predict accurate timing of applications with respect to on and off-field weeds and non-target arthropods, especially bees. This poster introduces a European Crop Protection Association (ECPA) project to collate a crop development database, largely from product efficacy and residue field trials held by individual members, in order to meet this important regulatory requirement and further develop exposure science to allow for more accurate and realistic risk assessment.

3.10.24

Specific Model Application to Superhydrophobic Substances in the Context of 1107/2009 and Low Risk Aspects

l. philippe, Total; S. Jubault, TOTAL Fluides; S. Gony, E. Beltran, S. Segelle, B. Journel, P. Adrian, CEHTRA SAS

A range of phytosanitary paraffin oils are used as insecticide, acaricide or fungicide. These paraffin oils are also substances authorized for organic farming in Europe. These substances were registered according to the “pesticides” directive (91/414/EEC then regulation 1107/2007) thanks to the purity criteria of the European pharmacopoeia. The composition and the very high purity of the oils including the absence of aromatic compounds lead to non-classification for both health and environmental hazards .lead to both health and environment non classification. (je propose : “”) These substances are accepted in organic farming because of their very specific physical and non narcotic mode of action which consists in the creation of an oil film a few microns thick which is deposited on the surface of the plant, asphyxiating the eggs and larvae of insects or inhibiting the growth of insect and fungi spores, for instance. This physical mode of action makes these oils suitable for use on many crops and does not result in the development of resistance phenomena in the target species. In addition, they do not have any adverse effect on the plant health or on the quantity and quality of the harvest (exemption of MRL). At the regulatory level in EU (Reg (EC) 1107/2009), legislation introduces the concept of "low risk" for certain substances with specific criteria. Paraffin oils satisfy almost all the criteria, but for environmental criteria related to bioaccumulation, paraffinic oils as UVCB substances, present problems to fulfill the mandatory regulatory endpoint. The quasi insolubility in water of these substances as well as their multiple constituent composition induces specific difficulties with regard to environmental risk assessment even if these are easily biodegradable. This presentation is focusing on the use of the transformation rates in fish to assess its ecological risks and take advantage of the AQUAWEB model (Arnot, Gobas) as used by MacKay for superhydrophobic organic compounds. Discussion of the input parameters to be used is included before conclusion on the lack of bioaccumulation property.

3.10.25

Types, Levels and Risks of Pesticides in the Ave River and Adjacent Atlantic Coastline (Northwest Iberian Peninsula, Portugal)

M. Rocha, S. Santos, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Portugal.; E. Rocha, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto (U.Porto), Porto, Portugal

Riverine and coastal environments of Vila do Conde experience anthropogenic stresses due to human activities, including intensive agriculture practices, which affect the Ave River and the coastal areas close located to the estuary of this stream. Therefore, we aimed to investigate the presence of 56 pesticides from different categories (fungicides, herbicides, and insecticides) in those ecosystems and evaluate the potential risk for biota. For this study, water samples were collected for one year (2019) at sites located in both the river's margins and at the coastline. The analyses involved an extraction step using solid-phase (OASIS HLB cartridges) and pesticide quantification/identification by gas chromatography-mass spectroscopy (GC-MS/MS). Data showed the presence of 11.8% of fungicides, 15.1% of herbicides, and 73.1% of insecticides, some being above the 2013/39/EU Directive levels. In this respect, the total loads of these compounds were ≈ 0.4 µg/L, ≈ 0.5 µg/L, and ≈ 2.5 µg/L, for fungicides, herbicides,

and insecticides, respectively. For some compounds, differences were found between seasons, being spring the one with higher amounts. However, no differences were detected between the river and the coastline, which raises potential health problems, not only for local biota but also for humans that use local beaches for recreational purposes. In this context, the concentration-addition model (CA) model was applied to predict the toxicity of mixtures of pesticides, using a two-tiered approach based on CA, independent action models, and three trophic levels (algae, invertebrates, and fish). The data revealed invertebrates as the most impacted trophic level, leading us to make acute toxicity assays, using the brine shrimp *Artemia salina* (ARC-test) and mixtures of pesticides. Exposure to the latter decreased the mobility of the tested organisms. In conclusion, despite the past depollution and remediation actions in the targeted habitats, our data support that further interventions are needed to comply with Portuguese and European and legislation. Funding: ICBAS, FCT (UIDB/04423/2020, UIDP/04423/2020), Project ATLANTIDA (NORTE-01-0145-FEDER-000040), by NORTE 2020/PORTUGAL 2020, via ERDF.

3.10.26
Evaluation of TROPHY, a Modelling Approach for Assessing the Risk of Surface and Groundwater Contamination by Pesticides in the French West Indies
P. Campan, INRAE; A. Samouelian, D. Crevoisier, Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE); A. Duboisset, E. Farama, J. Fostier, Anses - French Agency for Food, Environment and Occupational Health & Safety; M. Voltz, Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE)
The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) is in charge of the risk assessment of plant protection products before their placing on the market. The risk assessment of surface and groundwater contamination currently relies on the estimation of environnemental concentrations of pesticide in water for several scenarios representing the typical variation in climate, soil and agriculture across continental Europe [1], [2]. However, these scenarios were not developed to represent the particular agropedoclimatic conditions of the overseas tropical regions of France. Thus, specific tropical agropedoclimatic scenarios representing the two main crops, banana and sugarcane, and the climatic and pedological diversity were developed ; coupled to a new pesticide transfer model, WATSFAR, estimating simultaneously solute concentration in percolation and surface runoff waters for row crops. The transfer model and the scenarios are grouped together in the software TROPHY [3].In this paper, we present i) a sensitivity analysis of WATSFAR to pesticide properties and ii) an evaluation of TROPHY to predict worst case conditions of water contamination by comparing TROPHY's predictions to field runoff and percolation data [4]. The differences observed between the active substances in their simulated temporal concentrations changes in soil and water are in accordance with their sorption and degradation rates differences. Whatever the active substance, simulated concentrations in percolation and surface runoff are of the same order of magnitude, highlighting the importance of preferential flow in Antillean soils and similar risk of surface and groundwater contamination. A high interannual variation in simulated concentrations was observed as related to annual climate conditions, application dates and cropping cycles. The comparison of simulations to field concentrations confirmed that TROPHY provides generally worst case predictions. Further improvements of TROPHY are needed, for example extend the scenarios time length in order to better account for climate and cropping variations. **References** : [1] FOCUS. 2000. EC Document Reference SANCO/321/2000-Rev.2, 122. [2] FOCUS. 2001. EC Document Reference SANCO/4802/2001-rev.2, 245. [3] Voltz et al., 2015. Convention de recherche et développement n° 2012-CRD-03 ANSES/INRA.[4] Ponchant et al., 2020. Convention AFB / INRA 2016-2018, INRA.

3.10.27
Developing a MACRO Meta-Model for Swedish Drinking Water Abstraction Zones
S. Reichenberger, Knoell France SAS / Environmental Fate / Modelling / GIS; S. Multsch, knoell Germany GmbH / Environmental Fate / Modelling / GIS; N. Jarvis, SLU / Soil and environment; M. Gönczi, SLU / Centre for Chemical Pesticides; J. Kreuger, Swedish University of Agricultural Science / Centre for Chemical Pesticides
In Sweden farmers are legally obliged to apply for permits for pesticide use if their land is located within a drinking water abstraction zone. The standalone modelling tool MACRO-DB4 developed by the Swedish Agricultural University (SLU) is available for risk assessment and decision support. MACRO DB-4 is used by local authorities, farmers/landowners and consultants, and is based on the well-established leaching model MACRO 5.2 (Larsbo and Jarvis, 2003). However, the software is costly to maintain and slow for end users. The aim of this project was to develop a robust meta-model of MACRO-DB 4 and to integrate it in a web-based tool which should be fast, easy to maintain, and easy to understand for stakeholders. In a pilot study, 18720 leaching simulations were performed for a test region in Southern Sweden (Reichenberger et al., 2019) for a simulation period of 26 years (6 years warm-up + 20 years evaluation period). The target variable was the mean leaching flux concentration over 20 years at 2 m depth. Subsequently, two alternative methods for meta-model development were tested: Classification and Regression Trees (CART) and trilinear interpolation. Trilinear interpolation (in the three-dimensional space of Koc, DT50 and Freundlich exponent, for $\log_{10}$ of leaching concentrations) yielded better results than CART. Hence, we decided to use trilinear interpolation for the meta-model for the whole of Sweden. In the second phase of the project, a large number of MACRO simulations were performed for the whole agriculturally relevant area of Sweden. The simulations comprised 18 climates, 72 soils, 1 typical crop (spring cereals), 3 application seasons (spring, summer and autumn), and 150 dummy compounds consisting of a grid of Koc, DT50 and Freundlich exponent. The resulting 583200 simulations were run on the knoell IT infrastructure within 4 months. Simulations were postprocessed automatically in R, and the results were stored in a database. In the final phase of the project a web application will be developed to replace the standalone tool. The application will include a graphical user interface, the result database and the trilinear interpolation tool.

Micro(Nano)Plastics and Associated Chemicals (I): Occurrence and Analysis

3.11.01
Following Microplastic Fibers Down the Production Line
B. Pinlova, EMPA / 506; R. Hufenus, Empa Swiss Federal Laboratories for Materials Science and Technology; B. Nowack, Empa - Swiss Federal Laboratories for Materials Science and Technology / Technology and Society Lab
Textile industry of the 21st century has been dominated by synthetic fabrics, polyester in particular. Concurrently, microplastics in the environment has been observed and many studies have reported microplastic fibers (MPF) as a frequent constituent of the microplastic debris. MPF are shed from synthetic textiles during their user phase lifetime. However, there is little known about the origin of the MPF. Textile washing studies showed a decrease of MPF released with increasing number of washing cycles and based on these results the hypothesis was formulated that the MPF are already contained in the textiles prior to washing and have originated during textile production. The aim of this work was therefore to examine the formation of MPF during the process of yarn production from the bale opening step, through carding, sliver handling, to spinning into a yarn. Starting with bale of black polyester fibers, different machine set ups and spinning methods were used: the traditional ring spinning and its improved version of compact spinning, as well as the more novel and higher throughput methods of rotor and air-jet spinning. The impact of the changes in the production was evaluated by extracting the MPF from different materials and fibers and yarns sampled at different production stages. Using image analysis, MPF number and size distribution was determined. In addition, SEM analysis was performed on the raw yarns as well as the released MPF to obtain a better understanding of the mechanics affecting the results. As a result of this study we are able to propose recommendations of best practices for the yarn production line in order to minimize the number of MPF contained in the yarns, and textiles, and therefore also in the environment.

3.11.03
Microplastic Fiber Discharge From the Washing Machine for Realistic Laundry Setup
K. Altmann, BAM- Federal Institute Materials Research and Testing; C. Bannick, German Environment Agency (Umweltbundesamt) / III 2.6; A. Kerndorff, Umweltbundesamt; C. Heller, M. Fuchs, Hochschule für Technik und Wirtschaft Berlin; U. Braun, UBA - Federal Environment Agency
Microplastic (MP) particles (1 - 1000 µm) can be found worldwide. These are mainly caused by fragmentation of large carelessly into the environment released plastics or plastic products including textiles due to oxidation, water, radiation or mechanical abrasion. Until today the possible entry pathways for the plastic into the environment are often still unknown. One often mentioned entry path is the urban waste

water system. In addition to MP particles, there are also numerous reports about the findings of MP fibers. Especially polyethyleneterephthalate (PET) or polyamide (PA) fibers are often found as they are most commonly used in garments, which means that they are typically released into the environment during the production process and wearing. Another source of MP fibers can be the mechanical stress of textiles into the washing machine during the washing process. To this day, it is completely unknown how much fiber is discharged from textiles in the washing process under realistic conditions. For the first time, this presentation determines both textile physical parameters and MP mass contents as a mass for the MP fibre discharge during washing over a period of 30 washing cycles under realistic conditions. Waste waters from the washing machine are filterted by fractional filtration over various sieves with mesh sizes of 500, 100, 50 and 10 µm [1]. The mass content of textile MP is determined by ThermalExtraction/Desorption-GasChromatography/MassSpectrometry (TED-GC/MS) [2]. Specific attention was paid to compile a realistic laundry load, containing items, that are representative as textiles in most households (including natural fibers), under realistic conditions with dirt and washing powder in the washing machine. The aim is to find out whether washing machines make significant contributions to the MP fibre findings worldwide. A combination of eight t-shirts and 13 shirts consisting of 55% cotton and 45% PA (PA 6) or 65% cotton and 35% PET was determined as a realistic laundry load of households and washed with an easy-care program at 40 °C with standard detergent and standard dirt. The textiles or the washing water were analysed after 1, 3, 5, 10, 20 and 30 cycles. For both tested laundry items the air permeability decreases from the first to the last washing cycle. Length dimensions are shortened more than width dimensions. A total mass of 492 mg PET was discharged over 30 washing cycles in the size fraction of 50-100 µm, compared to 28 mg for PA. References 1. Bannick, C.G., et al., Development and testing of a fractionated filtration for sampling of microplastics in water. Water Research, 2019. 149: p. 650-658 DOI: https://doi.org/10.1016/j.watres.2018.10.045. 2. Duemichen, E., et al., Automated thermal extraction-desorption gas chromatography mass spectrometry: A multifunctional tool for comprehensive characterization of polymers and their degradation products. Journal of Chromatography A, 2019. 1592: p. 133-142 DOI: https://doi.org/10.1016/j.chroma.2019.01.033 Acknowledgement - The authors thank the BMBF for financial support of the project RUSEKU (02WPL1442A).

3.11.05
Different Characteristics of Microplastics in Stormwater Runoff by Urban Land Use Patterns

M. Jang, Korea Institute of Ocean Science and Technology / oil and POPs reserch group OPRG; S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; S. Ha, Korea Institute of Ocean Science and Technology; G. Han, Korea Institute of Ocean Science and Technology (KIOST); W. Shim, Korea Institute of Ocean Science and Technology (KIOST) / Oil and POPs Research Group (OPRG); Y. Cho, Korea Institute of Ocean Science and Technology / Oil and POPs Research Group (OPRG); J. Jeong, University of Ocean Science and Technology, KIOST-UST Campus / Oil and POPs Research Group (OPRG)

Terrestrial environment is known for important source of microplastics (< 5 mm) to the aquatic ecosystems. The point source such as discharge of microplastic from waste water treatment plants has been studied in detail, while there are only few studies on non-point source of microplastic such as stormwater runoff. In this study, the microplastics in stormwater runoff from urban area was identified. Residential, industrial, commercial and road areas has been selected for sampling sites, in order to evaluate whether the land use influenced the characteristic of microplastics in the stormwater runoff. Stormwater runoff were sampled during single rain event (total rainfall: 6.4 mm) at 4 sites of rainwater outlets in Hannea river of Gumi city, South Korea in Agust 2020. Stormwater runoff samples (n=5, 50L) from each sites were collected at an hourly interval after beginning of the rain event. Freshwater (50L) was also sampled before one day prior to rain event for determine contamination of microplastics in baseflow. Microplastics was detected in all samples of baseflow and stromwater runoff. The microplastic concentration in first one hour of rainfall samples (66-823 n/L) was 2-80 fold higher than its in baseflow samples (1.8-676 n/L), and the concentration was decreased by rainfall time. After 4 hours from rainfall start, the concentrations of microplastic (1.8-11 n/L) were similar between 4 sites. PE and PP were dominant type of microplasitc in commercial area, while PEVA was abundantly detected in industrial and residential area. In case of samples from road area, ABS, SB, BR, BA, rubber, polystyrene copolymer, and acrylate copolymer (22%) was identified more than common polymer types, unlike other regions. Rubber was found only in the samples from road area. Abundant number of PP pellets (5.5 n/L, 3.8±0.5 mm, 12±3 mg) was found in base sample from industrial area. The different polymeric composition of microplastics between regions might be related to diverse sources such as degradation of plastic products, spill of plastic raw materials and fragmentation of tire wear. The results of this study indicate that land use is an important parameter in microplastic contamination of stormwater runoff. High concentraion of microplastic in the samples show that urban runoff is an important source of microplastics to aquatic environment.

3.11.06
Microplastic Sequestration by a Highway Stormwater Filter

L.L. Iordachescu, Aalborg University / Department of Civil Engineering; A. Vianello, Aalborg University / Civil Engineering Department - Section of Water and Environment; K. Papacharalampos, J. Kelényi, J. Vollertsen, Aalborg University / Department of the Built Environment

Urban and highway stormwater runoff are direct sources for land-based microplastics (MP) into receiving water bodies. In Denmark, a large fraction of the urban runoff is treated with the use of stormwater ponds which is the most widely used practice for stormwater management. (Liu et al., 2019) found that surface waters in stormwater retention ponds contains a relatively large number of microplastics, which could potentially require an extra purification step before further discharge into receiving waters in the near future. In this study, we investigate the retention of MP of a post-polishing filter system that is purifying highway runoff (from a stormwater pond) before discharge into a sensitive stream. The filter installation consists of a layer of sand mixed with peat, followed by a layer of limestone and finally a stratum of drainage sand. Water samples were taken from the inlet and outlet of the filter system using a filtration device as seen in (Rist et al., 2020), where large amounts of water were filtered on 10 µm steel filters. Additionally, several areas of the soil surface of the filter system were also sampled. Prior to analysis, the samples underwent extensive purification steps such as enzymatic digestion, hydrogen peroxide oxidation, density separation. Subsequently, the concentrated samples were analyzed with state-of-the-art FPA-µFTIR and Pyrolysis-GS/MS methods, where MP's were quantified in terms of particle number and mass, polymer composition and size distribution. Bibiliography Liu, F., Olesen, K.B., Borregaard, A.R., Vollertsen, J., 2019. Microplastics in urban and highway stormwater retention ponds. Science of the Total Environment 671, 992–1000. https://doi.org/10.1016/j.scitotenv.2019.03.416 Rist, S., Vianello, A., Winding, M.H.S., Nielsen, T.G., Almeda, R., Torres, R.R., Vollertsen, J., 2020. Quantification of plankton-sized microplastics in a productive coastal arctic marine ecosystem. Environmental Pollution 115248. https://doi.org/10.1016/j.envpol.2020.115248

3.11.07
Fragmentation of Plastic Food-Packaging Objects in Semi-Terrestrial Zones

F. Konig, German Environment Agency (UBA); Y. Wiesner, Federal Institute for Materials Research and Testing (BAM); U. Braun, S. Meinecke, German Environment Agency (UBA)

Plastic are used in a multitude of products with a wide range of practical applications for example in the food-packaging industry. Plastics are economical, lightweight, chemically stable and used ubiquitously. Unfortunately, this widespread use and subsequent inappropriate disposal (i.e. littering as well as inadequate waste and waste water management) in combination with the described intended chemical properties, has led to their unintentionally global presence in the environment. Plastic pollution and contamination are one of the most important environmental issues of our time. Under the influence of environmental factors (UV radiation, oxidation, biodegradation etc.) larger plastics break down into smaller pieces and finally into microplastics (particles 1-1.000 µm, TSO/TC 21960). Beside break-down due to the above-mentioned factors, mechanical stress and abrasion can also be an important process for microplastic formation in shore lines. Within the scope of the BMBF (Federal Ministry of Education and Research, Germany) project ENSURE ("Development of new plastics for a clean environment by determining relevant entry paths), fragmentation processes in semi-terrestrial zones are currently being investigated in the artificial wave mesocosm system of the German Environment Agency´s field station, where semi-natural freshwater conditions can be

simulated in large scale. In first preliminary tests, defined plastic food packages consisting of Polyethylene terephthalate (PET), Polyethylene (PE) and Polypropylene (PP) were tested in a mesocosm system with a stone-gravel shore line to select suitable test specimens and measurement methods for abrasion effects. Three non-destructive detection methods were chosen and investigated (change in surface roughness, material thickness and loss of mass). In this contribution the result of the mass loss is presented. The loss of mass depends on the shape of the test specimen, polymer type and its specific, mechanical properties, density of the material and the structure of the shore line. For larger test specimens such as PET bottles, significant loss of mass was found in comparison to PE foils, before the test specimens showed visible damage. These mass losses can largely be attributed to abrasion. Microplastic was found in the water phase of the shore line with ThermalExtractionDesorption-GasChromatography/MassSpectrometry (TED-GC/MS).

3.11.08
Sources of Microplastics in Surface Waters of the Great Lakes
J.T. Yu, University of Toronto / Department of Earth Sciences; P.A. Helm, Ontario Ministry of the Environment, Conservation and Parks / Environmental Monitoring and Reporting Branch; M.L. Diamond, University of Toronto / Department of Earth Sciences
Microplastics are of increasing concern due to their widespread occurrence across the Great Lakes basin. Microplastics are diverse and enter lakes from a variety of sources which are not well understood. Particle morphologies can help indicate from where microplastics originate. To date, microplastics are commonly grouped into five categories: fragments, films, foams, fibers/lines and pellets/spheres. These categories broadly allude to potential sources and do not provide sufficient information for source identification. The goal of this study was to first assess the sufficiency of these categories for source identification and then to augment the current categorization scheme by introducing a source-based categorization framework. Our motivation is the need to reduce plastic emissions at their source. This framework is applied to 48 surface water samples collected in 2018-19 using manta trawls (335µm net) from sites around Lake Ontario, Lake Erie and Lake Superior. Sampling locations were chosen to capture a range of influences from urban areas and remote settings in each lake. Microplastics are visually counted and grouped according to source-based categories including fragments, pellets, commercial fragments, spherical microbeads, irregular microbeads, tire wear, paints, films, and different types of foams. Microplastics from 14 Lake Ontario samples have been visually identified and counted using a stereomicroscope. Preliminary results show wide spatial variability, with abundances ranging from 3205 to over 2,000,000 particles/km^2. Greater proportion of microplastics are consistently found within the 0.300-0.999 mm size range compared to 1-4.75 mm. Majority of particles are fragments, followed by irregularly shaped microbeads and commercial fragments. For each sample, a subset of particles from each category are chemically analyzed using micro-FTIR. This study informs on sources and their relative contributions to the overall microplastics load in the Great Lakes. Improving our knowledge on sources is necessary to target management decisions and prevent future microplastics pollution.

3.11.09
Occurrence of Selected Microplastics in Australian Road Dust
S. OBrien, The University of Queensland; E.D. Okoffo, C. Rauert, The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); J.W. O'Brien, QAEHS - The University of Queensland / QAEHS; F. Ribeiro, Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); S.D. Burrows, T. Toapanta, X. Wang, K. Thomas, The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS)
Microplastics are known to occur in a variety of environmental matrices, globally. Prior research has identified the occurrence of microplastics in road dust, however concentrations and the chemical composition of microplastics in road dust remain unknown. An Australian urban to rural transect from South East Queensland, Australia to a rural location, from two separate sampling events was analysed using pressurized liquid extraction (PLE) and Pyrolysis Gas Chromatography - Mass Spectrometry (Pyr-GC/MS) for microplastic quantification. Microplastics (polypropylene, polystyrene, polyethylene terephthalate, polyvinyl chloride, poly(methyl methacrylate) and polyethylene) were detected in road dust at varying concentrations ranging from ~9 mg/g/m^2 at the rural location to 117 mg/g/m^2 in the city. Polyvinyl chloride was the most abundant plastic. No statistical significance was observed regarding the total plastic concentrations between the two sampling events. Size fractionation of the road dust into five different size classifications (< 250µm, 250 to 500µm, 0.5 to 1mm, 1mm to 2mm and 2 to 5mm) indicated that the chemical profile of the microplastics remained consistent across all size fractions, at each location. Importantly, linear regression affirmed that the presence of microplastics in road dust was associated with the volume of vehicles each site received per day (r^2=0.91, p=0.05), suggesting traffic as a proxy for human movement is positively correlated with increased microplastic concentrations in the terrestrial environment.

3.11.10
Quantifying Intertidal Microplastic Distributions: Implications for Sampling Designs and Invertebrate Exposure
P. Vermeiren, C. C. Muñoz, Radboud University / Environmental Science
Microplastic contamination in the intertidal of beaches and estuaries has been reported worldwide. Nonetheless, evidence of microplastic distribution patterns along and across the intertidal is often contradictory, and the use of different sampling protocols limit comparison among studies. When such conflicting evidence is used to inform sampling campaigns, it increases uncertainty in resulting data. Moreover, the conflicting patterns hamper efforts in spatially explicit risk characterization of microplastic pollution to intertidal organisms. This study aimed to guide sampling designs for monitoring of microplastics in the intertidal, and to quantify the exposure of invertebrates to microplastic pollution. Despite many methodological advances in microplastic extraction, quantification, and identification from sediments, integrating these fragmentary advances into a cost-effective protocol remains challenging. We conducted a series of experiments to integrate methodological advances and clarify their applicability to organic-rich sediments with fine grain size, characterizing many estuarine intertidal areas. Pre-treatment with Fenton's reagent was efficient in reducing organic matter, and compatible with micro-Fourier Transform Infrared Spectroscopy (µFT-IR) for polymer identification, although it affected polyethylene (PE) and polyethylene terephthalate (PET) sizes. Density separation with $ZnCL_2$ in a Top Overflow Column (OC-T) obtained recovery rates > 90 % across polymers. Automated microscopic image analysis of Nile Red stained samples allowed quantification down to 125 – 66 µm, and > 90 % of validated particles to be determined as plastic polymers in both estuarine and beach samples. Microplastic abundances varied at 1 m^2 scales, and their distribution across and along the intertidal related to dominant physical gradients, including sediment grain size and structural complexity. Differences among sites were additionally influenced by proximity to anthropogenic sources. These results stress the importance of sampling with sufficient replicates spread across the intertidal, and highlight the importance of local environmental covariates when comparing among sites. Microplastic exposure landscapes to intertidal invertebrates vary at local, within home ranges, and larger, among population, scales. Nevertheless, risk assessment needs to account for species-specific differences to relate body burdens to sediment levels.

3.11.11
Occurrence of Microplastics in Coastal Sediments From the Inner Oslofjord, Norway
L. Bronzo, University of Pisa / Earth Science Department; A. Lusher, NIVA Norwegian Institute of Water Research / Environmental Contaminants; M. Schoyen, Norwegian Institute for Water Research (NIVA) / Centre for Coastal and Marine Research; C. Morigi, University of Pisa / Earth Science Department
Globally, microplastics (MPs) are expected to accumulate at high concentrations in coastal sediments because these are readily linked with both land- and sea-based inputs, and changes in density and buoyancy facilitate sinking to the seabed. The Fjordic systems on the Norwegian coast have received some attention, reporting the presence of MPs in sediment samples, although explanations for the reported values are mostly lacking. This study presents records of MPs (< 1 mm) in coastal sediments sampled in the Vestfjord, the western part of the Inner Oslofjord, from 100 m of water depth. Our aim was to understand the scale of which the release of MPs in the Vestfjord was influenced by a WWTP system (VEAS) and how the re-distribution of MPs is driven by oceanographic currents acting in the fjord. Thus, the upper 0—5 cm of sediment cores were analysed from three areas of interest: two stations (ST4, ST5) in proximity of the VEAS diffuser and one (ST2) from the Vestfjord's inlet. MPs were extracted from sediments using a NaI solution and were validated with µFT-IR. The distribution of MPs' shape (fibre, fragment,

film) and polymers in sediments was compared with grain-sizes (clay, silt, sand) via Canonical Correspondence Analysis (CCA).The most polluted area was ST4 with a total of 1.042 MPs/g dw, followed by ST5 with 0.524 MPs/g dw, both of which are close to the WWTP outlet. ST2 had the lowest concentration, 0.084 MPs/g dw. Fibres were the most common morphology found (76%), whilst fragments and films occurred with lower concentrations (18%, 6%). Common polymers were PES (50%), PP (18%), PMMA (9%), elastane (4%) and cellulosic materials (4%). MPs appear to preferentially accumulate according to their morphology and polymeric origin. Such that clays had preferential accumulation of PMMA, PA, PC, elastane and cellulosic materials; in silt PES, LD-PE, PS, PVC and films; in sand PP, fibres and fragments. The analyses on the vertical distribution of MPs showed that the occurrence of plastic particles decreased from 2002 and 2015 for ST2 and ST5 whilst increased for ST4. The input of plastic debris at ST4 and ST5 appears to derive from the release of treated waters from the WWTP diffuser with ST4 as the main accumulation zone - located down current from the diffuser. Due to the position of ST2 it is uncertain if the main input of MPs hails from the Inner or the Outer Oslofjord. Further analyses are needed to understand the main inputs of plastic debris in this area.

3.11.15

Characterization and Identification of Microplastics by Quantum Cascade Laser Based Rapid Infrared Chemical Imaging

D. Robey, Agilent Technologies

The prevalence of microplastics in the environment is a growing focus around the world. Environmental agencies are increasing monitoring and government agencies are seeking to act. To adequately assess the prevalence of microplastics in the environment, researchers need to measure the size, shape, and chemical identity of plastic particles, including those at the micron level. Traditional techniques such as visual inspection are slow, manually intensive and may be subjective. Likewise, existing vibrational spectroscopy techniques such as FTIR and Raman microscopy each face some limitations such as fluorescence in Raman analysis. Recently, an alternate source for infrared (IR) spectroscopy has emerged based on a rapidly tunable Quantum Cascade Laser (QCL) opening the possibility for alternate methodologies and workflows. While QCL based instruments may be configured to mimic the setup of an FTIR microscope by employing a Focal Plane Array (FPA) detector, which operates essential like a camera, an alternative is to pair the QCL with a single point detector and rapidly scanning optics. This approach facilitates distinct modes of operation that can address key limitation with FPA based FTIR and QCL systems for example very long time of analysis and very large data sets. One potential limitation however lies in the wavelength coverage of existing QCL instruments given they operate only in the fingerprint region of the infrared spectrum ($1800cm^{-1}$ - $975cm^{-1}$) rather than a broader range commonly available with FTIR instruments. While this fingerprint region is information rich, there remains a need to validate the performance of these systems to ensure results generated are comparable with existing techniques. To address this issue, several studies have been conducted. Due to the lack of suitable certified reference materials for microplastics, this validation was conducted using in-house reference materials. They included analysis of pure known component samples, mixed known component samples and known component particles spiked into natural samples. Samples were prepared for analysis and processed using automated workflows and spectral libraries within the instrument and its operating software. At the conclusion of the studies it was found that the instrument was able to locate and correctly identify particles of all polymers contained in the sample. In addition, reproducibility was acceptable as was recovery in spiked samples.

3.11.17

High-Resolution Vertical Distribution of Microplastics Down to 10 Mm and 100 m Depth With AAU - KRAKEN. a Novel High-Throughput Device for Sampling Microplastic in the Water Column

A. Vianello, Aalborg University / Civil Engineering Department - Section of Water and Environment; C. Lorenz, Aalborg University / Department of the Built Environment; L.L. Iordachescu, Aalborg University / Department of Civil Engineering; K. Papacharalampos, Aalborg University / Department of the Built Environment; D.A. Stephansen, Aalborg University / Civil Engineering; A.H. Nielsen, Aalborg Unversity / Department of the Built Environment; T.G. Nielsen, Technical University of Denmark DTU / National Institute of Aquatic Resources; K. Syberg, Roskilde University / Dept of Science and Environment; G. Kuddithamby, Aalborg University; J. Vollertsen, Aalborg University / Department of the Built Environment

Microplastic (MP) pollution has been nowadays documented in all marine environments, but the data related to the vertical distribution, cycling and fate of MPs in sub-surface water are still scarce[1,2,3]. To fill knowledge gaps and improve the methods for MP sampling, we developed and tested a novel sampling device (AAU – KRAKEN) based on an active filtration system capable of filtering a large volume of water at several depths. The device is composed by a borehole deep-well pump contained in a steel cylinder with six filtering units (UFO)[4] attached, each containing a 10 µm steel filter with 167 mm diameter. The water is filtered through the UFO units and then expelled through the outlet of the device equipped with flowmeter. The KRAKEN is powered by lithium batteries held in a sealed steel cylinder while the electronics (inverter, modem to transmit data, and a data logger to control the pump and collect data) are contained in a second container. An integrated CTD probe provides accurate on-line measurements of conductivity, temperature and depth. Before deployment, we primed the KRAKEN with pre-filtered sea water to avoid particle contamination from undesired water during the descent to a specific depth and pressure-related damages. The KRAKEN was deployed using an underwater cable for on-line CTD systems to a specific depth, activated and monitored remotely via computer, filtering around 3 m^3 of water in 30 minutes (timed sampling). We performed two high-resolution depth profiling in the Skagerrak area (DK) by collecting samples at six different depths, precisely at 5, 25, 40, 60, 80, and 100 m. In addition, we collected three consecutive samples at the same depth to test the repeatability of the sampling procedure. The collected samples were then processed by performing a multi-step purification treatment[4], and analysed by FPA-µFTIR-Imaging combined with automatic MP analysis (siMPle)[5]. The activities were conducted within the project MarinePlastic (Velux Foundation, Project no. 25084). 1. Choy, C. A. *et al. Sci. Rep.* **9**, 1–9 (2019). 10.1038/s41598-019-44117-2 2. Zobkov, M. B. et al. *Mar. Pollut. Bull.* **138**, 193–205 (2019). 10.1016/j.marpolbul.2018.11.047 3. Tekman, M. B. *et al. Environ. Sci. Technol.* **54**, 4079–4090 (2020). 10.1021/acs.est.9b06981 4. Rist, S. *et al. Environ. Pollut.* **266**, 115248 (2020). 10.1016/j.envpol.2020.115248 5. Primpke, S. *et al. Appl. Spectrosc.* 000370282091776 (2020). doi:10.1177/0003702820917760

3.11.18

A System for Measuring the Sinking Velocities of Microplastic Particles (10-300 Micrometers)

S. Dittmar, TU Berlin / Water Quality Engineering; J. Pries, TU Berlin / Chair of Water Quality Control; A.S. Ruhl, German Environment Agency (UBA); M. Jekel, TU Berlin / Chair of Water Quality Control

Various studies investigated sinking velocities of microplastic (MP) particles in aquatic environments. Different polymer types, shapes and sizes were tested in laboratory experiments, yet there is only sparse empirical data on sinking velocities of MP particles smaller than 300 µm despite their potential ecotoxicological relevance. We present a setup for determining sinking velocities of particles within a size range of 10 to 300 µm via optical imaging. To conduct an experiment, 10 mL of suspension containing the target particles are placed in a cone. The particles then enter a cylindrical sedimentation column (130 mm, 21 mm ⌀) via opening a ball valve. The measurement of low sinking velocities is prone to vibrations, flow pulses or currents caused by temperature variation. For thermostatic control, the column is encapsulated in an additional vessel connected to a circulating thermostat. Moreover, the experimental setup is incorporated in a cooling incubator to regulate ambient air temperature as well. To avoid particle contamination, ultrapure water is used to fill the column and prepare suspensions, respectively adding 5 mg/L of a mix of ionic and non-ionic surfactants to stabilize target particles. Embedded parallel glass panes allow for optical inspection of the column. Monochrome images can be captured at a rate of 1 to 15 fps covering a field of view of 3.65 x 4.36 mm (2048 x 2448 pixels). Raw images are processed with Python scripts for single particle tracking considering size, movement as well as contour sharpness. Velocities and morphological descriptors are derived from the obtained particle trajectories. Spherical PMMA particles with a mean diameter of 48 µm and a wide size distribution were used to validate the experimental setup by comparing measured to computed velocities (Stokes' law for spheres with $Re < 1$). Experimental runs revealed an effect of particle dose on the measured velocities, indicating interactions between particles at higher concentrations, e.g. the acceleration of smaller particles by larger particles passing them. At a low particle dose of 100 µg per run such effects could be minimized and the results showed very high agreement with the corresponding Stokes velocities. For 127 spheres with diameters between 14.0 and 89.6 µm the measured velocities showed an average absolute deviation

of 0.0017 mm/s ± 0.0096 and a mean relative deviation of 2.5% ± 11.0 from the respective calculated velocities.

3.11.20

A Novel, Automated Method to Identify Microplastic Polymers Based on Fluorescent Staining With Nile Red

N.S. Meyers, ILVO, VLIZ / Aquatic Environment and Quality Research Area, The Ocean and Human Health department; A.I. Catarino, Flanders Marine Institute / Ocean and Human Health; C. Janssen, Ghent University / Blue Growth Research Lab; B. De Witte, ILVO Institute for Agriculture and Fisheries Research; G. Everaert, Flanders Marine Institute / Ocean and Human Health

There has been a growing concern about the microplastic levels in the marine environment and their potential adverse effects for ecosystems and human health. Due to the increased demand for microplastic pollution monitoring at national and global levels, there is an urgent need for a standardized, reliable method that reduce the time, efforts and costs spent on plastic identification in the marine environment and allow for long-term monitoring.An optical microplastic detection method that has gained considerable attention lately because of its time- and cost-effectiveness makes use of the hydrophobic fluorescent dye Nile red (NR). This method has proven useful for discriminating plastic particles from non-plastic materials in several matrices, but the possibility to use a fluorescent dye to obtain polymer-specific identification has not been explored intensely. In this ongoing study, an automated approach is being developed that makes use of the solvatochromic nature of Nile red and allows for the identification of individual plastic polymers using fluorescence microscopy.The theoretical principle behind the new method is that the fluorescence emission spectrum of NR shifts depending on the polarity of its environment. The latter means that based on a large and qualitative training set of emission spectra of different polymers (collected under standardized conditions) automated algorithms can be developed for identification of polymers. Hence, the new method combines imaging of fluorescent particles with machine learning algorithms for the accurate and automated detection of different plastic polymers in various matrices at microscale. Based on the developed preliminary algorithms, this study shows that polymers can be categorized based on their coloration following staining with Nile red. We demonstrated that the identity of individual plastic particles can be predicted using this new technique. Based on the results so far this method could be a promising approach for the rapid, cost-effective and reliable identification of microplastics, and it could support the long-term monitoring of microplastics in various matrices.

3.11.22

Hydrological Variations As a Key Driver of the Temporal Dynamics of Microplastic Pollution in a Freshwater Ecosystem

A.R. Carvalho, University of Toulouse, CNRS / IMRCP/ EDB laboratory; L. Riem-Galliano, L. Tudesque, University of Toulouse, CNRS / EDB; M. Albignac, University of Toulouse, CNRS / IMRCP laboratory; A. ter halle, CNRS / Chemistry; J. Cucherousset, University of Toulouse, CNRS / EDB

Microplastic (MP) pollution has emerged as a novel source of concern for freshwater ecosystems. Improving our understand of the temporal dynamics of MP pollution is essential to mitigate their ecological impacts. Weather conditions and precipitations increase MP concentration; however, a comprehensive analysis of how these drivers act at different temporal scales is needed. Our first objective was to quantify seasonal variability of MP pollution (concentration, polymer type and size) and identifiy its drivers across seasons. Our second objective was to assess how strong hydrological changes caused by a flood episode modulate MP concentration and characteristics. Water surface was sampled in 14 sites in the Garonne river (France) in 4 sampling events across 2019. Two sites in the main stream were sampled in 4 occasions (2-weeks interval) before and after an important flood episode. Across the year, MP concentration averaged 0.15 $MP.m^{-3}$ (± 0.46 SD). MP concentration and size significantly differed between sampling events, with MP concentration increasing in periods of low discharge while MP size decreased. The three main polymers were polyethylene (44.5%), polystyrene (30.1%) and polypropylene (18.2%). The color distribution differed between polymer types, with more abundant white PS and black PE. During the flood episode, MP concentration and MP size were positively correlated with river discharge. The proportion of the three main polymers significantly changed during the flood episode. Particle color and shape differed between polymer types, with PS particles presenting a higher sphericity. Preliminar analysis of IR spectrum revealed important changes in oxidation level of MP particules during the flood episodes, helping to identify their origin. The increase in MP concentration during a flood episode did not exceed the level of variation measured between seasons, but the positive relationship between river discharge and MP concentration might represent temporal peaks of pollution and particular risks to freshwater organisms. Across the year, higher MP concentration of smaller particles was observed in low flow conditions, while in a flood an increase in MP concentration and larger particles was detected. Understand catchment scale-processes is therefore crucial for the mitigation of MP pollution in freshwater ecosystems and further quantification of MP hydrodynamics are needed.

3.11.23

Influence of Anthropogenic Stressors in Microplastic Abundance in Urban Rivers - a Case Study of Boulder Creek, Colorado, USA

A.T. Kukkola, U. Schneidewind, H.A. Nel, University of Birmingham / School of Geography, Earth and Environmental Sciences; R.L. Runkel, U.S. Geological Survey / Colorado Water Science Center; G. Sambrook Smith, I. Lynch, J. Drummond, S. Krause, University of Birmingham / School of Geography, Earth and Environmental Sciences

Microplastics have been found in all environmental compartments and their presence is established across the globe. However, the degree to which different anthropogenic stressors affect microplastics distribution and occurrence in rivers is still largely unknown. Here, we study microplastic abundance in Boulder Creek, Colorado, USA. River water and riverbed sediment samples were collected in October 2019 along Boulder Creek near the city of Boulder, CO (population of 107,353 in 2018) as well as along its tributary, South Boulder Creek. At each location, 100L of river surface water was filtered through a 64µm mesh (n=20) and approximately 100g of streambed sediment (n=40) was collected near both streambanks close to the water line. Samples were stored in glass vials (water) and glass jars (sediment) prior to transport back to the UoB lab. Sediment samples were oven-dried at 50°C before microplastics were extracted via density separation using $ZnCl_2$ and Sediment-Microplastics-Isolation units without ball valve closure as described by Nel et al., 2019. Water samples were backwashed into glass beakers using H_2O_2 and a 64µm mesh to remove any small organic matter such as algae. Fe^{2+} (aq) (as 0.05 M $FeSO_4 \circ 7H_2O$) was then added into H_2O_2 in concentration ratio of 1:10 to catalyze Fenton's reagent and to digest and oxidize any organic matter. Extracts were then stained with Nile Red (5µg ml^{-1}) in DI-water for an hour and filtered over Whatman GFD filters (0.45 um) as described as Nel et al., 2020. A combination of fluorescent and light microscopy was used to identify the microplastics and their abundance. Key-characteristics, such as color, size and morphology were also recorded. First results hint towards a variable distribution of microplastics along the river course that could potentially be linked to different input sources. Future efforts will provide further insight into how different anthropogenic stressors might affect microplastic abundance in urban rivers and as such inform decision-making for mitigation efforts.Nel et al 2019. Simple yet effective modifications to the operation of the Sediment Isolation Microplastic unit to avoid polyvinyl chloride (PVC) contamination. MethodsX, 6,2656-2661. doi:https://doi.org/10.1016/j.mex.2019.11.007Nel et al 2020. Detection limits are central to improve reporting standards when using Nile Red for microplastic quantification. Chemosphere, 127953. doi:https://doi.org/10.1016/j.chemosphere.2020.127953

3.11.24

Biodegradability Assessment of Organic Surface Treatments of Nanoforms Using the OECD 301F Respirometric Test

E. Cerro-Gálvez, M. Esponda, LEITAT Technological Centre / Human & Environmental Health & Safety; V. Gonzalez-Andres, LEITAT Technological Centre; M. Diez-Ortiz, Leitat Technological Center

In the context of the environmental risk assessment of nanoforms (NF), the NF surface, and thereby the stability of surface treatments and their biodegradation, is a key determinant of environmental fate and toxicity. Differences in surface chemistry can lead to different attachment coefficients, dissolution, and reactivity, and modulate environmental concentrations in sewage treatment plant effluents and in the aquatic *versus* sediment compartments, overall resulting in different environmental risks. The ready biodegradability of twenty common NF´s organic surface treatments (including synthetic and natural polymers, non-ionic and ionic surfactants, and stabilisers) was investigated using activated sludge as inoculum and OECD 301F respirometric test. According to the results, polyethylene glycol (0.2 kg mol^{-1}), citric acid, sodium dodecyl sulfate, chitosan, gelatin and hexamethylenetetramine can be considered

readily biodegradable, as they passed the 60% of biodegradation during the 10-day window. These compounds displayed a typical S-shaped biodegradation curve observed for growth-supporting biodegradation of a pure compound as sole source of carbon. Partial biodegradation was observed for polyethylene glycol (8 and 35 kg mol^{-1}), gum arabic, tannic acid, tween 80, dextran 70 and 11-mercaptoundecanoic acid. These compounds reached the pass level (>60% biodegradation) during the 28-day period but failed the 10-day window. In contrast, no biodegradation was observed for the other seven compounds (polyvinylpyrrolidone (10, 40 and 360 kg mol^{-1}), methyl cellulose, pluronic F127, tetramethylammonium hydroxide and dispex A4040) during 28 days of incubation. If we assume an average residence time of 14 days in the secondary treatment process of a sewage treatment plant, we consider that the ready biodegradation test provides key information to evaluate stability of NF surface treatments in sewage treatment plants. A ready biodegradability test is only a screening test, further biodegradation experiments may be needed to investigate biodegradation under different conditions. Further studies should also investigate how biodegradation of these surface treatment materials as bulk substances compare to the biodegradation of these substances when applied as surface treatment of NFs.
Acknowledgement – GRACIOUS is funded by the European Commission, Grant Agreement 760840.

3.11.25
Occurrence of Microplastics in Freshwater and Marine Fish Samples of Bangladesh
M. Shoeb, K. Tasnim, S. Akter, M. Islam, M. Mamun, University of Dhaka / Department of Chemistry
Microplastics are a growing threat for freshwater and marine ecosystems. The aim of this study is to analyze the exposition of microplastics in different commercial freshwater and marine fish samples collected from different super shops in Dhaka city, Bangladesh. To know the original source of this plastic pollution the microplastics were tried to identify and characterize from gastrointestinal tract (GIT) from 11 freshwater fish and 12 marine fish samples by digestion method. For the analysis of microplastics, the GIT was digested with 10 M NaOH at 60^0 C for 24 hours and the dried samples were analyzed for microplastics using FT-IR and the spectrum was set 4000-400 cm^{-1} and observed with a microscope for determining the presence of plastic materials. A variety of microplastics was identified including fiber, foam; elastomer, and polyethylene visually observed under microscope, and images were taken with Axio Cam digital camera at different magnification (4-40). Among them, fiber is the most prevalent microplastic found in both freshwater and marine fish samples. About 64% of the freshwater fish and 66% of the marine fish are reported to be contaminated by microplastics.

3.11.26
Detection of Heavy Metals in Microplastic Samples From Japanese Riverine and Coastal Environments
A. Abeynayaka, Tokyo City University / Graduate School of Environmental and Information Studies; M. Ohgaki, T. Fukai, Hitachi High-Tech Science Corporation; F. Kojima, Pirika Inc.; N. Itsubo, Tokyo City University / Graduate School of Environmental and Information Studies; M. Takenaka, Hitachi High-Tech Science Corporation
Marine plastic pollution is considered as one of the most challenging global environmental issues. Freshwater resources such as rivers carry plastic litter into marine environments, where the local and regional pollution becomes a global concern. Rivers are considered as a main contamination pathway of microplastics to the oceans. Microplastic contaminants in freshwater itself a threat to the ecosystem and a potential health hazard to humans. Moreover, plastics are a complex suite of chemicals with polymer and additives. The chemical composition of environmental microplastics is much more complex with the adsorbed chemicals in the environmental matrices. Advanced information such as microplastic concentration and other constituents associated with microplastic is useful for pollution load calculation and risk assessment studies. The potential human toxicity or ecological risk due to microplastics associated with the plastic itself and the associated chemicals. The present study focussed on the presence of heavy metal in microplastics collected from the environment. Microplastic samples were collected in riverine and coastal water environments of Japan at 95 locations by using with 300 µm net coupled device "Albatross" . The collected samples were first purified by density separation. The floating organics were digested (this step probably removed the heavy metals on the surface of the microplastic pieces. Then the samples were observed through a light microscope. Then polymer identification was conducted by using FT-IR. Then 50 microplastic pieces were analyzed for heavy metals with X-ray fluorescence analysis (XRF). The three main polymers detected as polyethylene (PE), polypropylene (PP), and polystyrene (PS). The sampling was focused on floating and suspended microplastics. Heavy metal concentrations in PE, PP, and PS were detected. There is a significant variation of concentrations of Cr in all three polymer types. The observations indicated that certain products, such as artificial grass having a higher concentration of Cr. Similar identification has been done for the PP microplastic products as well. Pd was predominant in both artificial grass and a few other common microplastics. The estimation of annual Cr, Pb, and PS flow to the oceans with microplastics to the oceans from Japanese rivers are 0.84, 0.25, and 0.13 kg respectively. The leachability and the exposure studies are in progress to obtain data for risk assessments aplications.

3.11.27
Microplastics Pollution in Saudi Arabia Artificial Water Ecosystems: Their Role As Vector of Persistent Contaminants
Y. Pico, University of Valencia / Environmental Quality and Soil; J. Campo, CIDE-CSIC / Degradación y Conservación de Suelos; A.H. Alfarhan, King Saud University; M.A. El-Sheikh, King Saud University / Department of Botany and Microbiology, College of Science; D. Barcelo, ICRA- G17920851
Artificial water ecosystems are regarded as sinks and pathways of microplastic (MP) pollutants from terrestrial environments to various other aquatic systems such a, groundwater, lakes and oceans. Although extensive research has been conducted in recent years on microplastic pollution in inland water, knowledge on the occurrence, transport and fate is still considered an under-researched area. Furthermore, the influence of the presence of microplastics in the behaviour of persistent organic compounds have not been fully understanding yet because some results are apparently contradictory. In this study, the occurrence and distribution of microplastics in different points of Al-Jubail, the most important industrial city, and the second biggest one after Riyadh have been studied. The sampling points included those of the runoff channel system that transports treated water from different industries, and that cross longitudinally the city up to the sea. MPs were visually identified after filtration and digestion of the organic matter and the chemical composition was identified by FTIR. Furthermore, using polycyclic aromatic hydrocarbons (PAHs) and perfluoroalkyl substances (PFASs) as a model for toxic hydrophobic organic contaminants, the current laboratory study investigates the relative bioavailability of dissolved and MP-sorbed phenanthrene, fluoranthene, prefluoroctane sulfonic acid (PFOS) and perfluoroctanoic acid (PFOA) under co-exposure conditions representing of those occurring in the aquatic environment. To this, high density polyethylene (PE) microspheres were purchased from "Gran Velada" in dry powder form, in 180–221 µm ('PE-200′) range. These spheres were put in contact with PAHs and PFASs dissolved in water according to their solubility capacity. Results pointed out the presence of MPs of different sizes, colours and forms in the water samples taken. The most common plastics were whit fibers >0.5 mm. However, there were many different types of plastics mostly < 200 µm. Regarding the type of polymer, only 4 polymer types, PE, PP, PET and PS were determined based on a comparison with infrared spectrogram databases. The sorption experiments demonstrated that both PAHs and PFASs are absorbed in the surface of the MPs. Then, the effects of this adsorption in the aquatic biota needs further This study can be used as a reference to better understand the impacts of MPs in artificial surface channels and ponds affected by the discharges of wastewater treatment plant. Furthermore, results confirmed the importance of polymer type, particle size and temperature as determining factors for the degree and mechanism of hydrophobic organic contaminant sorption from water to MP. *Acknowledgement* - The authors thank the financial support from the project number (RSP-2020/11) King Saud University, Riyadh, Saudi Arabia.

3.11.28
How Dangerous Are Additives in Microplastics? The Direct Analysis of Brominated Flame Retardants in Plastics Via Pyrolysis GC-MS
C. Sowa, Shimadzu Deutschland GmbH / Marketing Manager; S. Schröder, Shimadzu Deutschland GmbH / Specialist GC/GCMS / Food Market; U. Potyka, Shimadzu Europa Gmbh / Center of Innovation & Product Support
Some additives in plastics are highly toxic and difficult to break down in the environment. These are often present in microplastics and continue to pollute the environment. Using pyrolysis GC-MS, a reliable analysis of the additive load and the determination of the type of plastic can be carried out. Microplastics can now be found almost everywhere. Approximately 12 million tons of microplastics

find their way into the oceans every year, and the smallest plastic particles are carried around the globe in the air. Is there a danger emanating from these plastics? The experts do not agree on this. What is clear, however, is that additives are used in plastics; these additives are intended to change or improve product properties. Knowledge of the quantity and type of additives in the plastic is crucial for assessing potential toxicity. Additives widely used in industry include plasticizers, UV stabilizers and flame retardants. In particular, the brominated flame retardants are classified as toxic with a high potential for health hazards. Brominated flame retardants are used to delay the ignition of flammable materials and to slow down the flame propagation. They can be easily combined with a wide range of plastics and are relatively inexpensive. When these plastic products are released into the environment after their life cycle, they decompose into minute plastic particles, including microplastics. The additives contained in the plastic can get into water, and sediments, through leaching and evaporation and become a risk there. Using Pyrolysis GC-MS, a statement about used additives and the type of plastic can be made very quickly and easily. The sample preparation is minimized to a punching out or grinding and weighing of the sample.

3.11.29
Realistic Model for Environmental NanoPlastics (ENPs)
F. Blancho, Géosciences / OSUR; J. Gigault, TAKUVIK CNRS, Université Laval; M. Davranche, Géosciences Rennes / University of Rennes 1
Despite the increase in nanoplastic studies , their physico-chemical properties are still poorly studied. Based on proved ENPs occurrences, ecotoxicology studies have been intensively performed using perfectly spherical polystyrene nanoparticles (PS-NPs) as model. However, polystyrene is not the most produced plastic materials (compared to PE, PP and PVC) and spherical shaped ENPs are not thermodynamically favourable in aqueous environment. For these reasons, the use of PS-NPs to study behaviour of ENPs observed in the environment is still questionable. As a consequence, one of the major challenge in the nanoplastics studies is to develop nanoplastic models to mimic ENPs especially regarding their physico-chemical properties.For this purpose, we proposed a protocol to produce realistic ENPs in size, structure and surface properties from aged plastics. The protocol was divided in three consecutive steps. First, ENPs were released from aged plastics by agitation and sonication. Secondly, biofilm and organic matter residues associated to ENPs were removed by applying a combined UV/H2O2 treatment. Then, the final step was dedicated to the concentration of ENPs without OM. Our extraction protocol allowed to reach an important concentration (i.e. 40ppm) of nanoplastics assessed via dynamic light scattering for particle size characterization, total organic carbon analysis for plastic/OM quantification and Py-GC/MS for polymer identification. Moreover, fluorescence spectroscopy was suitable to monitor OM degradation.

Micro(Nano)Plastics and Associated Chemicals (II): Effects, Risks and Mitigation

3.12.02
Effect of Shoe Sole Leachate on Daphnia magna
H. Kim, D. Kim, T. Lee, Konkuk University / Department of Environmental Health Science; Y. An, Konkuk University / Department of Environmental Heath Science
Shoe soles are considered a new pollutant of microplastics and discharge microplastics of various shapes and sizes. Microplastics originating from shoe soles can enter soil and water ecosystems, and adversely affect the ecosystem. This study investigated the effect of leachate of microplastics derived from shoe soles on Daphnia magna in freshwater ecosystems. D. magna is a representative test species of aquatic organism for toxicity assessment. Four different types of shoes were selected which are commonly used. Shoe sole leachate were made that 1 g of shoe sole fragments and 500 mL mixture of distilled water and dmsNaHCO3 then shacking 45 rpm for 30 days. Each shoe sole leachate including control and blank were exposed for 48 hours. The results showed a significant lethal effect in the sneakers exposure group. After 48-h exposure, D. magna were stained with calcein-AM, DCFH-DA, and Nile red to measure esterase activity, oxidative stress, and lipid storage, respectively. This study is considered to be the first case to report the effect of microplastic leachates derived from shoe soles on the ecosystem at the screening level.
Acknowledgement- This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2020R1A2B5B02001734). This study was also funded by the Ministry of Environment (MOE) through the Graduate School of Specialization for Safe Management of Chemicals.

3.12.03
Toxicity of Tire Rubber, PVC and PE Leachates to Freshwater Organisms
V. Carrasco Navarro, University of Eastern Finland / ENVIRONMENTAL AND BIOLOGICAL SCIENCES; S. Seppänen, University of Eastern Finland / Department of environmental and biological sciences; J.V. Kukkonen, University of Eastern Finland / ENVIRONMENTAL AND BIOLOGICAL SCIENCES
The contamination of worldwide waters with plastics in general and microplastics in particular is a very important chapter in the history of anthropogenic alterations caused to our planet. In addition to the polymers that are the core of every plastic material, chemicals added to give certain properties (called additives) also enter water bodies. Depending on the conditions, plastic additives have the potential of leaching to these water bodies. Studies about the consequences of such leaching, covering pollution and toxicity in aquatic ecosystems are still scarce. In our study, the toxicity of three leachates, resulting from tire rubber, polyvinyl chloride (PVC) and polyethylene (PE), is assessed using the non-biting midge *Chironomus riparius* and the duckweed *Lemna minor* as model organisms. *Chironomus riparius* is a widely used organism in toxicity tests and an important prey of fish, birds and even terrestrial organisms. *Lemna minor* is an aquatic plant distributed widely in lentic and slow lotic freshwater bodies and an established model organism in the testing of chemicals. The plastic particles of tire rubber, PVC and PE were shaken in each of the organisms' growing medium for 15d to obtain the leachates. After filtering the resulting leachates to eliminate the plastic particles, the experiments are conducted with relative concentrations of 0, 10, 25, 50, 75, 90 and 100% of the extracted leachate. Chironomids were added as first instar larvae and they are exposed during 10d following the existing guidelines on growth tests. Monitored endpoints are mortality, length and head capsule length and width. *Lemna minor* are exposed for 7d, also following standard guidelines and the monitored endpoints are total frond area, fresh weight and root growth. Preliminary results conducted in our laboratory with *C. riparius* indicate that the leachates are toxic, causing a delay in the growth. However, the delay only occurred at the highest concentrations of leachate, results that suggest a more detailed investigation regarding the leachates and their chemical content. Additionally, information about the toxicity of PVC and PE leachates will be also presented. Overall, the present study adds valuable and missing information about the toxicity of plastic additives of three widely used materials to two representative species of the aquatic environment.

3.12.05
Environmentally Relevant Concentrations and Sizes of Microplastic DO Not Alter Marine Diatom Growth
z. niu, VLIZ / Ocean & Human Health, Research department; M.B. Vandegehuchte, Flanders Marine Institute / Research Department; A.I. Catarino, G. Everaert, Flanders Marine Institute / Ocean and Human Health
Microplastic (MP), i.e. plastic debris smaller than 5 mm, are widely distributed in the global ocean. Considering such wide distributions of MP in marine environments and their high availability to marine biota, concerns have been raised about their ecological impacts on marine life. The current knowledge about the ecological effects of MP remains limited, and to-date ecotoxicity tests often utilize standard MP with one or two distinct size classes and expose the organisms to unrealistically high MP concentrations. We exposed the marine diatom *Phaeodactylum tricornutum* to microplastic particles by mimicking a realistic size frequency distribution complemented with serial experiments with distinct size classes. To do so, we exposed this diatom to a concentration series of different-sized polyethylene (PE) microbeads (sizes: 10 – 106 µm; 1.25 x 10^2 - 1.25 x 10^7 particles / L) in a 72-hour growth inhibition test. No significant effect was observed on the growth of *P. tricornutum* by virgin PE microbeads up to 1.25 x 10^7 particles / L (or 499 mg / L). Our results indicate that environmentally relevant concentrations and sizes of MP do not alter the growth of marine diatoms. Results of smaller sized MPs (10 - 20 µm) did not differ from those obtained with larger MPs (90 – 106 µm) and mix sized MPs (10 - 106 µm), i.e. no impact on the microalgae growth. As a pioneer work, our results contribute with high quality dose-response data to an improved risk assessment of microplastic under realistic present and future marine MP pollution.

3.12.06

Ciliated Protozoans in the Face of Microplastics and Drugs Pollution of the Aquatic Environment

J. Chojnacka, Z. Wojciechowska, Medical University of Warsaw / Department of Environmental Health Sciences Faculty of Pharmacy; A. Drobniewska, Medical University of Warsaw Faculty of Pharmacy / Department of Environmental Health Sciences; G. Nałęcz–Jawecki, Medical University of Warsaw / Department of Environmental Health Sciences Faculty of Pharmacy

Pollution of freshwaters with microplastics is of increasing global concern. It include: primary microplastics from resins used in the industry and secondary microplastics generated during fragmentation of larger pieces. Microplastics, especially nanoplastics may remain in suspension in the water bodies for a long time. Ingestion of micro- and nanoplastics has been observed in many species of invertebrates, especially in filter feeders crustaceans and mussels. In addition to the release of toxic components e.g. monomers, plasticizers, microplastics have been suspected of adsorbing hydrophobic organic compounds e.g. aromatic hydrocarbons and polychlorinated biphenyls. Neuroleptics and antidepressants are one of the most dangerous groups of pharmaceuticals detected in the aquatic environment. They act not only on the nervous system of vertebrates, but also on lower organisms, including protozoans.Ciliated protozoans are ubiquitous in freshwaters and play a very important role in the environment due to purification of water from an organic suspension. In the first part of the project the effects of 4 kinds of microplastics towards ciliated protozoans *Spirostomum ambiguum* was assessed. The microplastics were not acutely toxic to the protozoans. However, the small particles 1-10 microns in diameter were picked up by the test organisms, and can be observed in their food vacuoles. The kinetics of absorption and release of the microplastics by the protozoans were studied and discussed.In the second part of the project the influence of the microplastic particles on the toxicity of the selected antidepressants toward the protozoans was studied. Concomitantly, the concentration of the tested pharmaceuticals in water and in the protozoans were determined with the use of UPLC-MS/MS. The antidepressants differ in their hydrophobicity from the least hydrophobic fluoxetine to the most hydrophobic sertraline. The relationships between the toxicity, sorption on microplastic particles and in the protozoans cells, and the physicochemical properties of the microplastics and antidepressants tested were discussed.

3.12.07

Comparative Effects of Three Types of Microfibers to Daphnia magna

D. Kim, H. Kim, Konkuk University; Y. An, Konkuk University / Department of Environmental Heath Science

Microplastics have become rising pollutants in aquatic environment for decades. Especially, in aquatic ecosystem, the microfibers, which come from textile products, were commonly detected. Based on previous studies, there are numerous microfibers including synthetic and natural fibers in aquatic environment. However, the studies on comparison of effects of natural fibers and synthetic fibers are very limited. This study compared the effects of natural and synthetic microfibers on *Daphnia magna* using lyocell, polyester (PET) and polypropylene (PP) microfibers. We exposed *D. magna* to three types of microfibers for 48 hours and recorded immobilization. Then, we transferred them into clean medium and incubated for 48 hours to observe depuration of microfiber. As a result, the immobilization rate of PET and PP exposure groups were higher than that of lyocell exposure group. The depuration rate of lyocell exposure group were faster than the synthetic microfiber group at initial duration. However, the largest number of organisms in the PP exposure group were found to have depurated microfiber. Moreover, all individual that failed to depuration for 48 hours were confirmed to be dead. It was also confirmed that natural fibers were also toxic to aquatic organisms, though the toxicity of synthetic microfibers were higher than natural microfiber. This study focused on the effects after exposure to microfibers by tracing the post-exposure depuration. This study could serve as a basic study on the effects of microplastics immediately after exposure, as well as the subsequent effects that may occur if exposure is continued. *Acknowledgement-This study was funded by the Ministry of Environment (MOE) through the Graduate School of Specialization for Safe Management of Chemicals.*

3.12.08

Long-Term Effects of Microfragments of Virgin and Recycled LDPE to Freshwater and Terrestrial Crustacean Daphnia magna and Porcellio scaber

A. Jemec, University of Ljubljana, Biotechnical Fac. / Department of Biology; J. Titova, National Institute of Chemical Physics and Biophsyics; H. Vija, National Institute of Chemical Physics and Biophysics / Laboratory of Environmental Toxicology; M. Visnapuu, V. Kisand, University of Tartu, Faculty of Science and Technology, Institute of Physics,; A. Dolar, Biotechnical faculty / Biology; D. Drobne, University of Ljubljana, Biotechnical Faculty / Department of Biology; M. Heinlaan, Nat. Inst. of Chemical Physics and Biophysics (Estonia)

Low density polyethylene (LDPE) is one of the most used polymers in the packaging industry. Virgin LDPE is considered safe food contact material yet the current unknowns may lie in the multitude of plastic additives, present in the final products. Adhering to European Plastics Strategy, one of the aims of European Green Deal is achieving reusability/recyclability of packaging by 2030. This on the other hand will potentially increase exposure to microplastics (MPL) arising from recycled polymers and hence implies the need for the respective hazard assessment. The aim of this study was to provide environmentally relevant toxicity data of virgin and recycled LDPE for freshwater *Daphnia magna* and terrestrial crustacean *Porcellio scaber*. LDPE MPL were tested as an average of 40 µm (virgin) and 204 µm (recycled) fragments. In 21-day *D. magna* exposure at 1-100 mg LDPE/L (OECD211), mortality, reproduction, parental body length and lipid content were recorded. In 21-day woodlice *P. scaber* exposure in soil at 0.02-1.5% w/w, mortality, feeding rate, and immune response were followed. In *D. magna* assay, the only observed adverse effect was minor neonatal mortality in 10 and 100 mg/L recycled LDPE treatments that however occurred sporadically and in very low share. For *P. scaber*, feeding activity was increased in the case of virgin LDPE. At the highest exposure concentration (1.5% w/w), a slight immune response (higher for virgin MPL) of woodlice was observed for both LDPE. To conclude, our study showed that during the 21-day exposure, neither LDPE MPL induced severe adverse effects on life-history traits of *D. magna* and *P. scaber*. Contrary to our expectations, recycled LDPE did not pose a higher hazard to test organisms than virgin LDPE. However, the fact that some adverse effects did manifest at higher concentrations is an alarming sign for the future as nothing indicates the decrease in the use of plastics.

3.12.09

Increasing the Environmental Relevance of Microplastic Toxicity Studies Changes the Degree of Toxicity to Daphnia magna

K. Reilly, The University of Birmingham; J. Sadler, The University of Birmingham / Department of Geography and Environmental Science; I. Lynch, University of Birmingham / School of Geography, Earth and Environmental Sciences

With an increasing awareness of the presence of microplastics in all environments studied there has been a concurrent increase in laboratory studies undertaken to further our mechanistic understanding of the potential toxicity that may arise due to microplastic exposures. Laboratory assays have historically used artificially dispersed microbeads (using a range of surfactants and antimicrobials) in pristine culturing medium with high concentrations and short-term exposures to determine toxicity. This study looked at how changing some of these parameters to be more environmentally realistic changes how *Daphnia magna* are impacted by the presence of microplastics. The first part of this study looked at dispersion protocols for microplastics, as recent studies have highlighted how the presence of artificial surfactants leads to an increases in toxicity compared to beads that have been dispersed washed of these surfactants. By removing this confounding element, we found negligible toxicity in daphnia when using 1-5µm polyethylene spheres. Following this we undertook toxicity assays in a range of media which are more representative of the natural environment that daphnia may be found in. This changed the surface conditioning of the plastic through the presence of natural organic matter and altered how the protein corona developed on the particles over time. The resulting variations in protein assays, retention in daphnia over time, increase in lipid deposits and chronic toxicity (OECD 211) endpoints such as growth and total neonate reproduction, demonstrated the importance of daphnia “fitness” and biomolecule interactions on microplastic toxicity. Finally, we assessed mixture toxicities of polyethylene microbeads and various co-pollutants including surfactant (sodium lauryl sulphate), antimicrobial (triclosan) and pharmaceutical (diclofenac). We looked at changes to the chemicals EC50 values with the addition of plastics and how the protein corona can impact this increasingly complex mixture. Again, toxicity varied during mixture scenarios based on the realism of the testing conditions. Discussion within the microplastics community highlights the need for more environmentally relevant concentrations and morphologies. In addition, this study highlights the importance of increasing the environmental relevance of exposure scenarios, such as dispersion protocols, testing medium and test

endpoints to further our understanding of this complex environmental issue.

3.12.10
Heterogeneous Mixture of Microplastics: Impact on the Freshwater Ecosystem Engineer (Tubifex tubifex) and Its Role in Biogeochemical Processes
M. Wazne, Université Claude Bernard Lyon1; F. Mermillod-Blondin, M. Vallier, H. Frédéric, UMR 5023 LEHNA, University of Lyon 1; H.A. Nel, A.T. Kukkola, S. Krause, University of Birmingham / School of Geography, Earth and Environmental Sciences; L. SIMON, UMR 5023 LEHNA, University of Lyon 1
Deposit-feeding bioturbators are key players in sediment functioning of aquatic ecosystems. Bioturbators, such as *Tubifex tubifex*, contribute to organic matter degradation and nutrient recycling in freshwater sediments. They provide these services via their ability to rework sediment and to produce and irrigate biogenic structures (galleries). By interacting with microorganisms, tubificid worms significantly stimulate biogeochemical cycles occurring at the water-sediment interface. However, the presence of contaminants in streambed sediments can significantly modify these processes by impacting the survival, the physiology and the bioturbation activities of tubificid worms. Among these contaminants, microplastic pollution has become a global environmental problem which is increasingly studied but poorly understood in freshwater ecosystems. The ingestion of microplastic particles by *Tubificidae* has been reported in literature, but the impact of microplastics on the bioturbation activities and biogeochemical cycle has never been studied. Therefore, the objective of this study is to investigate the effects of sediment contamination with a heterogeneous mixture of irregular shapes, sizes and different polymer types of microplastic pollutants (i.e. polymers of polystyrene and polyamide) on the survival, the physiological state and the bioturbation activity of tubificid worms. For this purpose, a laboratory experiment was developed in glass mesocosms allowing to (1) reproduce a water-sediment interface, (2) control microplastic concentrations and (3) control the presence of the worm *Tubifex tubifex*. Four concentrations of microplastics (0, 1000, 10000, and 100000 particles Kg-1 dry weight) and two worm conditions (no worm and 180 individuals per mesocosm) were tested. After 2 months of exposure, the survival and the physiological state (oxidative stress and energetic reserves) of worms, the surface sediment reworking (using non-fluorescent tracers) induced by worms, and the biogeochemical processes (nutrient fluxes, CO2 and CH4 degassing rates) were measured in mesocosms. While tubificid worms indirectly stimulated the mineralization of organic matter in sediments (CO2 efflux) by increasing the microbial activity in absence of microplastic contamination, this stimulation was inhibited by the presence of microplastics. Thus, this study highlights the potential impact of microplastic contamination on ecosystem processes through affecting bioturbation species.

3.12.11
Potential Toxic Effects of Environmental Plastic Litters on Wharf Roach (Ligia exotica): Two Omics Approaches
Y. Choi, Korea Institute of Ocean Science & Technology / Risk Assessment Research Center; D. Shin, Korea University of Science and Technology/Korea Institute of Ocean Science and Technology / Risk Assessment Research Center; C. Hong, Theragen Etex Bio Institute Inc.; S. Cho, Korea Research Institute of Chemical Technology (KRICT) / Chemical Analysis Center; S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; M. Jang, Korea Institute of Ocean Science and Technology / oil and POPs reserch group OPRG; Y. Cho, Korea Institute of Ocean Science and Technology / Oil and POPs Research Group (OPRG); G. Han, Korea Institute of Ocean Science and Technology (KIOST); W. Shim, Korea Institute of Ocean Science and Technology (KIOST) / Oil and POPs Research Group (OPRG); J. Jung, Korea Institutue of Ocean Science and Technology (KIOST) / Risk Assessment Research Center
This is first report to evaluate the toxic effects on wharf roach (Ligia exotica) exposed to environmental plastic litter: L. exotica, is an important ecological position as plastic detritus consumer. An experimental survey was conducted on May–June 2019 at two nearshore areas of South Korea including Maemul-do [microplastic (MP) contaminated site] and Nae-do [MP uncontaminated site]. The average MP abundances (>20 μm) was higher in L. exotica from Maemul-do (107.83 particles/individual) than those of Nae-do (1.00 particles/individual). Composition of MPs was dominated by the expanded polystyrene (EPS) in L. exotica from Maemul-do (93%). The comparative results of the transcriptomic analysis revealed significantly altered expression of genes associated with metabolic process, mitotic cell cycle regulation and immune system (cutoff: q< 0.05) in L. exotica sampled at Maemul-do. Four neurosteroids were also detected in brain, concentration of cortisol and progesterone significantly variated in L. exotica sampled at Maemul-do. Our findings highlight the importance of management of MP pollution and toxic effects of translocated MP using the bio-indicator species.

3.12.12
Trophic Transfer of Nanoplastics in the Food Chain With Three Marine Organisms
L. Kim, Konkuk University; R. Cui, Konkuk University / Department of Environmental Health Science; J. Kwak, Konkuk University; Y. An, Konkuk University / Department of Environmental Heath Science
Plastic is one of the useful materials in modern society. Small-size plastics such as micro- and nanoplastic have been generated and emitted to marine ecosystem. There are many studies on the direct exposure and effects of nanoplastics on test species, but the actual transfer of nanoplastics could include both waterborne and foodborne exposure in marine environment. This study designed the three-chain trophic level consisting algae, small crustacean and fish in the marine food web and the adverse effects on each test species were assessed. Nanoplastics were adsorbed to algae and transferred to small crustacean. Small crustacean was provided to fish as food and nanoplastics were found in the intestine of exposed fish. The small crustacean got a little damage on its gut tract and the digestive enzyme of fish got decreased. Designated trophic transfer of nanaoplastics in marine ecosystem was applied to assess the toxicity. These results could be used as the fundamental data for understanding the movement of micro- or nanoplastics in aquatic environment. *Acknowledgement. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2020R1A2B5B02001734). This study was also funded by the Ministry of Environment (MOE) through the Graduate School of Specialization for Safe Management of Chemicals.*

3.12.13
Main Drivers of Microplastic Toxicity in the Marine Polychaete Capitella Spp. Effects of Physical and Chemical Exposure From Different Plastic Materials
I.L. Lyngstad, Norwegian University of Science & Technology (NTNU) / Department of Biology; M. Wagner, Norwegian University of Science and Technology / Department of Biology; A. Booth, SINTEF Ocean / Environmental Technology; S. Piarulli, SINTEF Ocean; J. Farkas, SINTEF Materials and Chemistry / Environment and New Resources; A. Rodriguez, SINTEF Ocean; T. Gomes, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; L. Sørensen, SINTEF Ocean / Climate and Environment
Microplastics (MP) can adversely affect several aquatic species. However, it is still unclear whether the physical or chemical toxicity is the main driver of any observed effects and whether this depends on the toxicological endpoints. MP not only differ in their physical properties, such as size, shape, and surface morphology, but also in their chemical composition. This includes their polymer types, as well as the vast range of chemicals that can be incorporated into the polymer matrix. Toxicity from MP exposures derived from different plastic materials might therefore be profoundly different, also when derived from the same bulk polymer. In this study, we will investigate the effects of exposure to MP and their leachable chemicals from multiple plastic materials on the benthic deposit-feeding polychaete Capitella spp. Initially, 50 plastic products were screened using gas chromatography coupled to mass spectrometry and the Bacterial Luminescence Toxicity assay. Based on results from these experiments, a subgroup of materials representing both high and low chemical toxicity and chemical content will be selected. The selected materials will be cryomilled into irregular, polydisperse MP and aqueous seawater leachates will be produced. MP "stripped" of chemical content and silica particles as natural particle reference will also be used. Capitella spp. will be exposed for a substantial part of its lifecycle (30d), before survival and a range of sublethal endpoints (body mass, onset of reproduction, etc.) will be determined. For this ongoing work, we hypothesize (i) that MP and leachates from plastic material with higher baseline toxicity and chemical content will be more toxic to Capitella spp., (ii) that MP with associated chemicals and their chemical leachates will induce higher effect than "stripped" MP, and (iii) that "stripped" MP will induce the same effect as the natural particles.

3.12.15
Combined Toxicity of Plastic Additives in PLHC-1 Cell
T. Wang, CSIC - Spanish National Research Council / Environmental Chemistry; E. Perez-Albaladejo, Institute of Environmental Assessment and Water Research (IDAEA-

CSIC) / Department of Environmental Chemistry; C. Porte, IDAEA-CSIC / Department of Environmental Chemistry
Plastic additives are widespread environmental contaminants whose use and release to the aquatic environment is likely to increase in the next coming years. They are known to alter the endocrine and immune systems, and to act as metabolic disrupters. As aquatic organisms are constantly exposed to complex mixtures of plastic additives and other pollutants, this raises questions about their combined toxicity. Thus, this work investigates the toxicity, ability to generate reactive oxygen species (ROS) and to alter the lipid metabolism of a mixture of 10 common plastic additives -several bisphenol A derivatives, phthalates, alkylphenols, triclosan, and tritolyl phosphate- at an equimolar concentration. PLHC-1 cells were used as a model and exposed to the plastic mixture for 24 hours. Significant cytotoxicity (90 %) was observed at a mixture concentration of 20 µM (2 µM each compound). ROS generation was significantly enhanced (2.5-fold) at concentrations as low as 5 µM (0.5 µM each compound). Alterations in the lipid signature of the cells analyzed by flow injection coupled to high-resolution mass spectrometry (FIA-ESI(+/-)-Orbitrap-Exactive) were evidenced at concentrations far below those detected in single exposures. Overall, this study highlights the need for studying combined toxicity to protect environmental safety.

3.12.16
Microplastics and Health
M. Herrala, M. Huovinen, E. Järvelä, M. Lahtela-Kakkonen, J. Rysä, University of Eastern Finland / School of Pharmacy
Microplastics are small plastic particles (size < 5 mm) which are produced to be in a micro-size range or results from fragmentation of larger plastic pieces in the environment. Plastics have the tendency to carry and collect different types of hazardous substances that originate from the manufacturing process or from the environment. However, there is little reliable information on how microplastics and plastic associated chemicals (e.g. plastic additives, flame-retardants) affect the human health. Therefore, we will combine analytical tools, molecular modeling, and toxicity studies to assess the effects of microplastics on human health. Microplastics will be prepared from reference materials (e.g. polyethene, polystyrene, and polyethylene terephthalate from Sigma-Aldrich) or from environmental samples (plastic products collected from the environment). To explore the leaching of toxic plastic additives from plastics, extraction with solvents is used to mimic degradation process of plastics in the environment. Chemicals extracted from microplastics will be identified by analytical methods (NMR and MS). Molecular modeling is used to study the permeability of the nanosized microplastics, to explore whether plastic particles could transfer from human gut into circulation. The ability of plastic-associated chemicals to cause harmful effects in human health will be studied *in vitro*. Cell viability, cytotoxicity, and reactive oxygen species (ROS) production will be analysed in human colorectal adenocarcinoma Caco-2 and hepatic HepG2 cells. In addition, skin sensitisation potential is assessed using InstaCELL KeratinoSens assay kit (accCELLerate). Finally, modeling data is compared with experimental permeability and toxicity studies to determine microplastics' potential to cause hazards on human health. Our preliminary results showed that 48 h exposure to polyethylene (PE, ultra-high molecular weight, 40-48 µm particle size) decreased cell viability 40% compared to controls at 1 mg/ml ($p < 0.01$), and 30% with 0.5 mg/ml concentration ($p < 0.05$) in Caco-2 cells. Also, slight but not statistically significant increase was detected in ROS production. IC50 value of PE was determined from five repeated MTT measurements to be 1.6 ± 0.5 mg/ml. The experiments will be continued with other plastic particles with extractions that mimic the leaching of additives of plastic material.

3.12.18
The Role of Sand Filters As Tertiary Treatment in Microplastic Retardation Within Wastewater Treatment Plants
M. Funck, Institut für Energie- und Umwelttechnik e.V. (IUTA) / FA/UHS; M. Al-Azzawi, Technical University Muenchen / Chair of Urban Water Systems Engineering; O. Knoop, Technical University of Munich / Chair of Urban Water Systems Engineering; F. Itzel, Institute of Energy and Environmental Technology e.V. (IUTA); T.C. Schmidt, University of Duisburg-Essen / Chair of Instrumental Analytical Chemistry; J. Drewes, Technische Universität München / Chair of Urban Water Systems Engineering; J. Tuerk, Institute of Energy and Environmental Technology e.V. (IUTA)
Beside spectroscopic methods different thermoanalytical methods are used for microplastic analysis. An advantage of the thermoanalytical methods is the simple and comparatively fast analysis, which requires less effort in sample preparation than the spectroscopic methods. The main methods used in thermoanalytical analysis are pyrolysis GC-MS (Py-GC-MS) with its various versions and Thermo-Extraction-Desorption-Gas-Chromatography-Mass Spectrometry (TED-GC-MS), which was introduced in 2014. The advantage of TED-GC-MS compared to the Py-GC-MS is allowing higher sample masses up to the g range, whereas Py-GC-MS allows sample application in the µg or lower mg range. One discussed point source for microplastics (MPs) emission into the environment are wastewater treatment plants (WWTPs). The impact of tertiary wastewater treatment for MP removal has not been investigated reliably Secondary and tertiary effluents from three WWTPs were investigated to compare the effect of sand filters on microplastic retention. A cascadic MP filtration system using steel basket filters with mesh sizes of 100 µm, 50 µm and 10 µm was used for sampling in combination with TED-GC-MS. Four common polymers were investigated in this study (polyethylene (PE), polystyrene (PS), polypropylene (PP) and polyethylene terephthalate (PET)). Results showed that sand filters offered high additional microplastic retention capabilities, with the sand filters in two of the WWTPs offering extra 86% ± 8% and 84% ± 12% for the investigated polymers respectively. The presented scientific work was funded by the Federal Ministry of Education and Research (BMBF) within the framework of the project: "Plastics-Interconnected Project SubµTrack: Tracking of (Sub)Microplastics of Different Identities - Innovative Analysis Tools for Toxicological and Process Evaluation, Subproject 5", funding number 02WPL1443E as well as a grant from the State of North Rhine-Westphalia using funds from the European Regional Development Fund (ERDF) 2014-2020 "Investment in Growth and Employment" for the research project "Investigation of the influence of polymers on a terrestrial ecosystem using the example of mulch films used in agriculture - iMulch", funding code EFRE-0801177

3.12.19
Impact of Biofilm Formation on Biodegradable Microplastics in Aquatic Ecosystem
P. Ramesh, IITB Monash Research Academy / Metallurgical Engineering and Material Sciences Department; S. Shukla, S. Saxena, Indian Institute of Technology Bombay, Mumbai, India / Metallurgical Engineering and Material Sciences Department; T.M. Adyel, Monash University / Department of Civil Engineering
Biodegradable plastics are recent most-sought engineered materials disrupting single-use plastics worldwide. It implies onto commercial applications such as packaging, cosmetics, consumer electronics and biomedical industries. These polymers are either made up of aliphatic polyesters with ester units or aliphatic-aromatic polyesters with few aromatic units. The rate of degradation of such polymers in aquatic system is lesser due to lower temperature exerted compared to terrestrial environment. Generally, plastic biodegradation occurs in a two-step process: fragmentation and microbial mineralization. The fragmentation process directly contributes to microplastic generation, whereas microorganisms play a significant role in the degradation of such biodegradable polymers. It was hypothesized that this fragmented size (< 5 mm) of biodegradable polymers known to be persistent and ubiquitous, imposes the same ecotoxicological effect as their non-biodegradable microplastic counterparts. When the extracellular matrix of a biofilm forms a layered sheet that either feeds on or interacts with that microplastic provides additional survival attributes such as gain of function, specific resistance mechanisms, and metabolite interactions. Thus, microorganisms colonizing microplastic are of greater interest. However, the role of active microplastics degrading capable microorganisms over microplastics and nanoplastics in a water environment is still under study. This work highlights the fate and occurrence of biofilm formation with its functional attribute to biodegradable microplastics in aquatic environments. It also enumerates the list of microbial biofilms that exerts pressure on biodegradable microplastic polymers with their relevant conserved genetic makeup.

3.12.20
Physio-Chemical Properties of Microplastics Influence Egestion by Predatory Fish
A. Dawson, Australian Institute of Marine Science; M. Santana, AIMS@JCU, James Cook University & Australian Institute of Marine Science; M. Perez, James Cook University; K. Meehan, F. Kroon, Australian Institute of Marine Science
The ingestion of microplastic particles by fish is thought to occur frequently throughout the marine environment. Although microplastics are often isolated from with the gastrointestinal tract of fish sampled from the environment, the concentration and detection frequency tend to be extremely variable amongst organisms within sample populations. Thus far little is known

regarding the persistence of microplastics within fish, particularly predatory fish. Predatory fish are likely to be exposed tropically to microplastics through contaminated prey rather than passive ingestion. This study compared depuration of model microplastics with varying physio-chemical properties. Microplastic dosed food pellets were offed to juvenile predatory fish, *Lates calcifer*, in a pulse feeding, thereafter, stomach contents were sampled at 0, 2, 6, 12, 24, 48 and 96 hours. Depuration rates for microplastics were calculated based on size (500µm and 50µm, PET fragments), shape (500µm PET fragments and 500µm PET fibres), and polymer (500µm PET fibres and 500µm PA fibres). The ability of PET fragments and fibres to be taken up into the liver and muscle tissue was also investigated, where fish were offered pellets dosed with PET fragments (≤300µm) and PET fibres (500µm). To quantify depuration rates of each MP variable the gastrointestinal tract was chemically digested, filtered and enumerated. To determine the ability and size range of PET to translocate from the GIT, the liver and a portion of muscle from each fish were chemically digested, filtered and enumerated. This poster presents the results of these experiments.

3.12.21

Factors Impacting Microplastic Ingestion of Fish Species

i. koraltan, Akdeniz University - Graduate School of Natural and Applied Sciences; O. Guven, Akdeniz University Faculty of Fisheries; S. Mavruk, Çukurova University Faculty of Fisheries

Micro size plastic particles (MPs), either directly released or created as a consequence of the fragmentation of macro plastics, is an emerging threat to aquatic ecosystems. Due to their sizes they are easily accessible to marine life and disperse great distances. They are known to ingested by fishes yet many questions remain about the factors affecting microplastic ingestion. Here investigate the impacts of season, depth, fish size, trophic level and habitat on the MPs exposure of commercial fish species in the Mediterranean Sea. A total of 1364 fish individual belonging to four species were analysed in order to determine the state of MPs exposure to fishes. 275 MPs were detected in gastrointestinal tract of examined fishes. All the species found to be impacted from the MPs pollution and MPs exposure rate of the fish species in the study was %20.5. Highest exposure rate was detected for *Saurida lessepsianus* (%35.5). Within the five different MPs types, most common type was Fiber (%94.9). Black was the most common MPs colour (%48,8). The average length of detected MPs 1.49±1.485mm. Although depth and fish size reported to be the two factors that have impact on MPs ingestion of fish, our results indicated no impact for the evaluated species. On the other hand, in addition to seasonal environmental changes causing MPs dispersal in the marine environment, which accumulates in the terrestrial area, biotic factors such as habitat and food preference has a significant influence on MPs ingestion of fishes.

3.12.23

Now You See Them, Now You Don't - Fate and Retention of Microplastic Particles in Stream Mesocosms

T. Schmitt, Universität Koblenz-Landau / iES, Institute for Environmental Sciences; M. Wieczorek, R. Schulz, University of Koblenz-Landau / iES Landau, Institute for Environmental Sciences; B. Mckie, Swedish University of Agricultural Science / Aquatic Science and Assessment; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment

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 intentional produced MP for industrial applications and personal care products, and secondary MP (sMP). Secondary sMP 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 ecosystem either during surface run-off events or via sewage and domestic 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, we investigated the impact of macrophytes on the fate of MP particles using the "Landau Stream Mesocosm Facility" (LSMF) comprising 45 m long stream channels. With polystyrene (PS), polyvinyl terephthalate (PET) and car tyre powder (CT), three common MPs were chosen as model sMPs. We used three different vegetation densities (0 %, 25 %, 100 %) of the macrophyte *Elodea nuttallii*. We hypothesize 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.

3.12.24

Relevance and Reliability of Evidence for Microplastic Contamination in Seafood: A Case Study Using Australian Consumption Patterns

A. Dawson, Australian Institute of Marine Science; M. Santana, M. Miller, AIMS@JCU, James Cook University & Australian Institute of Marine Science; F. Kroon, Australian Institute of Marine Science

Seafood contamination with, and human consumption of, microplastics (MPs) have recently been highlighted as an emerging concern for global food security. While there is evidence that commercial marine species are contaminated with MPs, it is unknown if seafood can act as a vector for MP transfer to human consumers. Microplastics have been reported in the digestive tract, gills and in select internal organs of marine animals. However, many of these tissues are not typically eaten by human consumers but discarded. In this critical review, we examined the peer-reviewed literature for evidence of MP contamination in seafood, and its potential transference to human consumers. Based on known seafood consumption patterns in a typical Australian diet, we assessed the relevance and reliability of the current body of literature to examine the prospect and risk of MP transference. The relevance of data was considered based on the organism studied, origin of the samples, and the tissues analysed, while reliability was assessed based on procedural methodologies used to derive the data. A review of 94 studies found no evidence of MP contamination in edible tissues from fresh fish or crustaceans. MP presence was confirmed in packaged fish, as well as in fresh and packaged bivalve molluscs. The limited number of studies satisfying the relevance and reliability criteria (n=16) precluded a quantitative assessment of the potential risk associated with MP transference. While consumption of packaged fish and bivalve molluscs may result in the consumption of MPs by humans, it is currently unknown whether this presents a health risk.

3.12.25

Plastic Debris Ingested by Sea Turtles From the Korean Waters: Quantity, Shape, Origins, and Polymer Composition

Y. Moon, Korea Institute of Ocean Science & Technology (KIOST); S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; G. Han, Korea Institute of Ocean Science and Technology (KIOST); W. Shim, Korea Institute of Ocean Science and Technology / Oil and POPs research group; Y. Cho, Korea Institutue of Ocean Science and Technology (KIOST); M. Jang, Korea Institute of Ocean Science and Technology (KIOST); I. Kim, M. Kim, National Marine Biodiversity Institute of Korea (MABIK)

Globally, all seven species of sea turtles and about 52% of them were estimated to have ingested plastic debris. The literature from East Asia are scarce even though more than half of mishandled plastics are emitted to ocean from this region. Notably, no report has been published in the Korean waters. This study examined debris ingested by sea turtles stranded or by-captured from 2012 to 2018 in the Korean coastal waters for the first time. Plastic debris in the gastrointestinal (GI) tract of 34 turtles, including 21 loggerheads, 9 greens, 2 olive ridleys, and 2 leatherback turtles, were analyzed. The plastic ingestion frequency of greens, loggerheads, olive ridleys, and leatherbacks were 100%, 81%, 50%, and 50%, respectively. The overall amount of plastics were in the range of 0－1.31 g/kg turtle (0－229 pieces/turtle). One of the juvenile loggerhead had been found stranded on beach with 10.24 g of plastics in GI tract, which was released with satellite transmitter 11 days before. Various types of polymer were detected in sea turtles, for example, polyethylene, polypropylene, polystyrene, etc. Overall, film and fiber type constituted the majority of the debris. Some debris were labeled with Korean (n=9) or Chinese (n=10). This implies that these sea turtles had used Korean and Chinese waters as foraging areas. About 15% of total pieces could be identified what their origin was. Among them, single-use and fishing items were dominant, which is similar to the Korean beach monitoring result. Green turtles ingested more plastics (0.26 ± 0.44 g/kg) than loggerheads (0.07 ± 0.16 g/kg). The shape, origin of ingested debris varied between loggerhead and green turtles. Green turtles ingested commonly fiber (51%) such as rope, twine, and net. Contrarily, loggerheads ingested frequently film (61%) such as plastic bag, and packaging. It is plausibly assumed that the difference is related to feeding habit of each species. The shape patterns of plastic debris differed between sea turtles and coastal beaches. Sea turtles preferred to ingest film (43%), which relatively infrequent

in coastal beaches (12%). The result of this study demonstrates that sea turtles foraging around the Korean waters are widely affected by marine plastic debris. It is crucial to reduce mega-, macro-, and mesoplastic debris which promote generation of microplastic especially putting priority on the plastic that are widely detected in marine waters and biota.

3.12.26
Assessing the Bioaccumulation Potential of Nanoplastics in Oysters
F. Ribeiro, Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); D.M. Mitrano, ETH Zurich / Environmental Systems Science; K. Brigden, University of Exeter / Greenpeace Research Laboratories; C. Hacker, P. Cherek, University of Exeter / Bioimaging centre; S. Kaserzon, University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); K. Thomas, The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); T.S. Galloway, University of Exeter / Department of Biosciences
The potential bioaccumulation and excretion of nanoplastics in bivalves is still a knowledge gap in the literature due to analytical limitations. Doping polystyrene nanoparticles with palladium (Pd NPs) enables their detection by standard methods of trace metal analysis such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Transmission Electron Microscopy (TEM) supported by energy dispersive X-ray spectroscopy (EDS). The aims of this study were to (1) assess the concentration of Pd NPs in the different tissues of the oyster *Crassostrea gigas* following exposure, (2) understand the retention time of the particles after a long depuration period and (3) determine their accumulation and elimination rates. The oysters were exposed to two treatments of Pd NPs ("Smooth" and "Raspberry" particles; approximate size of 200nm) at a concentration of 4 µg Pd L^{-1} for 6 days followed by a 30 days depuration period. Oysters were sampled at the start of the experiment (T0H) and after 3H, 8H, 24H, 3 and 6 days of uptake and 24H, 6, 12, 19 and 30 days of depuration. Different segments of the oysters were collected at each sampling day: haemolymph, gills, digestive gland, remaining tissues and biodeposits (faeces and pseudofaeces). During the uptake period it was observed an increase in the pseudofaeces, reaching its maximum production on the 3rd day of exposure. The TEM analysis of the collected pseudofaeces showed an abundance of Pd NPs indicating the oysters are excreting the particles. Gills and digestive gland were fixed and processed for TEM analysis. The TEM analysis revealed the presence of Pd NPs in the haemolymph after 24H of uptake and in the gills and digestive gland after 6 days of exposure to the particles. A considerable decrease in the number of particles was observed after 25 days of depuration in the same tissues. Tissues and biodeposits will be dried, digested and analysed for total Pd concentrations by ICP-MS to infer the exact concentration of Pd NPs in the different compartments of the oyster through the uptake period and to infer if a complete excretion occurs at the end of the depuration period.

3.12.28
Microplastics in Marine Bivalves Produced in Aquaculture to Human Consumption
D.C. Silva, MARE - University of Coimbra / Department of Life Sciences; J.C. Marques, University of Coimbra, MARE - Marine and Environmental Sciences Centre / Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal; A. Goncalves, MARE - Marine and Environmental Sciences Centre, University of Coimbra / Life Sciences
Marine bivalves have a wide distribution around the world, play vital roles in the structure and function of the ecosystems and show high nutritional value. Some bivalve species are widely appreciated by humans, therefore having an important commercial value. In the past few decades aquaculture production expanded worldwide to meet the growing demand for marine bivalves. Most of the production of marine bivalves comes from extensive aquacultures placed in transitional waters systems. These systems are highly impacted by a vast number of pollutants, including microplastics, that may be ingested and bioaccumulated, causing negative impacts in the cultured marine bivalves. Therefore, this research aims to understand the occurrence and seasonal variation of microplastics in different tissues (visceral mass, digestive system, gills and muscle) of two marine bivalves – the Pacific cupped oyster, *Crassostrea gigas*, and the Japanese carpet shell, *Ruditapes philippinarum* – produced in an aquaculture located in the ria de Aveiro coastal lagoon, in Portugal, and in their surrounding environmental matrices (water and sediment surfaces). The methodology comprised wet peroxide oxidation, density separation and filtration. Samples were inspected by stereomicroscopy and the number of suspected microplastics found and their physical properties (type, size and colour) were registered and analysed. The chemical properties (polymer type) of the microplastics were identified by FTIR. The results revealed that microplastic particles were found in the water and sediment surfaces and in the tissues of the marine bivalve species. The abundance of microplastics was higher in sediment samples than in water samples. *Ruditapes philippinarum* showed higher microplastic abundance than *Crassostrea gigas*. Microplastics were mostly found in the digestive system, followed by the gills and muscle of both marine bivalve species. The microplastic size class predominant in water, sediment and bivalve tissues samples was 1-500 µm, 100-1000 µm and 100-500 µm, respectively. The most common types of microplastics found in water samples were fibers, in sediment samples were fragments and fibers, and in bivalve tissues were films and fibers. Our findings emphasise the major concern of microplastic pollution and its implications to seafood quality and thus to human health.

3.12.29
Accumulation of Environmentally Relevant NanoPlastics in Bioindicators
A. Valsesia, European Commission DG Joint Research Centre / Directorate F Health, Consumers and Reference Materials; J. Ponti, European Commission, Joint Research Centre / Directorate F Health, Consumers and Reference Materials; D. Cassano, R. La Spina, JRC, European Commission, Joint Research Centre; C. Desmet, D. Gilliland, European Commission, Joint Research Centre / Directorate F Health, Consumers and Reference Materials
The unintentional release of plastic litter into the environment is increasingly being recognized as a major threat not only for terrestrial and marine ecosystems but potentially also for human health[1]. These concerns are strongly related to their potential degradation into plastic micro and nanoparticles (pMP, pNP), both of which may exhibit size-related effects in relation to bioaccumulation and uptake levels. Among plastic particles found throughout the environment, polyethylene (PE) and polypropylene (PP) are two of the most abundant.[2] While these polymers are recognized as being chemically inert, a biological hazard, which may arise from the pMP and pNP is connected to their chemical composition.[3] The few data available in literature[4], show that the environmental contamination level from pNP is very low. A possible solution to overcome these limitations resides in the use of bioindicators and bioaccumulators, for example fito-zooplankton, mussels, ascidians, etc at different level of the trophic chain. In this work, bioindicators are exposed *in vitro* to environmentally relevant nanoplastics (PE, PP and Polyvinylchloride together with standard Polystyrene beads) which has been produced and characterized in our lab. We then describe a full analytical protocol for studying the efficiency of retention of environmentally relevant pNP by biondicators (mussels). The method described includes the protocol for mussels' exposure and the analytical protocol for the purification of the pNP and their detection and quantification. The analysis is based on an enzymatic digestion of the mussel's whole body and the spotting of the sample on a chip characterized by an array of nanocavities. The chip serves as a support for SEM counting of the particles and for the chemical identification of the pNP by Confocal Raman Microscopy. The combination of these two techniques enables a quantification of the pNP retained by the bioindicator. The work demonstrates the potential use of mussels (and others) as bioindicators of the presence of nanoplastics in the environment and describes analytical protocol to detect and quantify them.
1. Vethaak, A. D. & Leslie, H. A. Plastic Debris Is a Human Health Issue. *Environ. Sci. Technol.* **50**, 6825–6826 (2016). 2. Bond, T., Ferrandiz-Mas, V., Felipe-Sotelo, M. & Van Sebille, E. The occurrence and degradation of aquatic plastic litter based on polymer physicochemical properties: a review. *Crit. Rev. Environ. Sci. Technol.* **48**, 685–722 (2018). 3. Avio, C. G., Gorbi, S. & Regoli, F. Plastics and microplastics in the oceans: from emerging pollutants to emerged threat. *Mar. Environ. Res.* **128**, 2–11 (2017). 4. Ter Halle, A. *et al.* Nanoplastic in the North Atlantic subtropical gyre. *Environ. Sci. Technol.* **51**, 13689–13697 (2017).

3.12.30
One-Year Monitoring of Microplastics in Mussels (Mytilus edulis) From an Industrialized Bay of South Korea
J. Jeong, University of Ocean Science and Technology, KIOST-UST Campus / Oil and POPs Research Group (OPRG); S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; Y. Cho, Korea Institute of Ocean Science and Technology / Oil and POPs Research Group (OPRG); G. Han, Korea Institute of Ocean Science and Technology (KIOST) / Oil and POPs Research Group (OPRG); M. Jang, Korea Institute of Ocean Science and Technology / oil and POPs

reserch group OPRG; W. Shim, Korea Institute of Ocean Science and Technology (KIOST) / Oil and POPs Research Group (OPRG)
Microplastics are prevalent in the marine environment, but little information is available about microplastic contamination from a long-term perspective. Long-term monitoring can reveal contamination trend and reflect environmental changes over time. The present study conducted a long-term monitoring using mussels (Mytils edulis) as a bioindicator to understand the monthly variations of microplastic contamination from an island in Masan Bay, South Korea. Mussel is suitable species for a long-term monitoring of microplastic contamination due to its abundance, widespread distribution and sessility which results in high accessibility. In addition, its capacity of filtering seawater leads to accumulation of microplastics. Mussels were collected monthly from February 2018 to January 2019. After a series of sample treatment process, all plastic-like particles were analyzed using micro-Fourier transform infrared microscope. The detection frequency of microplastics in mussels was 100%, confirming that microplastic contamination was widespread throughout the year. The annual mean concentration was 0.38 ± 0.16 n/g and 1.29 ± 0.61 n/individual. The microplastic concentration showed a decline trend from March to August, and increased through fall and winter. The monthly change of microplastics in mussel tissues was not large, but was slightly related to condition index of the mussels. Polyacrylate copolymer, polypropylene and polyethylene were found as dominant polymers, accounting for 21.4% to 72.2% of the total microplastics. The high abundance of polyacrylate copolymers could be due to frequent ship activities inside the bay as it is mainly used for ship paint resin. Fragments smaller than 300 µm were dominant (65-89%). The overall contamination characteristics of microplastic (shape, size, polymer type, and color) was consistent throughout the year. The results of this study provide information about monthly change in microplastic concentration in mussel, which would be useful in planning biomonitoring study of microplastics using bivalves.

3.12.31
Ecological Risk Assessment of Micro- and Nano- Plastics in San Francisco Bay Using a Bayesian Network Relative Risk Model
E. Sharpe, W.G. Landis, Western Washington University / Institute of Environmental Toxicology and Chemistry
Micro- and nano- plastics are a complex and abundant emerging contaminant. Recent advancements in monitoring technology have allowed us to see that plastic particles are widely distributed in the environment. Increased public interest in this topic indicates a need for a comprehensive ecological risk assessment on the environmental impacts of micro- and nano-plastics. This study is part of a larger initiative funded by the National Science Foundation Growing Convergence Research Grant program that seeks to fill data gaps on micro- and nano-plastic toxicity, fate and transport and enhance our understanding of the risk micro- and nano-plastics present to ecological structures. The present study aims to conduct an ecological risk assessment for micro- and nano- plastics using a Bayesian Network Relative Risk Model (BN-RRM) and the San Francisco Bay as a case study. The BN-RRM has proven to be a successful framework for ecological risk assessment, allowing for the creation of a model with predictive capability and adaptive potential as new data becomes available. The data that are currently available on micro- and nano- plastics have yet to be applied to management strategies of these contaminants in the environment and previous risk assessments for microplastics are limited in scope or inadequate due to the methods they used to calculate risk. Using microplastic abundance data collected by the San Francisco Estuary Institute, micro- and nano- plastic toxicity data generated by Oregon State University, and water quality and chemical monitoring data, risk due to micro- and nano-plastics exposure is determined for Chinook salmon (Onocorhynchus tshawytsha), Olympia oysters (Ostrea lurida), and Pacific herring (Clupea pallasii). This study will lay the groundwork for future risk assessments of micro- and nano- plastics in the environment and help to identify key uncertainties that need to be addressed. This work is supported by a National Science Foundation grant (# 1935018).

Per-and Polyfluoroalkl Substances (PFAS): a Persistent Challenge for the 21st Century

3.13.02
Background PFAS Contamination of German Soils - Using the Top Assay to Uncover Unknown PFAS
N. Bandow, German Environment Agency / Laboratory for Water Analysis; J. Koschorreck, Umweltbundesamt / Inland Surface Waters; J. Wellmitz, German Environment Agency / Laboratory for Water Analysis
Per- and polyfluorinated alkylsubstances (PFAS) are highly persistent and have been found worldwide in many compartments. The persistence in combination with toxic effects on environmental and human health has resulted in a ban of individual compounds and to an ongoing substitution by other PFAS. Today, PFAS are estimated to consist of more than 4700 compounds including precursors and polymers making analysis of all single compounds impossible. To overcome this analytical gap, sum parameters, e.g. the total oxidisable precursor assay (TOP assay) have been developed. The application of the TOP assay on various samples types has indicated that the amount of unknown PFAS compounds in the environment is in general higher than what is revealed with the analysis of well-known PFAS. We used soil samples from the German environmental specimen bank representing different ecosystem types to investigate the background contamination of German soils in the time period 2002-2018. The sum of PFAS increased by around 20% in all samples when the TOP assay was applied. Increase up to 50% for PFOA indicate predominant exposure of soils to PFOA-precursors. In comparison, much higher increases have been reported at highly contaminated soils from, e.g. paper sludge or firefighting foams. The change in concentration from 2002 to 2018 is rather small and not statistically relevant. However, in some samples the sum of unknown PFAS decreased from 2002- 2018. The forestal samples showed higher PFAS concentrations and also higher diversity of single PFAS compounds detected than the samples from agricultural and urban areas. Higher deposition rates of contaminants transported via air at wood lands is a known phenomenon and may be one explanation. PFOA and PFOS are the most dominant PFAS in all samples before TOP assay. PFAS were detected in all samples in this study and it can be concluded that besides the numerous contamination hotspots PFAS are ubiquitously present at levels in the lower µg/kg soil range all soils. The TOP assay revealed the presence of unknown PFAS in all samples. This underlines the need for a more comprehensive strategy in PFAS regulation including precursors and substitutes.

3.13.06
Orbitrap HRAM LCMS for routine wastewater effluent analysis with simultaneous non-target screening and an insight into wastewater based environmental epidemiology
N. Llewellyn, ThermoFisher Scientific
The occurrence and effects of micro-organic pollutants in aquatic environments and subsequent risk to sustainable to ecology and human health is a significant concern. To protect and restore clean water across Europe and ensure its long-term sustainable use, in December 2000 the EU Water Framework Directive (2000/60/EC) came into force. Amongst other criteria, the Directive stipulates defined maximum levels for a number of Priority Substances to ensure waterbodies maintain 'Good Chemical Status'. Whilst generally comparable with triple quadrupole LCMSMS performance, data acquisition by full scan high resolution accurate mass (HRAM) has a number of benefits beyond direct targeted quantitation, which can greatly expand the meaningfulness of the data. This includes retrospectively expanding the initial quantitation target list as well delivering qualitative sample information including unknown identification and through using multivariate statistical tools, allowing differences and similarities between datasets to be identified. Building on a single data acquisition run, presented here is a stepwise data processing strategy that demonstrates: • Targeted analysis • Semi-quantitation of targets returned from the unknown identification workflow • Using statistical tools, a metabolomic approach to show how the data can be viewed holistically to highlight differences and similarities between sampling sites. Analysis was achieved using an EQuan large-volume on-line SPE autosampler coupled to a Orbitrap Exploris 120 with acquisition and quantitation performed with TraceFinder 5.1 and qualitative work using Compound Discoverer 3.2, utilising the Thermo Scientific™ mzCloud™ mass spectral library.

3.13.07
Quantitative Analysis of PFAS by HRMS: Multi-Lab Validation for Performance Evaluation
T. Anumol, Agilent Technologies, Inc.; R. Hindle, K. Hunt, Vogon Labs; D. Szabo, B. Clarke, University of Melbourne / School of Chemistry; J. Pyke, Agilent Technologies
Per/Polyfluoroalkyl substances (PFAS) have uniquely desirable properties for use in various industries. However, their wide-ranging use along with persistence results in their long-term presence in the environment. Traditionally, analysis of PFAS has focused on quantification of a selected set of carboxylic acids and sulfonic acids ranging from 15-40. Indeed, Standard and

Consensus methods developed by USEPA, ASTM, ISO etc are designed to monitor a small and discrete number of PFAS compounds using tandem quadrupole LC/MS. These methods strive to quantify selected PFAS at low ng/L levels but can be limited in scope. However, from a regulatory perspective, this quantification information is critical.To understand the complete scope of PFAS contamination, The total fluorinated compounds in a sample may be underestimated by not monitoring the precursor compounds and newer classes of PFAS of which there are >4,000 known to have been manufactured globally. Other techniques such as the Total Oxidizable Precursor Assay attempt to measure the total fluorinated compounds by forcing degradation of precursors into measurable end-products. However, this technique is time consuming and may not degrade all precursors into measurable end-products while giving no information on the specific PFAS in the sample. Identifying PFAS present in an environmental sample may impact decisions in treatment processes at remediation sites. Traditional instruments like LC-MS/MS, are targeted to quantify commonly monitored PFAS. This study conducted a multi-lab validation across 4 labs using high-resolution LC/MS to evaluate environmental samples for quantification of target PFAS and also then report additional PFAS screened for by HRMS. The ability of HRMS instruments to be able to retrospectively mine for PFAS present in samples previously run is critical, however the ability to also gain reproducible and robust quantitative data for a sunset of PFAS is a huge benefit too. This study aims to determine the sensitivity, reproducibility, robustness and accuracy of quantitative data on HRMS with methods optimized for PFAS screening in multiple labs. We will present method performance criteria for quantification of PFAS like spike recoveries, reproducibility, accuracy and blank evaluation seen in the labs and also an evaluation of the additional PFAs identified using non-target workflows. This is the first known multi-laboratory evaluation study for analysis of targeted and non-targeted PFAS using HRMS.

3.13.08
Use of Green Technologies for Studying PFAS: Supercritical Fluid Extraction of ENvironmental and Food Samples
R. Marfil-Vega, W. Hedgepeth, Y. Fujito, Shimadzu Scientific Instruments; S. MOREAU, Shimadzu Europa Gmbh / Product & Marketing Management
Supercritical Fluid Extraction (SFE) presents several advantages when compared to traditional solvent extractions: it can be automated and applied to broad classes of compounds. SFE also uses minimal amounts of organic solvents, hence it is a greener technique. In this study we used offline SFE for extracting 18 PFAS compounds from fish samples of different origin. Concentrations of the targeted compounds were then quantified by Liquid Chromatography/Mass Spectrometry, with Limits Of Quantification ranging between 0.5 and 2 ng/g. In this presentation, we will demonstrate the suitability of SFE for studying the occurrence of PFAS in fish tissue, as an example of biota and food samples. Extraction efficiency, reproducibility, and dynamic range will be discussed together with the automated workflow that was implemented for the analysis.

3.13.11
Strategies for Measuring Per- and Polyfluoroalkyl Substances in Water by Direct Injection Liquid Chromatography Tandem Mass Spectrometry Analysis
A. Ganci, PerkinElmer; J. Wu, F. Qin, PerkinElmer Inc.
Per- and polyfluoroalkyl substances (PFAS) represent a diverse group of man-made synthetic fluorinated organic compounds that have been widely used in industrial applications and consumer products. The unique physical and chemical properties of these compounds, along with their ubiquitous use, have led to the accumulation of PFAS in the environment, with growing concern over human exposure to these chemicals. Various PFASs have been found around the world in different water resources, including drinking, surface, ground and wastewater. To aid in the monitoring of PFAS in environmental matrices, several health advisory guidelines and recommended methods have been established. Following current regulatory levels, the determination of low PFAS concentrations in the ng/l range typically requires either a highly sensitive mass spectrometer, or a sample preparation technique that includes a concentration step. Coupling SPE with liquid chromatography tandem mass spectrometry (LC-MS/MS) has been a popular approach to PFAS analysis in aqueous samples. Recently, with the advancement and availability of highly sensitive mass spectrometers, a trend towards developing a high throughput analytical method for the determination of PFAS by direct injection, without SPE, has been appreciated. The direct injection approach can not only aid in achieving the highest sample throughput, save time and reduce cost, but also minimizes potential analyte loss and contamination resulting from SPE sample preparation. In this work we will demonstrate a direct injection approach to an extensive PFAS analysis, utilizing a PerkinElmer QSight® mass spectrometer coupled to an ultra high performance liquid chromatography (UHPLC) system for the analysis of trace amounts of PFAS in various types of water samples. Initially, the simple sample preparation procedure, steps in method optimization, as well as strategies to avoid interferences from used consumables and equipment will be illustrated. Further, performance parameters including method linearity, LOQs, recoveries and repeatability will be presented and discussed in accordance with regulatory values and method performance criteria. Finally, the result of the method applied to the analysis of different types of water sources will be presented as prove of its applicability to real-world samples.

3.13.12
Monitoring of Perfluoroalkyl Substances (PFASs) in Tagus River Basin (Spain)
I. Navarro, CIEMAT; A. de la Torre, CIEMAT / Environmental; P. Sanz, M. Martínez, CIEMAT
Perfluoroalkyl substances, PFASs, are emerging organic pollutants which present persistence, toxicity, potential for bioaccumulation and remarkable ubiquity in the environment. In fact, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been included to Annex B (May 2009) and Annex A (May 2019), respectively, as persistent organic pollutants in the Stockholm Convention (SC), and perfluorohexane sulfonic acid (PFHxS) is presently proposed for listing. Thus, under the leadership of the Spanish Ministry for the Ecological Transition and Demographic Challenge (SMETDC), a Spanish monitoring program framed in the SC National Implementation Plan was developed to evaluate the current status and temporal trends of PFOS in river water. A study area of high representativeness was selected and evaluated to reflect the ecological status of the Tagus River watershed in its Spanish section. A total of 92 river water samples were collected in 23 monitoring campaigns during 5 years (from February 2013 to August 2018) to determine the presence of 20 PFASs (4 perfluoroalkyl sulfonic acids, 13 perfluoroalkyl carboxylates and 3 perfluoroalkyl sulfonamides). Four sampling points were selected to represent different typologies of the Tagus River watershed. PFASs were detected in 76 water samples, with mean levels of 15 ± 13 ng/L. Positive correlations were found among the PFASs studied, suggesting that they may be from similar sources. Important differences between sampling points were found. ΣPFAS concentrations at points located in urban and industrial areas were statistically higher than those at background or remote areas. A statistically significant decrease of some PFASs was observed from 2013 to 2018. Annual average environmental quality standard (AA-EQS) for PFOS was above the established in the Directive 2013/39/EU (0.65 ng/L for inland surface waters) but below the maximum allowable concentration (36 µg/L). The mean mass flow rate of PFASs were 14 ± 16 kg/y, being PFOS, PFHxS and PFOA the compounds with higher contribution. 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 PFASs could have human health implications. PFOS study was funded by the SMETDC, projects EG042010 and 15CAES003. The authors thank to Confederación Hidrográfica del Tajo for providing the water samples and information related to mean daily flows.

3.13.13
Perfluoroalkylated Substances in European Household Dust
A. de la Torre, CIEMAT / Environmental; I. Navarro, P. Sanz, M. Martínez, CIEMAT
Perfluoroalkylated substances (PFASs) have been extensively used since the 1950s as intermediates or ingredients of surface protectors and surfactants in a wide variety of applications. However, evidence that they cause harm to the people health and ecosystems have triggered the implementation of regulations (perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonic acid (PFHxS) listed under Stockholm Convention) and a worldwide trend towards replacement of long-chain PFASs by shorter ones. Major historical and current uses are easily associated with home environments (electronics, construction products, household products, fire-fighting, metal plating, or even biocides) where they could slough off from applied materials via physical processes such as abrasion or weathering and accumulate in the dust and could enter into the human body via inhalation, ingestion and dermal contact. Therefore, house dust monitoring is a reliable tool to evaluate substitution tendencies and to perform human exposure assessment for dust intake. The present study evaluates the presence of twenty

PFASs, including perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and perfluoroalkane sulfonamides (PFOSAs) in sixty-five household dust samples from Belgium, Italy and Spain. Samples were obtained by vacuuming the entire floor home (settle dust). The three European countries presented similar ∑PFASs levels (10.2, 19.7 and 11.3 ng/g; median Belgium, Italy and Spain) and in all cases, ∑PFCAs concentrations (5.92 ng/g; median) were higher than those obtained for ∑PFSAs (2.19 ng/g). Compared to previously published data, Belgian samples presented higher PFBS levels, but Spanish dust showed lower PFOS concentrations. A positive association was found between PFOS concentrations and building edification age suggesting a decrease in the use of this PFAS in Europe. Estimated daily intakes (EDIs) were calculated for toddlers and adults at the median and worst-case scenarios. In all cases, EDIs were below oral Reference Dose (RfD) and tolerable daily intakes (TDI). Nevertheless, in the PFOA case, dust ingestion significance to total dietary exposure reached values of 51% for toddlers in the worst-case scenario. This work has been funded by the Organization of Consumers and Users (OCU Ediciones SA, Project number: 7653/2016).

3.13.14

Presence and Distribution of Perfluorinated Contaminants in the Amazon River

B. Gonzalez-Gaya, ESTACIÓN MARINA DE PLENTZIA. UPV/EHU / Analytical Chemistry; A. Huarte, A. Prieto, N. Etxebarria, University of the Basque country (UPV/EHU) / Analytical Chemistry; R. de Oliveira, State University of Campinas; G. Silva de Souza Nunes, Federal University of Pernanbuco; A. Waichman, Federal University of the Amazon; A. Rico, IMDEA Water Institute

Perfluorinated alkyl substances (PFASs) are receiving an increasing worldwide attention due to their persistence, toxicity and widespread occurrence. These emerging contaminants have high water solubility and lower lipophilicity compared to legacy persistent organic pollutants, being their global fate still uncertain due to their chemical features. This study provides the first wide-spread assessment of the occurrence and distribution of these contaminants in the Amazon River, the largest drainage basin in the world. A monitoring campaign covering more than 1500 km of the Brazilian Amazon River was carried out in November-December 2019. Thirty-nine water surface samples were taken in urban streams (Manaus, Santarem, Macapá, Belém), the dilution areas of such streams and along the Amazon river (including three of its major tributaries). One liter of water was prefiltered and extracted using Oasis WAX, 200 mg cartridges on site, and the rest of the analysis was performed at the UPV/EHU labs on the following month after sampling. Identification followed all the QA/QC constraints including field and laboratory blanks, internal standards quantification and an in house method validation for the recoveries. Extracts were injected on a ACE UltraCore 2.5 SuperC18 (2,1mm x 100nm, 2,5 µm) column with a precolumn (0,5 µm, Vici Jour) in an HPLC 1260 Infinity coupled to a triple quadrupole mass spectrometer (QqQ) 6430 (Agilent Technologies). Twenty-four PFAS including carboxilates, sulfonates, phosphonic, propanoic and ethanoic acids, fluorotelomers and phosphates were evaluated. Out of these, 12 compounds were identified for the first time in Amazon waters, being PFPeA, PFOS and PFHxS the most abundant ones, and being all short chained. The ∑PFAS ranged between 1.0 and 84 ng/l but significant differences were found between PFAS concentrations in urban areas (tens of ng/L) and the open river (few ng/L), pointing at the streams of Manaus and Belem as pollution hot-spots. At those sites PFOS, PFHxS and PFOA were the predominant species and showed the highest occurrence rate. ACKNOWLEDGEMETS: This study has partly been funded by the National Geographic Society (EC-59809C-19), www.silentamazon.com and by the Agencia Estatal de Investigación (AEI) of Spain and the European Regional Development Fund (ERDF) through project CTM2017-84763-C3-1-R. A.R. is supported by a postdoctoral grant provided by the Spanish Ministry of Science, Innovation and University (IJCI–2017–33465).

3.13.19

Distribution of Perfluoroalkylated Acids (PFAAs) in ENvironmental Matrices and Self-Cultivated Food in Antwerp

R. Lasters, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp / Biology; T. Groffen, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp / Department of Biology; M. Eens, University of Antwerp / Department of Biology, Behavioural Ecology and Ecophysiology Group (BECO); L. Bervoets, University of Antwerp / Department of Biology, Systemic Physiological and Ecotoxicologal Research (SPHERE)

Perfluoroalkylated acids (PFAAs) can enter the human food chain due to their persistence, widespread use and global distribution. Field research has demonstrated that these substances can bioaccumulate and biomagnify in wildlife. Hence, it is very plausible that PFAAs can biomagnify to high concentrations in humans. For these reasons, PFAAs may pose a significant risk to human health as residents may inadvertently consume PFAA contaminated food. Over the last decade, humans consuming food products from self-cultivated origin has become a remarkable trend in rural, urban and even industrial environments. However, little is known on the contribution of home-produced food to the total PFAS burden in humans throughout each of these environments. Furthermore, knowledge gaps exist on the role of main environmental matrices, such as water and soil, in the potential transfer of these chemicals to home-produced food. Therefore, the main objective of this study was to analyze and examine the distribution of PFAAs in home-produced chicken eggs and vegetables from volunteers within a distance radius from a known fluorochemical point source in Antwerp (Belgium). Secondly, we examined potential transfer of PFAAs from soil and rain water to self-cultivated food, taking into account the role of relevant soil characteristics. Finally, we investigated possible human health risk implications based on consumption scenarios. Up to 12 and 14 PFAAs could be detected in the food items and in the environmental matrices. The contribution of PFAAs to the total sum of PFAAs in soil and rain water was dominated by PFOS (54%) and PFOA (55%), respectively. In home-produced eggs, PFOS dominated the contribution pattern with 71% to the total sum PFAAs, whereas PFBA (20%) and PFBS (23%) contributed mostly in home-produced vegetables. Most PFAS concentrations followed a clear decreasing pattern from the fluorochemical plant onwards, which indicates that ingestion of soil particles and organisms through pecking behavior might be a dominant source of PFAA contamination in the eggs. Some main soil characteristics, such as total organic carbon content (TOC), were negatively related with ≥C10 PFCAs. Based on a consumption scenario of two eggs per week (Flemish reference guideline), the PFOS intake exceeded the available tolerable weekly intake (TWI) values in > 40% of the households. Summarized, some PFAAs may represent a possible health-risk to local residents via the consumption of home-produced eggs.

3.13.20

Ecological Risk Assessment of PFAS - Contaminated Sites Using Avian Eggs As a Monitoring Tool

C. Ferrario, IRSA-CNR (Brugherio) / Water Research Institute; M. Morganti, S. Polesello, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute; M. Parolini, University of Milan / Department of Environmental Science and Policy; S. Pascariello, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute; D. Rubolini, University of Milan / Department of Environmental Science and Policy; S. Valsecchi, Water Research Institute - Italian National Research Council IRSA-CNR / Water Research Institute

For many years, avian eggs have been used as monitoring tools to investigate PFAS contamination, especially in marine and remote areas. Eggs are a favourable monitoring matrix because they are relatively easy to collect and their mostly lipidic and protoeic yolk efficiently stores a wide range of PFAS, whose concentrations are a good proxy of maternal exposure, allowing the assessment of the potential risk for birds. In this study, we explored the PFAS content in eggs of three common insectivorous passerines (great tit, blue tit and European starling) breeding near a perfluoropolymer factory site in the Po river valley (Northern Italy), and compared it to those of eggs collected in a rural site not directly impacted by PFAS contamination. The target analytes included 9 perfluoralkylcarboxylic acids, perfluorooctanesulfonate (PFOS), perfluorooctanesulfonamide (FOSA) , 1H, 1H, 2H, 2H-perfluorodecane sulfonic acid (8:2 FTS) and an PTFE processing aids namely the acetic acid, 2,2-difluoro-2-((2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl)oxy (C6O4). PFAS concentrations in eggs of all the three species collected around the perfluoropolymer factory were at least one order of magnitude higher than those collected in the rural site. For all fluorinated compounds except PFOA and C6O4, concentrations in the sampled eggs collected around the perfluopolymer factory were independent of species. In contrast, PFOA concentrations in starling eggs were two order of magnitude higher than those measured in the tit eggs. The egg fingerprint of all PFAS, except PFOA and C6O4, in the eggs collected near the fluopolymer factory were analogous among bird species with PFOS occurring as the other PFAS, unlike the eggs collected in the rural site. Considering all PFAS measured in the eggs collected near the fluoropolymer factory,

PFOA dominated the PFAS composition in the starling, accounting on average for the 87% of the fingerprint, whereas C6O4, its recent substitute, had the highest prevalence (40%) in tit eggs. These discrepancies can be explained by the different feeding strategies of bird species; blue and great tits are mostly canopy feeders and they are probably exposed to different PFAS source with respect to the starling that mostly feed on ground. Our results support the use of avian eggs as an important biomonitoring tool in environmental risk assessment (ERA) of PFAS-contaminated sites.

3.13.21
Perfluoroalkyl Acids (PFAAs) in Eggs of Black-Tailed Gull From Its Breeding Sites Along the Korean Coast
G. Han, Korea Institute of Ocean Science and Technology; S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; M. Jang, Korea Institute of Ocean Science and Technology / oil and POPs reserch group OPRG; Y. Moon, Korea Institute of Ocean Science and Technology (KIOST); W. Shim, Korea Institute of Ocean Science and Technology / Oil and POPs research group
The widespread occurrence of perfluoroalkyl acids (PFAAs) in wildlife has spurred monitoring efforts and regulatory concerns regarding these emerging contaminants. The physicochemical properties of PFAAs make them very useful for application in various commercial products such as surface protectors for carpets and leather, active components in fire-fighting foams, and processing aids in the production of fluoropolymers. Seabird egg have long been used as a biomonitor for long-term monitoring of environmental contaminants in the Europe and North America. In South Korea, national monitoring program on environmental contaminants has been run primarily for coastal sediment and bivalves, while biomonitoring program for high-tropic marine species such as seabird has not been established yet. This study was performed to investigate the best approach and strategy for seabird monitoring, and to identify the levels and profiles of PFAAs 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, and egg content was used for PFAAs analysis. Among the target analytes, PFOS showed highest level and followed by PFUdA and PFTrDA, indicating their great bioaccumulation and biomagnification potential. The overall level of PFAAs was highest in the western coast and lowest in the eastern coast where level of urbanization could be the potential source. The concentrations of PFAAs are relatively lower than or similar to other legacy POPs such as PCBs and DDTs but are significantly higher than emerging flame retardant compounds such as HBCDs and PBDEs.

3.13.22
Per- and Polyfluoroalkyl Substances Leves in Spanish Adolescents Urban Population
J.R. Rodríguez, M. Bartolomé, S. González, C. Grande, E. Soto, A. Cañas, M. Esteban, A. Castaño, National Center for Environmental Health, Instituto de Salud Carlos III
Human Biomonitoring (HBM) is an important tool to assess the actual body burden in population encompassing chemical exposure from multiple sources (e.g. air, diet) and routes (e.g. dermal absorption, ingestion), by measuring the concentration of chemical compounds and/or their metabolites in human samples. Furthermore, HBM studies can provide a valuable support for the implementation and development of environmental and health policies and their effectiveness. In 2016, the Instituto de Salud Carlos III in cooperation to the Spanish Ministry of Agriculture, Food and Environment, funded the BEA study (*Biomonitoring in adolescents*), the first nationwide HBM survey carried out in Spain to study the levels of environmental pollutants in adolescents (14-16 y). In this cross sectional HBM study, the voluntaries were recruited through 20 secondary school centers from 11 cities with more than 150,000 inhabitants. Per- and polyfluoroalkyl substances (PFAS) were one of the chemicals included in BEA Study due to the toxicological relevance of these persistent organic pollutants, their ubiquity in the environment and their presence in consumer products. In addition, in previous studies with adults, the levels found for some of these compounds showed slightly high in some of the Spanish regions studied (1).The analysis of serum levels of 12 PFAS *i.e.* perfluoro-1-butanesulfonate (PFBS), perfluoro-1-hexanesulfonate (PFHxS), perfluoro-heptanesulfonate (PFHpS), perfluorooctane sulphonate (PFOS), perfluoro-n-pentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluoro-n-nonanoic acid (PFNA), perfluoro-n-decanoic acid (PFDA), perfluoro-n-undecanoic acid (PFUdA), perfluoro-n-dodecanoic acid (PFDoA), from 327 adolescents was carried out using solid-phase extraction on-line coupled liquid chromatography with tandem mass spectrometry LC-MS/MS. The compounds with the highest concentrations were PFOS (geometric mean, 1.33 ng/ml and 95th percentile, 3.04 ng/ml) and PFOA (geometric mean, 0.643 ng/ml and 95th percentile, 1.04 ng/ml). These descriptive results show differences between cities, with Huelva and Albacete presenting the highest levels. The concentrations of PFAS in serum were in clearly lower than those reported for Spanish adult population (1).The study was partially funded by ISCIII-Spanish Ministry of Agriculture, Food and the Environment project nº SEG 1321/15. References: 1. Bartolomé M, *et al.* Sci Total Environ, 2017, 603-604:352-360

3.13.23
Are Accumulated PFAS Concentrations in Aquatic Macroinvertebrates Related to the Ecological Quality of Water Courses?
C. Byns, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp / Biology; T. Groffen, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp / Department of Biology; L. Bervoets, University of Antwerp / Department of Biology, Systemic Physiological and Ecotoxicologal Research (SPHERE)
Perfluorinated alkyl substances (PFAS) are synthetic chemical compounds with a highly persistent character and a widespread usage, making them ubiquitous in the environment. They are of ecological concern due to their bioaccumulation potential and toxicological characteristics. However, monitoring micropollutants in aquatic ecosystems is still mainly based on measurements in the abiotic environment. Sediment and water samples represent only a momentary pollution status and do not take into account differences in bioavailability driven by fluctuating abiotic (pH, water hardness, temperature) and biotic factors (physiological status, feeding mode, etc.). Consequently, derived water and sediment quality criteria might not always be adequate for the protection of aquatic communities. This study aims to investigate the relationship between accumulated PFAS levels in benthic invertebrates (*Chironomus* sp., *Asellus* sp. and *Gammarus* sp.) and the aquatic ecological status. Therefore, resident aquatic invertebrates are collected at 30 sites in rivers and water courses across Flanders, Belgium. To assess the invertebrate community responses a set of ecological endpoints such as the Multimetric Macroinvertebrate Index Flanders (MMIF) is calculated. Simultaneously, sediment and water samples are collected to investigate possible relationships between accumulated PFAS in the biotic and abiotic environment. In addition, we investigate if critical body burdens can be defined in order to protect aquatic communities.

3.13.27
Adsorption, Desorption, and Bioaccumulation Behaviors of Cationic and Zwitterionic Per- and Polyfluoroalkyl Substances in the Soil Environment
F. Xiao, P. Challa Sasi, A. Alinezhad, S. Mallula, University of North Dakota / Civil Engineering; B. Jin, University of North Dakota
Sorption linearity and reversibility are implicit in models for the fate and transport of per- and polyfluoroalkyl substances (PFAS). In this study, however, we found that the sorption of cationic and zwitterionic PFAS in natural soils was highly nonlinear. The nonlinearity was so severe that it led to a variation in the coefficient of sorption by several orders of magnitude over the experimental concentration range. This implies a considerable increase in sorption as concentration falls in the natural environment. Sorption of cationic PFAS correlated strongly with the soil organic matter (SOM) content and was reversible in all soils. Sorption of zwitterionic PFAS, on the other hand, displayed concentration-dependent hysteresis in soils with a low SOM content. The irreversibility, which was associated with neither SOM, pore deformation, nor surface complexation, was likely caused by the entrapment of molecules in porous structures within inorganic components of soil aggregates. Furthermore, electrostatic interactions with negatively charged soil constituents and the hydrophobic effect were found to be major sorption driving forces for cationic/zwitterionic PFAS at low and high concentrations, respectively. The maximum electrostatic potential of PFAS ions, computed using density functional theory, was found to be a useful predictor of the sorption of ionic PFAS species. We further demonstrate here the formation of PFOA and PFOS in earthworm (*Lumbricus terrestris*) from a group of four zwitterionic/cationic polyfluoroalkyl amides and sulfonamides. In bioaccumulation tests, the zwitterionic PFAS compounds were metabolized within ten days to PFOA/PFOS at yields of 3.4–20.8 mol % by day 21 and several infrequently reported PFAS species for which chemical structures were determined using high-resolution mass spectrometry. Cationic PFAS, on the other hand, were found to be much less metabolizable in terms of the number (n = 2) and yields (0.9–5.1 mol %) of metabolites. Peak-shaped bioaccumulation profiles were

frequently observed for studied PFAS. Residual zwitterionic/cationic PFAS in earthworm were detected at end of the elimination phase, indicating that not all zwitterionic/cationic PFAS molecules *in vivo* are available for enzymatic degradation. Lastly, the relative importance of different exposure routes (i.e., waterborne and dietary exposure) was investigated.

3.13.29
Does PFAS Toxicity Occur Via Their Surfactant Properties?
T.F. Webster, Boston University / Dept Environmental Health; J. Sørli, National Research Centre for the Working Environment; X. Trier, European Environment Agency EEA / Health, Sustainability and Resources
A number of human health outcomes have been associated with exposure to PFOA, PFOS and other PFAS, including increased serum cholesterol and reduced antibody titers. The toxicological mechanisms and molecular initiating events are not known, but the focus has been on mediation by receptors such as PPARa. This focus does not consider other potential classes of mechanisms based on the surfactant properties of PFAS, some of the strongest known surfactants, as measured by their ability to lower surface tension. The combination of potency and persistence/ bioaccumulation of many PFAS suggests that exploration of this mode of toxicity is warranted. Can PFAS, at environmentally relevant internal concentrations, cause toxic outcomes via surfactant-related mechanisms? Here we examine two cases: 1) Cholesterol is necessary for normal biological function. Being insoluble in water it is carried in serum in vesicles, e.g., VLDL, LDL and HDL. We hypothesize that PFAS may alter the size distribution of such vesicles in vitro through its surfactancy. However, the results of Butenhoff et al (2012) suggests no change in the vesicle size distribution in plasma with vehicle added vs. 0.19 – 19 uM of PFOA or PFOS. 2) There is some epidemiologic and toxicologic evidence for effects of PFAS on lung function. Recently, fluorine was found in the fibrotic lesions in lungs from a person with lifelong occupational exposure to PTFE and PFOA (Butnor et al 2020). Natural lung surfactants play a critical role in respiration regulating surface tension, a function potentially disrupted by PFAS. Sørli et al (2019) examined the potential for acute inhalation toxicity by PFAS using an in vitro lung surfactant function assay measuring surface tension. LOAECs were found for PFOA and PFOS at 500 uM, 1000 uM for PFHxS. However, it is difficult to compare these concentrations with those encountered by people. While these preliminary experiments do not demonstrate surfactant-related toxicity by PFAS at concentrations experienced in the general population, further research in this area is warranted as surfactants and vesicles, such as exosomes, play extremely important roles in biology. As risk assessment methodologies increasingly move towards in-vitro and in-vivo tests, it is important that they capture effects surfactants may have on transport of substances in biological systems as well as other effects on e.g. bioavailability and protein functioning.

3.13.33
Emissions and the ENvironmental Distribution of PFASs and Fluorine-Free Alternatives in Textiles, Upholstery, Carpets, Leather and Apparel
W. Gebbink, PFA Brussels; M. Crookes, Peter Fisk Associates Ltd.; I. Keyte, L. Nicol, Wood group UK; M. Schopel, Ramboll Deutschland GmbH; R. Whiting, Wood group UK
Perfluoroalkyl and polyfluoroalkyl substances (PFASs) have been used for a wide range of functional applications within textiles, upholstery, leather, apparel, and carpets (TULAC). Emissions can occur from product manufacture and their uses. The aim of this project was to determine emissions and the environmental distribution of PFASs themselves and fluorine-free alternatives. Based on a literature review on PFASs and fluorine-free alternatives used in TULAC and an EU market analysis, a total of 20 substances were selected for which emissions were estimated based on ten exposure scenarios including manufacturing of the substance, formulation into textile or leather treatment products and industrial/consumer application of treatment to textile or leather. The environmental distribution of the emissions was performed using EUSES 2.2.0. The selected 15 PFASs included various chain length PFSAs, PFCAs and FTOHs as well as MeFOSA and EtFOSE. The selected five fluorine-free alternatives were stearamidomethyl pyridine chloride, polydimethylsiloxanes, di-2-ethylhexyl sulfosuccinate, naphtha and polyethylene oxide mono-C12-16-alkyl ether. Information on physico-chemical properties and environmental fate parameters were obtained from publicly available sources or from the peer reviewed literature. If no information on a property was found, either a modelled value was generated or a read-across value or a default value from EUSES was used. For PFASs, the combined releases to air, water and soil was estimated to range between 823 and 3506 t/y with the highest releases to water for most PFCAs and PFSAs and air and soil for FTOHs. The combined releases to air, water and soil for the five alternatives ranged between 79 and 1161 t/y. The amount of substance released into the environment relative to the amount used from the market analysis ranged between 27 and 56% for the PFASs and between 24 and 68% for the fluorine free alternatives. The exposure scenario covering widespread use of articles over their service life was identified as the scenario with the highest releases compared to the other scenarios. Once released into the environment, most of the mass for PFASs and alternatives was estimated to be present in the freshwater and agricultural soil compartments in the regional scale of the model.

Plastics in Terrestrial Ecosystems: Key Considerations in the Assessment of Fate and Exposure

3.14.02
Development of Selective Detection Method for Microplastic in Soils Using Ted-Gc-Ms and Raman Spectroscopy
M. Wenzel, Institut für Energie- und Umwelttechnik e. V. (IUTA); M. Funck, Institute of Energy and Environmental Technology e.V. - IUTA; F. Itzel, Institute of Energy and Environmental Technology e.V. (IUTA); B. Fischer, Fischer GmbH; D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry; K. Weinfurtner, IME Fraunhofer; A. Yildirim, Institut für Energie- und Umwelttechnik e. V. (IUTA); T.C. Schmidt, University of Duisburg-Essen / Chair of Instrumental Analytical Chemistry; J. Tuerk, Institute of Energy and Environmental Technology e.V. (IUTA); C. Wolf, Institut für Energie- und Umwelttechnik e. V. (IUTA)
As plastic production continues to grow worldwide, plastics are becoming increasingly relevant to the environment and many researchers developed concepts for the determination of microplastics in almost all environmental compartments. However, the research on microplastics in soils is still uncommon in literature. The overall objective of the "iMulch" project is to investigate the effect of agricultural mulch films consisting of polyethylene (PE) (not biodegradable) and polylactic acid/ polybutylene adipate terephthalate (PLA/PBAT) copolymers (biodegradable) on organisms and soil ecosystems. The aim was to establish a method that allows both, thermal extraction-desorption gas chromatography mass spectrometry (TED-GC-MS) and Raman spectroscopic analyses of the mentioned polymers in agricultural soils. In this study the detection method, including sample preparation for RAMAN spectroscopy and TED-GC-MS, as well as the quantification of different field soils are presented. In order to obtain an assessment of what plastic contamination is present in soils and whether this can be linked to soil management, soil samples from fields with different crops and known frequency of previous use of mulch films were analysed. For sample preparation a density separation through NaI and NaCl was used and validated. Therefore, a reference soil was spiked with PE and PLA/PBAT mulch foils. After the density separation the supernatant was filtered and digested with a Fenton reagent in order to minimise interferences by the organic components of the soil. Following a second filtration step, Raman spectroscopy could be used to obtain a particle number distribution. Additionally, the sample could be analysed with TED-GC-MS for the determination of the recovery rate. In both cases (NaI and NaCl), a recovery higher than 92% for PLA/PBAT and PE could be observed. The field soil samples were treated in the same way as the reference soil for the quantification of the microplastic load. For quantification TED-GC-MS was used. The overall content of polymers for the first three soil samples is 3E-05 wt% (polypropylene (PP), polystyrene (PS)) in soil sample 1, 3E-02 wt% (PE) in soil sample 2 and 6 wt% (PE, PS) in soil sample 3. **Acknowledgement** This work was carried out as part of the iMulch project. The authors thank the European Fund for Regional Development for funding.

3.14.06
Effects of Industrial Microplastic on Plant Growth
T. Khan, M. Hodson, University of York / Department of Environment & Geography
Plastic debris is a prevalent contaminant in environments, but the interaction between plastics and plants remains largely unknown. To date scant attention has been paid to investigating impacts of microplastics (MPs) in terrestrial ecosystems, though MPs have recently been detected in agricultural soils, urban cities and industrialized areas. It is believed that the soil tends to act as a long-term sink for MP used in industries and these MPs will ultimately enter the terrestrial ecosystems. The aim of this study was to assess the effect of

high-density polyethylene microplastic on an important plant species (*Lolium perenne*). We incubated a low organic matter (LOMS) and a high organic matter (HOMS) soil with MPs incorporated at concentrations of 0, 0.05, 0.50 and 5.00 % w/w MP at 25 °C for 28 days (n = 4 for each treatment). Subsequently, a greenhouse experiment (minimum day/ night temperature of 20 °C/ 15 °C) was conducted for seven weeks using the incubated mixtures. We determined microbial respiration, enzyme activity, Olsen P, soil water holding capacity (WHC) and hot and cold water extractable C in both the incubated and greenhouse soils. In addition, we determined plant height, chlorophyll content, root and shoot biomass, and root: shoot ratio of the *L. perenne* at the end of the greenhouse experiment. In both the incubation and greenhouse experiments, the high dose of MP (5.00 %) significantly ($p < 0.001$) increased respiration rate (2.2-fold in LOMS and 3.2-fold in HOMS) and enzyme activity (2.5-fold in LOMS and 1.5-fold in HOMS) compared to other treatments. In contrast, plant height, leaf chlorophyll, shoot biomass, root: shoot ratio, WHC and organic C extracted with hot and cold water were significantly ($p < 0.001$) decreased in the 0.50 % and 5.00 % treatments compared to the 0.05 % and control treatments. No significant ($p > 0.001$) difference between treatments was observed for root biomass. Overall, MP did not show any negative effects on soils and plants when applied at a small concentration (0.05 %), whereas a concentration of 0.50 % and 5.00 % did cause negative responses, which is unlikely to occur in the natural environment since typical concentration of MP is about 0.002% w/w in the natural soil.

3.14.07
Life Cycle Assessment of Rice Farming Considering Plastic Pollution Aspects
H. Suzuki, Tokyo City University / School of Environmental Studies; A. Abeynayaka, Tokyo City University / Graduate School of Environmental and Information Studies; Y. Fukunaga, F. Kojima, Pirika Inc.; N. Itsubo, Tokyo City University / Graduate School of Environmental and Information Studies
Plastic pollution in the ocean has been attracting attention. In addition, plastic materials are indispensable for agriculture and will continue to generate waste in the future. Therefore, it is necessary to understand the outflow of plastics and its effects to prevent the seriousness of pollution in the future. In this study, we investigated plastic runoff from the Arakawa and Tone rivers and assessed the life-cycle environmental impact of agricultural services in order to clarify the environmental impact of agriculture including plastic pollution.

3.14.08
Plastic Pollution in Terrestrial Protected Areas - Evaluating Effectiveness of In-Situ Vs Ex-Situ Conservation Habitat in Managing the Plastic Problem
A. Baroth, wildlife institute of india / Habitat Ecology; S. Kumari, wildlife institute of india
Plastic pollution is a major concern throughout the world. The consequences of toxic adore of man for plastic is spreading even to the protected areas of the environment. This research is an attempt to create a baseline for quantification of plastic in a terrestrial protected area and evaluating the management of plastic pollution in the in-situ and ex-situ conservation habitats. The study was conducted in one of the Tiger reserves (Rajaji Tiger Reserve (RTR) located in Western Himalayan state of Uttarakhand, India. For control, the results were compared with ex-situ conservation habitat known as Dehradun Zoo in the same area. 17 line transects were laid within different Ranges of Rajaji Tiger Reserve, by predicting and priortising areas with high human activities and conducting systematic surveys. A total of 2126 plastic items were recorded in these 17 line transects along with 92 metal items, 58 glass items, 389 items of processed lumber, 25 rubber items and 60 other items, giving an average plastic density of .125 plastic litter/m2 of RTR. A total of 46800 meter area of RTR tourist tracks was surveyed. Plastic density inside the tourists tracks was found to be 0.00024 plastic litter/m2, much lower than commercially active zones of RTR. On the other hand, 4 line transects were laid inside Dehradun Zoo. A total of 67 plastic items were recorded along with 1 Rubber item and 13 items of processed lumber. No metal, glass or clothing item were found inside the Zoo which gives the plastic density of 0.016 plastic litter/m2. Pan Masala wrappers and food wrappers were found to be the most abundant plastic contaminants in the RTR with a density of 0.060/m2 and 0.033/m2 respectively. Plastic was omnipotent in all the line transect surveyed. Major factors contributing to plastic pollution in RTR was careless attitude of the locals and tourists, lack of knowledge about environmental and health impacts of plastic, and insufficient waste disposal facilities. Ex-situ model was clearly the better model between the two, with defined plastic policies for visitors that are enforced strictly. Key Words: Plastic, in-situ, ex-situ, Rajaji Tiger Reserve, Dehradun Zoo

POPs and Emerging Pollutants: Developments in Sampling, Targeted and Non-Targeted Analysis, Modeling and Reporting to Support Policy and the Protection of the Environment and Human Health

3.15.06
PHARMARINE - Transport Via Ocean Currents of Human Pharmaceutical Products and Their Impact on Marine Biota in the European Arctic
I. Overjordet, SINTEF Ocean / Climate and Environment; K. Smolarz, University of Gdansk; R. Kallenborn, Norwegian University of Life Sciences / Chemistry, Biotechnology and Food Sciences; S. Kwaśniewski, IO PAS; A. Sarno, SINTEF Ocean / Environment and New Resources; L. Sørensen, T. Nordtug, SINTEF Ocean / Climate and Environment; A. Hallmann, Medical University of Gdansk; M. Caban, University of Gdansk; M. Włodarska-Kowalczuk, IO PAS; I. Ellingsen, J. Skancke, SINTEF Ocean / Environment and New Resources; D. Walecka, F. Spiralski, A. Sokołowski, University of Gdansk
The increased use and large disposal volumes of pharmaceuticals in Europe has raised concern about their impacts in marine ecosystems. Though pharmaceuticals undergo degradation, some compounds can escape through sewage treatment and remain in the environment over time. As a pristine system, the Arctic is particularly susceptible to environmental change and stressors such as pharmaceutical pollutants. This issue has recently received attention following the detection of pharmaceuticals in Arctic waters. However, the knowledge of presence, exposure, and effects of pharmaceuticals on Arctic fauna is limited. PHARMARINE will contribute to risk assessment and policy making for pharmaceuticals by providing new knowledge on 1)Transport pathways of pharmaceuticals from populated mid-latitude Europe towards the European Arctic as well as local sources; 2) Bioaccumulation and biomagnification processes of pharmaceuticals in the Arctic; 3) Biological impacts of pharmaceutical pollutants on Arctic species The transport range of pharmaceuticals may be enhanced by association to biotic and abiotic particles like phytoplankton and zooplankton. Transport along a northward transect from the Baltic Sea and the North Sea through the Norwegian Coastal Current and North Atlantic Current to Spitsbergen fjords will investigated through field sampling. These results will feed into oceanographic models for predictions of present and future scenarios for oceanic pharmaceutical transport to the Arctic. Initial modelling of horizontal transport of selected model pharmaceuticals based on literature and coastal population densities will aid determining the most suited sampling areas. Diclofenac (non-steroidal anti-inflammatory drug), tetracycline (antibiotic), fluoxetine (antidepressant) and simvastatin (lipid lowering drug) are initially selected as model pharmaceuticals. This presentation is aimed at showing initial results of source and transport predictions. Bioaccumulation and biomagnification of pharmaceuticals in benthic food webs will be studied in Svalbard fjords. Selected species will be exposed to pharmaceuticals to assess potential harmful effects through a series of biological end points. This will provide novel empirical information on biological impacts and the threat pharmaceuticals pose on the Arctic marine ecosystem. The resulting biological stress indicators will provide a tool to forecast and assess environmental risk of pharmaceuticals in the European Arctic.

3.15.09
HCHs, PeCB and HCB in Air Close to a Historical Spanish Lindane Production Site
I. Navarro, CIEMAT; A. de la Torre, CIEMAT / Environmental; P. Sanz, CIEMAT; M. Arjol, Sociedad Aragonesa de Gestión Agroambiental (SARGA); J. Fernández, Department of Rural Development and Sustainability, Government of Aragón; M. Martínez, CIEMAT
The landfilling of persistent organic pollutants (POPs) and other hazardous compounds can have adverse environmental consequences. More concretely, the widespread dumping of γ-HCH (lindane) and a large amount of α-, β-, δ- and ε-HCH isomers as by-products and chlorobenzenes have caused contamination in soil, water, and atmosphere systems. In Spain, 65% of the lindane manufacture (115,000 t) was generated by the INQUINOSA Factory located in Sabiñánigo (Aragón, Spain). HCH wastes were mainly dumped at two unlined landfills, Bailín and Sardas, located in the surroundings of the production site, becoming a threat to the environment. Since 2007, an important investment has been done in activities focused on the remediation and containment at the production site as well as to secure landfills, framed in a project plan approved by the

Government of Aragón (GA). Further pollution control activities are still ongoing to assess the potential impact on the local environment. This study is focused on monitoring of α-, β-, γ-, δ-, ε-HCH, PeCB (pentachlorobenzene) and HCB (hexachlorobenzene) in the air of Sabiñánigo area, and Sardas landfill to assess the current status and evaluate the existence of pollution sources, in which it may be necessary to carry out remediation works in the future. A total of 200 air samples were collected in 40 passive sampling campaigns from winter 2016 to autumn 2019 in 5 selected sites. ΣHCH concentration ranged from 0.07 to 19.2 ng/m^3. Positive relationships ($r > 0.331$, $p < 0.05$) were obtained among HCH isomer levels in all sampling points, evidencing a major source in the area associated to the historical manufacture of lindane and the uncontrolled dumping of wastes in Sardas landfill. ΣHCH air concentrations obtained at INQUINOSA Factory (4.21 ng/m^3) and Sardas landfill (5.11 ng/m^3) showed statistically higher values than those for the other locations (0.50, 0.58 and 0.30 ng/m^3), pointing out these facilities as currently HCH air pollution sources. Positive correlations were found between PeCB and HCB, suggesting a major common origin for both chlorobenzenes. Results highlight that despite of remediation and containment measures implemented in Sabiñánigo, further pollution control activities should be carried out to protect the environmental compartments and the human health. This work has been funded by the GA, through the public company SARGA under the contracts 5506079-17, 5506079-29 and 5507001-18.

3.15.11

Emerging and Legacy Flame Retardants in European House Dust

A. de la Torre, CIEMAT / Environmental; I. Navarro, P. Sanz, M. Martínez, CIEMAT

Flame retardants are human-made chemicals added to a wide variety of consumers and industrial products like textiles, electric and electronic equipment, for the purpose of retarding flammability and reducing the human and material damages of fire. However, evidence that they also cause harm to the people health and ecosystems have triggered the implementation of regulations like the inclusion of polybrominated diphenyl ethers (PBDEs) c-penta, c-octa, and c- decaBDE commercial formulations in the Stockholm Convention or even the proposal to list Declorane Plus in Annexes A, B or C. Nonetheless, the industry is still forced to meet safety standards, so restrictions on the use and/or production of a substance must be implemented by the use of an alternative. Among these proposed substitutes, organophosphate flame retardants and plasticizers (OPs) or novel brominated flame retardants (NBFRs) like decabromodiphenyl ether (DBDPE) raise a concern about their potential toxicity and could become emerging pollutants in future years. The present study evaluated the presence of 52 pollutants including OPs, PBDEs and NBFRs in sixty-five household dust samples obtained from Belgium, Italy and Spain. Home occupants were asked to vacuum the entire floor home (settle dust) and fill in a questionnaire to evaluate the influence of building characteristics and/or occupant habits on the dust pollutant content. The three European countries presented indistinguishable ∑OPs and ∑PBDEs concentrations with a chemical pattern dominated by ∑OPs (14.1, 12.1 and 10.0 µg/g; median Belgium, Italy and Spain) followed in decreasing order by ∑PBDEs (210, 283 and 176 ng/g), DBDPE (142, 119 and 85.6 ng/g), BTBPE (0.87, 1.67 and 1.58 ng/g), HBB (0.26, 0.36 and 0.22 ng/g) and finally PBEB (0.02, 0.06 and 0.03 ng/g). OP concentrations were in the same range of house dust collected since 2006 in these countries. On the other hand, data evidenced a significant decrease in c-pentaBDE and c-octaBDE use. Interestingly, c-decaBDE concentrations increased in Spanish dust. Estimated daily intakes (EDIs) via house dust ingestion and dermal adsorption were calculated for toddlers and adults at the median and worst-case scenarios. In all cases, EDIs were below oral Reference Dose (RfD) values, but in the TBOEP case it was only 9 times below. This work has been funded by the Organization of Consumers and Users (OCU Ediciones SA, Project number: 7653/2016).

3.15.14

Prioritization, Identification and Trend Monitoring of Organic, Cationic Contaminants in Suspended Particulate Matter Using Non-Target Screening

T. Köppe, Federal Institute of Hydrology / G2 - Water Chemistry; K. Jewell, German Federal Institute of Hydrology / G2 - Water Chemistry; B. Ehlig, A. Wick, Federal Institute of Hydrology / G2 -Water Chemistry; J. Koschorreck, Umweltbundesamt / Inland Surface Waters; T.A. Ternes, Federal Institute of Hydrology

Organic cationic substances are used in domestic as well as agricultural and industrial applications and are discharged into the environment via wastewater treatment plants or via diffuse sources. Examples are various quaternary ammonium compounds, which are used as biocides or cationic surfactants and organic phosphonium compounds, used as starting materials or intermediates in the chemical industry. Due to their positive charge, these compounds have been detected not only in surface water but also in sediment and suspended particulate matter (SPM). Regulatory monitoring programs include only selected Priority Substances (PS) and river basin specific pollutants (RBSP). To support the regulatory prioritization process, non-target screening was performed to identify previously unknown or overseen cationic organic contaminants and evaluate their concentration changes over a 14-year period. Annual mixed samples of SPM from the German rivers Rhine and Saar from 2005 to 2018 provided by the German Environmental Specimen Bank were analyzed with high resolution LC-QToF-MS/MS. In order to identify permanent cationic compounds, prioritization strategies were implemented taking advantage of the physicochemical properties of this compound class. Two approaches were applied in combination. Compounds were prioritized which i) showed interactions with cation exchange resins and ii) were detected with the same m/z in both deuterated and non-deuterated solvents during chromatography, i.e. showing no adduct formation. According to this approach, 123 of almost 2700 detections were prioritized and used to identify 22 permanent cationic compounds. The identified substances include the organic dye Basic Yellow 28 and the Fluorescent Brightener 363, which to the best of our knowledge have not been detected in environmental samples so far. The temporal trend for Basic Yellow 28 in Rhine SPM shows that from 2005 to 2011 the concentration decreased from about 300 µg/kg to 50 µg/kg and has remained constant at this level since then. In the same period, the concentrations of Basic yellow 28 in the Saar remained almost constant with an average of 17 µg/kg over the entire period. The concentrations of Fluorescent Brightener 363 did not display a clear trend, but show two concentration peaks in 2007 and 2015 of about 900 µg/kg and 1200 µg/kg, respectively.

3.15.16

Use of Passive Samplers to Determine Emerging Contaminants and Persistent Organic Pollutants in Todos Os Santos Bay (Bahia, Brazil)

A.B. Martins, UFBA / Institute of Geosciences of UFBA; A. Barbosa, Federal University of Bahia / Department of Oceanography; F. Barros, Federal University of Bahia / Biology Institute; T. Combi, Universidade Federal da Bahia / Instituto Oceanográfico

In modern societies, the use of large amounts and a broad variety of chemicals (such as persistent organic pollutants (POPs), emerging contaminants, polycyclic aromatic hydrocarbons (PAHs), and others), which will end up released into the aquatic environment, has resulted in contamination with complex chemical mixtures that are potentially harmful to aquatic and human life. The presence of such a high number of compounds in the environment leads to interferences during chemical analysis, hindering the assessment of the occurrence and distribution of contaminants in environmental matrixes. The development of methods that can detect multiple classes of compounds at low levels are among the most complex issues in environmental chemistry, especially in less developed countries as Brazil. Thus, the aim of this study is to implement a passive sampling method for the simultaneous analysis of emerging contaminants, POPs, and PAHs in Todos os Santos Bay (BTS), Bahia, Brazil. BTS is the second largest bay in Brazil and is subject to several anthropogenic stressors, including oil refinery, ports, industrial activities, and intense urbanization. The bay comprises ecosystems with significant ecological importance, including mangroves, and coral reefs. Considering the multiple sources of contaminants, compounds with different physico-chemical characteristics (e.g., octanol-water coefficient) are constantly entering the bay. To overcome the challenges related to the analysis of such a wide range of compounds in seawater, passive sampling devices (PSDs) made of silicone rubber will be employed in this study. PSDs have the capability for measuring aqueous concentrations of most nonpolar compounds (log Kow > 4) and generate an integrated contaminant signal over a determined period (i.e, the time for which they are exposed in the environment). The analytical methodology for the in tandem analysis of the selected contaminants will also be optimized and implemented. This is an ongoing work, which is part of a PhD project where the PSDs are currently being prepared and tested before field exposition. The final results of this work will allow the implementation of a state of the art methodology for multi-target analysis in seawater and contribute with data on the occurrence, spatial-temporal variations and potential sources of contaminants in areas of interest in the BTS, as well as contribute with

unprecedented data for regional and global inventories.

3.15.18

Environmental Risks of PAHs in Seawater and Suspended Particulate Matter From the Northwest Iberian Peninsula (Portugal)

M. Rocha, A. Ribeiro, D. Campos, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Portugal.; E. Rocha, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto (U.Porto), Porto, Portugal

The industrial growth and human occupation of coastlines in the northwest of Portugal are producing anthropogenic stresses in aquatic habitats, including coastlines. Despite past monitoring surveys that covered xenobiotics from distinct natures, up to this moment, few data exists about the levels of the 16 priority PAHs in the Portuguese Atlantic seacoast. This fact applies to both the fractions dissolved in the aqueous phase (DAP) and suspended particulate matter (SPM). To tackle the knowledge gap, in 2017 and 2018, seawater samples were taken from the sea at four locations from the Porto coastline (Northwest Portugal), including areas adjacent to an ornithologic reserve. Water and SPM extracts were obtained by solid-phase and ultrasonic extractions and analyzed by gas chromatography-mass spectroscopy (GC-MS/MS). The PAHs showed seasonal fluctuation patterns, reaching global annual average concentrations of ≈ 8 ng/L in DAP and ≈ 97 ng/g *dw* in SPM. The type of PAHs revealed that their possible origins in this seashore are related to pyrogenic (forest fires) and petrogenic activities linked to maritime transports. The calculation of the toxicity equivalent quotient (TEQ) for PAHs in these samples was, on average, of » 0.3 ng/L and » 4 ng/g *dw*, for DAP and SPM. Evaluation of risk coefficients revealed the presence of "Low-risk" for both matrices and all sampling areas. Nevertheless, *Artemia salina* (ARC-test) acute bioassays testing environmental concentrations of PAHs, as found in DAP and SPM, revealed differences between control and exposed groups, suggesting that this coastline is not "Risk-free". Funding: ICBAS, FCT (UIDB/04423/2020, UIDP/04423/2020), Project ATLANTIDA (NORTE-01-0145-FEDER-000040), by NORTE 2020/PORTUGAL 2020, via ERDF.

3.15.19

First time non target screening of organic and inorganic pollutants of concern in Lebanese marine sediments

M. Zayter, CNRS-L, IPREM / Institut Pluridisciplinaire de Recherche en Chimie Analytique et Physico-Chimie pour l'Environnement et les Matériaux; H. Preud'homme, IPREM-UMR5254 / Institut Pluridisciplinaire de Recherche en Chimie Analytique et Physico-Chimie pour l'Environnement et les Matériaux; C. Mahfouz, National Centre for Marine Sciences-Lebanon

The marine environment and in particular coastal areas are an important receptor of multiple and emerging pollutants. The urbanization and the overexploitation of coastal resources along the Lebanese coast are constantly increasing. In particular, the presence of uncontrolled solid waste dumpsites and the discharges of domestic and industrial effluents into the sea without prior treatment are threatening the quality of the coastal marine environment. Moreover, the 2020 Beirut explosion, classified one of the world's most significant explosions, added extra damage to the surrounding marine ecosystem, due to the scale of the blast shock wave and the potential leakage and spread of hazardous pollutants in the marine area. Hence, the objective of the present study was to assess the state of the Lebanese marine environment by determining the occurrence of emrging Persistent Organic Pollutants (POPs) in marine sediments collected from Beirut before and after the 2020 explosion. Sediments were sequentially extracted using a semi-pressurized solvent extraction device (EDGE from CEM (France)), if required a fat clean-up is applied and then screened after their concentration for volatile organic pollutants such as organo-chlorine pesticides, brominated flame retardants and their metabolites, halogenated dioxins & furans, PAHs and PCBs using gas chromatography GC-TimsTOF. For the first time in Lebanon, these contaminants are identified, quantified (sub-ppb level) and reported on the following 5 criteria (exact mass, true isotopic pattern, HR-MSMS, retention time & ion mobility (CCS)). The results of the present study show that the sediments collected near the explosion site, the landfills and the effluent discharges show considerable concentrations of various contaminants. Total PAHs concentrations ranged from 15.08 to 2132.88 ng/g dw, while total PCBs levels ranged from 0.044 to 32.13 ng/g dw. Intense release of POPs especially in coastal areas are threatening the marine environment and could be accumulated along the food chain. Sediments can act as sink for various pollutants and may be a second source of contamination due to biogeochemical processes influencing pollutants mobility in the sediments and therefore, can have on long terms, serious effects on living organisms and ecosystems which highlight the importance of this study.

3.15.20

Can Sediment Stratigraphy Tell a Success Story of the Regulation of Contaminants?

V.A. Junttila, Finnish Environment Institute SYKE / Centre for Sustainable Consumption and Production; P. Ruokojärvi, National Institute for Health and Welfare / Department of Health Security; N. Perkola, Finnish Environment Institute / Laboratory Centre; V. Vartti, Radiation and Nuclear Safety Authority; J. Mannio, Finnish Environment Institute SYKE / Centre for Sustainable Consumption and Production

There is an urgent need in practically all international conventions (Stockholm Convention on POPs, Minamata Convention on Mercury, LRTAP, OSPAR, HELCOM) and EU legislation to show the effectiveness of the already implemented regulation of the most dangerous substances. Sediment core studies are a cost-effective method to reveal the recent history of substances with high affinity to particle phase. This method has been used in different fora for decades but has been somewhat forgotten at least in Europe in regulatory monitoring in the 2000's, due to focusing on quality standards based on water and biota. However, sediment core sampling and analyses may actually be much easier to harmonise between countries, compared e.g. to differences in food web structures affecting the species selection for monitoring. The concept is based on sampling of short sediment core (height of ca. 10 to 30 cm) which is cut to1–2 cm thick subsamples. Based on chemical analysis and Pb^{210} and/or Cs^{137} -dating of these samples the concentration trends of contaminants and their sedimentation rates can be assessed. We have analysed and dated sediment cores both in pristine areas in northern Finland and Baltic Sea for Hg, PAHs, PCBs, PCDD/Fs, PBDEs and PFASs. The results show declining concentrations for most of the contaminants, especially for PCDD/Fs and PCBs over the last couple of decades. However, results also reveal recent increase in concentrations of several PFASs, e.g. PFOA, PFNA and PFHxS, which together with PFOS are addressed in EFSAs scientific evaluation of risks caused by PFASs in food. Also, the results indicate the substitution of restricted PFOS by other PFASs and support the need of regulation of PFASs as a group, rather than as individual compounds.

3.15.21

Occurrence of Highly and Medium Polar Pharmaceuticals in Groundwater of Barcelona City (Spain) Using Lc-Hrms/Ms Orbitrap

N. Montemurro, Jordi Girona 18-26, null / Dipartimento di scienze agro-ambientali e territoriali; F.L. Roig, CSIC - Spanish National Research Council / Department of Environmental and Food Chemistry (ENFOCHEM); D.M. López, IDAEA CSIC Barcelona / Environmental Chemistry; O. Gómez Navarro, IDAEA- CSIC / Environmental Chemistry; L. Scheiber, IDAEA-CISC / Groundwater and Hydrogeochemistry; E. Vazquez-Sune, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Groundwater and Hydrogeochemistry; S. Perez, CSIC / Department of Environmental and Food Chemistry (ENFOCHEM)

Water samples may contain thousands of anthropogenic synthetic chemicals. Sewage treatment plants are the main source of contamination of the aquatic environment. Indeed, emerging persistent, mobile, compounds (PMCs), can be detected in surface waters far from where they entered. Among these, pharmaceutical residues, traveling with/without degrading, can contaminate water resources including groundwater sources, with impact on humans, animals, and plants. Due to their high polarity which makes them very soluble in water, their removal through drinking water treatment with existing methods is often ineffective. Furthermore, the identification of these highly polar substances can be very complex and expensive. In this study, we aim to work on the detection of polar substances (mainly drugs) that potentially seep through the water of urbanized environments. The city of Barcelona was chosen as a pilot area due to the deterioration of the quality of the aquifers of the past years and the proven presence of numerous organic and inorganic pollutants. In this regard, 54 groundwater samples taken from the entire Barcelona urban aquifer network were tested and extracted by using solid phase extraction (SPE) and a homemade multilayer mixed-bed cartridge with four different specific absorbents as weak anion and cation exchangers (WAX and WCX), Oasis HLB commonly used for wide-scope screening, and Bond Elut PPL to retain even the most polar classes of analytes. For the separation of a wide range of highly polar

substances, various analytical separation techniques have been evaluated, including polarity enhanced chromatography methods, such as hydrophilic interaction liquid chromatography (HILIC) or mixed-mode liquid chromatography. More than 160 pharmaceutical active compounds with a wide range of polarity and diverse physicochemical characteristics were screened using liquid chromatography high-resolution mass spectrometry Q-Exactive Orbitrap system and data-independent acquisition. After an initial screening, several samples tested positive for the presence of 17 persistent contaminants including acetaminophen, caffeine, carbamazepine, cocaine, iohexol, sulfamethoxazole, and trimethoprim.

3.15.22

Bromoanisoles in Nordic Macroalgae, 2018 Versus 2017

T.F. Bidleman, Umea University / Chemistry; A. Andersson, Umea University / Department of Ecology and Environmental Sciences and Umeå Marine Science Centre; S. Brugel, Umea University / Dept. of Ecology and Environmental Sciences; L. Ericson, Umea University / Department of Ecology and Environmental Sciences; M. Tysklind, Umea University / Department of Chemistry

Marine macroalgae are used worldwide for human consumption, animal feed, cosmetics, agriculture and biofuels. In addition to beneficial nutrients, macroalgae contain halogenated natural products (HNPs), some of which have toxic properties similar to those of well-known anthropogenic contaminants (1). Climate change is likely to impact the production and environmental pathways of HNPs (2). Macroalgae comprising brown, red and green species were collected from the Bothnian Sea (northern Baltic) and west coast of Sweden in 2017 and 2018 for determination of 2,4-dibromoanisole (2,4-diBA), 2,4,6-tribromoanisole (2,4,6-triBA) and other natural bromophenolic compounds. Compounds were extracted by soaking in methanol or methanol-dichloromethane, cleaned up on Florisil and determined by capillary gas chromatography with mass selective detection (GC-MSD) (1). Concentrations ranged from < 0.02 to 18 ng g^{-1} wet weight and were higher for 2,4,6-triBA compared to 2,4-diBA. Year 2018 was marked by higher summer temperatures, and some (but not all) macroalgae showed higher concentrations of bromoanisoles in 2018 compared to 2017. 1. Bidleman, T.F.; Andersson, A.; Brugel, S.; Ericson, L.; Haglund, P.; Kupryianchyk, D.; Lau, D.C.P.; Liljelind, P.; Lundin, L.; Tysklind, A.; Tysklind, M. (2019). Bromoanisoles and methoxylated bromodiphenyl ethers in macroalgae from Nordic coastal regions. *Environ. Sci. Proc. Impacts 21*, 881-892. 2. Bidleman, T.F.; Andersson, A.; Haglund, P.; Tysklind, M. (2020). Critical review: Will climate change influence production and environmental pathways of halogenated natural products? *Environ. Sci. Technol. 54*, 6468-6485.

3.15.23

Quantitative Determination of 80 Contaminants of Emerging Concern in the Freshwater Amphipod, Gammarus pulex

L. Birkitt, Imperial College London / Environmental Research Group; S. Owen, AstraZeneca / Global Sustainability; T. Miller, Brunel University London / Environmental Sciences Division; L. Barron, Kings College London / School of Public Health

Many contaminants of emerging concern (CECs) have been detected in the aquatic environment, including pharmaceuticals, illicit drugs and pesticides. While occurrence in water has been widely examined, CECs present in aquatic wildlife is less understood. In particular, extracting ultra-trace concentrations of large numbers of CECs, especially from small low trophic level organisms, is very challenging and time consuming. The aim of this investigation was to develop and validate an analytical method to extract and rapidly determine a large number of compounds of different classes, including pesticides, pharmaceuticals, and illicit drugs, from the freshwater amphipod *Gammarus pulex*. A total of 200 CECs were extracted using liquid-liquid extraction followed by solid phase extraction. The extracts were then analysed using a rapid and highly sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method in under 5.5 minutes. Out of all compounds tested, the method was successfully validated according to ICH guidelines for 80 compounds in whole body homogenate matrix to the low ng/g level. In addition, performance was excellent for direct analysis of water, without the requirement for extraction or pre-concentration. Limits of detection (LOD) and limits of quantification (LOQ) were satisfactory at the low ng/g concentration level in matrix. For analysis of freshwater using direct injection of only 10 µL of sample LOD and LOQ were excellent at 4±1 and 13±3 ng/L on average. This rapid method enabled the quantitative analysis of ~50-60 biota or water extracts in triplicate in a single day (~260 injections/day). Exposure to CECs in *G. pulex* sampled from four sites across the south of England was then performed, encompassing urban, rural, coastal, and inland sites. Differences were observed particularly between the urban and rural sites. A total of 41 compounds were detected, with more pharmaceuticals found at the two urban sites (n = 22, 22) compared to the two rural sites (n = 16, 20). There were also differences observed in the types of pharmaceuticals found at each location. Overall, biomonitoring using the developed method represented a robust means to detect and quantify a large number of CECs in small organisms a short period of time and at high sensitivity to help understand any risks they may pose to aquatic fauna.

3.15.25

Investigating the Source of Recently Discovered PCB Metabolites in Soil: Sulfonated and Hydroxy-Sulfonated PCBs

E. Terzaghi, University of Insubria (Como) / Department of Science and High Technology; R. Bagnati, A. Passoni, Istituto di Ricerche Farmacologiche Mario Negri / Department of Environmental Health Sciences; E. Davoli, Mario Negri Inst / Environmental Health Sciences; E. Fattore, Instituto di Ricerche Farmacologiche Mario Negri / Department of Environmental Health Sciences; A. Maspero, G. Palmisano, A. Penoni, University of Insubria / Department of Science and High Technology; E. Zanardini, University of Insubria / DISAT; C. Morosini, A. Di Guardo, University of Insubria / Department of Science and High Technology

Recently, the identification of two new classes of contaminants (sulfonated-PCBs and hydroxy-sulfonated PCBs) was described. More specifically, these new PCB metabolites were detected, for the first time, in soil samples collected from a site located in Northern Italy, highly contaminated by the industrial production of PCBs (Caffaro factory). The total PCB concentration in soil was around 12000 ng g^{-1} dw, while sulfonated-PCB and hydroxy-sulfonated-PCB levels were approximately 0.40 % (50 ng g^{-1} dw) and 0.25% (30 ng g^{-1} dw) of the native PCB levels in soils, respectively. Their physico-chemical properties were also estimated using a QSAR approach (EPISUITE), showing a scarce mobility toward the air compartment and suggesting their *in situ* formation from PCB degradation. The aim of the current study was to investigate the origin of these recently discovered PCB metabolites. Four sampling campaigns were organized to collect samples of soil at increasing distance and in four opposite directions (NW, NE, SW, SE) from the Caffaro PCB production plant. More specifically, samples were collected at 2, 4, 42 and 91 km distance along four 100 km transects. Samples were analyzed with GC-MS and HPLC-HRMS (Orbitrap Q Exactive mass spectrometer) for the determination of native PCBs and PCB metabolites, respectively. Native PCBs showed a decreasing trend with distance with average concentrations that ranged from ~100 ng/g dw (2 km) to ~3 ng/g dw (91 km). PBC metabolites were found in all samples, including the most far away points (42 and 91 km from the Caffaro factory). Their concentrations in soil ranged from 0.001 to 2.85 ng/g dw for sulfonated-PCBs and from 0.001 to 3.79 ng/g dw for hydroxy-sulfonated-PCBs. Regression analysis was used to compare the concentrations of native PCBs and the metabolites at each point. The results confirmed a correlation, which shows an in-situ formation of these metabolites. Further studies are necessary to understand the role of sulfonated metabolites in the degradation pathways of PCBs and the organisms responsible for degradation.

3.15.26

Identification of Sulfonated and Hydroxy-Sulfonated Polychlorinated Biphenyl (PCB) Metabolites in Soil: New Classes of Intermediate Products of PCB Degradation

A. Di Guardo, University of Insubria / Department of Science and High Technology; R. Bagnati, Istituto di Ricerche Farmacologiche Mario Negri / Department of Environmental Health Sciences; E. Terzaghi, University of Insubria (Como) / Department of Science and High Technology; A. Passoni, Istituto di Ricerche Farmacologiche Mario Negri / Department of Environmental Health Sciences; E. Davoli, Mario Negri Inst / Environmental Health Sciences; E. Fattore, Instituto di Ricerche Farmacologiche Mario Negri / Department of Environmental Health Sciences; A. Maspero, G. Palmisano, University of Insubria / Department of Science and High Technology; E. Zanardini, University of Insubria / DISAT; S. Borin, University of Milan / Department of Food, Environmental and Nutritional Sciences

Polychlorinated biphenyls (PCBs) are an important class of persistent organic pollutants ubiquitous in the environment. In Italy, they were produced and sold as mixtures (Fenclor and Apirolio) by Caffaro S.p.A. until 1984, when their production was stopped. These chemicals have been shown to undergo biodegradation in the laboratory and in the

environment under aerobic (mainly less chlorinated PCBs) and anaerobic (mainly high chlorinated PCBs) conditions via distinct mechanisms. The study of PCB metabolism had received increasingly attention in the last few decades focusing, not only on metabolites produced by microbial degradation, but also on those derived from plant, human and wildlife metabolism, and other reactions in the environment. Three classes of metabolites were mainly identified containing: 1) hydroxy group (-OH) - hydroxylated PCBs, 2) methoxy group ($-OCH_3$) - methoxy PCBs, 3) sulfur such as - SCH_3 (methyl thio PCB), $-SO_2CH_3$ (methyl sulfonyl PCBs) and $-OSO_3H$ (sulfate PCBs). Recently, sulfonated PCBs ($-SO_3H$) were discovered in polar bear blood. These metabolites have different physico-chemical properties and biological activities compared to the parent PCBs and there is still a lack of knowledge about their environmental fate and toxicity. In the present work the identification of two new PCB metabolites i.e., sulfonated-PCBs ($-SO_3H$) and hydroxy-sulfonated- PCBs ($-OH$, $-SO_3H$) is described. The identification was achieved after analysis by HPLC-HRMS (Orbitrap Q Exactive mass spectrometer) and comparison with reference standards, obtained through the chemical sulfonation of an industrial mixture of PCBs. Sulfonated-PCBs and hydroxy-sulfonated- PCBs were found for the first time in soil samples collected from a site historically contaminated by the industrial production of PCBs (Caffaro s.p.a) and in background soils (at 2 and 4 km distance). Sulfonated-PCB levels were approximately 0.4−0.8% of the native PCB levels in soils and about twice the levels of hydroxy-sulfonated-PCBs and hydroxy-PCBs. Being novel chemicals, no CAS number or description of their physico-chemical properties are available; therefore Episuite (version 4.11) was used to preliminary estimate these properties. Results indicated that, also given the low pKa of sulfonated- and hydroxy-sulfonated-PCBs, they possess negligible volatility, leading to speculate about their *in-situ* formation from PCBs. The mechanisms driving this biotransformation have still to be studied.

3.15.27
Obsolete Pesticides: Former Storehouses and Shops
A. Aleksandryan, Ministry of Environment of the Republic of Armenia / Head of Division; Y. Bunyatyan, A. Khachatryan, Hydrometeorology and Monitoring Center SNCO / Division of waste inventory, classification and technology investigation
Despite the 40-year period of prohibition to apply organochlorine pesticides (OCPs) from Persistent Organic Pollutants (POPs) group in agriculture, they are still present in environmental media (matrices) and in foodstuff. This latter is explained by their long persistence, preservation in soil and secondary pollution of the ground layer of air followed by their subsequent air-transfer to considerable distances. In the Republic of Armenia (RoA) the areas contaminated by OCPs are former storehouses of pesticides and the surrounding sites. Until 1985 Armenia received DDT, HCH, Hexachlorobenze OCPs. At that time 550 warehouses were functioning in the republic. After the ban to use OCPs, their accumulation began in warehouses. The condition of these storages was found to be substandard. At some locations the pesticides are still spilled out on the ground with contaminated soils or construction materials. It is highly probable that the buildings of these storages and the surrounding environment are potentially contaminated sites. This poses significant threat to the environment and humans. The largest numbers of probably OCP-contaminated sites are in Ararat (89), Shirak (89) and Armavir (86). Other provinces have approximately by 40 contaminated sites. Analyses for OCPs and summary POPs were performed and summary data obtained on POPs concentrations in soil samples taken near former warehouses of obsolete pesticides. Concentrations of HCH isomers, DDT isomers and metabolites, and other POPs were of interest, as well as generalizations of data obtained. *Strengthening the management of POPs-contaminated sites requires:* - Elaboration of mechanisms for identification of POPs-contaminated sites;- Development of risk assessment methodology, formulation of criteria for sites contaminated by POPs; - Decontamination of POPs-contaminated area in the vicinity of obsolete pesticides burial;- Development of a plan for assessment of POPs-contaminated sites- Mapping of POPs-contaminated sites;- Creation and maintenance of Register on POPs contaminated sites; - Assessment of identified sites, implementation of analytical investigations;- Implementation of decontamination/remediation measures at POPs-contaminated sites;- Development of projects/programmes on modern, environmentally sound, and profitable use of resources for decontamination/remediation of contaminated sites; arrangement of workshops;- Working-out measures on decontamination/remediation of contaminated sites.

3.15.28
Characterising the Organohalogen Iceberg: Comprehensive, Multi-Halogen Mass Balance Determination in Sludge
K. Spaan, Stockholm University, ACES / Department of Environmental Science; M.M. Plassmann, Stockholm University / Department of Environmental Science; J. Benskin, Stockholm University / Department of Environmental Chemistry; C. de Wit, Stockholm University / Department of Environmental Science
Organohalogen compounds (OHCs) comprise a wide range of fluorine-, chlorine-, bromine- and/or iodine-containing substances which are mostly of anthropogenic origin and often have persistent, bioaccumulative and toxic properties. OHCs are manufactured for use in numerous applications, including flame retardants, pesticides, refrigerants, and plasticisers. New OHCs are constantly introduced onto the market, much faster than analytical methods can be developed to measure them. Currently, more than 30,000 individual OHCs, containing one or more halogen, are registered with a unique Chemical Abstracts Service Registry Number (CASRN). Capturing this large number and diversity of chemicals in a single targeted method is currently not possible, leading to concerns that exposure to OHCs may be underestimated. For this reason, there is growing interest in so-called "organohalogen mass balance" experiments, which seek to quantify the fraction of unidentified organohalogen in samples through a combination of mass spectrometry-based targeted analysis and combustion ion chromatography (CIC)-based total- or extractable organohalogen measurements. In this study we build on previous organofluorine mass balance studies by developing a new multi-halogen CIC method for simultaneous determination of total- and extractable organo-fluorine, -chlorine and -bromine mass balance. The new method was tested on sludge samples collected in November 2019 from the Henriksdal wastewater treatment plant (WWTP) in Stockholm, serving 750,000 people (656,000 person equivalents). Targeted analysis included 19 per- and polyfluoroalkyl substances (PFASs) and 29 halogenated flame retardants (HFRs). For the flame retardants, BDE209 predominated in sludge samples (131 ± 16 ng/g, dw). Known extractable organobromine concentrations ($\Sigma Br_{_HFRs}$) were calculated by summing the bromine concentrations from all individually measured halogenated flame retardants, corresponding to ~240 ng Br/g dw, which accounted only partly for the total extractable organobromine (EOBr) concentrations. To the best of our knowledge, this study represents the first multi-halogen mass balance experiment conducted on sewage sludge samples. These data provide a comprehensive picture of both the known and unknown portions of the OHC "iceberg" in sludge. Future work will apply high resolution mass spectrometry-based non-target screening to elucidate this unknown fraction.

3.15.29
Occurrence and Analysis of Targeted Phthalates in Fish and Sediment in Belgian Rivers
G. Vangampelaere, Flemish Environment Agency / Environment
A new analytical method is being developed for the analysis of phthalates in fish (DEHP) and sediment (DEHP, DiByFt, DnByFt, DEyFt). Samples were collected in several important rivers in Flanders. After freeze drying, 1g of sample was spiked with 13C6 internal standards, prior to ASE-extraction with dichloromethane. Clean-up was performed simultaneously using aluminium oxide (and florisil for biota samples). Using TurboVap500, the extract was concentrated to 1ml, hereby switching solvent to n-hexane. Using splitless injection and GCMS-EI in SIM mode, 1µl of extract was analyzed. All fish samples (perch and eel) were tested positive for DEHP with concentrations varying between 10 and 250 µg/kg dw. The highest concentrations were found in eel samples. Targeted phthalates in sediment also tested positive in all analyzed samples, with DEHP being most abundant. Concentrations varying from 20-100 µg/kg dw for sandy soils, up to a few hundred µg/kg dw for sediments. Levels of DEHP peaked in some highly contaminated soils up to several mg/kg dw. These preliminary results indicate that phthalates are omnipresent in the environment. Because of their endocrine disruption properties and the lack of knowledge on the occurrence and spread of these compounds throughout the environment, there is a definite need to map out these results and assess the ecological value. Despite the fact that DEHP is included in the WFD, there are no well-defined rules for toxic concentrations in biota and sediment so far.

3.15.30
Exposure to Persistent Organic Pollutants Is Linked to Over-Wintering Latitude in a Pacific Seabird, the Rhinoceros Auklet, Cerorhinca monocerata

J.E. Elliott, Environment and Climate Change Canada / Science and Technology Branch Ecotoxicology and Wildlife Health Division; M. Hipfner, M. Drever, Environment and Climate Change Canada; K.G. Drouillard, University of Windsor / Great Lakes Institute for Environmental Research; K. Studholm, Dalhousie University

Seabirds are wide-ranging organisms often used to track marine pollution, yet the effect of geographical differences on exposure over the annual cycle is often unclear. We used solar geolocation loggers and stable isotope analysis to study the effects of post breeding dispersal and diet on persistent organic pollutant (POP) and mercury (Hg) burdens in rhinoceros auklets, Cerorhinca monocerata, breeding on islands along the Pacific Coast of Canada. Mercury and four classes of POPs were measured in auklet eggs: organochlorine insecticides (OCs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and perfluoralkyl substances (PFASs). Stable isotope values of adult breast feathers grown during winter were used in conjunction with geolocation to elucidate adult wintering latitude. Previous seabird studies identified only relatively small effects of wintering location on contaminant exposure compared to breeding location. Here, however, wintering latitude was the most consistent and significant predictor of some POP and Hg concentrations in eggs of rhinoceros auklet. The magnitude and pattern of exposure varied by contaminant, with ∑PCBs, ∑PBDEs and DDE decreasing and Mirex, the PFAS, perfluoro-n-tridecanoic acid and mercury increasing with auklet wintering latitude. We suggest that concentrations of these contaminants in rhinoceros auklet eggs are influenced by variation in uptake at adult wintering locations related to anthropogenic inputs and oceanic and atmospheric transport.

3.15.31

Persistent, Mobile, Toxic (PMT) and Very Persistent, Very Mobile (vPvM) Chemicals Used in Food Contact Materials

K.J. Groh, Eawag - Swiss federal Institute of Aquatic Science and Technology / UTOX Environmental Toxicology; B. Geueke, J. Muncke, Food Packaging Forum Foundation

Substances which are persistent in the environment, mobile in the aquatic environment, and toxic (PMT), as well as substances which are very persistent in the environment and very mobile in the aquatic environment (vPvM), can pose a significant environmental threat. This has been recognized in the EU's Chemicals Strategy for Sustainability, published in October 2020, where the European Commission has announced its intent to introduce the new PMT and vPvM categories for identification of substances of very high concern (SVHCs) under REACH. While the methodology and evidence base for identification and management of PMT/vPvM substances and their uses in the REACH-covered products have been growing, much less attention has been given so far to food contact materials and articles (FCMs/FCAs) as a potential source of PMT/vPvM substances' emissions. Therefore, we here present a first analysis of potential PMT/vPvM substances which might be used in different types of FCMs. For this, we searched our recently developed Food Contact Chemicals database (FCCdb), which contains over 12'200 FCCs possibly used to make FCMs worldwide, against the list of PMT/vPvM substances identified in the 2019 report by the German Environment Agency (UBA) based on their analysis of over 15'000 REACH-registered substances. We found that 149 FCCs have been identified as PMT and/or vPvM substance in the UBA's report. Among these substances, 45, 91, and 13 were identified following the assessment defined as being of high, medium, and low quality, respectively (the level of assessment quality has been assigned by the UBA itself based on data availability). Among the high-quality assesed PMT/vPvM FCCs, five are explicitly authorized for use in food contact plastics in the EU: melamine (CAS 108-78-1), isophorone diamine (CAS 2855-13-2), chlorendic anhydride (CAS 115-27-5), dapsone (CAS 80-08-0), and benzoguanamine (CAS 91-76-9). Overall, the highest numbers of FCCs identified as PMT/vPvM appear to be used in printing inks, plastics, coatings, and paper/board FCMs. Because single-use, disposable products have a high potential for littering and hence high probability of contributing to environmental emissions of PMT/vPvM substances, we next collected information on the use of PMT/vPvM FCCs in disposable FCAs, such as packaging or cutlery. PMT/vPvM substances confirmed to be used in FCMs should be targeted by substitution efforts in order to reduce the burden on the aquatic environment.

3.15.34

Simultaneous GC-MS Analysis of PCBs and PAHs in Soil Using a Modified QuEChERS Method

A. Lamb, A. Ladak, J. Renpenning, Thermo Fisher Scientific / CMD; C. English, C. Rattray, Restek; C. Cojocariu, Thermo Fisher Scientific / CMD

The work consists of a consolidated approach for the rapid and cost-effective quantitative determination of persistent organic pollutants (POPs) (PAHs, PCBs, oxyPAHs, methylPAHs and NSO-PAHs) in soil and was developed to increase sample throughput and laboratory testing capacity. For this a modified QuEChERS extraction and clean up method was selected as recent literature had demonstrated it to be quicker and more cost effective than traditional Soxhlet extraction. Chromatographic separation of target compounds was achieved by a TraceGOLD TG-5 SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column and was optimised for a < 20 min/sample method using the Pro EzGC™ chromatogram modeler. Detection of compounds was achieved using a ISQ 7000 single quadrupole GC-MS instrument operated in electron ionisation mode (EI). The performance of the ISQ 7000 single-quad MS system with PCBs, PAHs and their derivatives were evaluated in terms of selectivity, sensitivity, dynamic range, extraction & clean up performance (accuracy & precision) and system robustness. Instrument detection limits (IDLs) were calculated using a two tailed student t test at the 99% confidence interval from the peak area % RSD, t-score of 2.681 and injected amount on column (OC) of 13 replicate injections at the lowest concentration that the peak area was < 15% RSD. LOQs were determined through the injected amount, ion ratio % deviation ±30% (from calibration average) and peak area < 15 % RSD. Instrument sensitivity was demonstrated down to tens of fg on column (OC) and LOQs ranged from 0.4–5.0 µg/kg (ppb). Linearity R2 values were >0.998 and residual % RSDs < 10% over a range of 0.1-500 pg/uL. The extraction performance of the QuEChERS method was evaluated in terms of accuracy and precision through the analysis of 7 technical replicates and gave an average recovery of 75% with % RSD of the replicates < 15%. Use of the modified QuEChERS workflow was shown to save 22 hours versus typical Soxhlet extraction workflows. Suitability for routine analysis was assessed over 12 days of unspiked sample extract analysis (250 matrix injections) with low calibration level QCs spaced every 20 sample injections with no inlet maintenance (other than periodic septa change) or MS tuning. The QCs remained constant across the continual analysis with relative repose factor % RSD and ion ratio % deviation for all analytes being < 15% & ±15% of the original calibration average, respectively.

3.15.37

Empirical Prediction of PPLFER Equations; First Steps Towards a New Tool for Chemical Mixtures Assessment

T.N. Brown, TNB Research

Exposure and risk assessments are conducted as part of regulatory schemes to protect humans and the environment from potential adverse effects of chemicals. Mechanistic mass balance exposure models have been developed to estimate human exposure to chemicals. While these models are commonly applied for single constituents there is a need to expand their capabilities to include mixtures. Interactions between components may be particularly important for estimating exposure to products/substances containing multiple constituents spanning a wide range of physico-chemical property values (e.g., industrial or consumer product formulations, UVCBs). A key challenge in improving exposure estimates for constituents of mixtures for any existing mass balance exposure model is to account for changes in mass transport (diffusion, volatilization) from these complex matrices. Polyparameter Linear Free Energy Relationships (PPLFERS) provide a method for predicting the partitioning properties of solutes in various solvents; empirical equations and descriptors have been calibrated for about 100 solvents, and for thousands of solutes. The goal of this research is to develop empirical regressions to predict PPLFER equations from the solute descriptors. This would expand the number of PPLFER equations to thousands of solvents and allow PPLFER equations to be used to apply both Henry's Law and Raoult's Law approximations, which is a first step towards using PPLFER equations for assessing the partitioning properties of chemicals in mixtures. A standard dataset of PPLFER equations has been recalibrated from partition coefficients (log *K* values) and solute descriptors collected from the literature and the UFZ LSER Database. Using these data as a training set, empirical regressions have been calibrated and externally validated on a separate dataset to predict log *K* values, as well as the vapor pressure and water solubility of pure solvents.

3.15.38

IFSQSAR - an Interface Integrated With Eas-E Suite for Applying Group Contribution QSARs to Predict Chemical Properties Related to Chemical Fate and Exposure

T.N. Brown, TNB Research; A. Sangion, University of Toronto Scarborough /

Department of Physical and Environmental Sciences; J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology
Thousands of new and existing chemicals require hazard and risk assessment under existing regulations. Reliable chemical property data are critical to establishing confidence in legislated chemical evaluations. Most new and existing chemicals do not have measured property information and predictive methods such as Quantitative Structure Activity (Property) Relationships (QSA(P)Rs) are required to address data gaps and uncertainty. The Iterative Fragment Selection (IFS) QSAR group contribution method has evolved over the last 10 years, with a focus on key properties for chemical assessment for which few prediction tools were publicly available, for example biotransformation half-lives in fish and humans. All IFS QSARs are developed and validated according to the OECD guidelines for QSAR applications in regulatory contexts using critically evaluated (curated) training and testing datasets. A key feature of the IFS QSARs is that the applicability domain (AD) has been defined and metrics quantifying the AD are included with every chemical prediction. This includes estimations of the potential errors of the QSAR predictions which can be used in uncertainty analyses of model outputs. IFSQSAR can function as a stand-alone python package, with a simple graphical user interface, a command line interface, and a python API. Moreover, IFSQSAR is one of the QSAR application packages included within the new Exposure And Safety Estimation (EAS-E) Suite platform. EAS-E suite is a free and publicly available platform developed to facilitate the application of exposure models for non-experts and to bridge the gap between evolving scientific research and assessment challenges. EAS-E Suite includes databases of key properties and QSARs to autoparameterize various chemical emission, fate, PBPK, exposure and risk estimation models that are included in the platform. In EAS-E suite, each QSAR prediction is provided with an estimate of the Applicability Domain and uncertainties to aid users to evaluate the reliability and consistency of the predictions fostering confidence in the application of these data for applications in EAS-E Suite or for external applications.

3.15.40
A Biota Monitoring Network to Determine Priority Substances Concentrations in Walloon Rivers
D. Leroy, ISSeP / Ecotoxicology; P. Libert, Service Public de Wallonie SPW / DGARNE-DEE; C. Nadin, ISSeP / Organic Chemistry Unit; I. Hardy, M. Canisius, ISSeP; Y. Marneffe, Inst. Scientific de Service Public / Ecotoxicology
The European Water Framework Directive (WFD) aims to achieve and ensure a good quality status of water in each Member State. Most of the Environmental Quality Standards (EQSs) are defined for water itself. However standards have also been set in biota for some substances (Directive 2008/105/EC and Directive 2013/39/EU). This is the case of mercury, hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), PAHs (benzo-a-pyrene and fluoranthene), Polybrominated diphenylethers (PBDEs), Perfluorooctane sulfonic acid and its derivatives (PFOs), dioxins and dioxin-like compounds, heptachlore and heptachlore epoxyde, hexabromocyclododecane (HBCDD), and dicofol. To answer these recommendations we developed methods to measure the concentration of these pollutants in freshwater biota sampled in Walloon Rivers. A preliminary study conducted from 2013 to 2015 on four fish species (*Leuciscus cephalus, Abramis brama, Cottus gobio* and *Barbatula barbatula*) and macroinvertebrates species (crustaceans and/or molluscs) on 54 sampling sites allowed us to implement a monitoring network on freshwater biota that began in 2016. In this frame, 60 sites are sampled every year, some of which are subject to time monitoring. Differences in contamination between the studied sites, and over time for some sites, are discussed and obtained values are compared to EQSs. Sentinel species are not present in every sampling site. To overcome this problem, a caging technique with the crustacean *Gammarus pulex* has been developed. Gammarids coming from a clean site are acclimatized for two weeks in the laboratory and caged for two weeks in several sites. Benzo-a-pyrene and fluoranthene are searched in these organisms after caging. Results are discussed.

Extended submission 3 - Environmental chemistry and exposure assessment: analysis, monitoring, fate and modeling

3.16.01
The Hormetic Effects of Polystyrene (PS) NanoPlastics on the Growth of Bacillus inaquosorum
F.A. Olabemiwo, Wesleyan University / Biology; A.A. Hagan, F.M. Cohan, Wesleyan University / Department of Biology
Plastic waste build-up in the environment is exponentially increasing. When plastic breaks into tiny, nanoplastic particles, diverse consequences on organisms become possible. Numerous studies have investigated the harmful effects of nanoplastics on microbes. However, it is unclear whether the nanoplastics can be both beneficial and harmful to bacteria depending on the dosages (hormesis). In this study, we exposed *Bacillus inaquosorum*, a mesophilic soil bacterium isolated from Death Valley soil, to 25 nm polystyrene (PS) nanoplastics (0 mg/L, 300 mg/L, and 800 mg/L) for 24 hours. The results indicated immediate death during short-term (60 minutes) exposure of the bacteria to nanoplastics. Despite this immediate killing from plastic exposure, over a period of 24 hours, the bacteria grew to a higher density at 300 mg/L than with no plastics. At a higher dosage of 800 mg/L PS nanoplastics, the bacteria grew to a lower density than with no plastic. Gas chromatography-mass spectrometry (GCMS) analysis of the growth culture at 24 hours revealed by-products of polystyrene degradation. In general, these findings highlight a beneficial effect of nanoplastics on the growth of *Bacillus inaquosorum* and suggest a possible role of this species as a bioremediator of polystyrene.

3.16.03
Transformation and Leaching Behaviour of Polyfluoroalkyl Phosphate Diesters (diPAPs) in Unsaturated Soil Columns
E. Weidemann, University of Kassel / Hydrology and Substance Balance; R. Lämmer, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; T. Stahl, Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe (CVUA-MEL); B. Göckener, M. Bücking, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; J. Breuer, Agricultural Technology Centre Augustenberg; J. Kowalczyk, H. Just, German Federal Institute for Risk Assessment BfR / Safety in the Food Chain; M. Gaßmann, University of Kassel / Hydrology and Substance Balance
In the last decades considerable scientific attention has been drawn to the chemical group of PFAS (per- and polyfluorinated alkyl substances), fluorinated anthropogenic compounds with various industrial applications such as the surface treatment of paper. Many PFAS are classified as PBT (persistent, bioaccumulative, toxic) and therefore of environmental concern. Recently, precursor substances such as polyfluoroalkyl phosphate diesters (diPAP), which can transform into PFAA (perfluoroalkyl acids), a persistent group of PFAS, have been detected in the environment.The project PROSPeCT (PFAA and Precursors Soil Plant Contamination) deals with the behaviour of PFAAs and precursors in soils and the associated plant uptake in a South-west German study site. Six soil were set up for two years, each containing 1.16 mg of either 6:2 diPAP (n=3) or 8:2 diPAP (n=3), which were mixed into the upper half of sandy soil. Unsaturated soil conditions were created by watering the columns with 35 ml/week, which corresponds to the average natural rainfall of the study site. After 59 weeks, PFAA metabolites were found in the leachate, however, no diPAPs were detected. The main PFAA metabolite of 6:2 diPAP in the leachate was PFPeA (15.4 %) followed by PFHxA (8.5%), PFBA (1.6%) and PFHpA (< 1%). In sum, 33.1% of the initial mass of 6:2 diPAP was found in the leachate due to transformation. Non-PFAS transformation product masses originating from the precursors were also included. The major PFAA metabolite of 8:2 diPAP found in the leachate was PFOA (12.1%) followed by small amounts of PFHpA (2.1%) and PFAA with a shorter chain length (< 1%). 18.6% of the initial 8:2 diPAP mass was found in the leachate, including non-PFAS transformation product masses. The breakthrough of all measured PFAA in both experiments was already over after 59 weeks, yet small concentrations of PFAA are continuously found in the leachate to date. According to published values of one study, after 59 weeks 99% of 6:2 diPAP should have already been degraded (half-life: two months) as well as 25% of 8:2 diPAP (half-life: > 1,000 days). Thus, larger quantities of metabolites of 6:2 diPAP would be expected in the leachate than were detected. This discrepancy could be related to an overestimation of the half-lives, not determined metabolites or the formation of non-extractable residues in soil, which is intended to be verified after analysing the soil at the end of the study.

3.16.04
Impact of Climate Change on the Relevance of OECD Crosswalks
C.G. Hoogeweg, Waterborne Environmental, Inc. / Modeling; A. Ritter, R. Vamshi, Waterborne Environmental, Inc. / Exposure Modeling; D. Desmarteau, Waterborne Environmental, Inc.
Climate change studies confirm that the earth is

warming up and that shifts in rainfall patterns is occurring. Various computer models predict that Earth's average temperature will rise between 1.8° and 4.0° Celsius (3.2° and 7.2° F). Consequently, these warmer temperatures will cause a higher rate of evaporation, resulting in a predicted increase of average global rainfall by 3-5%. Under OCED guidance, TFD studies conducted in one country can be applicable to other countries if the characteristics of overlapping ecoregions are the same. These assessments are conducted using the OECD ENASGIPS tool. If these characteristics change, so will the similarity assessment and therefore the potential relevance of TFD sites. With increase rainfall, TFD sites in the Southeastern US, which typically have few similar areas, may become more relevant because other areas are getting wetter. Likewise, TFD sites in arid ecoregions may see a decrease in similarity scores. In this presentation we show that TFD studies in arid regions in the US remain relevant for foreseeable future.The objective of this assessment was to determine if climate change will impact the relevance of TFD studies and OECD ENASGIPS crosswalks ecoregion similarity scores. Specifically, we were interested in determining if ecoregions overlapping TFD sites remain the same over time in terms of precipitation, temperature, and ecoregion similarity. Both statistical analysis and ecoregion crosswalk assessments were conducted. In this presentation we will focus on an arid in California's Central Valley using the intermediate IPCC RCP4.5 and worse case RCP8.5 climate change scenarios. Results indicate that under moderate climate change scenario the arid ecoregion has an increase in the number of similar ecoregion over time but a decrease is observed under the worse case scenario. In the short-term, 30 to 40 years, TFD studies are not predicted to see an impact by of climate change and data obtained from these TFD studies can be used for registration and reregistration of pesticides for the foreseeable future.

3.16.07

Assessment of Diesel Distribution in Undisturbed Soil Cores After Simulated Rain Episodes Using Analytical Chemistry Techniques and X-Ray Computed Microtomography

M. Balseiro-Romero, IRNAS CSIC; A. Mazurier, Institut de Chimie des Milieux et Matériaux de Poitiers-UMR CNRS 7285; B. Calviño-Vázquez, C. Monterroso, CRETUS (Cross-Research in Environmental Technologies), Universidade de Santiago de Compostela / Departamento de Edafoloxía e Química Agrícola; P. Baveye, AgroParisTech, Université Paris-Saclay

The behaviour of fuel compounds in soil and their fate in the environment have raised much concern in recent years due to the environmental and ecological associated risks. A detailed study of the migration of a simulated diesel spill using analytical and tomographic techniques was carried out using undisturbed soil cores (5.5 cm height x 4 cm diameter) from an agricultural soil (first 5-10 cm) and a forest soil (topsoil, first 5-10 cm; and B horizon 50-60 cm). Packed cores of coarse sand were used as a control. A surface spill of diesel was simulated by adding 3 mL of commercial diesel on top of the cores. Every 48 h, artificial rain episodes were simulated in two batches (8 mL and 7 mL) spaced by 6 hours. Rain intensity was controlled using rain reservoirs with ten hypodermic needles homogeneously distributed and situated at 3 cm over the core surface. Leachates were sampled at the bottom of the cores and analysed for total diesel compounds (TDH) and diesel range organics (DRO, alkanes from 10 to 25 carbons) by gas chromatography coupled to mass spectrometry. The experiment was kept for 4 weeks and the soil in the cores was analysed for TDH and DRO at four different depths, in order to characterise the diesel distribution. In parallel, cores were scanned using an X-ray computed microtomograph (μCT), before and after the experiment, in order to characterise, respectively, the pore space (macroporosity, distribution and connectivity of the pores, tortuosity, etc.) and the probable distribution of residual diesel within. The results showed that the leaching rate of water, the distribution of residual diesel and its migration behaviour inside the cores, were closely related to the characteristics of the pore space. On the other hand, the soil composition (in terms of organic matter and other colloidal components) had a strong influence on the rate at which the diesel abandoned the cores (generally delayed in soil samples with a higher content of organic matter or clays, which exerted a stronger retention), and on the composition of residual diesel (mainly enriched on high-molecular weight alkanes in the upper layers, more strongly retained, and due to evaporation losses of the lightest). These results are of a high significance for understanding the behaviour of fuel spills in real soils, and reflect how the internal structure of the pores and the soil colloidal components play a role in determining the final fate of fuel compounds in the environment.

3.16.08

Potential Application of Surface-Active Ionic Liquids and Surfactants in the Remediation of HCH - Contaminated Soils Through Solubility Enhancement

Z. Chaos-Carballo, CRETUS (Cross-Research in Environmental Technologies), Universidade de Santiago de Compostela / Departamento de Edafoloxía e Química Agrícola; M. Balseiro-Romero, IRNAS CSIC; B. Calviño-Vázquez, CRETUS (Cross-Research in Environmental Technologies), Universidade de Santiago de Compostela / Departamento de Edafoloxía e Química Agrícola; A. Soto, CRETUS (Cross-Research in Environmental Technologies), Universidade de Santiago de Compostela / Departamento de Enxeñaría Química; C. Monterroso, CRETUS (Cross-Research in Environmental Technologies), Universidade de Santiago de Compostela / Departamento de Edafoloxía e Química Agrícola

Hexachlorocyclohexane (HCH) has been one of the most used pesticide from 1950 to early 2000 and has led to a current global soil pollution. HCH isomers are considered persistent organic pollutants (Stockholm Convention, 2009) and restoration of HCH-contaminated soils is urgently required. Bioremediation is one of the most promising remediation techniques and its effectiveness can be enhanced through the application of surfactants, which solubilize the contaminant, and hence increase its bioavailability. However, their application *in situ* is challenging due to their reported toxicity. In this context, we evaluated the micellar effect of a wide structurally diverse group of Surface-Active Ionic Liquids (SAILs) and conventional surfactants (non-ionic, cationic and anionic) on the modification of the apparent solubility of HCH in water. The synergistic effect of a combined system of a SAIL and a surfactant was verified using two blends with different SAIL/surfactant proportions. SAILs/surfactant solutions of different concentrations (>critical micelle concentration) were contaminated with HCH isomers (at a concentration *ca.* 10 times their solubility limit) and left under constant agitation until equilibrium (24 h). Then, the solubilized fraction of HCH isomers was analysed by GC/MS, and the solubility enhancement caused by SAILs/surfactants was calculated by normalization with a SAIL/surfactant-free control. Additionally, two ecotoxicological assessments were carried out: a) germination and early growth of *Lolium perenne* and *Trifolium pratense*; and b) inhibition of bacterial bioluminescence of *Aliivibrio fischeri*. HCH solubility enhancement varied depending on the concentration and the structure of SAILs/surfactants, inducing also different effects among individual isomers. In general, anionic compounds showed a higher effect on the solubilisation of HCH, reaching up to a *ca.* 7-fold increase with regard to the control. However, most of them were toxic to plants and/or bacteria. Non-ionic compounds were less toxic and performed better at lower concentrations, what may be an advantage for *in situ* bioremediation procedures. Moreover, the use of blends demonstrated an improvement over individuals. This study evidences the potential of SAILs and surfactants for their application in the remediation of HCH-contaminated soils, particularly, for bioavailability enhancement, and set the basis for their application in further research with contaminated soil samples.

3.16.11

Variation in the Levels and Composition of Selected Semi-Volatile Organic Pollutants Based on the Size of Dust Particles From Various Indoor Microenvironments

n. ali, King Abdulaziz University / Center of Excellence in Environmental Studies; M. Kadi, I.M. Ismail, King Abdulaziz University / Department of Chemistry; A.S. Summan, King Abdulaziz University / Department of Environmental Sciences; G. Malarvannan, University of Antwerp / Toxicological Center

In this study, we studied the distribution of three different classes of semi-volatile organic chemicals (SVOCs) namely phthalates (PAEs), organophosphate flame retardants (PFRs), and polycyclic aromatic hydrocarbons (PAHs) in four different size fractions of dust (25, 50, 100, 200 μm) collected from various indoor microenvironments namely AC filter dust, household, hotel, mosque, and car floor dust. For each microenvironments 10 samples were collected and then pooled to get four fractions of dust using sieving apparatus with required mesh size. The samples were extracted using solvent mixture of n-Hexane: Acetone (1:1 v/v) and ultrasonication. Quantitative analysis of the selected SOVCs were performed using gas chromatography- mass spectrometry in electron impact (EI) mode using a fused silica capillary column (TR5 30 M × 0.25 mm × 0.25 μm). Overall dust from car parking was the least contaminated with chemicals with ∑SOVCs 72, 48, 40, 21 μg/g of dust in 25, 50, 100, 200 μm dust fractions, respectively. While ∑SVOCs in car floor dust were 845, 590, 470, 335 μg/g of dust in 25, 50, 100, 200 μm dust fractions, respectively. Among studied microenvironments car dust and car parking

dust exhibited a trend of increasing concentration with decreasing dust particles. For all other microenvironments the levels of SVOCs varied in concentrations but there was not clear trend in increasing or decreasing concentrations of SVOCs in different fractions of dust. PAEs was the major contributor in the profile of ∑SOVCs with contribution of >90% followed by PFRs and PAHs. However, for car parking dust PAHs contributed more than PFRs in the profile of SVOCs. Levels of PAHs and PFRs were highest in car dust, which is understandable since many interior parts of the cars are possibly treated with PFRs as flame retardants to fulfil the fire safety regulations. While, petroleum products are major source of PAHs in the surrounding environment and all the sampled cars in study were fitted with combustion engine therefore there are higher chances of car dust to be contaminated with PAHs realizing from the combustion of petroleum projects. Few studies in literature have reported increasing levels of certain chemicals such as heavy metals and brominated flame retardants with decreasing dust particles. However, in present study except two microenvironments other studied microenvironments did not show such trends of increasing concentrations with decreasing dust particle size, this might indicate there are other factors influencing the presence of SVOCs in dust particles.

3.16.12

Release Kinetics and Stability Study of a Nanoformulation of Tebuconazole Encapsulated in Polycaprolactone Nanoparticles

M. Eghbalinejad, Masaryk University; M. Lopez-Cabeza, Masaryk University, Faculty of Science, RECETOX; J. Hofman, RECETOX, Faculty of Science, Masaryk University / Research Centre for Toxic Compounds in the Environment (RECETOX), Faculty of Science

Excessive use of pesticides in agriculture and its consequent damage have been economically, environmentally and morally controversial issue for years, leading to an increasing demand to find safe alternatives. Nanotechnology in agriculture, by offering chances to develop more effective agrochemicals, may have less impact on the environment by increasing the functionality and benefit of active ingredients while reducing the risk profile of them. In the present study, poly(e-caprolactone) (PCL), as biocompatible and biodegradable polymeric carrier, was selected to prepare a nanoformulation of tebuconazole (TBZ). Interfacial deposition of preformed polymer method was used to prepare PCL nanocapsules loaded with the pesticide. After preparation, the nanoformulation was characterized by the determination of particle size, polydispersity index and zeta potential using dynamic light scattering, and particle morphology by scanning electron microscopy. Encapsulation efficiency of TBZ in the PCL nanoparticles was determined by centrifugal ultrafiltration as well. The results showed that we obtained a suspension of spherical particles in the nanometric size range (particle size = 241.1 nm), whose particle distribution was moderately polydisperse (polydispersity index = 0.242) and the zeta potential was -37.5 mV. Encapsulation efficiency was very high (95%) meaning most of the TBZ was associated with the nanoparticles. To find out more feasible evaluation of nanoformulation behaviour in real medium compared to milli Q water, the release kinetics of TBZ from PCL nanoparticles in reconstituted water (RCW) over 672 h for 3 different dilutions whose total concentrations of TBZ including 50000, 5000 and 500 ng/ml was measured by the sample and separate method using centrifugal ultrafiltration. The results showed an increase in the release rate with the increase in the dilution factor. Thus, the release of TBZ in 500 ng/ml dilution reached 100% of released TBZ much faster compared to 50000 ng/ml dilution. The stability of the nanoformulations during the release experiment was also evaluated by determining changes in particle size and polydispersity index in RCW over 672 h. No significant changes were observed in these two parameters during the time period studied, with the exception of a slight increase in PDI in 500 ng/ml dilution, but without aggregation. The results showed that the nanoformulation was stable in RCW during the 672 h. The increase in dilution leading to an increase in the rate is important in real situation where the formulation of pesticide is diluted in a great amount of water such as in application tanks or in the field. Consequently, tebuconazole with PCL has got promising results in different aspects especially environmentally which may help to reduce the adverse effect of pesticides.Keywords: Release kinetic, Centrifugation, Ultrafiltration, Polymeric nanocarrier

3.16.13

Brominated Flame Retardants in PM10 and Indoor Dust of Saudi Children's Room: Concentration, Composition and Health Risk Assessment

n. ali, King Abdulaziz University / Center of Excellence in Environmental Studies; M. Kadi, I.M. Ismail, King Abdulaziz University / Department of Chemistry; A.S. Summan, King Abdulaziz University / Department of Environmental Sciences; N.A. Alhakamy, King Abdulaziz University / Pharmaceutics Department; M.I. Rashid, King Abdulaziz University / Center of Excellence in Environmental Studies; G. Malarvannan, University of Antwerp / Toxicological Center

Children spend most of their daily time indoors, especially in their rooms. Many of the stuff used indoors, such as furniture, electronics, textile and children toys, are treated with chemicals to provide longevity and fulfil the safety standards. Various studies have reported brominated flame retardants (BFRs) in indoor environments; however, few focused on the environment specified for young children. In this study, we collected air (PM10) and dust samples from rooms (n=30) of Saudi children. Samples were analyzed for different congeners of polybrominated diphenyl ethers (PBDEs) and three important alternative flame retardants using a TSQ™ 8000 Evo triple quadrupole GC-MS/MS (Thermo Fisher Scientific) in the selected ion monitoring (SIM) mode. A fused silica capillary column (Rxi-5silMS 15 M x 0.25 mm x 0.10 µm) used for the separation. Decabromodiphenyl ether (BDE-209) was the most identified BFR in dust and PM10 samples with a median value of 3150 ng/g of dust and 75 pg/m^3, which indicates the wider application of BDE-209 has still implications for its occurrence, although its use in regulated for specified use since 2014. Among alternative BFRs namely 2-Ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), Bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (TBPH), 1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE) were found with a median levels of 10, 15 and 8 ng/g of dust, respectively. However, alternative BFRs were present in < 50% of the PM10 samples. Calculated hazard quotient (HQ) and hazardous index (HI) for all studied chemicals was < 1 which indicate indicating a low non-carcinogenic risk to the Saudi children from exposure to indoor dust and PM 10 from their room. The probabilistic Incremental lifetime cancer risk (ILCR) assessment for BDE-209 was 2.05E-07 which is well below the USEPA recommended safe limit (1.00 E-4) for long term cancer risk. This indicates Saudi children have low carcinogenic risk linked to BDE-209 from its presence in their rooms. The calculated low and high end daily exposure *via* indoor dust and PM 10 using average and 90th percentile concentrations were below the reference dose values for Saudi children from their room. Nonetheless, the study highlights BDE 209 at higher levels than previously reported from Saudi Arabia's household dust and warrants further extensive studies to estimate the different classes of chemical exposure to children from their rooms.

3.16.14

Mercury Accumulation in Foodstuffs From Artisanal Goldmining Areas of Madre De Dios, Peru and Estimated Human Exposure

M.J. Marchese, Duke University; J.R. Gerson, Duke University / Biology; K. Lansdale, E. Letourneau, Duke University; H. Hsu-Kim, Duke University / Department of Civil Environmental Engineering; W.K. Pan, Duke University; E. Bernhardt, Duke University / Biology

Artisanal and small-scale gold mining (ASGM) is the largest global source of anthropogenic mercury (Hg) emissions. This practice occurs in over 70 countries and is particularly widespread in the Peruvian Amazon. As a result of ASGM, people can be exposed to this potent neurotoxin by consuming Hg-laden foods. While Hg levels in aquatic ecosystems and human exposure from consuming fish have been previously studied, accumulation in commonly consumed plants and animals from the terrestrial environment is often overlooked. To form a more complete picture of Hg uptake in food staples from the Peruvian Amazon, we measured the Hg content of local crops, fish, chicken, and eggs from communities heavily impacted by mining and from remote communities surrounded by preserved forest. Stable carbon and nitrogen isotope signatures of fish and chicken meat were also analyzed to assess trophic magnification. Chicken meats, feathers, and crops from communities heavily impacted by mining had significantly higher Hg content than those from remote communities ($p< 0.001$). Average THg content of tissues and feathers from mining areas was 44 and 620 µg/kg, respectively compared to 7 and 220 µg/kg in tissues and feathers from remote areas. Though few crops exceeded international food safety recommendations, methylmercury (MeHg) proportions in rice were very high (94%). As expected, trophic level was a key predictor of fish Hg content, and 81% of carnivorous fish from Madre de Dios exceeded WHO guidelines. In chickens, egg whites and livers were most likely to exceed international Hg recommendations. Trophic magnification was not a significant factor in chicken Hg content but proximity to mining was, indicating that accumulation in chicken results from exposure

to mining rather than differences in diet. Our results demonstrate that both terrestrial and aquatic foods can accumulate Hg from mining activity, leading to additional routes of human exposure to Hg.

3.16.16
Determination and Computational-Based Study of Disinfection By-Products of Alloxydim Herbicide During Chloramination Process
P.S. España, INIA - National Institute for Agricultural and Food Research and Technology / Unit of plant protection products; J. Villaverde, B. Sevilla-Morán, M. Mateos-Miranda, INIA(National Institute of Agricultural and Food Research and Technology / Plant Protection Products; J.L. Alonso-Prados, INIA National Institute for Agricultural and Food Research and Technology / Plant Protection Products
During water supply management, chlorine is one of the most common disinfectant agents employed in water treatment to control pathogenic bacteria. Chloramination is a disinfection method less effective than free chlorine but more persistent, so it is very used for the maintenance of a stable distribution system of big cities. However, chloramines, as well as chlorine, can react with natural and synthetic compounds present in the water to form degradation products. Little is known about the reactions of chloramines with anthropogenic chemicals. This fact is very important because during water treatments, residues of pesticides may also suffer chemical transformations to form emerging compounds with different environmental behaviour and/or worse (eco)toxicological profile than the parent substances. Therefore, the study of DPs with the potential to be originated through chloramination treatments should be a priority. In this context, the objectives of this work were; (1) to study the reaction between alloxydim, a cyclohexanedione herbicide, and chloramines in water and (2) to study the by-products formed and their environmental and ecotoxicity impact by means of QSAR analysis and the Microtox assay. The kinetic evolution of aqueous solutions of alloxydim in the presence of chloramines and the detection of the by-products formed were performed by HPLC-MS. The herbicide was degraded by chloramines with a half-life of 15 hours. During the degradation, different by-products were detected and the main by-product was isolated and identified as chlorinated-alloxydim. Structure-activity relationships model pointed out that the environmental and ecotoxicological effects of by-products can differ from its parent herbicide alloxydim. Besides, chlorinated-alloxydim was isolated to study its ecotoxicity on Vibrio fisheri bacterium using Microtox assay. Chlorinated by-product exhibit higher toxicity that its parent herbicide, showing a EC50 of 6.55 mg L-1 versus 341 mg L-1 of alloxydim, which is a significant increase of toxicity. This work demonstrates that further studies will be necessary to fully evaluate the environmental impacts of pesticide residues in chlorinated treated drinking waters.

Artificial Intelligence Approaches in Environmental Risk Assessment: Bayesian Networks, Machine Learning and Predictive Modelling

4.01.02
Precaution: Predicting the Chemical Sensitivity of Aquatic Organisms
S. van den Berg, Wageningen University & Research / Environmental Risk Assessment; C. Rivetti, Unilever / Safety and Environmental Assurance Centre SEAC; B. Campos, Unilever / Safety and Environmental Assurance Centre; P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences
It is impossible and ethically undesirable to experimentally determine the sensitivity of all species present in rivers, lakes and streams everywhere around the world to all chemicals to which they can possibly be exposed. Therefore, a valuable method to help with the assessment environmental safety of chemicals is to extrapolate the known chemical sensitivity of species, for which toxicity has been determined, to species for which the toxicity is unknown. This method is also known as cross-species extrapolation of chemical sensitivity and is aimed at predicting the sensitivity of realistic species assemblages to chemicals. Creating a cross-species extrapolation modelling tool, flexible to different taxonomic and chemical groups, would allow for its incorporation into aquatic risk assessment, where it can assist in the protection of biodiversity. Therefore, the two main objectives of this new project are: i) to transform an already existing cross-species extrapolation modelling framework into a user-friendly tool that can construct predictive models for multiple aquatic taxonomic and chemical groups, and ii) to deliver a set of case studies that demonstrate how this tool can directly be applied for aquatic water quality and risk assessment purposes. We expect that this tool could be used to evaluate important modelling aspects for the application of trait- and lineage-based models (e.g. exposure duration, effect endpoints), and facilitates their incorporation into ecological risk assessment.

4.01.04
Development of a Physiologically-Based Toxicokinetic Model of BPA in Fish Including Its Two Main Metabolites, Bpa-Glucuronide and Bpa-Sulfate
C. Mit, INERIS / Models for Ecotoxicology and Toxicology METO; A. Bado-Nilles, INERIS / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); R. Beaudouin, INERIS / Models for Ecotoxicology and Toxicology METO
Physiologically-based toxicokinetic models (PBTK) are useful tools to improve understanding of the fate of a chemical inside an organism. Recently, Grech et al. (2019) proposed a generic PBTK model for four different fish, including zebrafish (*Danio rerio*), threespine stickleback (*Gasterosteus aculeatus*), rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*). Meanwhile, bisphenol A (BPA) is considered as very high concern substance due to its endocrine disruptor function, its high production volume and its persistence in the aquatic environment. In this work, the PBTK will be adapted to accurately model the ADME processes (absorption, distribution, metabolization and excretion) of BPA. A particular effort will be carried out to predict internal concentration of its two main metabolites, BPA-monoglucuronide (BPA-g) and BPA-monosulfate (BPA-s), in various organs. *In vitro* data from zebrafish, rat (Sprague-Dawley) and mouse (CD-1) hepatocytes will be used to calculate Michaelis-Menten parameters related to metabolism. Moreover, TK data in zebrafish and rainbow trout for BPA, BPA-G and BPA-S in different organs will be retrieved from literature to calibrate the model. The objective will be to accurately predict TK data measured *in vivo* in external datasets. Further development will later add TD sub-models to predict the dynamic of biomarkers measured in organs.

4.01.05
Constructing Conceptual Models for Ecological Risk Assessment Using Bayesian Networks
W.G. Landis, A.J. Markiewicz, Western Washington University / Institute of Environmental Toxicology and Chemistry
The building of a proper conceptual model is foundational step in ecological risk assessment. However the process of constructing the framework is not discussed in detail in most publications, ours included. Now that Bayesian networks (BNs), case learning and datamining tools are being used it is even more important. In the case of BNs there are practical considerations as to how many connections can be made to a node and the resulting size of the conditional probability table. There are also trade-offs between having more or less categories in the discretization of the various nodes. Additionally, it is also key that if the model is assumed that it represents a causal relationship, that the lines of influence are drawn only when the interaction is supported by evidence. In the simplest case the transformation can appear to be straight forward. In Landis et al. (2020) the basic relative risk model was made specific to the risk due to organophosphate pesticides and water quality parameters to Chinook salmon in four watersheds. The presentation will focus on those more detailed cases such as we are now experiencing on our Upper San Francisco Estuary risk assessment and the risk assessment for microplastics in San Francisco Bay. The final conceptual model had different pathways for the toxicological effects and the water quality effects. The effects from each pathway were combined into a Leslie matrix age structured population model for Chinook salmon. While Landis et al. 2020 only involved one pesticide, Mitchell et al. (2021) had multiple OPs and focused on just the Yakima river and was able to break it up into further risk regions and examined the metapopulation dynamics as well. The presentation will focus on more detailed cases such as we are now experiencing on our Upper San Francisco Estuary risk assessment and the risk assessment for microplastics in San Francisco Bay.

4.01.07
A Prototype Bayesian Network for the Environmental Risk Assessment of Pharmaceuticals in Norway
S. Welch, NIVA - Norwegian Institute for Water Research / Ecotoxicology; K. Olsen, Norwegian Institute of Public Health / WHO Collaborating Centre for Drug Statistics Methodology; J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; M. Nouri Sharikabad, Norwegian Institute of Public Health / WHO Collaborating Centre for Drug Statistics Methodology; K. Tollefsen, NIVA -

Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; M. Grung, NIVA / Ecotoxicology and Risk Assessment

Despite rising awareness of the role of pharmaceutical pollution in the fractured and growing environmental risk landscape, the environmental risk assessment of this class of chemicals is marred by the sparsity and fragmentation of effects data and measurements. Current predictions of risk must, therefore, contend with the inclusion of considerable uncertainty, creating a situation where different assessments of the same chemicals can produce wildly different results. Bayesian Networks – causal networks of nodes linked by conditional probability distributions – have since their formal inception in the 1980s presented a potential alternative for modelling relationships between pollutants, environmental conditions and risk, permitting for a more nuanced prediction that includes quantified uncertainty. Here, we present a prototype Bayesian network for the prediction of environmental risk in Norwegian surface water from pharmaceuticals, using sales data from the Norwegian Drug Wholesales Statistics, Norwegian Institute of Public Health to predict environmental concentrations and publicly available Predicted No-Effect Concentrations as a measure of toxicity. In the future, we intend to expand this Bayesian network to incorporate the effects of projected population, demographic and climate change, as well as mixture effects between substances with similar modes of action.

4.01.09

Probabilistic Risk Assessment of Pesticides for a Norwegian Case Study: A Bayesian Network Approach

S. Mentzel, NIVA - Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment; M. Grung, NIVA / Ecotoxicology and Risk Assessment; K. Tollefsen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; M. Stenrød, Norwegian Institute of Bioeconomy Research NIBIO / Department of Pesticides and Natural Products Chemistry; K. Petersen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment

The aquatic environment is constantly exposed to various anthropogenic chemicals caused by activities, such as the use of plant protection products in agricultural practices. Often traditional Environmental Risk Assessment is based on a calculated risk estimation, usually quotient representing a ratio of exposure to effects, in combination with assessment factors to account for uncertainty. In this study, we explore a more informative approach using probabilistic risk assessment, where conditional probability distributions for exposure and effects are propagated throughout the model and enabling better accounting for variability and uncertainty. We focus on the risk assessment of various pesticides in a representative study area in the south east of Norway. Exposure data in this research was provided by the Norwegian Agricultural Environmental Monitoring Programme (JOVA)/ or predicted exposure concentration from a pesticide exposure model, and effect data was derived from the NIVA Risk Assessment database. A Bayesian Network model is used as an alternative probabilistic approach to assess the risk posed by pesticides. Bayesian Networks can serve as meta-models that link selected input and output variables from several separate project outputs and offer a transparent way of evaluating the required characterization of uncertainty for Environmental Risk Assessment. They can predict the probability of several risk levels, while facilitating the communication of estimates and uncertainties.

Assessment and Management of Wastewater Effluents

4.02.01

What's New in New Test Methods for Assessing Whole Effluent and Receiving Water Toxicity

J.M. Lazorchak, U.S. Environmental Protection Agency / Office of Research and Development Cincinnati; T. Norberg-King, U.S. EPA ORD NHEERL/Mid-Continent Ecology Division Laboratory / National Health and Environmental Effects Research Laboratory

EPA currently has aquatic toxicity testing methods for effluent and ambient testing that were promulgated in 2002. Currently, EPA's Whole Effluent Toxicity (WET) test methods include the freshwater toxicity test methods for acute lethality include cladocerans (*Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*) and three fish species (fathead minnow (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), and brown trout (*Salvelinus fontinalis*)). EPA's WET short-term chronic freshwater toxicity test methods include three species, a cladoceran (*C. dubia*), the fathead minnow (*P. promelas*), and a green alga (*Raphidocelis subcapitata*). Beginning in 2020, ORD has been working on two test species to expand the possible options for species sensitivity selection for ambient and effluent toxicity testing. In the first stage, EPA will design and develop test methods for the short-term chronic test with the cladoceran, *D. magna*, and acute and short-term chronic test protocols for the freshwater mussel (fatmucket, *Lampsilis siliquoidea*). Both the Duluth (mussels) and Cincinnati (*D. magna*) laboratories within ORD will be developing these test protocols and refining the various test conditions and test acceptability criteria for each test species within the EPA/ORD laboratories. A second phase will include plans to develop test methods for additional species. These will potentially include acute and short-term chronic methods for the mayflies, amphipods, and midges; a short-term chronic test procedure using trout; and possibly acute and/or short-term chronic testing methods for additional algae species and aquatic plants. The development of WET tests for these species will provide significant new tools to aid in the detection of both known and unknown chemical and biological contaminants in wastewater and ambient water quality testing for aquatic life protection. The eventual standardization and laboratory(s) validation of these new WET tests will support testing programs within EPA's Office of Water, EPA Regions, States, and other aquatic regulatory programs. This presentation will present the latest progress on the development of a short-term *Daphnia magna* chronic method which is a modification of a previous publication by Lazorchak, Smith and Haring, 2009 and a short term survival and growth method with *L. siliquoidea*.

4.02.03

Eco-Toxicological Characterization of Municipal Wastewater Treatment Plant Effluents in South Africa

K. Apetogbor, O.K. Pereao, Cape Peninsula University of Technology / Environmental And Occupational Studies; B. Opeolu, Cape Peninsula University of Technology / Faculty of Applied Sciences

Wastewater treatment plants (WWTPs) discharge complex mixtures of industrial and domestic effluents into receiving waters. This often leads to the contamination of receiving waterbodies; the effective management of surface waters therefore necessitates the acquisition of empirical data for informed decision-making. Some physicochemical indicators of water quality were measured to determine effluent quality relative to regulatory limits. The potential risk of WWTPs discharge into waterbodies was evaluated using toxicity bioassays. A battery tests using a primary producer - *Raphidocelis subcapitata*, a consumer -*Daphnia magna* and a decomposer - *Tetrahymena thermophile* were conducted. Physicochemical parameters' values were all within the regulatory limits. The pH values ranged between 6.8 – 8.38, temperature was between 18.8 – 22.2°C and dissolved oxygen (DO) was 1.9 – 23 mg/L. The respective values for chemical oxygen demand (COD), total dissolved solids (TDS) and electrical conductivity (EC) were 21–25 mg/L, 394 – 410 mg/L and 60.6 – 63 mS/m. The effluent was eutrophic in the first 24 h of algae exposure but growth was inhibited after 48 h until 72 h of up to ten orders of magnitude over a sampling period of 3 months. There was 10% mortality of daphnids exposed to the whole effluent. *T. thermophile* growth was inhibited on exposure to the effluent. The NOEC value for the effluent was > 6.25% effluent v/v. Results of the effluent toxicity units (TUs) were calculated and effluent classified as Class II (Slight acute toxicity). This suggested that the effluent was not acutely toxic to algae and *D. magna*. More studies are needed to study ecological effects of chronic exposure of aquatic biota.

4.02.04

Developing and Implementing an Effluent Management Program to Support Pharmaceutical Manufacturing

K. Block, J. Brenchley, K. Kenny, MSD / Global Safety & the Environment; J.G. Tell, Merck & Co., Inc. / Global Safety and the Environment; J. Vestel, MSD / Global Safety and the Environment; L. Ziv, MSD / Global Safety & the Environment

Since 1990, MSD has had an EHS management program in place to assess and control unsafe water discharges from our manufacturing plants. Over time, our program has grown and evolved to address new challenges like corporate mergers and acquisitions, expanding supply chains, advances in chemical treatment and analysis, new regulatory guidance and changing portfolios of products. Practices that were once considered "above and beyond" are common best practices today. However, it is critical that companies remain agile in order to rapidly respond to new threats, such as the environmental dimension of antimicrobial resistance (AMR). This poster presents a collection of tools and resources that can inform a successful EHS management program for water discharges in today's rapidly changing

climate.

4.02.05
Investigating Predictive Tools for Refinery Effluent Hazard Assessment Using Stream Mesocosms
C. Gelber, TOTAL S.A. / PERL; J. Postma, Ecofide; K. Cailleaud, Total Petrochemicals France / Centre scientifique et technique Jean-Féger; A.D. Redman, Exxon Mobil; P. Leonards, Vrije Universiteit Amsterdam / Environment & Health; M. Hjort, CONCAWE
Managing oil refinery effluents using risk-based approaches is challenging due to their compositional complexity. Hydrocarbons (HCs) are key components of these effluents and a weight of evidence approach is often required to assess their risk contribution. Several predictive tools are proposed, from chemical characterisation assessment (e.g. Biomimetic Extraction Solid Phase Microextraction [BE-SPME]), to modelling approaches linking HC composition to toxicity (e.g. PETROTOX and two-dimensional gas chromatography [GCxGC] analysis), and whole effluent toxicity assessments using standard bioassays. This study aims at investigating the value of these tools with regard to ecological shifts observed in stream mesocosm studies. Artificial streams were dosed with six different loadings, mimicking oil refinery effluent HC composition. Mesocosms were treated for 21 d followed by a 21 d recovery period. BE-SPME and GCxGC analyses were done to follow actual exposure, while shifts in ecosystem communities (e.g. diatoms and macro-invertebrates) were monitored to determine ecological effects. In addition, a laboratory study with a low and high energy mixing with similar loadings was performed to compare experimental approaches. In the mesocosms, strongest effects were observed for macro-invertebrates in all treatments ≥ 0.15 mg/L. The laboratory high-energy mixing exposure showed comparable or slightly lower effects. The Lowest Observed Effect Concentration (LOEC) for in-stream exposure of 0.15 mg/L corresponded on average to a BE-SPME concentration of 2.5 mM and an acute toxic unit of 0.08 (based on an organism of median sensitivity). The findings are in line with expectations from previous studies (e.g. Cailleaud et al., 2019). Overall, the community shifts were correlated with the HC bioavailable fraction. BE-SPME measurements were used to extrapolate results of the laboratory and field datasets, since they represent different exposure conditions. The study demonstrated the potential of the predictive tools and the robustness of the stream mesocosm design to improve understanding of environmental effects posed by refinery effluents.

4.02.10
Fluorescence-Based Detection of Optical Brighteners in a Peatland
M. Pons, UL-CNRS-LRGP; M. Laviale, University of Lorraine / LIEC UMR 7360 CNRS; V. Felten, LIEC / LIEC CNRS UMR
The Morte-Femme peatland (Vosges Mountains, Northeastern France) is a protected area receiving treated wastewater from several textile industries. In spite of the treatment, these effluents still contain non-biodegradable micropollutants related to the textile process. Composed of highly substituted aromatic structures, optical brighteners (OBs) are water-soluble substances with a high affinity for cellulose fibers. OBs are used by the textile industry to mask the yellowing of cotton fabrics, as the blue light (400 – 440 nm range) they emit after UV excitation makes the fabrics appear whiter to the human eye. OBs can be quantified by HPLC-based techniques but this requires an expensive equipment. Furthermore, the exact nature of the OBs is generally unknown. Fluorescence-based methods are preferred for routine monitoring (Cao et al., 2009). For this purpose, yhe presence of OBs in the different watercourses running across the peatland has been assessed by differential synchronous fluorescence (i.e. before and after irradiation by UV (365 nm), with synchronous fluorescence spectra collected with a gap of 50 nm between excitation and emission) (Assaad et al., 2014). In order to discriminate OBs from the humic substances naturally present in the water samples, different irradiation durations have been tested. If a 15min irradiation is sufficient to detect OBs in solution in pure water, the presence of other elements (salts, dissolved organic matter, etc.) seems to hinder the irradiation effect. Therefore the effect of the irradiation duration has been tested on synthetic solutions of several OBs in presence of salts, organic matter and other potential textile additives. The final procedure has then been applied in the framework of the monitoring of the quality of the peatland surface water over two years. The results are discussed with respect to the streams natural discharge rate, which is highly dependent upon the season, and the textile industry activity. References: Cao, Y., Griffith, J. F. & Weisberg, S. B. (2009) Evaluation of optical brightener photodecay characteristics for detection of human fecal contamination. Water Res. 43, 2273–2279.Assaad A., Pontvianne S., Pons M.N. (2014) Photodegradation-based detection of fluorescent whitening agents in a mountain river, *Chemosphere* 100, 27–33.

4.02.12
Self-Cleaning Capacity of Lotic Ecosystems: Can Biofilms Retain Nitrogen of Anthropogenic Origin?
T. Loeffler, Institute for Environmental Sciences, University of Landau, Germany / Functional Aquatic Ecotoxicology; E. Bollinger, University of Koblenz-Landau, iES Landau, Institute for Environmental Sciences / iES, Institute for Environmental Sciences; A. Feckler, University Koblenz-Landau / iES, Institute for Environmental Sciences; R. Schulz, Institute for Environmental Sciences, University of Koblenz-Landau; S. Stehle, University Koblenz-Landau / iES, Institute for Environmental Sciences; J.P. Zubrod, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment
Natural biofilms are of central importance for carbon and nutrient cycling in lotic (i.e., stream) ecosystems. From a human-centric perspective, biofilms can retain nutrients from anthropogenic sources and thus contribute to the ecosystem service of clean drinking water. We assessed the retention capacity of biofilms along two second order stream systems within the biosphere reserve Vosges du Nord/Palatinate forest (at the boarder region between France and Germany). The study took place in eight stream reaches of which six receive discharge from wastewater treatment plant (WWTP) effluents and two served as unimpacted control reaches. The uptake of nitrogen (N) in biofilms from anthropogenic sources, which is enriched with the heavy isotope ^{15}N, was estimated by determining the isotopy of nitrogen ($\delta^{15}N$) after 4 weeks of deployment. Downstream of continuously working WWTP´s, a substantial increase in $\delta^{15}N$, exceeding 7‰, was detected, with the signal already being reduced within less then 300m further downstream and a reduction of up to 67% within 1km distance (maximum distance investigated). In contrast, at the two references reaches, no change in $\delta^{15}N$ was detected. These data highlight the ability of biofilms to retain anthropogenic N and thus to contribute to the self-cleaning capacity of streams, with the reduction of $\delta^{15}N$ signals further downstream of WWTP´s suggesting an immediate uptake of anthropogenic N. Based on these promising results, we argue that a better understanding of the dynamics of N-retention would additionaly promote the interpretation of biofilms as a sink of anthropogenic nutrients. These data could ultimately help to develop models for the nutrient retention and thus self-cleaning capacities of lotic ecosystems which support environmental management.

Bioaccumulation Assessment and BiER (Biotransformation and Elimination Rate) in Regulation of Chemicals

4.03.02
Importance of Dietary Uptake for In Situ Bioaccumulation of Systemic Fungicides Using Gammarus pulex As a Model Organism
J. Raths, Eawag / Department of Environmental Chemistry; J. Schnurr, University of Koblenz-Landau / iES Institute for Environmental Sciences; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment; J. Hollender, Eawag / Environmental Chemistry
Bioaccumulation from contaminated allochthonous food sources, such as detritus from pesticide treated plants, might pose a currently underestimated risk for detritivore invertebrates such as *Gammarus pulex*. This assumption is substantiated by environmental monitoring data pointing to discrepancies of tissue concentrations modelled solely based on water concentrations compared to measured internal concentrations. This discrepancy spanned several orders and was mostly observed for systemic pesticides, compounds that are absorbed and circulated through the tissues of treated plants. In order to assess the relative importance of aqueous and dietary exposure, gammarids were exposed to leaves from trees treated with a systemic fungicide mixture (i.e., azoxystrobin, cyprodinil, fluopyram and tebuconazole). The treatment of trees was realised by soil drenching simulating runoff from adjacent agricultural fields or direct treatment for tree protection. Two groups of gammarids were simultaneously exposed in one basin containing contaminated leave material. One group was allowed to feed on the leaf material, a second group restrained from feeding by cages. Medium and gammarid tissues were sampled in regular intervals and analysed by LC-HRMS/MS. Thereby the dynamics of fungicides in the test system were characterised.

Internal fungicide concentrations of gammarids with access to leaves were significantly higher than in caged gammarids. Medium concentrations of leached fungicides differed depending on the initial leaf concentrations and physico-chemical properties. In accordance with literature, additional food choice experiments showed that gammarids did not discriminate between control and contaminated leaves pointing to the relevance of this pathway in the field. The present study demonstrated the potential impact of the dietary uptake on *in situ* body burdens of benthic invertebrates. The obtained uptake and elimination rates also provide a helpful aid for modelling of environmental exposure. More importantly, the uncovered significance of the dietary uptake for detritivores raises concerns for higher trophic levels by trophic magnification. This warrants further consideration from a scientific but also regulative perspective.

4.03.03
One and Multi-Compartments Toxicokinetic Predict Metal Organotropism in Gammarus fossarum at Environmental Concentrations
O. Gestin, Université Lyon 1, UMR CNRS 5558; O. Geffard, INRAE Lyon / UR RIVERLY Laboratoire Ecotoxicologie; C. Lopes, Université Lyon 1, UMR CNRS 5558 / Laboratory of Biometry and Evolutionnary Biology; T. Lacoue-Labarthe, LIENSs CNRS & Université de la Rochelle UMR 7266
Previous work (see abstract of Gestin et al., 2020) demonstrated the interest to apply one and multi-compartments toxico-kinetic models to describe metal organotropism in gammarids (*Gammarus fossarum*). Toxico-kinetic modelling, coupled to Bayesian inference, revealed the transient and storage roles of organs, such as intestine and caeca, respectively. However, these previous works are based on elevated dissolved metal concentrations and did not include the gills despite of their key role in metal bioaccumulation. Indeed, in waterborne contamination conditions, gills are supposed to be the main organs for influx and efflux of metals in the ADME (Accumulation, Distribution, Metabolization and Elimination) processes. To be more relevant from an environmental point of view, we had set up experiments with ^{109}Cd-labelled water in exposure phase to work at a concentration lower than 10 ng Cd.L^{-1}. Indeed, radio-isotopic metals allow at quantifying very low trace element concentrations in small organisms and their organs at environmental conditions. Gammarids were exposed for 7 days to dissolved radiolabeled Cd of 24 ± 17 kBq.L^{-1} (i.e. 4.36 ± 3.13 ng Cd.L^{-1}) and then placed for 10 days in depuration conditions (clean water). All along the experiment, at several sampling days (days 1, 2, 3, 4, 7, 8, 9, 11, 15 and 17), 20 gammarids were dissected and separated in four pools for each organ (caeca, cephalon, gills, intestine and remaining tissues) (n=5) before gamma counting. A one-compartment TK model was fitted, by Bayesian inference, to each organ dataset separately, leading to an estimation of TK parameters (i.e. uptake and elimination rates known as k_u and k_e) specific to each organ. Then, a complete multi-compartments TK model was developed, associated to an iterative inference process to select the more parsimonious multi-compartments model that best fits all organ datasets simultaneously. These results provided information on the *in vivo* Cd management by gammarids at environmentally relevant concentrations that were discussed regarding models previously computed following exposure at high concentration of 11 µg Cd.L^{-1}. Finally, this approach will be applied to ^{110m}Ag and ^{65}Zn allowing discussion on contrasting metal management with respect to the essential or non-essential character of trace element. Based on the first experimentation data, gills could be a relevant candidate in uptake of dissolved Cd.

4.03.04
Toxicokinetics of Ag Nanomaterials in the Soil Model Enchytraeus crypticus (Oligochaeta) - Impact of Aging and Concentration
F. Santos, Department of Biology & CESAM - University of Aveiro / Biology &CESAM; P.d. Tourinho, VSCHT Praha / Biology &CESAM; J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience Terrestrial Ecology; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science; M. Amorim, Universidade de Aveiro / Biology &CESAM
Silver (Ag) is among the most used nanomaterials (NMs), introduced in many products given its properties, e.g. antimicrobial. Their wide use leads to an increasing release into the environment hence, it is important to assess its environmental impact. The aim of this study was to investigate the toxicokinetics of Ag materials (Ag NM300K and $AgNO_3$) using the soil invertebrate *Enchytraeus crypticus* (Oligochaeta, Enchytraeidae). The standard bioaccumulation test was performed (OECD, 2010) consisting of 14 days for uptake followed by 14 days in clean soil. Two concentrations were selected based on sub-lethal effects on enchytraeid reproduction, and two aging periods, 3 and 14 days after spiking. During the test, animal and soil samples were collected at several sampling times. Body concentrations were related to total and 0.01 M $CaCl_2$ extractable Ag concentrations in the Lufa 2.2 test soil. In all cases, the enchytraeids showed increasing Ag uptake with time, as well as a decrease when transferred to clean soil. The toxicity and toxicokinetics of silver differed depending on the Ag material, aging and concentrations, e.g. for NM300K at the highest concentration survival was only possible in the aged soil. As to uptake, for $AgNO_3$ and NM300K, aging reduced the available fraction of Ag. For $AgNO_3$ uptake was higher for the higher concentration and seemed less variable when exposed to aged soil. Uptake of Ag from NM300K was overall more variable and without reaching equilibrium within the test period. These observed differences are showing the importance of toxicokinetics studies.

4.03.05
Temperature-Dependent Toxicokinetics of Phenanthrene in Enchytraeus Albidus (Oligochaeta)
W. Dai, Aarhus University (AU) / Department of Bioscience; S. Slotsbo, Aarhus University / Department of Bioscience; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science; M. Holmstrup, Aarhus University / Department of Bioscience
Although the toxicokinetics of organic pollutants in soil invertebrates under optimal and constant temperature has been widely reported, their uptake, elimination and bioaccumulation under sub-optimal temperatures, and especially daily fluctuating temperature (FT) regimes have received only little research attention. In this study, the uptake, elimination and bioaccumulation of phenanthrene (PHE) in *Enchytraeus albidus* (Oligochaeta) under different constant temperatures (CT) and a FT regime were investigated in a natural soil. In general, the PHE concentrations in worm tissues reached steady state within 14 days at different temperatures. The uptake (k_u) and elimination (k_e) rate constants and the bioaccumulation increased with increasing temperature likely because of an increased diffusivity of PHE into the worms and an increased metabolic rate. Interestingly, the bioaccumulation factor of PHE in *E. albidus* showed a positive relationship with temperature because the slope of the k_u-temperature relationship was larger than that of the k_e-temperature relationship. Further, the uptake and elimination rate constants were larger under the FT regime than at the constant average of the fluctuating temperature. These findings suggest that climatic conditions, especially daily fluctuating temperatures, should be considered for the assessment of the toxicokinetics of organic pollutants in terrestrial organisms.

4.03.07
The Role of Cytochrome P450 Metabolism in the Clearance of Antipsychotic Drugs in Rainbow Trout In Vitro
T. Pihlaja, University of Helsinki / Faculty of Pharmacy, Drug Research Program; J. Sinisalo, University of Helsinki / Faculty of Pharmacy; E. Ollikainen, T. Sikanen, University of Helsinki / Faculty of Pharmacy, Drug Research Program
The residues of antipsychotic drugs in the environment have been shown to bioconcentrate and cause behavioral changes in fish. Xenobiotic metabolizing enzymes and hepatic clearance pathways in fish are similar to those in humans, including cytochrome P450 (CYP), UDP-glucuronosyltransferases, sulfotransferases, and glutathione-S-transferases. The CYP mediated oxidoreductive reactions form a critical bottleneck in the clearance of such pharmaceuticals that cannot readily undergo conjugation via the other pathways. In the environment, fish are simultaneously exposed to a variety of chemical residues, which may interfere with the metabolic clearance, and thus elevate the bioconcentration, of one another. In the present study, we examine the importance of CYP metabolism in the clearance of selected antipsychotic drug residues in rainbow trout in vitro. The hepatic clearances of haloperidol, levomepromazine, and risperidone in rainbow trout *(Oncorhynchus mykiss)* were determined using S9 liver fractions in accordance with the OECD 319b test guideline, and diclofenac as the positive control. The intrinsic clearances were determined with and without NADPH (the critical cofactor for CYP reactions). The cofactors of the transferase reactions were included in the enzyme incubation in both cases. In addition, the half-maximal inhibitory concentrations (IC50) of ethoxyresorufin *O*-deethylation (EROD, CYP1A) and benzyloxy-4-trifluoromethylcoumarin *O*-debenzylation (BFCOD, CYP3A) were determined for each compound. The time-dependent nature of EROD and BFCOD inhibition was examined based on IC50 shift upon pre-incubation of the drug substances with the cofactors before initiation of the model reactions. Similar to diclofenac (control compound), the hepatic clearance of levomepromazine was shown to be

dependent on CYP metabolism being 26 mL/h/g liver and 1.4 mL/h/g liver in the presence and absence of NADPH, respectively (n=9 timepoints each, in duplicate). Levomepromazine was also a fairly potent EROD inhibitor (IC50 < 100 µM). Haloperidol and risperidone were not metabolized in vitro, but haloperidol was a potent and time-dependent inhibitor of EROD (IC50 < 100 µM). None of the test compounds inhibited BFCOD at concentrations below 100 µM. The results suggest that antipsychotic drug residues are poorly metabolized in rainbow trout in vitro and may affect the hepatic clearance of other pharmaceuticals by inhibiting particularly their CYP1A dependent metabolism.

4.03.08

Comparison of Biotransformation Rates of Fragrance Ingredients in Liver S9 Sub-Cellular Fractions From Common Carp and Rainbow Trout

H. Laue, L. Hostettler, Givaudan Schweiz AG / Fragrances S & T; G. Sanders, Givaudan International SA / Regulatory Affairs & Product Safety; K. Jenner, Givaudan / Global Regulatory Affairs & Product Safety; A. Natsch, Givaudan Schweiz AG / Fragrances S &T

Bioaccumulation in aquatic species is a critical endpoint in the regulatory assessment of chemicals which usually involves the determination of the bioconcentration factor (BCF) in fish (OECD TG 305). In vitro systems measuring biotransformation rates of chemicals (OECD TGs 319A/B) have been established as alternative methods to refine in silico BCF models which are based on hydrophobicity (i.e. log Kow). Whereas different fish species are recommended in TG 305, the in vitro methods have been validated for hepatocytes and liver S9 fractions (RT-S9) from rainbow trout, although these in vitro methods can also be used for other fish species as outlined in the Guidance document GD 280. 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 determine in vitro biotransformation rates of 10 different fragrance chemicals in Common Carp-S9 fractions and compare these data to RT-S9. Enzyme activities of the carp-S9 were measured using model substrates for CYP1A (7-ethoxyresorufin-dealkylation, EROD), uridine diphosphate glucuronosyltransferase (UGT) and glutathione transferase (GST). Additionally, substrate depletion assays were performed with pyrene as reference and 10 fragrance chemicals in carp-S9 according to TG 319 B with two modifications: 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.Whereas EROD and UGT activities were within our historical range of activities for different RT-S9 batches, GST rates were lower in carp-S9. In vitro intrinsic clearance of pyrene was around 14-fold lower in carp-S9 compared to RT-S9. For 9 fragrance chemicals, the difference was within a factor of 2 except for a 3-fold higher rate for one isomer of Javanol in RT-S9 with no general trend that the rate was in one system higher than in the other. Only for one fragrance chemical (Cyclohexyl Salicylate) a 6-fold lower rate was observed in carp-S9. Since there is currently no specific IVIVE model for carp available, BCFs were predicted using the IVIVE model for rainbow trout with some modifications and compared to the in vivo BCFs which were determined in carp. This dataset gives a first estimate on the effect of in vitro/ in vivo species matching for bioaccumulation prediction of industrial chemicals.

4.03.09

Environmental Risk Assessment of Pharmaceuticals: Development and Evaluation of a Generic Physiologically Based Kinetic Model in Fish

J. Wang, Radboud University Nijmegen / Environmental Science; A.M. Ragas, Radbound University / Environmental Science; J.A. Hendriks, Radboud University / Environmental Science

With the increase in medical and veterinary use, pharmaceuticals are detected in aquatic organisms such as fish, potentially imposing adverse effects on organism survival and fitness and the entire ecosystem health. Physiologically based kinetic (PBK) models have become important risk assessment tools since they allow a mechanistic approach to understanding chemical effects within organisms. Fish is often selected as a model organism in PBK modelling of pharmaceuticals. However, PBK models require a large number of species- and chemical-specific data that are not readily available. Meanwhile, fish PBK models designed for ionisable pharmaceuticals are rare. The fate and toxicity of pharmaceuticals could vary substantially in fish depending on environmental pH. Therefore, our study aimed to develop and evaluate a generic fish PBK model for pharmaceuticals, integrated with allometric scaling and quantitative structure-activity relationship (QSAR) models. An extensive literature search and meta-analysis was performed for anatomical and physiological parameters in fish. Organ weights were successfully related to fish body mass through allometric scaling. Cardiac output and oxygen consumption rate were successfully related to temperature and fish mass through the Boltzmann-Arrhenius kinetics. Blood flow parameter values in fish were scarce. Chemical-related parameters (e.g. various partition coefficients) were estimated and compared through reported QSARs based on octanol-water partition coefficient (K_{ow}), taking the water pH influence into account. Consequently, only the fish body mass, the octanol-water partition coefficient and the exposure scenario were required for input. Overall, the acquired mechanistic-based regressions relieved intensive data requirements and were used in generic fish PBK models. We apply the model to several pharmaceuticals covering $\log K_{ow}$ values from -0.9 to 4.5. Model performance is evaluated by comparing estimated concentrations with measured data in respective organs in fish. A sensitivity analysis is carried out to determine the most influential parameters on model outputs. Our mechanistic-based approach to minimising input parameters can be applied in investigating any fish species and pharmaceutical of concern. The development of the generic fish PBK model for pharmaceuticals will facilitate ERA of emerging pollutants.

4.03.10

Critical Evaluation of In Vitro and In Vivo Rodent Biotransformation Databases

A. Sangion, University of Toronto Scarborough and ARC Arnot Research and Consulting Inc. / Department of Physical and Environmental Sciences; J.M. Armitage, AES Armitage Environmental Sciences, Inc / Physical and Environmental Sciences; K.L. Foster, A.B. Looky, ARC Arnot Research and Consulting. Inc; M.R. Embry, Health and Environmental Sciences Institute (HESI); E. Papa, University of Insubria / Department of Theoretical and Applied Sciences; J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology

Toxicokinetic (TK) processes such as chemical absorption, distribution, biotransformation and excretion (ADBE) are fundamental to better understand relationships between external and internal exposures, and to assess bioaccumulation in various organisms. Mathematically these processes can be represented using Physiologically-Based Biokinetic (PBK) models that simulate ADBE processes. PBK models are required to examine, interpret, and apply in vitro and in vivo TK data and to address extensive data gaps for chemical hazard, exposure and risk assessment. Traditionally PBK models have required many compound-specific inputs that mainly rely upon animal studies; however, New Approach Methodologies (NAMs) for hazard and TK data are being developed including high-throughput TK models for humans and other species. In this context, we have complied and critically evaluated databases of *in vivo* and *in vitro* TK parameters for chemicals tested in rodents to provide a comprehensive basis for further investigation of the In Vitro-In Vivo Extrapolation (IVIVE) and the evaulation of PBK models. *In vitro* data comprise > 8,000 biotransformation rate estimates ($CL_{int, invitro}$) derived from microsomal, S9 homogenate, and hepatocyte-based assays for >4,000 organic chemicals. The *in vivo* database includes > 1,000 TK parameters for > 600 compounds tested in rats and mice. Each entry was obtained from experiments on healthy animals (i.e., not toxicity tests) and reported at least one relevant TK parameter (i.e. area under the curve (AUC), rate of elimination (k_{el}) or clearance rate (Cl)). In addition to the collection of the TK parameters, complementary information was collected to aid the analysis and the evaluation of the data quality. Data quality assessment methods based on the OECD Guidelines for the Testing of Chemicals have been developed and applied to address variability and uncertainty in the data. Compounds present in both *in vivo* and *in vitro* rodent TK databases were used to test different assumptions in the IVIVE and to evaluate a general PBK model. Reliable *in vitro* and *in vivo* data are required for the development and application of reliable *in silico* tools (i.e., PBK and Quantitative Structure-Activity Relationship (QSAR) models). This work seeks to identify datasets that are most appropriate to test and evaluate PBK models, to address uncertainty IVIVE modeling and PBK models for high throughput screening-level bioaccumulation, exposure, and risk assessment.

4.03.12

Assessment of Toxicity, Bioaccumulation and Biotransformation Products of Selected Antidepressants by Using the Organisms From Different Trophic Levels

M. Wawryniuk, Medical University of Warsaw; A. Drobniewska, A. Maciejczak, P.

Stankiewicz, G. Nałęcz-Jawecki, Medical University of Warsaw Faculty of Pharmacy / Department of Environmental Health Sciences
For many years antidepressants have been characterized by very high dynamics of consumption growth. Constantly increasing sales of these drugs may recently be additionally enhanced by rise of depressive disorders cases observed during pandemic of COVID 19. It is also estimated, that concentrations of antidepressants in the aquatic environment will continue to increase. Aquatic organisms have a very important role in the transformation of chemicals in the environment. Many studies show that the pharmaceuticals are toxic to aquatic organisms and their bioaccumulation could lead to long-term exposure of organisms on pharmaceuticals and even at low concentration, may have an adverse effect on population or whole aquatic ecosystems. Furthermore, the biotransformation products may be characterized by high biological activity. The environmental risk related with bioaccumulation of antidepressants and also their mechanisms of biotransformation in aquatic organisms have not been fully recognized.The aim of the research was a comprehensive evaluation of toxicity, bioaccumulation and biotransformation products of selected antidepressants: fluoxetine, mianserin, paroxetine and sertraline. The samples were tested using the biological and physicochemical methods. First point of research was the analysis of the toxicity and bioaccumulation by using three organisms: protozoa (*Spirostomum ambiguum*), crustaceans (*Daphnia magna*) and plants (*Lemna minor*). The organisms came from different taxonomic groups and trophic levels to reflect conditions that occur in ecosystems. Second step was the assessment of biotransformation products of antidepressants in the cells of organisms by UPLC-MS.The results show that the tested antidepressants were toxic for aquatic organisms used in the study. On the basis of the bioaccumulation tests, it was concluded that uptake and elimination kinetics of antidepressants in organisms varied greatly between the tested pharmaceuticals. It was observed that the protozoa were unable to excrete the accumulated antidepressants. On the basis of UPLC-MS/MS results 2-6 biotransformation products were detected in the organisms and the media. However, their concentrations were low in the relation to the parent compounds. Received results suggest that the future research should focus on antidepressants metabolism in aquatic organisms, accumulation in intracellular structures and impact of antidepressants on the next generations of organisms.

4.03.14
Bioaccumulation Screening of Neutral Hydrophobic Organic Chemicals in Air-Breathing Organisms Using In Vitro Liver S9 Biotransformation Assays
Y. Lee, Simon Fraser Univeristy / School of Resource and Environmental Management; T.R. Cole, Simon Fraser University / School of Resource and Environmental Management; M. Jhutty, Simon Fraser University / Department of Biological Sciences; B. Chee, Simon Fraser University / School of Resource and Environmental Management; M. Cantu, Simon Fraser University / Resource and Environmental Management; F. Gobas, Simon Fraser University / Resource & Environmental Management
To reduce effort, cost and animal testing and speed up bioaccumulation assessment, in vitro assays for measuring biotransformation rates in combination with in vitro-in vivo extrapolation (IVIVE) has been applied for bioaccumulation assessments of chemicals in fish. The Organisation for Economic Co-operation and Development (OECD) has developed test guidelines (TG 319A and 319B) to determine in vitro biotransformation rates using primary hepatocytes or liver S9 of rainbow trout for bioaccumulation assessment in fish. However, similar protocols for bioaccumulation screening in air-breathing organisms are currently not available. The objective of this study is to develop and test a refined bioaccumulation screening framework following OECD TG 319B for neutral hydrophobic organic chemicals in air-breathing organisms using rat liver S9 biotransformation assays. The refined framework consists of four main components, including (i) S9 preparation and characterization to determine the metabolic capacity of S9, (ii) preliminary experiments to optimize test conditions, (iii) final experiments to determine in vitro biotransformation rates in a standardized way, and (iv) analysis of assay results for bioaccumulation screening. The framework also includes the determination of the unbound fraction of the test chemical in the incubation, which is a key parameter in IVIVE but not included in the OECD test guidelines. The refined protocol was tested and evaluated using 14 test chemicals with a variety of chemical structures and biotransformation capacities, and 5 test chemicals were tested using both in-house prepared and commercial S9. The results indicate that (i) the measurement of lipid content of S9 is useful for the estimation of unbound chemical fractions in the incubation; (ii) significant differences in biotransformation rates were observed among S9 sources; and (iii) the use of a reference chemical in final tests can assist in the comparison and interpretation of the test results. The refined bioaccumulation screening framework can inform a weight of evidence approach to bioaccumulation assessment.

4.03.16
PBT Assessment of Saturated -alkanes, -iso, -Cyclics in the Frame of UVCB Substances . Discussion on Modeling End Points and Weight of Evidence
P. Adrian, CEHTRA SAS; S. Jubault, TOTAL Fluides; S. Gony, S. Segelle, E. Beltran, B. Journel, CEHTRA SAS; l. philippe, Total
PBT assessment is a key hazard assessment for chemicals. For certain chemicals like UVCBs the use of models allow to conclude on certain environmental end points. This presentation is dealing with important characteristics to take into account when choosing an appropriate strategy. An example is presented with the results of selected modelling characteristics of UVCB substance (saturated alkanes,-iso,-cyclics). One of the end point of interest concerns bioaccumulation. Indeed these saturated alkanes with high carbon range are leading to high calculated Kow values. This would lead to consider the substance as bio accumulable without taking into account that they are not bioavailable based on both very low water solubility and high steric size not allowing them to enter into biological cells. A simple approach is presented where the segregation of the substance in two categories i.e. one with isomers triggering high steric size and another pool of isomers with biodegradation potential to be assessed. Moreover, this approach is avoiding tests on aquatic vertebrate species.
Keywords: Bioaccumulation, Models, Alkanes

4.03.17
QSAR Advances in Biotransformation Assessment in Mammals: Main Results From the CEFIC-LRI ECO44 Project
E. Papa, University of Insubria / Department of Theoretical and Applied Sciences; L. Bertato, I. Casartelli, University of Insubria / QSAR Research Unit/Department of Theoretical and Applied Sciences; N. Chirico, University of Insubria / Department of Theoretical and Applied Sciences; A. Sangion, University of Toronto Scarborough and ARC Arnot Research and Consulting Inc. / Department of Physical and Environmental Sciences; J.M. Armitage, AES Armitage Environmental Sciences, Inc / Physical and Environmental Sciences; M.R. Embry, Health and Environmental Sciences Institute (HESI); J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology
The CEFIC-LRI ECO44 project “Integrating Bioaccumulation Assessment Tools for Mammals (iBAT-Mam)” has developed a state of the science toxicokinetic (TK) framework for bioaccumulation assessment for mammals by integrating multiple line of evidence (*in vivo*, *in vitro*, *in silico*). Critically evaluated empirical toxicokinetic data (e.g., *in vitro* intrinsic clearance and *in vivo* elimination half-lives) have been used for the implementation of several *in silico* models such as physiologically-based biokinetic (PBK) models and models based on quantitative structure activity relationships (QSARs) for multiple endpoints, i.e., biotransformation rates. These data sets and models can be used to-address uncertainty hazard, exposure and risk assessment for mammals. This poster summarizes the best outcome in terms of multiple linear regression QSAR models for key TK parameters. The poster describes a battery of QSARs based on CYP-450 mediated reactivity to predict *in vitro* biotransformation rates in hepatocytes and microsomes in rats, mice and humans. In addition, new molecular structure based models for the prediction of *in vitro* biotransformation rates in hepatic S9 fractions were developed for humans, rats and mice and are presented together with new QSARs to predict the total elimination half-lives (HL_T) in rats and mice, as well as the volume of distribution in humans. These new models have been developed following the OECD principles for QSAR models validation in order to guarantee model’s transparency, statistical validity, and reliable applicability, and represent a significant advancement in the field of biotransformation rate estimation science.

4.03.19
Understanding the Fluxes of Contaminants Between Water Column, Sediment and Biota by Means of Stable Isotopes Analysis
N. Garcia-Velasco, University of the Basque Country UPV/EHU / Dept. Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU); J. Carrero, University of the Basque Country UPV/EHU / Dept Analytical Chemistry; A. Gredilla, University of the

Basque Country UPV/EHU / Dept. Applied Chemistry; E. Urionabarrenetxea, University of the Basque Country UPV/EHU / Dept. Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU); J. Schäfer, 5UMR CNRS 5805 EPOC University of Bordeaux; A. de Diego, University of the Basque Country UPV/EHU / Dept. Analytical Chemistry, Research Centre for Experimental Marine Biology and Biotechnology; U. IZAGIRRE, UNIV BASQUE COUNTRY / Dept. Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU); M. Soto, University of the Basque Country / Zoology and Animal Cell Biology

The presence of high levels of ancient pollutants in estuarine sediments implies a potential environmental risk, mainly after physico-chemical alterations (i.e. dredging activities). The resuspension of contaminated sediments can compromise the survival of benthic species and alter the environmental quality of coastal zones. Hence, the determination of fluxes of pollutants between sediments, water and sentinel organism is crucial. Recently, stable isotope (SI) tracing has emerged as a useful tool to perform quantitative ecological assessments of pollutants among different biotic and abiotic compartments at environmentally relevant concentrations. This approach could improve the understanding of pollutants fate and impact in aquatic ecosystems. The aim was to determine sedimentation/resuspension rates and the bioavailability of pollutants for mussels and polychaetes after spiking SI into sediment and water column. For that, experimental tanks were filled with a sediment layer (6 cm, treated following ICES 2001) and seawater column. For the first experiment, water was dosed with 10 µg ^{65}Cu/L, 1 µg ^{109}Ag/L and 100 ng ^{194}Pt/L. In the second, air dried sediments were moisten with isotope solutions to obtain 500 µg ^{65}Cu/kg, 500 µg ^{109}Ag/kg and 50 µg ^{194}Pt/kg (stabilized for 4 m). 20 mussel (*Mytilus galloprovincialis*) and 10 polychaetes (*Hediste diversicolor*) were caged in a net and introduced into the sediment, respectively. In each experiment 3 treatments with 3 replicates each were performed: control, spiked (with SI and no organisms) and spiked with organisms. A continuous air flow, 18 ºC, 33 salinity, pH 7.5 and photoperiod (12 h:12 h light:dark) were maintained during 7 d in all tanks. After exposure, seawater, sediment, mussel and polychaete samples were collected for SI analysis by ICP-MS. Isotopically spiked sediments showed higher enrichments for ^{194}Pt than for ^{109}Ag and ^{65}Cu due to their natural amount in the samples. Mussels and polychaetes accumulated SI spiked in the different compartments. Hence, a flux of isotopes from the water column/sediment to the biota was established. SI analysis appeared to be an accurate method to decipher the environmental fate of removed pollutants and allow studying trace metal dynamics at environmentally relevant exposure levels; crucial for the management of polluted sediments and to regulate dredging activities.

Acknowledgements: Basque Gov. (IT1302-19, IT1213-19), U. Basque Country and MINECO (CTM2017-87766-R).

4.03.20

Bioaccumulation and Long-Term Biomonitoring of Mercury in the Terrestrial Food Chain

S. Mahat, University of Bern / Soil Science; L. Stanisic, University of Bern, Switzerland / Department of Soil Science; A.K. Weber, University of Bern / Department of Soil Science; M.P. Ryser, Vetsuisse / Center for Fish and Wildlife Health; R. Bühler, B. Almasi, Swiss Ornithological Institute; G. Heckel, University of Bern / Ecology and Evolution; A. Mestrot, University of Bern / Soil Science Group

The chemistry and biochemistry of mercury in aquatic environments has been studied extensively but the fate of mercury in the terrestrial food chain is understudied, although the majority of total mercury is estimated to be deposited in terrestrial environments. As a result, data on the transfer and bioaccumulation of mercury and methylmercury and the associated risks in terrestrial food webs is scarce, especially in Switzerland. Furthermore, the impact of the Minamata convention on mercury pollution signed in 2017 should be assessed, which implies the monitoring and quantification of the decline of mercury concentrations in terrestrial environments. The present study focuses on bioaccumulation of mercury and methylmercury in the terrestrial food chains in Switzerland and aims to develop a long-term biomonitoring plan. Firstly, an extensive literature review of European studies was conducted to determine the concentration of mercury and methylmercury in a range of animals and to assist for the selection of species, tissue samples and food chains for our study in Switzerland. Kidneys of roe deer were found as a potentially good indicator for mercury pollution and therefore, secondly, kidney, liver and muscle samples of roe deer were chosen for total mercury concentration (T-Hg) measurement. Among the tissues analysed for roe deers, kidneys had the highest concentration and few exceeded the maximum regulatory limit (1000 µg/kg) for total mercury in predatory fish species. Third, barn owl down feathers were analysed for T-Hg as a non-invasive long-term biomonitoring on a specific food chain consisting of soil – voles – barn owls. Results from unmolted barn owls indicate that intra-nest variation (i.e., mercury measurements in different juveniles from a same clutch) in mean T-Hg is in general higher than the inter-nest variation in mercury measurements. An observed positive linear relationship between the age of juveniles and T-Hg suggest that bioaccumulation is taking place over time. Finally, to determine the mercury concentration ratio between fur and organs, tissues of species of voles commonly eaten by barn owls were analysed. It is important to know this ratio in order to accurately assess the biomagnification in this food chain, hence, enabling us to determine the mercury load of the individual voles eaten by the studied barn owls by measuring mercury concentrations only in the fur contained in the pellets that are found in the barn owls' nests

4.03.22

Modelling Uptake of PAHs in Early Life Stages of Fish From Produced Water Sources in the Norwegian North Sea

R. Nepstad, SINTEF Ocean; B. Hansen, J. Skancke, SINTEF Ocean / Environment and New Resources

Oil extraction in the Norwegian North Sea region is associated with a continuous discharge of produced water to the sea.
The produced water will pass through a separation and cleaning process prior to discharge, but small amounts of hydrocarbons from the reservoir will remain, and these residual components may pose a risk to the marine environment.
Such components include polycyclic aromatic hydrocarbons (PAHs), some of which are persistent in the environment and have a potential for bioaccumulation.
Thus, studies of PAH fate and uptake pathways in marine life are relevant when assessing environmental risk of produced water discharges.
Assessment of environmental impacts from produced water discharges can make use of numerical models, which simulate the transport and dilution of the produced water plume in order to predict environmental concentration of the residual chemical constituents.
We have developed an individual-based model with a toxicokinetic component, coupled to an existing fate model for produced water, and have used this tool to investigate the exposure and uptake in fish early life stages (ELS) of 26 different PAH PW components from point sources in the Norwegian North Sea.
In our study we have run multi-year simulations of North Atlantic cod eggs and larve from three distinct spawning grounds in the North Sea, and have found that their trajectories may intersect the PW plumes from multiple sources.
The model results suggests that the internal composition of PAH components in the model super-individuals is different from the composition in the discharges, due to a combination of fate processes and toxicokinetics.
A large variability is found in the internal concentration levels among super-individuals in the model, while the total PAH levels are below thresholds commonly associated with acute narcotic effects.

4.03.24

The Effect of pH on Hydroponically Grown Wheat Plant Health and the Potential Impact on Plant Uptake Factor Testing

V.R. Peck, Fera Science Ltd / CCSS

The quantification of chemical uptake by plants can be used during exposure modelling to represent the proportion of a chemical taken up by a plant via the root system and transported to the shoots. These processes can reduce the mass of chemical available in the soil pore water and subsequently reduce the predicted concentrations in soil affecting the leaching potential to groundwater. A hydroponic study for quantifying uptake of chemicals via the root system of plants is currently being developed by the ECPA/IVA Working Group. The experimental design measures the principle ability for uptake and the availability of the compound in the soil pore water without the influence of adsorption or degradation. The methodology has previously been tested using a variety of plant species and requires the performance of the test at two different pH values if the test compound is ionisable (i.e. pKa between 2 and 8). There is a requirement that these two pH levels should however be tolerated by the test plant and not induce plant damage. An investigation into the health of wheat plants grown hydroponically at various pH levels has been performed using the proposed experimental design. Plants were grown from seed to ≥ BBCH 13, with

experiments performed at pH levels of 5.5, 6.0, 6.5, 7.0 and 7.5. Test conditions were optimised by modifying the nutrient solution through the use of chelating agents and alternative buffers, together with the testing of different wheat strains. Plant health demonstrated a consistent decline from pH 5.5 (the optimum pH level tested) to pH 7.5. Wheat grown at pH levels ≥ 6.0 demonstrated visibly poor health and a significant reduction in biomass when compared to plants grown at pH 5.5. Optimisation experiments improved the growth and health of the plants to some degree, however an overall poor plant health was observed for all wheat plants grown at pH ≥ 6.0. The effects of the test substance on plant uptake cannot be reliably compared between two pH levels if their plant health differs, and thus the applicability of the currently proposed range of pH levels may require further consideration.

Bioremediation and Phyto-Remediation of Contaminated Ecosystems

4.04.03

Role of Phyllosphere Bacteria in Degrading Pyrene on Holm Oak (Quercus ilex) Leaves

E. Terzaghi, University of Insubria (Como) / Department of Science and High Technology; R. Posada-Baquero, Instituto de Recursos Naturales y Agrobiologia de Sevilla CSIC; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiologia / Agroquimica y Conservacion del Suelo; A. Di Guardo, University of Insubria / Department of Science and High Technology

Improving the air quality in cities is currently one of the main challenges for the European Union. Many studies showed that urban trees could be "*Nature Based Solutions*" that, providing important ecosystem services, can contribute to air pollution reduction. For example, plants can uptake organic contaminants from air including Polycyclic Aromatic Hydrocarbons (PAHs). Moreover, these compounds can be used as carbon source by phyllosphere microorganisms contributing to their degradation. Few studies focused on the quantification of the actual contribution of these microorganisms to the natural attenuation of airborne PAHs in urban areas. Existing studies focused mainly on the composition of the phyllosphere microbial communities and on the isolation of PAH degraders rather than on the estimation of biodegradation half-lives for these chemicals. In this study we measured the partitioning of ^{14}C-pyrene (^{14}C-PYR) on leaves of an urban holm oak (*Quercus ilex*), and its mineralization by indigenous phyllosphere microorganisms and by a PAH degrader (i.e., *Mycobacterium gilvum*) inoculated on leaves. Mineralization was investigated in two environments (i.e., water vs. air) using two different experimental systems. Moreover, to evaluate the influence of traffic generated particulate matter (PM) (deposited on leaf surface) on partitioning and mineralization of pyrene, both unwashed and washed leaves were considered. Partitioning was evaluated by measuring leaf-water partition coefficient (K_{LW}) and estimating leaf-air (K_{LA}) partition coefficient. Moreover, pyrene mineralization rate (k) and biodegradation half-lives (HL) were calculated under different experimental conditions. The partition experiments provided a leaf-water partition coefficient (Log K_{LW} ~3.5 L kg^{-1}) and a leaf-air partition coefficient (Log K_{LA} ~1 m^3 g^{-1}) for holm-oak which were in the range of those reported in literature for other plant species. The mineralization experiments showed that degradation was faster on unwashed leaves resulting in biodegradation half-lives of 14 days and 127 days for unwashed and washed leaves respectively. The results highlighted that plant-PM-microbe interactions could have a role in air quality improvement. The data obtained in the current study are among the few data available on PAH mineralization on leaves and are important input parameters for multimedia fate models aiming at quantifying the "air quality improvement" ecosystem service provided by urban trees.

4.04.04

Sublethal Effects Caused by a Metal Mixture Exposure Are Mitigated in a Neotropical Teleost Through Lemna minor Phytoremediation

V. Bezerra, Universidade Estadual de Londrina; W.E. Risso, J. Rocha, State University of Londrina / Department of Physiological Sciences

Phytoremediation mediated by the aquatic macrophyte *Lemna minor* is a possible alternative to prevent that high metal concentration reaches aquatic ecosystems. However, no study has evaluated if this type of treatment really mitigates the sublethal effects caused by a binary mixture of cadmium (Cd) and nickel (Ni) in a neotropical fish. Thus, this study aims to assess whether phytoremediation mediated by *L. minor* really attenuates the sublethal effects induced by Cd and Ni mixture in the neotropical teleost *Prochilodus lineatus*. For this purpose, *P. lineatus* juveniles (n = 32; 27.2 ± 2.0 g, 14.2 ± 0.3 cm) were provided by a fish farm and were acclimated for 20 days in laboratory conditions. After acclimation, the animals were equally distributed into 4 groups: a control (CTR) group, exposed only to dechlorinated water; a phytoremediation control (PhytoCTR) group, exposed to dechlorinated water with *L. minor* on its surface; a group exposed to 20 µg L^{-1} of Cd and 1.5 mg L^{-1} of Ni, with *L. minor* on the water surface (PhytoMix group); and a group exposed only to the metal mixture (Mix group). After 96 h, the fish were anesthetized and a blood sampling was taken to evaluate micronucleus (MN) and erythrocytic nuclear abnormalities (ENA) frequency. The remaining blood was centrifuged to obtain the plasma, that was used to assess glycemia. Then, the animals were euthanized by cervical transection for removal of gills, liver, and muscle samples, which were used to evaluate Cd and Ni accumulation. Muscle samples were also used to assess acetylcholinesterase (AChE) activity. The reduction in dissolved metal concentration in the exposure media was not so different between PhytoMix and Mix group after 96 h. Cadmium and Ni reduced, respectively, 13.5% and 15.3% in PhytoMix group. In the Mix group Cd reduced 14.6% and Ni 14.5%. Perhaps, this occurred because the remediation mechanisms of *L. minor* in this exposure involved more phytostabilization than phytoextraction. In the animals there was accumulation of Cd and Ni in the gills and also only Ni accumulation in the muscle samples of PhytoMix and Mix group. However, hyperglycemia, a reduction in AChE activity and an increase in MN and ENA frequency were observed just in the Mix group. Therefore, we can conclude that a 96 h phytoremediation mediated by *L. minor* can attenuated hyperglycemia, neurotoxic and genotoxic effects, but not bioaccumulation, caused in *P. lineatus* due the exposure to Cd and Ni mixture.

4.04.05

Cd-Induced Alteration in Photosynthetic Performance of Rapes Affecting Cd-Phytoextraction Performance Under Warmer Climate

A. Dikšaitytė, Vytautas Magnus University / Department of Environmental Sciences; J. Zaltauskaite, Vytautas Magnus University / Department of Enviromental Sciences; I. Kniuipyte, Lithuanian Energy Institute / Laboratory of heat equipment research and testing; R. Juknys, Vytautas Magnus University / Department of Environmental Sciences

The world rapidly growing population, expanding economics and anthropogenic activities contribute to the pollution of heavy metals, which are extremely persistent elements in the environment and represent a threat to biota and ecosystem services. Moreover, because anthropogenic CO_2 and other greenhouse gases have reached an unprecedented level, at the moment, key ecosystems and ecological processes are simultaneously experiencing pollution-related and climate-related stresses. Cadmium (Cd) is one of the fourth most toxic trace elements in soils without a physiological function in higher plants that causes various symptoms of phytotoxicity. Rape is considered as an efficient phytoremediator of Cd under current climate conditions. Therefore, the growth chamber experiment with pot-grown oilseed rapes was conducted in this study under a controlled environment to evaluate the potential of rape, as a multipurpose fast-growing annual crop with high biomass production, to remediate Cd-contaminated soil under warmer climate conditions (elevated atmospheric CO_2 and temperature). As photosynthesis plays an essential role in plant growth and biomass production, the Cd-phytoextraction performance in rapes under warmer climate conditions was evaluated using leaf gas exchange and chlorophyll fluorescence parameters, in this study.

4.04.06

Potential Risk From Biological Treatment of Soils Contaminated by Organic Chemicals: Root-Mediated Bacterial Accessibility and Cometabolism of Pyrene

C. Fernandez-Lopez, University Center of Defence (CUD), Spanish Air Force Academy, MDE-UPCT; R. Posada-Baquero, J. Garcia, Instituto de Recursos Naturales y Agrobiologia de Sevilla CSIC; J. Castilla Alcantara, IRNAS CSIC / Agroquimica y Conservacion del Suelo; M. Cantos, Instituto de Recursos Naturales y Agrobiologia de Sevilla CSIC; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiologia / Agroquimica y Conservacion del Suelo

Healthy and resilient soil ecosystems are essential to help mitigate and adapt to climate change, but pressure on soil systems is increasing. Enhancing natural attenuation processes is a sustainable alternative for the restoration of soils contaminated by organic chemicals represented by PAHs and co-occurring compounds. The flow of hydrophobic contaminants to degradative microbial communities in soil can be significantly affected by microbial positioning along the contaminant paths, which may result in enhanced or diminished biodegradation rates. In this study,

the flagellated bacterium *Pseudomonas putida G7*, which degrades pyrene by cometabolism, was cultivated and prepared differently for soil slurry experiments, metabolite analyses and greenhouse experiments. Additionally, we integrated passive dosing with ^{14}C-labeled pyrene, inoculation of motile bacteria into soil and a complete sunflower (*Helianthus annuus L.*) ontogenic cycle to evaluate a new scenario related to pollutant transformation and risk in soil. A preliminary evaluation of possible risks to human and ecosystems were also done. Our results showed that the plants facilitated bacterial access to the distant pollutant source, possibly by increasing bacterial dispersal in the soil; this increased bacterial access was associated with cometabolism. Cometabolism of this PAH occurred immediately in the inoculated and shaken soil slurries, where the bacteria had full access to the passive dosing devices (silicone O-rings). Furthermore, the resulting metabolites were not only mobilized into the soils leachates but also taken up by the plants, accumulating in the roots at significantly higher proportions in inoculated samples than in uninoculated controls and acting differently on their way to the fruits. This new, proof-of-concept scenario successfully showed that bacterial cometabolism may contribute to the environmental risk from PAHs in soil by improving pollutant mobilization and uptake by plants. These results are relevant in the bioremediation field because they show how inoculated bacteria can be mobilized by plants to reach distant pollutant sources and how partial pollutant transformation may generate further issues. Our results may also contribute to other pollution management sectors, such as wastewater treatment and prospective risk evaluation of agrochemicals, where rhizosphere microorganisms play a relevant role.

4.04.07

Behavioural Influences on Mineralization and Cometabolism of PAHs by Motile Bacteria in Membrane Bioreactors With Restricted Porosities

J. Castilla Alcantara, IRNAS CSIC / Agroquimica y Conservacion del Suelo; A. Akbari, McGill University / Civil Engineering; S. Ghoshal, McGill University / Department of Civil Engineering; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiologia / Agroquimica y Conservacion del Suelo

The controlled dispersal of motile bacteria through contaminated soils is currently considered as one of the most promising aspects in bioremediation. The difficulty caused by the heterogeneous distribution of the pollutants and the degrading bacteria can be overcome by the use of different chemoefectors. These compounds provide the control over bacterial tactic motility, what results in an increase in the efficiency of the microorganisms dispersion and subsequently, enhanced biodegradation rates (*Environ. Sci. Technol*. 42: 1131-1137, 2008; *Environ. Sci. Technol*. 46: 6790-6797, 2012). The objective of this study was to evaluate the dispersal and biodegradation capacity through mineralization tests with ^{14}C-labelled napthalene and cometabolism tests [by synchronous fluorescence spectrophotometry (Appl. Environ. Microbiol. 76 (13): 4430-4437; Sci. Total Environ. 717: 137210)] with pyrene by the chemotactic bacterium *Pseudomonas putida* G7 (cell dimensions: 1 µm x 3 µm). The experimental design consisted in a bioreactor system with two chambers separated by a membrane with specific pore sizes that restrict bacterial dispersal (*Environ. Sci. Technol.* 49: 14368-14375, 2015). Different chemoffectors (GABA and artificial root exudates) were used to modulate the motile bacterial behavior throught restricted porosities (5 µm). The initial results were promising. The transport of *P. putida* G7 through the membrane occurred at higher rates than the control for both chemoeffectors, thus enhancing biodegradation rates. The cometabolism process was evaluated by fluorescence spectrophotometry determinations, measuring the effect of *P. putida* G7 on the removal of aqueous- dissolved pyrene in the presence of the chemical effectors. Progress in this field will undoubtedly open up new possibilities for bioremediation processes in contaminated soils, improving the existing techniques for the treatment of poorly bioaccessible contaminants.

4.04.08

Microbial Transformation of Organic Chemicals in Soil Through Cometabolism and Bioavailability Assessment of the Parent Chemical and Transformation Products

R. Posada, IRNAS CSIC / Agroquimica y Conservacion del Suelo; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiologia / Agroquimica y Conservacion del Suelo

The main objective in this study was to integrate the bioavailability assessment in an approach that followed the OECD 307 mineralization and biodegradation guideline, where polar metabolites of a model non-ionic compound (pyrene) were produced by cometabolism. The integration of bioavailability assessments in contaminated soils is necessary for risks assessments and remediation approaches. In these measurements, the persistence of organic compounds in the soils plays a very important role. The incomplete biodegradation of these compounds through cometabolism, leading to transformation products, could even increase the risks, that is also important. Therefore, integrating cometabolic transformations in bioavailability determinations will lead to more realistic risk assessment of the bioremediation processes. A standardized method (ISO 16751:2020), based on Tenax extraction at 20 hours was used to measure bioavailability. This is a chemical method, preferably used with hydrophobic chemicals (Kow > 3), in which the potential bioavailable fraction is the amount of contaminant present in the matrix that can be released from the solid phase to the aqueous phase in a well-mixed water soil mixture and in presence of a receiving phase (Tenax). For this reason, the physico-chemical properties of the compounds in the system, is a very important factor that can affect the results of bioavailability and it is the objective of this study. The first step was an experiment in which the microbial transformation of 14C-pyrene by cometabolism assured the presence of transformation products in the system. In this way, the soil bacterium Pseudomonas putida G7 was used, and a passive dosing device (silicone O-rings) in a soil slurry experiment to control the release of the parent compound to the aqueous phase. The results of this experiment indicated that water soluble metabolites were produced and some possible metabolites were analyzed too (Fernández-López, et al., 2020). Then, we performed a bioavailability assessment to evaluate the effectiveness of this Tenax-based ISO method, and possible ways of optimization, in presence of the metabolites of pyrene, which are more hydrophilic compounds and therefore tended to remain in the aqueous phase and to sorb back to the soil. The results could be applied in future experiments with other compounds, such as pharmaceuticals, which are usually transformed partially as a result of their biological processing in soil.

4.04.09

Bioaugmentation As an Effective Technique for Degradation of Foaming Agent Conditioned Soils

L. Rolando, National Research Council of Italy / Water Research Institute; A. Barra Caracciolo, National Research Council - Water Research Institute / Water Research Institute; T. Pescatore, J. Rauseo, National Research Council of Italy / Institute of Polar Science; A. Visca, G.L. Garbini, National Research Council of Italy / Water Research Institute; F. Spataro, L. Patrolecco, National Research Council of Italy / Institute of Polar Science; P. Grenni, National Research Council of Italy (CNR) / Water Research Institute

The addition of selected microbial strains promotes the catabolic potential of soil microbial communities for the degradation of pollutants. The anionic surfactant sodium lauryl ether sulphate (SLES) is the principal component of several commercial foaming agents (FA) used for soil conditioning in the tunnelling industry. Huge amounts of soil debris are produced during the excavation process and the presence of SLES can affect the re-use of the spoil material as a by-product in green areas close to water bodies. A prompt SLES biodegradation is a key point for the re-use of the excavated soil because it can be toxic for aquatic organisms. Soil debris from a real excavation site was sampled and enrichment cultures using SLES as the only carbon source at various concentrations were performed. A bacterial consortium capable to degrade completely this anionic surfactant in only 24 hours was identified and characterized by NGS and FISH methods. Most bacteria identified belonged to *Gamma-Proteobacteria* (99%) and the *Pseudomonas* genus (ca 90%) was the predominant one. Subsequently, bioaugmentation experiments on two different soils using a selected FA were performed. Soil sub-samples were collected at selected times to determine the SLES degradation (Blue Active Substances method) and microbial abundance (DAPI counts) and cell activity (dehydrogenase activity). The results of the application of the consortium on two soil debris consisting of two different and extreme soil texture faced in a tunnelling will be reported and discussed.

4.04.10

Enhancement of Microbial-Mediated Cr(VI) Reduction Processes for Recovering Contaminated Groundwater

M. Tumolo, Italian National Research Council - Water Research Institute (CNR-IRSA) / Water Research Institute (IRSA); C. De Palma, De Palma Thermofluid s.r.l. - Industrial projects division; D. De Paola, Italian National Research Council / Institute of Biosciences and Bioresources; V. Uricchio, Italian National Research Council / Water Research Institute / Water Research Institute (IRSA); P. Cotugno, A. Tursi, University of the Studies of Bari Aldo Moro / Department of Biology; V. Ancona, Water Research Institute - Italian National Research Council / Water Research Institute

Groundwater Cr (VI) pollution largely depends

on industrial applications in the field of energy production, manufacturing of metals and chemicals, tanning, and subsequent waste and wastewater management. Cr(VI) is a hazardous environmental pollutant, because of its mobility and toxicity, especially for mammalian cells, which do not have efficient chromate detoxifying pathways. On the contrary, chromate-reducing genes are widely present in prokaryotes which can reduce Cr(VI) to the less toxic and less mobile Cr(III). Bioremediation represents a valid alternative to chemical methods which are ineffective at lower concentrations of Cr(VI) in large volumes of wastewater and could generate secondary pollution. Nevertheless, the biological treatment of Cr(VI) contaminated groundwaters is limited, because most known species capable of chromate reduction are heterotrophic, and need extra nutrition addition during the reduction process, especially in an oligotrophic groundwater environment. The aim of this work was to evaluate the extent of Cr(VI) reduction by groundwater indigenous microorganisms at a laboratory scale, aimed at potential application for *in situ* recovery of the industrial area of Barletta Municipality (Southern Italy). In 2020, two monitoring campaigns carried out on the study site revealed a Cr(VI) concentration in groundwater above the legal limit (Italian environmental law, D.Lgs. 152/06), ranging from 50 to 102 $\mu g\ L^{-1}$. Microcosms have been set up with groundwater and water-saturated soil, collected from the above-mentioned industrial area. Different experimental conditions have been tested, as follows: biological batch, biological batch with two different amendments and, the corresponding sterile controls. Specifically, the amendments employed in the microcosms' set up, were: 1) yeast extract, that resulted efficient in promoting a quick Cr(VI) bioreduction in our previous laboratory study; 2) polyhydroxybutyrate, that is well-known in scientific literature as a slow-release substrate for enhancing biological reduction in bioremediation processes. A multi-disciplinary approach has been performed to elucidate microbial-mediated Cr(VI) reduction processes. Changings in microbial abundance have been evaluated via Real-Time PCR in parallel with chemical and spectrophotometric analyses for monitoring Cr(VI) concentration. FTIR spectroscopy has been used to investigate chromium forms in microcosms solid phase.

4.04.11
Can Fe2O3 Nanomaterials Improve Crop Tolerance to Contaminated Soils?
A. Rodriguez Seijo, CIIMAR - University of Porto / Department of Biology; C.F. Soares, S. Ribeiro, GreenUPorto & Faculty of Sciences, University of Porto; B. Ferreiro Amil, Universidade de Santiago de Compostela; C. Patinha, Universidade de Aveiro; A. Cachada, CIIMAR - University of Porto / Department of Biology; F. Fidalgo, GreenUPorto & Faculty of Sciences, University of Porto; R. Pereira, Faculdade de Ciências da Universidade do Porto / Biology
As a result of anthropic pressures, soil contamination is increasing and, consequently, compromising soil functions and impacting crop yields worldwide. Among all contaminants, potentially toxic elements (PTEs) are some of the most common and important, being able to negatively affect the growth and development of plants. Nanotechnology, an emerging field in technological sciences, offers multiple tools that can be used for this purpose. In this way, this work aimed to evaluate the potential of Fe_2O_3 nanomaterials (nano-Fe_2O_3) to alleviate metal-induced stress in barley plants (*Hordeum vulgare* L.), focusing on bioaccumulation patterns and on the redox homeostasis. To achieve this goal, plants grew under two agricultural soils, contaminated with different levels of PTEs, collected from an industrial area, and previously treated or not, with 1% (w/w) nano-Fe_2O_3. After 14 days of growth, biometric parameters, PTE bioaccumulation and biochemical endpoints, especially those related to the induction of redox disorders, were evaluated. The effectiveness of nano-Fe_2O_3 to reduce available PTEs in soil solution was also assessed. After exposure to contaminated soils, plant development was greatly affected, as evidenced by significant decreases in root length and fresh weight of roots and leaves. However, upon co-treatment with nano-Fe_2O_3 this phytotoxicity was partially recovered, with less inhibitory effects on biometric parameters, especially on the less contaminated soil. This pattern was also noticed for levels of total chlorophylls and carotenoids. Regarding the oxidative damage, both soils led to increases in Lipid Peroxidation (LP), though H_2O_2 levels were only increased in the most contaminated soil. In response to the co-treatment with nano-Fe_2O_3, barley plants exhibited less oxidative damage, which is evidenced by the decrease of H_2O_2 and LP in both soils. The present study revealed that nano-Fe_2O_3 can enhance the tolerance of barley plants to contaminated soils, possibly by limiting the occurrence of oxidative stress, through a set of modifications in the PTE (bio)availability.

4.04.15
Analysis of ecotoxicity and bioremediation of metal polluted soils with different humus content
V. Calero Herrera , Peoples Friendship University of Russia; I. Kozlova, J. Belfeg, Lomonosov Moscow State University / Faculty of Soil Science; P. Uchanov, Institute of Ecology and Evolution RAS / Laboratory for soil ecological functions; E. Prudnikova, Moscow State University / Soil Science; A. Kiruyshina, Institute of Ecology and Evolution; S. Kuznetsova, N. Nefedova, S. Kulachkova, Lomonosov Moscow State University / Faculty of Soil Science; V. Terekhova, Lomonosov Moscow State University / Lab of Ecotoxicological Soil Analysis
In this study, we analyzed the heavy metal (HM) contaminated soils with different humus content before and after adding of biochar (5%) and lignohumate (0.05%). Phytotesting, biotesting using organisms of main's trophic levels organisms, microbial structure community as well as microbial functional activities using chromatographic methods were performed with different soil types - agrosod-podzolic, chernozem and , agrozem (Moscow region, Ural and Kalmykia, Russian Federation). The addition of HM in poor-humus soil was more toxic and led to the complete death of test plants, while the plants in rich-humus soil continued to develop until flowering. The basal respiration and other indicators of microbial metabolic activity also changed to a greater extent in poor-humus soil. A decrease in the toxic phytoeffect of heavy metals during the remediation activity of biochar was observed in samples with a high humus content, while the addition of the tested dose of biochar did not affect plant development in poor-humus soils. The efficiency of biochar increased in some variants of humus-poor soils with the addition of lignohumate (0.05%) *Key words***:** metal polluted soil, organic carbon, microbial biodiversity, remediation, biotest
Acknowledgement.*This research was funded by the Russian Foundation of Basic Research (grant #18-04-01218a).*

4.04.18
Approaches for characterizing and neutralizing oil sludges
K. Ponogaybo, Centre for Strategic Planning of FMBA of Russia, Russia; L. Voronina, Lomonosov MSU, Center for Strategic Planning, Russian Ministry of Health, Russia
Pollution associated with the oil industry, both in scale and toxicity, is a global hazard. It causes soil degradation, pollution of water reservoirs and atmospheric air, death of living organisms, poisoning, diseases, etc.Recultivation of sludge pits, including *in situ* neutralization of oil-contaminated sludges that are complex in their composition and usually consist of several phases, is considered from a number of positions in accordance with the Russian and the international requirements: conducting preliminary studies of samples, planning the degree of restoration works, cost efficiency, use of safe technologies, etc. In nature, landfills with waste (sludge pits are sites with oil sludge waste) are subject to natural destruction. The intensity of these processes depends primarily on the composition of pollutants and external factors (climate, chemical, biological, etc.). All technological measures to introduce humans into these processes are aimed at regulating the activity of the factors established in nature and of the studied characteristics of waste. Therefore the study of drilling waste — namely its chemical characteristics (organic and inorganic components), the reaction of biological objects to complex pollution of natural objects by this waste and a number of methodological studies — remains relevant.As a result of interaction of the drilled rock with the reagents used in drilling, new complex compounds with unclear chemical composition are formed, so chemical analysis alone may not be enough. Bioassay on living organisms with the determination of chemical indicators is the main way to assess the hasard of drilling studge pits to the environment. Bioassay is based on the study of behavioral responses of living organisms that are able to detect stressful effects earlier than many commonly used methods. An aqueous extract from samples of oil drilling sludge in a ratio of 1:10 with distilled water was used for bioassay. According to the test results and the ratio of dilution of the water extract, which eliminated the toxicity of the waste, the toxicity of the samples was defined. The use of hydrobionts in assessing the hazard level of waste can be justified by the entry of toxic substances into the environment in the following chain: waste → soil → ground water and surface water → human body. The simulation of this input is the preparation of extracts from waste. Based on the conducted research, a scheme for the experimental study of the present type of waste was developed. Oil drilling sludge analysis scheme: 1) Separation of samples into solid and liquid phases, determination of the ratio of these phases; 2) Conduction of chemical analysis of each phase separately. Determination of the element

composition: active and gross forms. Determination of qualitative and quantitative organic composition; 3) Definition of toxicity using bioassay methods; 4) Drawing up an action plan for the neutralization of oil drilling sludges.

4.04.20

Nitrogen Removal in Biochar Supplemented Constructed Wetlands (CWs) From Secondary Wastewater Effluent

F.O. Ajibade, FEDERAL UNIVERSITY OF TECHNOLOGY AKURE / Civil Engineering; H. Wang, A. Guadie, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences; T.F. Ajibade, Institute of Urban Environment, Chinese Academy of Sciences; A. Wang, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences

Trace total nitrogen discharge is considered to be still significant to eutrophication, however, its deep removal is difficult from secondary wastewater effluent with low C/N ratio. Biochar was employed to intensify constructed wetland (CW) for further organic and nitrogen removal from secondary wastewater effluent. Five sets of non-aerated biochar modified vertical flow CW (VFCW) was designed to study the synergy between biochar and microbes for enhancing wastewater depuration. The results depicted that the mean COD and nitrogen removal efficiencies of VFCW1 (with 1% w/w biochar with microbe and plants) were observed as 89.1±5.6% and 90.2±3.1% respectively, and their corresponding removal rates of 10.2± 0.8 mg-COD/(m^3.d) and 3.57± 0.3 mg-TN/(m^3.d) which were 35 and 52.3% higher than the control. The dissolved organic carbon (DOC) extraction from the VFCWs portrayed that water and acidic extractants indicate the optimum conditions for the biochar's DOC release in the non-aerated CW system which enhanced the removal of nitrogen. The 16S RNA gene sequencing analysis indicated that in the biochar-modified VFCWs, bacterial phylum *Proteobacteria* (24.13 – 51.95%) followed by *Chloroflexi* (5.64 – 25.01%), *Planctomycetes* (8.48 – 14.43%), *Acidobacteria* (2.29 – 11.65%) and *Nitrospirae* (0.83 – 2.19) were abundantly enhanced compared to the control with 19.76, 8.55, 12.42, 9.9 and 0.45% respectively. On the whole, supplementing biochar in a non-aerated VFCWs is a credible and effective approach for enhancing nitrogen removal from secondary effluent which does not alter the ecological function and the vital features of VFCW such as low operational and maintenance cost, high microbial diversity, less adverse environmental impacts as well as the treatment efficiency.

4.04.21

Selenium Removal and Pollution Swapping Potential of Passive Anaerobic Bioreactors Receiving Coal Slurry Impoundment Wastes

M.A. Mahmud, University of Kentucky; E. D'Angelo, University of Kentucky / Plant and Soil Sciences

Worldwide, selenium is a major contaminant of concern for coal and metal ore mining industries. In coal mining regions of the eastern USA, for example, billions of liters of wastewaters with elevated Se oxyanion concentrations (primarily SeO_4^{2-} and SeO_3^{2-}) are generated during coal washing which is often stored in >700 man-made impoundments built into remote mountain valleys. Because impoundments are unlined systems, they tend to leak contents into nearby streams which have detrimental effects on sensitive biota, particularly fish. The primary goal of this project was to evaluate the capacity of passive anaerobic bioreactors (PAB) to remove Se from coal slurry leachate, while at the same time not contributing other pollutants (e.g. nutrients, trace elements, greenhouse gases) that could have detrimental effects on water or air quality ("pollution swapping"). Simply, PABs are trenches filled with organic substrates (e.g. wood chips) which support the growth of microorganisms that improve water quality as wastes move through the system. Over the last 20 years, PAB systems have been employed to remove nitrate from agricultural drainage by denitrification, but PAB have not been widely evaluated for other pollutants, such as SeO_4^{2-} reduction to insoluble Se (0). Selenate removal and pollution swapping were evaluated in PAB filled with biofuel plant substrates (either hardwood chips, switchgrass, Miscanthus, timothy hay) in triplicate laboratory batch reactors fed synthetic coal slurry at 32-d retention times over 7 months. PAB water and headspace gases were sampled with time and analyzed for pH, Eh, Se and trace elements, nutrients, and dissolved organic C, CO_2, CH_4, and N_2O. Pollutant concentrations were compared with those in pristine streams to calculate Canadian Water Quality Indices as a function of type of organic substrate and maturation time. In all but wood chip PAB, Eh and pH rapidly decreased and DOC rapidly increased which supported rapid denitrification and selenate reduction. In the wood chip PAB, extremely low pH (< 4.5) strongly inhibited selenate reduction and the final denitrification step, so high amounts of Se and greenhouse gas N_2O evolved from that system. In timothy hay PAB, effluent contained high dissolved phosphate and other nutrients that could adversely affect water quality of receiving streams. This study emphasizes the importance of organic substrate selection when designing and implementing PAB remediation technology.

4.04.22

Remediation of P-Rich Eutrophic Water Using Anionic Nanoclays

R. Correia Fernandes, Universidade de Aveiro / Department of Biology; F. Maia, Smallmatek - Small Materials and Technologies, Lda.; S. Loureiro, Universidade de Aveiro / Biology; A.M. Soares, Universidade de Aveiro / CESAM & Department of Biology; J. Tedim, University of Aveiro / Department of Materials and Ceramic Engineering & CICECO; R. Martins, Department of Biology, University of Aveiro / CESAM & Department of Biology

Eutrophication of water bodies is a common phenomenon resulting from the surplus of inorganic nutrients, usually related to anthropogenic activities, such as, agriculture, sewage discharges, mining, overconsumption, among others. Recent efforts to control and remediate eutrophication processes include the use of green nanotechnology. Zn-Al layered double hydroxides (LDH) are non-toxic hydrotalcite-like anionic nanoclays that can exchange the stabilizer anion by others that can be present in the water, such as phosphates. Therefore, the present study aimed to assess the efficacy of Zn-Al LDH-NO_3^- as a low toxic adsorbent for the remediation of P-rich water bodies (benchmarked with calcined LDH). For this purpose, three concentrations of LDHs (5, 50 and 500 mg/L) were added to P-rich solutions mimicking the maximum recommended concentration of phosphorus on drinking water (0.4 mg P/L) and wastewater (10 mg P/L), according to the Portuguese legislation (DL 236/98) and to water collected from an eutrophic artificial lake (Aveiro, Portugal), simulating a real scenario. Nitrates and phosphates were periodically measured to evaluate the nanomaterial capacity to remove phosphates' content for a period of 7 days. The toxicity of remediated water was also assessed at the end of the remediation period through growth inhibition tests using the freshwater microalgae *Raphidocelis subcapitata.* The tested materials showed a great efficacy on the removal of phosphates, in all tested media. Overall, the higher the LDH tested concentration, the higher the speed on P removal, the higher the nitrates released (below the recommended threshold for drinking water, 25 mg NO_3^-/L) and the higher the growth inhibition effects on the green microalgae *R. subcapitata*. The intercalation of phosphates was confirmed through X-ray diffraction (XRD). In conclusion, the results suggest that Zn-Al LDH-NO_3 is a technological and environmentally friendly promising solution for phosphates remediation, preferably if used for very short-periods (e.g. the addition of 500 mg LDH/L into the solution of 10 mg P/L removes 99.9% of the P in just 5 minutes, releasing only 6 mg NO_3^-/L).

4.04.23

How a World War 2 Shipwreck Still Influences the Surrounding Sediment 70 Years Later

J.W. Van Landuyt, Ghent University (UGent) / BW25 Biotechnology; K. Kundu, Ghent University - LabMET; S. Van Haelst, Flanders Marine Institute VLIZ; K.F. Parmentier, RBINS / OD Nature; M. De Rijcke, Flanders Marine Institute VLIZ; N. Boon, Ghent University - LabMET

Almost 300 shipwreck sites found in the Belgian part of the North Sea are registered in the database (www.maritieme-archeologie.be). These are mainly ships that were sunk during the First and Second World War. Shipwrecks often act as artificial reefs and are sometimes considered historical heritage. As solid heterogeneous substrates, shipwrecks are rapidly colonized by microorganisms, which allows other organisms to attach and form an assemblage with dynamic community interactions. These assemblages influence the shipwrecks' structural integrity and subject it to years-long (bio-)corrosion, which can ultimately cause leakage of fuels and heavy metals. Sediment samples were taken around a Second World War shipwreck at increasing increments further away from the wreck in different directions. Ideally, this corresponds to a decrease in concentration of fuels and heavy metals, and a differential microbial response in the affected sediment. Subsamples were taken for both chemical and microbial analysis. Polycyclic aromatic hydrocarbon levels, as well as heavy metal levels, were determined for all sediment samples to investigate if there was any leakage visible in the sediments. In addition, we performed DNA extractions on all sediment samples and on swaps taken from steel shipwreck fragments, after which we did 16S rRNA gene sequencing to map the microbial composition. The chemical and microbial fingerprint of the samples demonstrated that low-level leaking of the coal bunker still influences the surrounding sediments.

Correlations between the pollutant concentrations and the differential relative abundance of specific OTU's indicated the microbial genera and families that might play a role in the biodegradation of the leaking pollutants. By understanding better how, even after 70 years, World War shipwrecks can still significantly influence the surrounding sediment, better management strategies could be developed to preserve these artificial reefs and remediate the areas surrounding them.

Bringing Together Exposure and Effects: Towards a Mechanistic Understanding of the Environmental Risk of Chemicals in Aquatic Ecosystems

4.05.02
A New Approach to Determine the Appropriate Use of Time-Weighted Average Concentration in Aquatic Risk Assessment
Y. Wolf, Bayer Ag; F. Gabsi, Rifcon GmbH; E. Bruns, Bayer AG, Division Bayer CropScience / Ecotoxicology; S. Heine, Bayer AG Research & Development Crop Science; A. Solga, Bayer AG Crop Science Division / EnSA; J. Witt, T. Preuss, Bayer Ag / Environmental Safety
Chronic pesticide risk assessment is based on regulatory acceptable concentrations for surface water bodies (RACsw,ch) derived from standard studies with constant exposure of organisms to a test compound for days to weeks. This RACsw,ch is compared to the maximum concentration of complex exposure scenarios, whereby the exposure duration may be notably shorter (e.g. hours) than in the standard study. This can result in an overly conservative Tier 1 assessment. A risk assessment using time-weighted average concentrations (TWAc) in accordance with the complex decision scheme of the EFSA aquatic guidance document could address this discrepancy. There is still a controversial discussion on how to handle certain parts of this decision scheme. Before moving to the use of more complex models, we propose a simple approach to determine at Tier 1, whether the use of TWAc is appropriate in aquatic risk assessment. This approach is based on the idea that the TWA concept is a model for aquatic risk assessment, thus, we can apply standard model performance measures as the posterior prediction check (PPC) to the TWA. We propose a new method using the PPC approach that should clarify if the use of the TWA concept is appropriate or even conservative. As a minimum, two datasets (constant and pulsed exposure studies) are required for the suggested approach. We propose to use a 10 % acceptance threshold as the maximum for underestimated predictions. Overestimated effects are accepted since the conservatism of the TWA approach is evaluated and not the goodness of fit. The proposed approach specifically identifies that the use of TWA is not appropriate for early onset or latency of effects. We demonstrate that different pulse exposure scenarios, various intervals between pulses, different toxicity mechanisms and dynamics of pesticide concentrations can easily be tested. The TWAc method can be applied based on a case by case decision. For decision making conservativeness of the approach has to be demonstrated. If the proposed concept identifies that the use of 7d-TWA is not applicable, a shorter time window or the use of TK/TD models are suggested as potential refinements. For example, with the latter approach the (in)dependence of different pulse exposures can be demonstrated with a more mechanistic approach without a need for additional animal experiments.

4.05.04
Determining Acute to Chronic Ratios in Aquatic Toxicity
P. Thomas, P. Bicherel, KREATiS
Acute to chronic ratios (ACR) is a mainstay of environmental risk assessment and has been used to develop generic uncertainty factors for decades. For example, Predicted No Effect Concentration (PNEC) based on a battery of acute ecotoxicity studies (algae, daphnids, fish) will require an uncertainty factor of 1000, while the PNEC for a battery of chronic ecotoxicity studies on the same species will require a factor of 10 leading to a default, generic (conservative) ACR of 100. At the same time, many authors conclude on an ACR of 10 (or less) as being sufficient. Some authors propose different ACR for different species. Thanks to recent advances in toxic Mechanisms of Action (MechoA) as described by Bauer et al. (2018), we can consider not only species differences but also molecular interactions of organic substances across a range of hydrophobicities resulting in acute and chronic toxicity to aquatic organisms. By comparing the regression lines modelling the aquatic toxicity against the solubility, it is possible to consider ACR for each species for a specific MechoA. As demonstrated in Thomas et al. (2019), non-polar narcotic (MechoA 1.1) substances tend to result in regression lines which are close to parallel regardless of their hydrophobicity, with ACRs ranging from 5 to 10 which is very different from enzymatically hydrolysed (MechoA 2.1) substances. These latter substances have acute and chronic regression lines that are not parallel and ACRs vary from a factor of 2 to 15 depending on the hydrophobicity of the substance. ACRs of other than MechoA 1.1 or 2.1 have also recently been found to differ significantly both between species and with hydrophobicity. In conclusion, we propose linking ACRs to MechoA in order to obtain more appropriate assessment factors for hazard based reference values (e.g. PNECs or other tools such as USETOX in Life Cycle Analysis) from acute toxicity data.

4.05.05
Speed It up: Temperature Drives Toxicokinetic Processes in Aquatic Invertebrates
J. Raths, Eawag / Department of Environmental Chemistry; J. Hollender, Eawag / Environmental Chemistry
Studies for the environmentally risk assessment of chemicals are generally conducted under highly standardised laboratory conditions. However, environmental parameters and external stressors are known to exert a strong influence on organism fitness. The ongoing climate change draws a lot of attention towards the parameter temperature. Many studies found a higher sensitivity of aquatic invertebrates towards contaminants with increasing or dynamic exposure temperature. However, the exact mechanisms do remain unravelled. Few studies have been carried out to achieve a more structured and in depth understanding of this link. This study aimed to investigate the influence of temperature on toxicokinetic parameters in *Gammarus pulex*. Bioconcentration experiments at four different temperatures (6, 11, 16 and 21°C) with a mix of 12 compounds were carried out using the aquatic invertebrate *G. pulex*. Tissue and medium samples were taken in regular intervals and analysed by LC-HRMS/MS. From these data uptake and elimination kinetics, as well as toxicokinetic rate constants in dependence of the exposure temperature were modelled. A increase of the initial uptake from water was observed with increasing temperature. Similarly, faster formation of biotransformation products was found. Further, steady state conditions were reached faster with increasing temperature. However, the steady state bioconcentration factors of the compounds were similar between the different temperatures or even lower at higher temperatures. The later observation could potentially be explained by higher biotransformation rates. The present study demonstrated that temperature can be an important driver of toxicokinetic processes and provides data and methods for risk assessment implications. The obtained results help understanding the mechanisms of temperature on chemical uptake, biotransformation and elimination and their interaction resulting in different sensitivity towards chemicals. Further, generated toxicokinetic rate constants can support modelling of environmental exposure as well as toxicodynamic laboratory studies under different temperature conditions. Especially when very dynamic exposure profiles are present, such as high run off in summer, the toxicokinetics before reaching a steady state, i.e. the initial uptake are of high importance.

4.05.08
PPP Case Study: Increasing the Ecological Relevance of Chemical Risk Assessments Using Geospatial Approaches
C.M. Holmes, Applied Analysis Solutions, LLC; L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences; S. Marshall, Consultant; J.C. Otte, BASF SE; P. Sweeney, Syngenta / Environment Product Safety; P. Thorbek, BASF SE / Agricultural Solutions
This poster and its companion surfactant case study poster provide the foundation for the similarly titled platform presentation. Review of these posters prior to viewing the platform will provide helpful background information. A key rationale for making geo-referenced chemical risk assessment is that it provides assessments that can be tailored to local landscape/watershed abiotic characteristics and ecology to account for spatial heterogeneity within river basins. Since heterogeneity is often reflected in localised specific environmental objectives and protection goals, spatially explicit assessments can better relate to landscape/watershed scale environmental management objectives than can current generic chemical environmental risk assessment frameworks. In 2017 ECETOC initiated a Task Force to investigate current capabilities in making spatially explicit chemical risk assessment (from both an exposure and effects perspective). After comprehensive research for applicable and available data, we investigated techniques and methods for combining disparate data sets using 2 case studies and identified some of the challenges of using different levels of taxonomic, spatial and temporal resolution in geo-referenced risk assessments. The results of our case studies give an indication of the potential value of making geo-referenced chemical risk assessments as well as the

limitations to current capability. In this plant protection product (PPP) case study located in the German State of Hessen, georeferenced aquatic exposures were generated from the SYNOPS model using surveyed usage data randomly applied to >80 000 fields utilizing standard regulatory exposure modeling. We focused on 3 winter cereals and 3 PPPs commonly used on these crops (insecticide, herbicide and fungicide). Daily aggregate exposure-toxicity ratios (ETRs) were generated for 3 acute and 5 chronic endpoints across the PPPs for each field over a one-year period. The 90th percentile of 1 and 7 day ETRs for each field were assigned to the closest stream segment within 300m. Biological monitoring data (linked to the same stream dataset) obtained from local authorities was used to determine the ecological state for algae, macrophytes, macroinvertebrates and fish. Biomonitoring data and ETRs were spatially associated by stream segment and analyzed. This poster will present the source data, processing methods, and results from three levels of relating georeferenced exposure and biomonitoring data.

4.05.09
Surfactant Case Study: Increasing the Ecological Relevance of Chemical Risk Assessments Using Geospatial Approaches
C.M. Holmes, Applied Analysis Solutions, LLC; L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences; S. Marshall, Consultant; J.C. Otte, BASF SE; P. Sweeney, Syngenta / Environment Product Safety; P. Thorbek, BASF SE / Agricultural Solutions
This poster, along with its companion PPP case study poster, provide the foundation for the similarly titled platform presentation. Review of these posters prior to viewing the platform will provide helpful background information. A key rationale for making geo-referenced chemical risk assessment is that it provides assessments that can be tailored to local landscape/watershed abiotic characteristics and ecology to account for spatial heterogeneity within river basins. Since heterogeneity is often reflected in localised specific environmental objectives and protection goals, spatially explicit assessments can better relate to landscape/watershed scale environmental management objectives than can current generic chemical environmental risk assessment frameworks. In 2017 ECETOC initiated a Task Force to investigate current capabilities in making spatially explicit chemical risk assessment (from both an exposure and effects perspective). After comprehensive research for applicable and available data, we investigated techniques and methods for combining disparate data sets using 2 case studies and identified some of the challenges of using different levels of taxonomic, spatial and temporal resolution in geo-referenced risk assessments. The results of our case studies give an indication of the potential value of making geo-referenced chemical risk assessments as well as the limitations to current capability. In this surfactant case study, georeferenced aquatic exposures to a surfactant used in domestic cleaning products for 350 wastewater treatment plants (WWTPs) in the German State of Hessen were examined and compared to biomonitoring data. Predicted environmental concentrations (PECs) were generated from emissions based on per capita product usage, connectivity to municipal sewage treatment plants, and removal efficiency geographically linked to local river flow and dilution factors. PECs were converted to Exposure-Toxicity Ratios (ETRs) using available ecotoxicity data. Water Framework Directive biological monitoring data obtained from local authorities was used to determine the ecological state for algae, macrophytes, macroinvertebrates and fish. Biomonitoring data locally downstream of WWTP discharges (n=794) were spatially linked to surfactant ETRs and analyzed. This poster will present the source data, processing methods, and results from three levels of relating georeferenced exposure and biomonitoring data.

Effect Modelling for Regulatory Environmental Risk Assessment of Chemicals: Where Are We and What Comes Next?

4.06.02
A Mechanistically-Based QSAR Approach to Predict Aquatic Toxicity of Reactive Compounds
P. Bicherel, F. Bauer, P. Thomas, KREATiS
Unspecific reactive substances were initially categorised in the Verhaar scheme for the toxic Mode of Action (MOA) as class 3 (Verhaar et al., 1992). In spite of updates (Verhaar et al., 2000; Enoch et al., 2008) and other classification schemes (Russom et al., 1997), these tools are in disagreement when classifying the majority of reactive substances (Kienzler et al., 2017) and aquatic toxicity predictions are not accurate using existing ecotoxicity QSAR models available (Cappelli et al., 2015). The Mechanism of Action (MechoA) classification based on the Molecular Initiating Event (MIE) more accurately defines reactivity according to the type of reaction and the reaction products (Bauer et al., 2018a, 2018b). The three subcategories of reactivity: hard electrophiles (MechoA 3.1), soft electrophiles (MechoA 3.2) and radical-generating compounds (MechoA 3.3) are all expected to directly lead to specific toxicity. Recent work has demonstrated that aquatic toxicity can be more accurately predicted when based on MechoA rather than just on structural similarity. As suggested by MechoA theory combined with activity theory (Thomas et al., 2015), the compounds defined as MechoA 3.1 (aliphatic epoxides and aldehydes, benzaldehydes, benzyl halides, etc.) follow the same toxicity-hydrophobicity relationship while the compounds categorised in MechoA 3.2 (acrylates, unsaturated aldehydes and nitriles, etc.) follow a different relationship. Some exceptions have been noted, particularly regarding benzaldehydes. However these can be interpreted mechanistically notably for fish which have metabolic capacities compared to invertebrates or algae in order to explain excess or lack of toxicity. These observations can be accounted for in the MechoA scheme, as in other cases, as species-dependent specifications. The stronger aquatic toxicity of soft electrophiles compared to hard electrophiles (ca. factor of 10) demonstrates that the kind of reactivity that is involved is key to accurately predicting toxicity. In both cases, reactivity categories are associated with a significant excess toxicity compared to baseline as defined by non-polar narcotic toxicity (MechoA 1.1). As a conclusion, structural similarity alone is not sufficient to explain toxicity. Mechanistic interpretation, suggested by the OECD principles for QSAR validation as being facultative to obtain a reliable model, actually appears to be a critical factor, especially in the field of toxicology.

4.06.05
Study on Species Sensitivity Distribution of 35 Pesticides With Different Distribution Models
P. Huang, Tongji University, Shanhghai - China; S. Liu, Tongji University / State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering
Pesticides are often detected in soil and water environment because of their widespread use and persistent pollution characteristics. Considering the potential hazards and wide detection range, it is particularly important to evaluate the ecological risk of pesticides. However, an important step in ecological risk assessment is to determine the safety threshold for a chemical, that is, to obtain the predicted no effects concentration (PNEC) of the chemical. The most accepted method for derivation of PNEC is the species sensitivity distribution (SSD) method. The SSD method is more and more used in the derivation of water quality criteria. However, there is no authoritative research to prove that SSD curve belongs to a specific curve distribution, and in the field of ecotoxicology, the selection method of SSD fitting model has no specific principles. In this paper, the aquatic ecotoxicity data of 35 pesticides were collected and the SSD curves of each pesticide were fitted by 9 two-parameter models. The optimal fitting model is determined by the principle of minimum root-mean-square error with a cumulative probability of less than 50%. In terms of the fitting effect, the arctangent function appeared the most frequently in the optimal model, 15 times in total, accounting for 42.9%; the Gompertz function appeared 7 times, accounting for 20%. The optimal model was used to calculate HC5 (hazard concentration of 5% organisms) and HC10 (hazard concentration of 10% organisms) of 35 pesticides, and we found that HC5 and HC10 have a power function relationship. Through extrapolation of 35 pesticide PNEC and recalculation of evaluation factors, it was found that the PNEC5 derived by HC5 was consistent with the PNEC10 derived by HC10. Through SSD studies on 35 pesticides, this paper provides reference for the selection of SSD models, and the feasibility analysis of deriving PNEC by HC10 was discussed, which provided new ideas and basic data support for ecological risk assessment.

4.06.06
The SETAC Working Group "Model Acceptability, Version Control and Scenario Development (MAD)
A. Focks, University of Osnabruck / Institute of Environmental Systems Research; J. Axelman, Swedish Chemicals Agency; A. Barsi, Ctgb; J. Becker, UBA, German Federal Environmental Agency; E. Chaideftou, Benaki Phytopathological Institute; S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology; B. Goussen, IBACON GmbH; U. Hommen, Fraunhofer IME / Ecotoxicology; O. Jakoby, Rifcon GmbH; M. Kattwinkel, University of Koblenz-Landau / Quantitative Landscape Ecology; J. Klein, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecotoxicology;

J. Koch, gaiac Research Institute / Environmental Toxicology; T. Preuss, Bayer Ag / Environmental Safety; M. Reed, HSE / CRD; S. Reichenberger, Knoell France SAS / Environmental Fate / Modelling / GIS; C. Rendal, Syngenta / Product Safety; T. Strauss, Research Institute gaiac; P. Thorbek, BASF SE / Agricultural Solutions; P. Vermeiren, Radboud University / Environmental Science; M. Wang, WSC Scientific GmbH / .
There is a need for scientific input and advice for the use and evaluation of mechanistic effect models in regulatory environmental risk assessment of pesticides. Therefore, in 2019 the formation of a cross-sectoral working group was initiated under the umbrella of the SETAC Europe interest group "Effect Modeling" with the aim to develop scientific input on model acceptability criteria, version control and scenario development for mechanistic effect models. The working group, composed of scientists from academia, governmental authorities, contract research organizations, and industry identified a list of specific topics of relevance for model application and evaluation, including options for general model use and criteria for evaluation, scenario development, data evaluation, modular model evaluation, and the definition of qualitative and quantitative criteria for model calibration and validation. Evaluation of the acceptability of specific models is out of the scope of the group. This poster gives an overview of the work in the WG MAD and provides more details about the currently active topic groups. The aim of the WG is to compile a series of chapters to be published at the end of 2021 as a SETAC e-book.

4.06.07

Main Outcomes of the Symposium „ Evaluation of Toxicological and Ecological Effect Models for Risk Assessment of Plant Protection Products"

S. Duquesne, UBA, Federal Environment agency / Plant Protection Products; J.M. Becker, German Federal Environment Agency (UBA) / Plant Protection Products; T. Jager, DEBtox Research; S. Kramer-Schadt, Leibniz Institute for Zoo and Wildlife Research; M. Liess, Helmholtz centre for environmental research - UFZ / System-Ecotoxicology; S. Pieper, M. Solé, German Federal Environment Agency (UBA) / Plant Protection Products; S. Matezki, German Environment Agency UBA / Plant Protection Products
A symposium organized by the Federal Environmental Agency (UBA, Germany) and the Helmholtz Centre for Environmental Research (UFZ, Germany) was held in Berlin in September 2019 to discuss the main outcomes of a research project; the aim of this project was to review critically existing mechanistic and ecological models potentially suitable for the refined risk assessment of plant protection products (PPP). The symposium included a total of 38 participants from industry, consultants, academia and authorities (11 Member states and EFSA). It was divided in 2 sessions focused on individual level (ToxicoKinetic-ToxicoDynamic, TKTD) and population level (Individual-Based, IB) models, followed by an overall discussion which main aspects are presented here. The strengths and limitations of TKTD and IBM were discussed as well as the issue of these models being fit for purpose and ready to use in relation to scientific and regulatory aspects. Opinions were exchanged on the issue of validation of models and how this could be achieved. On the question on how population models could add to the risk assessment of pesticides, a number of topics were raised that included the understanding of mechanisms, the exploration of risk ranges, and the demonstration of intended uses with acceptability of risks. The possible use of models to identify risk mitigation measures was also discussed. Specific model developments and requirements to address prior to possible implementation were formulated. Finally, opinions were exchanged on how model evaluation and validation for environmental risk assessment of PPP could be handled at EU level.

4.06.08

Ready-To-Use Tools to Efficiently Model and Predict Effects in the ERA Framework

S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology
Among the consequences of the development of our societies, chemical pollutants and their impacts on living organisms have become a priority issue for regulatory agencies with guidelines reinforcing the requirements for the approval of active substances. Before any decision, information must be collected on their physico-chemical properties, toxicity and ecotoxicity, and the associated risks must be assessed. In this perspective, many toxicity tests are carried out within laboratories, according to standardized protocols whose data are then analyzed by appropriate and reliable mathematical models and statistical inference methods. Classical outputs are critical effect concentrations for the biological traits of interest, that may be obtained by fitting an exposure-response/effect model. Such a model consists of a deterministic part describing the mean tendency of the data, and a stochastic part depending on the type of the data (namely, binary, count or continuous data). Choosing the most appropriate model may thus appear awkward, but can be supported by ready-to-use tools. Such tools should be specifically designed for ecotoxicologists and risk assessors, in order to help them in easily performing statistical analyses of standardized toxicity test data, in a user-friendly way, with a freely available graphical web interface and without requiring to invest in the underlying statistics and computer technologies.Faced with this statement of fact, our team developed several ready-to-use tools offering a collection of services for statistical inference and mathematical modelling applied to ecotoxicology. This presentation gives an overview of these tools: (1) the R-package 'morse'; (2) the web-platform MOSAIC, in particular its GUTS module (*General Unifed Threshold model of Survival*) allowing to fit GUTS toxicokinetic-toicodynamic models directly online; (3) the GUTS Shiny Application to simulate GUTS models online and predict x% lethal concentration profils (LPx) as suggested by EFSA for time-variable exposure scenarios. All these tools give results associated with their uncertainty.

4.06.11

Reduced General Unified Threshold Model of Survival (GUTS-RED) for Regulatory Pesticide Risk Assessment - Applying Several Model Implementations and Data for Five Fish Species

A. Singer, B. Rall, D. Nickisch, L. Ibrahim, O. Jakoby, Rifcon GmbH; R. Ashauer, Syngenta Crop Protection AG / Environment
Toxico**k**inetic-**t**oxico**d**ynamic modelling (TKTD) can support **e**nvironmental **r**isk **a**ssessments (ERA) of pesticides. The 2018 published scientific opinion of the **E**uropean **F**ood **S**afety **A**uthority (EFSA) on TKTD models considers the **red**uced **G**eneral **U**nified **T**hreshold model of **S**urvival (GUTS-RED) as ready-to-use for the prediction of survival rates under untested exposure conditions. We present an aquatic Tier 2C case study in which we used GUTS-RED modelling to support aquatic vertebrate higher tier ERA. This study provides hands-on information on the operation of GUTS-RED for five fish species exposed to the fungicide benzovindiflupyr, using data from standard toxicity tests. We applied the models and predicted lethal effects under standard FOCUS exposure scenarios. We used several GUTS-RED software implementations, allowing us to compare results and to gain insight in the robustness of these effect modelling options in the risk assessment. Hence, this case study provides a complete example of an application of GUTS modelling in regulatory ERAs along with a rich information base for understanding the usefulness of that approach and its several available implementations.

4.06.12

openGUTS v1.1 - Update of a User-Friendly Open-Source Software for Guts-Red Modelling in Environmental Risk Assessment

A. Singer, B. Rall, D. Nickisch, Rifcon GmbH; R. Ashauer, Syngenta Crop Protection AG / Environment; T. Jager, DEBtox Research
The 2018 scientific opinion of the **E**uropean **F**ood **S**afety **A**uthority (EFSA) on **T**oxico**k**inetic-**t**oxico**d**ynamic (TKTD) models considers the **red**uced **G**eneral **U**nified **T**hreshold model of **S**urvival (GUTS-RED) as ready-to-use to support **e**nvironmental **r**isk **a**ssessment (ERA) of pesticides. The open-source software openGUTS (http://openguts.info/) supports GUTS-RED application in ERA by implementing the suggested standard process of GUTS modelling in a user-friendly Windows application. A step-wise approach guides users through the sequence of model calibration, validation and prediction. The open-source concept in conjunction with ample documentation, a version control log and a well-designed set of test cases ensures transparency and correctness of currently and future implemented processes. We present the updates of openGUTS in the recently published version 1.1. Updates comprised correction of a minor bug in the GUTS-RED-IT projection routine (see http://openguts.info/downloads/Error_IT_calculations.pdf), improvement of automatic results reporting, backwards-compatible project storage as well as an enhanced consideration of prediction uncertainty. Reliability of openGUTS v1.1 is demonstrated with a multitude of test calculations (including the ring test – Jager & Ashauer, 2018). The software openGUTS v1.1 is found to be a useful tool to support GUTS-RED analyses in ERA.

4.06.14

An Integrative Approach for the Sublethal Effects of Plant Protection Products: Deb-Tktd Model Calibration and Forward-Predictions

M. Trijau, B. Goussen, IBACON GmbH / Ecological Modelling; A. Gergs, Bayer Ag /

Research & Development, Crop Science Effect Modelling; S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology

The extrapolation of effects from controlled standard laboratory tests to real environmental conditions is one of the main challenges facing Environmental Risk Assessment (ERA) of chemicals. In that respect, Toxicokinetic-Toxicodynamic (TKTD) models play an important role in analysing and predicting effect of contaminants under untested and environmentally realistic exposure conditions. While the EFSA Scientific Opinion on the state of the art of TKTD effect models (EFSA PPR, 2018. EFSA Journal;16(8):5377) considers models that are based on the Dynamic Energy Budget theory adapted for ecotoxicology (DEB-TKTD models) as valuable tools for ERA, their full acceptance by stake-holders still requires the standardization of their calibration/validation framework. DEB-TKTD models provide a comprehensive and mechanistic framework for the analysis of sublethal effects of chemicals, by describing how chemicals disrupt the acquisition and the allocation of energy by organisms over their full life cycle. In order to enhance and facilitate the use of DEB-TKTD models, the R-package "rDEBtktd" was recently developed. This package is taking advantage of the Bayesian inference framework enabling the estimations of model parameters and their associated uncertainty, which can then be propagated to forward-predictions. Based on this package, this poster presents a full and real case study on the practical use of a DEB-TKTD model through model calibration, validation and forward-predictions steps, using length and reproduction data of *Daphnia magna* exposed to a Plant Protection Product.

4.06.15

rDEBtktd, an R-Package for the Analysis and the Forward-Prediction of Sublethal Effects

M. Trijau, B. Goussen, IBACON GmbH / Ecological Modelling; A. Gergs, Bayer Ag / Research & Development, Crop Science Effect Modelling; S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology

Environmental Risk Assessment (ERA) of chemicals is based on standard laboratory toxicity tests with living organisms which ensure controlled experimental conditions and reproducibility. These toxicity tests are usually carried out under constant exposure concentrations, which can be far from reality of environmental exposure regimes as foreseen by the practical use of chemicals. In that respect mechanistic effect modelling, such as Toxicokinectic – Toxicodynamic (TKTD) modelling, has recently been playing an increasing role in the extrapolation of effects from constant controlled exposure conditions to time-variable exposure, closer to real environmental conditions. Among TKTD models, models based on the Dynamic Energy Budget theory adapted for ecotoxicology (DEB-TKTD models) offer a comprehensive framework to analyse and extrapolate sublethal effects (growth and reproduction) of chemicals on individual organisms across their whole life cycle. While the EFSA Scientific Opinion on the state of the art of TKTD effect models (EFSA PPR, 2018. EFSA Journal;16(8):5377) considers DEB-TKTD models as valuable tools for ERA, their full acceptance by stake-holders still requires the development of standardized and user-friendly tools. To bridge this gap, we developed ready-to-use functions within a new R package "rDEBtktd". This package takes advantage of the general Bayesian framework thus enabling the estimation of probability distributions for physiological DEB parameters and TKTD parameters, from which uncertainties can be easily quantified to be then propagated to forward-predictions for untested time-variable exposure scenarios. The physiological part of the DEB-TKTD model we implemented follows the original definition of the DEB model, which allows using the parameter values available for more than 1000 species in the Add-my-Pet database as prior information for the Bayesian inference process. This poster illustrates: (1) how to simply simultaneously estimate all the parameters of the DEB-TKTD model from one or several growth and reproduction datasets, (2) how to produce informative summaries to assess the results of the Bayesian inference and check all goodness-of-fit criteria, (3) how to make growth and reproduction predictions for untested time-variable exposure scenarios, (4) and finally the influence of both data quantity and design on the precision of parameter estimates.

4.06.16

Enhancing the Risk Assessment of Plant Protection Products Thanks to TKTD Modelling: Concept and Challenges, a Case Study With DEB - TKTD

B. Goussen, IBACON GmbH / Ecological Modelling; A. Coors, ECT Oekotoxicologie GmbH; T. Jager, DEBtox Research; R. Ashauer, Syngenta Crop Protection AG / Environment

The current environmental risk assessment of plant protection products (PPP) is often based on controlled laboratory experiments with constant or refined simple pulse exposure scenarios. While this approach has the benefits of simplicity, the tested exposure patterns are far from the real world ones. In addition, the summary statistics derived from these traditional approaches do not allow meaningful extrapolations to non-tested exposure patterns or to different exposure durations for instance. Mechanistic modelling and, in particular, toxicokinetic - toxicodynamics (TKTD) models have the potential to overcome these challenges. These types of models make a holistic use of all data available by integrating them in a unique framework. Dynamic Energy Budget models coupled with TKTD modules (DEB-TKTD) are the leading approach to assess sublethal effects of PPP on individuals. However, its use still needs to be standardised and expended in order to increase confidence for use in risk assessment. In this project, we used DEB-TKTD modelling to predict the effects of a fungicide on four species (*Daphnia magna*, *Ceriodaphnia dubia*, *Hyalella azteca*, and *Chironomus riparius*) exposed under time-varying conditions. This project followed the recommendations of the EFSA Scientific Opinion on TKTD modelling. As such, experiments were developed for both the model calibration and validation. The validated models were then used for forwards predictions. We outline here the concept used as well as the challenges we faced and how these were overcome on both the experimental and the modelling parts of the project. We discuss the usage of this approach and its benefits to the regulatory risk assessment framework.

4.06.18

Robust and (Partially) Automated DEBtox Analysis of Chronic Toxicity Data

T. Jager, DEBtox Research; M. Trijau, IBACON GmbH / Ecological Modelling; N. Sherborne, Syngenta / Environmental Safety; B. Goussen, IBACON GmbH; R. Ashauer, Syngenta Crop Protection AG / Environment

Toxicokinetic-toxicodynamic (TKTD) models offer a mechanistic understanding of individual-level toxicity over time and allow for meaningful extrapolations from laboratory tests to untested exposure conditions in the field. Thereby, they represent a powerful tool for ecotoxicological studies, both in a regulatory context as well as for basic research. In contrast to mechanistic effect models at higher levels of biological organization (e.g., population and community models), TKTD models can be, and generally are, parameterized by fitting them to data (results from laboratory toxicity tests). Fitting models comes with a range of statistical and numerical challenges, which may hamper routine application of TKTD models in practical settings. Especially in the context of environmental risk assessment, there is a need for robust and user-friendly software tools to automatically extract the best-fitting model parameters and quantify their uncertainty from any data set. This study presents a general outline for TKTD model analysis, rooted in likelihood-based ("frequentist") inference. This general outline forms the basis for the specific algorithm that has been implemented into the openGUTS software for the robust and automated analysis of survival data. Here, we show that the presented approach is more broadly applicable to low-dimensional problems, using a DEBtox2019 analysis as example.

4.06.19

Estimating Biovolume of the Copepod Tigriopus Brevicornis From Automated Imaging in Light of Changes in Shape; a Prelude to Deb-Based Modelling

T. Jager, DEBtox Research; J. Heuschele, University of Oslo / Section for Aquatic Biology and Toxicology; T. Lode, K. Borgå, University of Oslo / Department of Biosciences

Dynamic Energy Budget (DEB) modelling offers numerous advantages for the analysis and prediction of stressor effects. These advantages result from the relative simplicity of DEB, the fact that it mechanistically links effects on different traits, and because of the explicit incorporation of the factor of 'time'. However, any bio-energetic analysis of growth and development requires accurate estimation of the animal's biomass over time. Length measures are often easy to obtain, but they are only useful if the organism retains a constant shape over ontogeny. For an isomorphic organism, there is a constant factor between the actual length and the cubic root of body volume (and thereby, most likely, body mass as well). Copepods offer several challenges in this respect. They have a major change in morphology at the metamorphosis from nauplius to copepodite, and for some species, more gradual changes in shape have been identified within the nauplius or copepodite phases as well. Therefore, before we can analyse effects of stressors with DEB models over time, we need to establish rules to provide an accurate estimation of biovolume. To this end, we analysed measurements from automated imaging on *Tigriopus brevicornis*,

where individuals were followed over their entire development (from nauplius stage I to adult copepodite stage VI). From the images, we obtained total body length, body width, and projected area. In addition, some images allowed estimation of the organism's height. The results confirm that *T. brevicornis* changes shape dramatically at the moult from nauplius to copepodite. Further, also within the nauplius and copepodite phases, a clear, gradual, change in shape over ontogeny was discernible. Accounting for these changes in shape, *T. brevicornis* grows almost linearly over time on length basis, as expected from previous DEB-based analyses for copepods, as long as body length is expressed as 'volumetric length' (cubic root of estimated volume). Another interesting finding is that this species stops growing, and even shrinks, before or during the metamorphosis to the first copepodite stage. We are not aware of previous observation of this phenomenon for other copepod species. After metamorphosis, growth continues again as expected, until the adult stage. These rules will help us analyse automated imaging results in later stages of the project, where we will expose the organisms to to multi-stress by combined predation risk and copper exposure.

4.06.21
Approach for Simplified TKTD Modelling of Less Toxic Compounds in Mixtures
J. Witt, Bayer AG / Environmental Safety; S. Heine, Bayer AG Research & Development Crop Science; T. Preuss, Bayer Ag / Environmental Safety
Toxicokinetic/Toxicodynamic (TKTD) effect models are gaining increasing importance as Tier 2C refinement tools. The setup of a compound-specific TKTD model requires a set of calibration and validation studies, which are often non-standard and labour-intensive. When modelling the effect of mixtures, certain compounds (e.g. metabolites) may contribute little to the overall toxicity. The effort required to generate the calibration and validation data for such a compound (as well as the effort for model development and evaluation) would then appear excessive. Nevertheless, in order to obtain the (scaled) internal concentration at an appropriate temporal resolution, a TKTD model for each component of the mixture is so far required for a calculation of mixture toxicity. We present a simple, straightforward, and conservative approach that allows for inclusion of less toxic compounds into the effect modelling of mixtures using only tier 1 data, no peak exposure studies. The underlying assumptions are the same as those implicitly underlying the standard tier 1 risk assessment: - Constant exposure corresponding to the PECmax - Rapid equilibrium between external and internal concentration We show that the approach is - More conservative than applying a tier 2C risk assessment for all compounds of the mixture - Equivalent to the standard tier 1 approach when applied for all compounds of the mixture This allows to focus the experimental efforts on the compounds contributing most to the overall risk. While the approach is presented for macrophyte models, it should be equally applicable to other TKTD models.

4.06.23
Global Sensitivity Analysis of the Lemna Model by Schmitt Et Al. 2013 Using R
J. Klein, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecotoxicology; U. Hommen, Fraunhofer IME / Ecotoxicology; J. Witt, T. Preuss, Bayer Ag / Environmental Safety
The toxicokinetic-toxicodynamic (TKTD) growth model by Schmitt et al. 2013 for the standard macropyhte test species Lemna sp. (duckweed) is considered ready for use in the risk assessment of plant protection products in a Scientific Opinion of the EFSA PPR panel. We investigated the general behaviour of the model parameters by conducting a global sensitivity analysis. The analysis is restricted to the simulation of laboratory growth tests, which can be used as Tier 2C approach in the EU risk assessment scheme (refined exposure test). To cover different exposure patterns, we evaluated a) constant exposure, b) one pulse exposure and c) two pulse exposure within the standard test duration of 7 days. The exposure level was chosen such that the area under the curve was the same for all three scenarios. As relevant endpoint, the growth rate over 7 days was used.

4.06.26
Individual Based Modelling to Extrapolate Early Life Stage Effects of Zinc to Brown Trout Populations
S.D. Janssen, Ghent University (UGent) / Vakgroep Dierwetenschappen en Aquatische Ecologie; K. Viaene, ARCHE; P. Van Sprang, Arche consulting; K. De Schamphelaere, Ghent University (UGent) / Environmental Toxicology
In the ecological risk assessment of metals, the aim is to protect populations and communities. However, it is not always possible to experimentally study toxic effects of metals and other toxicants in population level studies. In these cases, population models can be used to predict population-level effects based on individual-level toxic effects obtained with laboratory experiments. For fish, metal toxicity experiments are often performed on early life stages, because true chronic studies are laborious, expensive and increasingly unethical to perform. Therefore, the main aims of this study were: 1) to predict how zinc induced effects on early life stages of brown trout (*Salmo trutta*) influence population density and population structure of a brown trout population; and 2) to investigate which early life stage effects are most important to explain predicted population-level effects. In this study, we extrapolated early life stage effects of zinc using the spatially explicit Individual Based Model inSTREAM-Gen. We implemented zinc induced effects on median hatching time (HT50), emergence success and the size of newly emerged trout. For this we separated the redd phase in the population model into an egg phase and a yolk-sac larvae phase, which enabled us to implement relative effects of zinc at the correct life stage. We then compared individual-level NOECs reported in the original study with the predicted population-level effects. Furthermore, we compared predicted trout densities by a relative effect of 50% on HT50, emergence success and size of newly emerged trout separately. Although, the separate individual-level effects showed up to 10% effect, predicted trout densities showed no effects larger than 3% reductions in the simulations in which we combined all observed individual-level effects together. Furthermore, a 50% reduction in trout emergence showed to affect initial recruitment to the population, but no significant effects on trout densities later in the year were predicted. In addition, a negative effect of 50% on the daily development rate (i.e. a higher HT50) or the lengths of new recruits showed positive effects on the densities of young-of-the-year and adults. Overall, this study demonstrates a method how early life stage effects of trout can be extrapolated to an endpoint more relevant for ecological risk assessment i.e. population density.

4.06.27
A Dynamic Energy Budget Individual Based Model (DEB-IBM) for Lead (Pb) Toxicity to Lymnaea stagnalis
K. Weighman, Ghent University Laboratory of Environmental Toxicology and Aquatic Ecology / Faculty of Bioscience Engineering; J. Koch, gaiac Research Institute / Environmental Toxicology; K. Vlaeminck, Arche consulting / Laboratory of Environmental Toxicology and Aquatic Ecology; K. Viaene, ARCHE; P. Van Sprang, Arche consulting; K. De Schamphelaere, Ghent University (UGent) / Environmental Toxicology
Lymnaea stagnalis is a particularly sensitive species for a variety of metals. However, the observed sensitivity of *L. stagnalis* to lead (Pb) has been highly variable between studies. The exact mechanism of Pb toxicity to *L. stagnalis* currently 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 individual-based models (DEB-IBMs) can provide a mathematical framework connecting chemical stress to individual exposure effects and, ultimately, to population dynamics. This work aims to implement a DEB-IBM to better understand the mechanism of Pb toxicity to *L. stagnalis*, explain variation in observed sensitivity between studies, and estimate the effects of Pb exposure for *L. stagnalis* at the population level. The *Lymnaea* DEB parameters were based on the add my pet database and recalibrated to available growth and reproduction data. However, these predictions, while reasonably predicting growth, gravely underpredicted the egg production observed in our focal study. A sensitivity analysis of the parameters in the DEB-IBM was conducted to provide insight into which aspects of energy budget allocation could explain this discrepancy. Ultimately, a partial re-parameterization of the DEB parameters resulted in accurate reproduction predictions. Using the reparametrized model, our predictions for the PMoA of decreased assimilation most closely align with experimental observations for Pb effects on growth and reproductive output. Additionally, assuming decreased assimilation, the model was able to predict some of the experimentally observed recovery patterns in reproductive endpoints. These results suggest decreased assimilation as the most plausible physiological mode of action for Pb toxicity in *L. stagnalis*. In a final step, the effects observed in the toxicity studies were extrapolated to the population level using the DEB-IBM. Overall, the DEB-IBM enabled us to form a hypothesis on the mechanism of toxicity and evaluate the population level relevance of these effects.

4.06.29
Identification of Patterns in Mesocosm Data: An Analysis of Untreated Control Ecosystems Across Multiple Studies

F. Abiakar, A. Schmolke, Waterborne Environmental, Inc. / -; P. Ebke, J. Schmidt, MESOCOSM GmbH Institut für Gewässerschutz; N. Galic, Syngenta Crop Protection LLC / Environmental Safety; S.M. Bartell, Cardno Inc. / Science and Enviroment; I.A. O'Connor, S. Spycher, EBP Schweiz AG; N. Schuwirth, Eawag, Swiss Federal Institute of Aquatic Science and Technology; T. Strauss, gaiac Research Institute; D.V. Preziosi, R.A. Pastorok, Integral Consulting, Inc.; R. Ashauer, Syngenta Crop Protection AG / Environment

For risk assessment, experimental mesocosms provide valuable insights into the complex responses of aquatic ecosystems to stressors. Similarly, aquatic systems models (ASMs) represent food web interactions in an aquatic species community and interactions with abiotic environmental conditions. In the context of a study to simulate mesocosms using ASMs, an analysis of control mesocosm data was conducted to identify patterns in temporal dynamics in the species communities. Control data from six mesocosm studies were anonymized, collated, and characterized using visual and statistical analyses. The data were generated during studies conducted in 2016, 2018 and 2019 by MESOCOSM GmbH. During these studies, physical parameters of temperature, oxygen, pH, water level and conductivity were measured over the study duration. Nitrate, ammonium, and phosphate concentrations and water hardness were measured prior to the beginning of each study. Additionally, weekly samples were collected for taxon and species abundance evaluation. The resulting dynamics of phytoplankton, periphyton, macrophytes, zooplankton and macroinvertebrates were analyzed for each study data set and across studies. Correlation matrices were constructed and used to examine the data and identify consistent patterns of biotic and abiotic interactions. The trends observed within and across the studies show considerable temporal variability in species composition and abundance. The characterization and understanding of similar and repeated temporal patterns in the untreated aquatic mesocosms is an important foundation for the simulation of mesocosm studies using ASMs. We provide perspectives on the challenges associated with variability observed in mesocosm controls, and offer possible explanation and insights for managing these challenges in modeling.

4.06.30
Linking Time-Variable Exposure on Catchment Scale to Effects on Stream Populations in Realistic Landscapes
T. Strauss, gaiac Research Institute; S. Bub, University of Koblenz-Landau; J. Gerhard, gaiac - Research Institute for Ecosystem Analysis and Assessment; T. Schad, Bayer AG Crop Science Division / Environmental Modelling

Ecosystem models are powerful tools for predicting species population dynamics for different environmental scenarios under consideration of natural and anthropogenic stressors such as plant protection products (PPPs). By assessing the risk at landscape level, more realism can be achieved for the environmental risk assessment of aquatic ecosystems. The variable use of PPPs by different farmers on a spatio-temporal scale in a catchment area will be propagated into an exposure scenario taking into account weather conditions, chemical fate and hydrological processes. This exposure pattern then affects the organisms in the respective river sections of the catchment area. We follow a modular, tiered approach in this landscape model framework (Landscape Model), in which individual components can be added, improved or exchanged according to current needs and knowledge. The Landscape Model integrates hydrological (Catchment Modelling Framework (CMF)), PPPuse, exposure (eXDrift), e-fate (e.g. Cascade-TOXSWA), population (e.g. STREAMcom) and TKTD effect models (GUTS). For effect modelling, we use the aquatic stream community model (STREAMcom). STREAMcom simulates the population dynamics of aquatic macroinvertebrates depending on spatial explicit habitat maps for a stream section as well as temporal information on abiotic factors like water flow and temperature, food availability and chemical exposure. Additionally, functional trait databases, dynamic energy budget-based population models and process-based effect models (e.g. TKTD / GUTS) are included in the STREAMcom model. The goal of our study is to implement and simulate the dynamics of regulatory relevant species like mayflies as a function of PPP exposure, hydrological and thermal conditions, and landscape structure. This concept basically allows the combined assessment of anthropogenic stressors, such as pesticides, and natural stressors, such as flooding and drought at changing temperatures, as influencing factors on aquatic organisms. In this presentation, the conceptual and technical integration of STREAMcom into the modular structure of the Landscape Model is demonstrated and illustrated using exemplary simulations.

Environmental Risk Analysis and Regulation of Polymers

4.07.07
Algal Toxicity of Polyquaterniums - Do's, Don'ts and Interesting Results!
A. Brun, Aarhus University / Department of Environmental Science; J. Brill, Procter & Gamble Company / Global Product Stewardship; M. Hansen, Aarhus University / Department of Environmental Science; D. Arndt, The Procter & Gamble Company / Global Product Stewardship; K.A. Connors, Procter & Gamble Company / Global Product Stewardship; H. Sanderson, Aarhus University / Department of Environmental Sciences

Cationic polymers and their effect in the environment is to this day still not very well understood. Polymers are currently exempt from many regulatory frameworks and thereby lack publically available data. Though, literature suggests that water soluble cationic polymers may have adverse effects on aquatic organisms such as algae. Algae are often considered to be the most sensitive test organism and are of great importance to maintain a healthy ecosystem. Standard algal toxicity test guidelines such as the OECD guideline 201 are routinely used within regulatory frameworks. This guideline was however designed with soluble industrial compounds in mind. Cationic polymers can be inherently difficult to test given their strongly sorbtive nature. Additionally, given their large size, tendency to be sticky, and cationic charge, it is unclear if traditional test methods are appropriate for characterizing their hazard potential. This research investigated the toxicity towards green algae, *Raphidocelis subcapitata,* in a standardized manner. The cationic polymers investigated included polyquaterinum-6, -10 and -16. These compounds represent a range of charge density, monomer backbones and molecular weights varying from 60 kDa to more than 2000 kDa. Tests followed the OECD 201 guideline, with some modifications based on guidance for difficult to test substances and historical experience with similar compounds. Here we outline the "*do's and don'ts*" for testing algal toxicity of these compounds (e.g. solution preparation, homogenization considerations before biological measurements are made, etc.). Mitigating factors like addition of organic carbon in the form of humic acid were also explored. Our findings point toward the importance of specific steps and experimental considerations when testing polyquaterniums in algae. We also found toxicity differences between the different groups of polyquaternium compounds, where polyquaternium-10 has shown to impact the algae by causing it to clump, but not inhibit the growth substantially. Polyquaternium-6 and -16 where both considered to be more toxic since they inhibited the growth of algae. Humic acid was found to mitigate toxicity in low concentrations – which underlines the importance of adjusting the existing test guidelines for compounds like polyquaterniums.

4.07.08
Evaluation of the Chronic Toxicity of Polyquaternium-6 to Daphnia magna
H. Woods, A. Brun, Aarhus University / Department of Environmental Science; J. Brill, K.A. Connors, Procter & Gamble Company / Global Product Stewardship; H. Sanderson, Aarhus University / Environmental Science

Cationic polymers have been recognized as potential polymers of concern and have received increased regulatory attention due to mounting evidence of their documented potential toxicity to aquatic organisms. Polyquaterniums (PQs) are a water-soluble subset of cationic polymers that possess multiple positive charges due to their quaternary ammonium functional group. The intrinsic properties of PQs that are responsible for their use as water treatment flocculants and in personal care products, among other uses, are also responsible for making them notoriously difficult to study. Their strong and well documented affinity for an abundance of anionic surfaces necessitates careful consideration of biological availability in toxicity test systems as they are likely to sorb and interact with organic carbon and anionic surfaces in test solutions. For chronic testing, where feeding is required, this may significantly impact bioavailability. Furthermore, while explorations into acute cationic polymer toxicity to *Daphnia magna* have noted that toxicity is reduced in the presence of supplied food, implications for chronic toxicity mitigation are unknown. This study aimed to examine the chronic toxicity of two polyquaternium-6 (PQ6) compounds to the freshwater invertebrate *Daphnia magna*. Overall, the chronic toxicity of PQ6 on *Daphnia magna* reproduction, 21-day EC_{50} and 21-day EC_{10} values ranged from 0.28 mg/L - 0.32 mg/L and 0.09mg/L - 0.12 mg/L, respectively. Additionally, this study aimed to illustrate the challenges and barriers needed to consider and overcome in order to accurately assess the chronic toxicity of polyquaterniums to *Daphnia magna.* Emphasis was placed on appropriate test solution preparation with a

focus on minimizing sorption losses in the test system coupled with the sufficient reporting of TOC and water hardness parameters of exposure solutions.

4.07.09
Toxicity of Cationic Polymers to Fish Embryos
K.A. Connors, Procter & Gamble Company / Global Product Stewardship; J.M. Rawlings, Procter and Gamble Company / Global Product Stewardship; M. Lam, Procter & Gamble Company / Global Product Stewardship; S.E. Belanger, The Procter & Gamble Company (retired) / Global Product Stewardship
Cationic polymers have been highlighted as potential polymers of concern. It is likely that broad categories of cationic polymers will soon require further regulatory assessment under REACH. There is currently very limited data in the public domain on cationic polymer toxicity. Fish toxicity data is especially sparse. Given their large size, polymers are not expected to pass biological membranes and may therefore not follow the standard dose-response relationships. Two cationic polymers, Merquat and Luviquat, were used in the validation of the Fish Embryo Toxicity (FET) test. These compounds had little to no measurable toxicity at 48h but were clearly toxic to embryos by 96h upon hatching. The iTAP project aims to develop methods to understand the toxicological profile of cationic polymers in order to improve cationic polymer ecological risk assessments. In this work, we will present the results from FET toxicity tests from select PQ6, PQ10, and PQ16 cationic polymers that vary in molecular weight and charge density. FET studies were conducted with and without the chorion to explore the effects of compound bioavailability. The potential toxicity remediation effects of humic acid will also be highlighted.

4.07.10
Biodegradability and Toxicity Testing of a Cosmetic Liquid Polymer - Carbomer
U. Rozman, University of Ljubljana; G. Kalčíková, University of Ljubljana / Faculty of Chemistry and Chemical Technology
In the last decades, many researchers have focused on the evaluation of environmental impacts of various polymers and particularly on their fragments called microplastics. However, there are also other types of polymers that are extensively released into the environment, but their environmental impact is practically unknown. For example, various liquid (water-soluble) polymers are used as flocculants in wastewater treatment, in paints, for textile impregnation and in cosmetics. In this context the aim of our study was to evaluate toxicity and biodegradability of a cosmetic liquid polymer – carbomer (polyacrylic acid polymerized in cosolvent mixture of ethyl acetate and cyclohexane) that is extensively used as a thickener in cosmetics and for preparation of gels. The toxicity of selected carbomer was evaluated by testing of inhibition of heterotrophic and nitrifying microorganisms in activated sludge after 30 minutes of incubation. The ultimate aerobic biodegradability in aqueous medium was evaluated by the method by measuring the oxygen demand in a closed respirometer for 28 days. The investigated carbomer did not have any negative impact on heterotrophic and nitrifying microorganisms even at the highest concentration (100 mg/L). Further, the carbomer was subjected to biodegradability testing. Microorganisms in the biodegradability test showed a high activity by degrading 60% of a reference compound (natrium acetate) in 5 days. However, the results of the biodegradability test show no degradation of the carbomer after 28 days of incubation. The abiotic degradation (without microorganisms) also did not proceed. According to obtained results, investigated carbomer do not pose an acute threat to microorganisms in activated sludge, but these microorganisms are unable to use the polymer as a source of carbon and energy and therefore there is a potential for its persistency in the environment.

4.07.11
Ecotoxicological Characterization of a Microgel-Based Pesticide Release System
C.A. Bertold, Johann Wolfgang Goethe Univ Frankfurt am Main / E3T; S. Johann, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology; F.G. Weichert, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis; A. Töpel, T. Sassmann, A. Pich, F. Jakob, RWTH Aachen University and DWI – Leibniz Institute for Interactive Materials e.V., / Institute of Technical and Macromolecular Chemistry; C.L. Kämpfer, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis; S. Heger, M. Roß-Nickoll, RWTH Aachen University / Institute for Environmental Research; H. Hollert, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology
The increasing use of industrial chemicals and pesticides poses a threat to ecosystem health and biodiversity. Hence, in the context of a sustainable bioeconomy an aim is to reduce the pesticide usage while preserving efficiency by, e.g., optimization of technologies. Creating an effective and simultaneously sustainable pesticide requires a high level of development effort and quality assessment. Within the FocusLab *green*Release, a project funded in the context of the Bioeconomy Science Center, novel copper-based pesticide release systems for plant protection via microgel containers are developed and investigated, aiming to significantly reduce the amount of applied pesticide and thus resulting in reduced environmental contaminations. An integrated anchor protein makes the pesticide less prone to wash-off by rain, and the gradual release of the copper fungicide from the microgel allows a long-lasting effect. Since copper can reduce the productiveness of soils, and can have severe impacts on aquatic wildlife, a reduced copper influx is a promising aspect for sustainable bioeconomy. Besides an efficient and cost-effective product development it is crucial to investigate the ecotoxicological effects of such products already during early stages of development and optimization in order to focus on the least hazardous candidates (Green Toxicology strategy).Hence, the current project GreenToxiConomy aims at integrating the Green Toxicology strategy and bioeconomical concepts by applying an ecotoxicological bioassay battery that contributes to the risk characterization of the newly developed microgels. The novel copper-releasing system is assessed for acute toxicity in well-established ecotoxicological model organisms by means of the zebrafish (*Danio rerio*) embryo acute toxicity test, and *Daphnia magna* acute immobilization test. Additionally, mechanism-specific toxicity is addresses by selected *in vitro* based methods focusing on, e.g., chromosomal aberration (micronucleus test) and endocrine disruption (CALUX System). Furthermore, the ecotoxicity of the novel pesticide release system is compared to commonly used copper fungicides. Alongside the aquatic ecotoxicological assessment, another part of the project also examines the effects on terrestrial organisms in the effort to draft a holistic ecotoxicological characterization.

4.07.12
Framework for Polymer Risk Assessment and Considerations on Applicability of Standard Tools, Test Methods and Models
D. Allan, Firmenich SA; N. Vallotton, Dow Europe GmbH / Toxicology Environmental Research and Consulting
An ECETOC Task Force entitled ‘Assessing the human health and environmental safety of polymers’ has been working on the topic of polymer safety assessment since January 2018, and has published two reports (ECETOC, 2019; ECETOC, 2020). The conceptual framework for polymer risk assessment (CF4Polymers; ECETOC, 2019) provides guiding principles for safety assessment of polymers. The CF4Polymers consists of the following eight steps: • Step 1: Problem formulation (risk assessment scope and protection goal definition) • Step 2: Polymer identification • Step 3: Polymer component strategy • Step 4: Grouping approach evaluation • Step 5: Determination of exposure scenarios (first part of exposure assessment) • Step 6: Exposure characterisation (second part of exposure assessment) • Step 7: Hazard assessment (hazard identification and characterisation) • Step 8: Risk characterisation The sequence of these steps can be adapted as necessary depending on the risk assessment needs and/or data availability. The second report (ECETOC, 2020) provides a detailed review of the applicability of standard analytical tools, in vitro and in vivo test methods and in silico models to assess the physico-chemical, fate, exposure-related, ecotoxicological, and toxicological properties of polymers. During 2020 and 2021, the ECETOC Task Force is working to develop a series of case studies for a range of polymer types and applications. The case studies aim to put into practice the guiding principles of the CF4Polymers (ECETOC, 2019) and the considerations on the applicability of tools, test methods and models (ECETOC, 2020). Both reports, and the case studies under development, include considerations on polymers of low concern and grouping. ECETOC. 2019. The ECETOC conceptual framework for polymer risk assessment (CF4Polymers). May 2019. Technical Report No. 133-1. http://www.ecetoc.org/wp-content/uploads/2019/06/ECETOC-TR133-1CF4Polymers.pdf ECETOC. 2020. The applicability of analytical tools, test methods and models for polymer risk assessment. March 2020. Technical Report No. 133-2. http://www.ecetoc.org/wp-content/uploads/2020/03/ECETOC-TR133-2.Polymers-Risk-Asessment.pdf

4.07.13
Registration of Polymers Under REACH: Revisiting the Scientific Background and Its Implications
K.J. Groh, Eawag - Swiss federal Institute of

Aquatic Science and Technology / UTOX Environmental Toxicology; J. Ligthart, International Chemical Secretariat; J. Muncke, Food Packaging Forum Foundation; D. Romano, European Environmental Bureau
Polymers are used in multiple applications, resulting in a widespread exposure of humans and the environment. Despite the high exposure, polymers are now exempt from registration under REACH, hence little is known about the identity, volumes, uses, hazards, and risks of polymers in the EU. In order to start polymer registration but save resources by exempting the 'safer' polymers still, the European Commission seeks to develop criteria to identify the so-called "polymers requiring registration."
Unfortunately, the latest report produced by Wood/PFA consultancies failed to provide transparent and up-to-date scientific evidence in support of the approaches or thresholds selected in several of the proposed criteria, which thus appear to have been set arbitrary. Further, several types of potential hazards of polymers were not addressed at all. In response to these omissions, we here highlight several aspects requiring further discussion among the regulators and scientists, including (i) scientific justification (or lack thereof) for using the 1000 Da as a generic threshold to identify polymers as 'safer' by default and for establishing further thresholds related to molecular weight; (ii) selection of acceptable levels of low molecular weight oligomers; (iii) treatment of stability-preserving additives; (iv) treatment of (bio)degradable, 'natural,' and inorganic polymers; (v) the need to consider production volumes and exposure levels as a registration criterion; and (vi) the need to address polymers' hazards beyond those depending on (high-level) systemic exposure. The latter deficiency should be addressed by expanding the current assessment framework to include additional hazard classes and/or testing methods necessary to enable understanding of currently unaddressed aspects, e.g., adverse effects resulting from surface interactions without systemic uptake, such as local inflammatory responses or interactions with microbiota; the presence and effects of non-intentionally added substances; and the propensity to generate microplastics, which should be regarded as an inherent hazardous property of all synthetic polymers. Given the lack of systematic information on polymers in the EU, we call for a mandatory pre-registration procedure where a minimum set of data should be submitted for all marketed polymers, as this would enable broader analyses by scientists and other stakeholders, and help ensure transparent selection of polymers to undergo full registration.

Environmental Risk Assessment of Bees and Other Arthropods Pollinators: Regulatory and Scientific Challenges for PPP and Biocides

4.08.02
Summary of the Conclusions From the EFSA Technical Report on Bee Background Mortality; Review of Usefulness and Uncertainties
H. Crosland, Cambridge Environmental Assessments (CEA) / Ecotoxicology; A.C. Brooks, Cambridge Environmental Assessments / Ecotoxicology Risk Assessment; A. Lawrence, Cambridge Environmental Assessments / Ecotoxicology and Risk Assessment; K. Brown, Brown Environmental
An updated guidance document which aimed to provide a framework for the assessment of potential risks to bees from exposure to plant protection products (PPPs) has now been available for several years (EFSA, 2013). The 2013 EFSA bee guidance document has so far not been fully implemented due to insufficient support by EU Member States (MSs). The current use of the guidance document in the EU is sporadic. Many MSs have requested a review of the guidance before they would consider full implementation. As such, in 2019 the Commission mandated EFSA to review the EFSA (2013) guidance document (Mandate M-2019-0100). Of several issues EFSA were requested to address, one particular task was reviewing and summarising evidence relating to natural bee background mortality (considering also beekeeping techniques for honeybees). Previously, a limited data set had been used by EFSA to conclude bee background mortality rates, which were used in the calculation of trigger values for the risk assessments presented in EFSA (2013). In response to the Commission request, EFSA has recently published a Technical Report (EFSA 2020). In this report EFSA aimed to present a more thorough literature search than was previously used to assess bee background mortality, as well as to consider data on different bee life stages (where previously the focus had been forager bees). The intention of this Technical Report is to inform a revision of EFSA (2013), as well as to respond to the Commission mandate. As such, the results from this Technical Report could potentially have a large impact on the future of the bee risk assessment for PPPs.This poster aims to summarise the conclusions on background mortality rates for the different bee groups and their life stages, as outlined in the Technical Report. We will review how these values differ from those used to calculate trigger values in the current version of EFSA (2013), and therefore how they may impact a revised guidance document. Finally, we will consider any uncertainties associated with the mortality values concluded in the Technical Report, as a result of the approaches and data used.

4.08.07
Response of Different Bee Species to Exposure to Plant Protection Products in Relation to Nutritional Conditions
D. Castle, Julius-Kuehn-Institute / Institute for Bee Protection; A. Alkassab, Julius Kühn-Institute / Institute for Bee Protection
Dietary nutrition (e.g. amino acids, fat, carbohydrates, protein and phytochemicals) is important for bee development and health. Especially, amino acids are necessary for growth, development, gene regulation and immunity. Therefore they might be also relevant for the detoxification of PPPs. How far different diets can affect health of different bee species in combination with plant protection products has been little investigated. We investigated combined effects of oral exposure to insecticide Chlorantraniliprol and the EBI-fungicide Prochloraz in single and mixture and diet with different nutritional values on adult *Apis mellifera, Bombus terrestris* and *Osmia bicornis.* The bees were fed either sugar solution, sugar solution with amino acids or a diverse pollen mixture. Various parameters such as longevity, food consumption, protein content and enzyme activity were studied in a laboratory approach. Newly hatched *A. mellifera* and *O. bicornis* were observed for 44 days, *B. terrestris* were of different ages and therefore only observed for 15 days. The results showed interspecific differences among bee species in response to various factors. In general, providing additional pollen to *O. bicornis* and *B. terrestris* has a positive effect on longevity of the bees, whereas amino acids had no positive effect on *O. bicornis*. Interestingly, *A. melifera* benefitted more from additional amino acids, especially in combination with Prochloraz than from pollen. This may indicate that *A. melifera* benefits especially from high protein content and amino acid composition, whereas *B. terrestris* and *O. bicornis* may benefit from phytochemicals. Furthermore it is possible, that *B. terrestris* and *O. bicornis* have different essential amino acids requirements than *A. mellifera*. An appropriate nutritional composition seems to be necessary to reduce stress from orally consumed PPPs.

4.08.08
Determination of Pesticides Using QuEChERS in Samples of Honey, Beeswax and Honey Bees From Valencia, Spain
V. Soursou, Desertification Research Centre / Environmental and Food Safety Research Group, CIDE (UV, GV, CSIC); J. Campo, Centro de Investigaciones sobre Desertificación / Environmental and Food Safety Research Group, CIDE (UV, GV, CSIC); Y. Pico, University of Valencia / Environmental Quality and Soil
Honey is an important food produced by bees from floral nectar or honeydew and is consumed daily worldwide. It is widely known for its beneficiary properties. The quality control of honey samples is undoubtedly the most important step before the release of these products at the local and global market. The contaminants that mostly affect honey bee colonies globally are pesticides. Indeed, excessive use of pesticides has been linked to high mortality rate in apiaries and to the pollinator decline that has been observed the last few years [1]. As a result, the determination of possible contamination of honey, honey bees (Apis mellifera L.) and beeswax is necessary. Hence, this study mainly aims to examine the presence of pesticides and their metabolites in honey, beeswax and honeybee samples from Spanish apiaries. The extraction protocol used was QuEChERS since this method extracts pesticides from the matrices of interest and is, also, an economical and time-saving choice [2]. Samples were analyzed by high performance liquid chromatography-mass spectrometry (HPLC-MS/MS) in order to determine the occurrence of 64 pesticides and some of their degradation products. Pesticides (e.g. dimethoate and fenitrothion) and degradation products (e.g dimethylformamide and fenthion sulfoxide) were found at concentrations ranging 0.9-84.0 ng/g, 0.7-55.9 ng/g and 1.2-327.6 ng/g in honey, honey bees and beeswax samples, respectively. This is in accordance with the results of other studies and could be the cause of the observed reduction of pollinators [1,3]. Furthermore, the hazard of pesticide residues detected in honey samples for the human being needs to be assessed [1] Hakme E, Lozano A, Gómez-Ramos MM, Hernando MD, Fernández-Alba AR. 2017. Chemosphere 184:1310-1319 [2] Calatayud-Vernich P, Calatayud F, Simó E,

Picó Y. 2016. MethodsX 3: 452-458. [3] Kaczynski P, Hrynko I, Lozowicka B. 2017. Ecotoxicol Environ Saf. 139: 124-131.
Acknowledgement - The authors thank the financial support from the Spanish Ministry of Science, Innovation and Universities and the ERDF (European Regional Development Fund) through the project CICLIC -subproject WETANPACK (RTI2018-097158-B-C31), and the Generalitat Valenciana through the project ANTROPOCEN@ (PROMETEO/ 2018/ 155).

4.08.09

The Use of Transducer Technology for the Non-Invasive Assessments of Honeybee Colony Status in (Semi)Field Studies

M. Ramsey, Covance Laboratories Limited / Terrestrial Ecotoxicology

Honeybee toxicity studies are an essential requirement in today's regulatory climate. In particular, field and semi-field trials are very high value Terrestrial Ecotoxicology study types that continue to be in ever-increasing demand from global customers. Data collection for these studies is time consuming and very labour-intensive, and observations require the most invasive procedure, the regular deconstruction of the nest for visual observations, and even then still only offer a brief snapshot of the internal status of the colony. Previous publications, have shown how instantaneous vibrational spectra obtained from a single inexpensive accelerometer placed directly in the honeycomb of a frame of bees can be used, non-invasively, to accurately track the brood cycle, predict when the colony is going to swarm, and track multiple honeybee communication signals involved with queen death, colony agitation, mass exodus events and foraging. There is no other sensor in the world that can be placed inside a honeybee hive that will collect such detailed information that is immune to the bees' activities. In this current work, we first discuss how such technology can already be beneficial for inclusion in field and semi-field trials, and then present a methodology for a study designed to further the capabilities of these devices towards field trials for testing plant protection chemicals. This would be achieved through the collection of instantaneous vibration spectra from the heart of colonies exposed to varying levels of toxic pesticide (such as Dimethoate) in a controlled field trial. From this, using various machine learning techniques, we will be able to establish the measurable effects between the vibrational spectra of the affected and unaffected colonies for use as a benchmark in future studies of crop protection chemicals.

4.08.10

Differences in Activity of Honey Bee Colonies With Restricted Flight in Tunnels and Free Flying

G. Gonsior, EUROFINS; F. Kaiser, Eurofins Agroscience Services Ecotox GmbH / Bee Field Testing; S. Knaebe, EAS Ecotox GmbH / Ecotox Field

Honey bee tunnel studies according to EPPO170 and OECD75 are standard studies in the pollinator risk assessment. They allow testing under real environmental conditions reflecting a worst-case scenario. Usually, assessments of mortality, flight intensity and behaviour are performed at single time points. Based on experience, it is assumed, that honeybee colonies are stressed due to confinement and restricted availability of forage area. But up to now, no data were available proving this assumption. In this experiment we tried to evaluate the impact of the confinement in comparison to temperature and cloud cover in detail, using the apic.ai activity monitoring system. The apic.ai monitoring system is consisting of a visual monitoring device and analysis software. It was attached to the hive entrance. It is solar-powered and UMTS-connected. All bees entering and leaving were recorded with a camera. For the trial, 5 bee colonies of similar strength (approx. 11000 bees/colony) were equipped with an apic.ai monitoring system for a period of 28 days. The observation started over three days before set-up in the tunnel tents for accommodation of the system, continued over 8 days in the tunnels (100m²; 88m² crop area), and an additional free-flying period for 20 days at a monitoring site. In addition to common assessments of mortality, flight intensity, temperature, humidity and cloud cover also honey bee activity was recorded. Periods of free-flying and confinement were compared and evaluated in relation to the parameters observed. From the results, it can be concluded that colony activity is not only driven by temperature and cloud cover but also by confinement. Higher activity values were recorded during the period in the tunnels. Interestingly individual colonies show different sensitivity under confined conditions increasing thereby the variability even without the influence of a chemical disturbance.

Environmental Risk Assessment of Chemicals with Difficult to Test Properties: Challenges, Solutions, and Strategies

4.09.01

Improving Available Guidance for Persistence Assessment of Substances (CEFIC-LRI ECO52) - Influencing Parameter on Environmental Fate of Natural and Synthetic Polymer

S. Hahn, Fraunhofer ITEM / Chemical Safety and Toxicology; D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry; G. Whale, Whale Environmental Consultancy Limited; C. Hughes, Ricardo Energy & Environment / Chemical Risk

Chemical persistence plays a key role in the determination of environmental exposure making it an important component in risk assessment and regulation. However, existing frameworks under which persistence is assessed have shown some limitations. Chemical degradation rates are prone to wide variability depending on environmental conditions, which is difficult to address in a weight of evidence approach. In addition, many substances are problematic, or fall outside the applicability domain of existing frameworks due to their specific characteristics. Finally, evaluating degradation half-lives using a compartment-by-compartment approach is overly simplistic, and neglects dynamic multimedia exchanges and degradation processes that may have an important bearing on the overall persistence of a substance in the environment. This CEFIC-LRI project aims to address these issues by improving available guidance for persistence assessment of substances, including those considered problematic to evaluate under the current frameworks. Existing testing guidelines and persistence criteria have been primarily developed for liquid or soluble organic pollutants, and do not take into account critical substance properties. For this reason polymers are usually out of the scope of those guidelines and methodologies, and specific methods have bene developed. Standard methods on biodegradation testing of polymers are usually based on respirometry, and thus mineralization data are available for natural and synthetic polymers. However, often degradation studies for polymers provide information on mass loss only or loss of functionalities. The latter is often used also for accelerated aging testing. As natural polymers are usually not a concern under real environmental conditions, better understanding is necessary to improve our methodology of polymer persistence assessment and to provide potential benchmarks for man-made polymers. Available literature was reviewed for polymer specific methodologies to identify influencing testing parameters on the environmental fate, knowledge gaps and areas of improvement. Several issues might have an influence on degradation results such as material form (powder or film, etc.), particle size, solubility, availability of isotope labelled material for testing, etc. The project aims to provide information on essential elements to justify weight of evidence on persistence assessment of polymers, and to give suggestions for future needs and research.

4.09.02

Per- and Polyfluoroalkyl Substances Testing Methods: A Critical Review"

K. Parakal, The Ohio State University / Department of Evolution, Ecology, and Organismal Biology; R.P. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology

Per- and polyfluoroalkyl substances (PFAS) are a large group of environmentally persistent organic pollutants with diverse physical and chemical characteristics. This group of chemicals has recently drawn great concern due to their ubiquitous presence in aquatic environments and the potential hazard they present to aquatic organisms. While these chemicals have important consumer and industrial applications due to their unique properties of heat, grease, oil, and water resistance, these same properties also make them very stable in the environment and difficult to biodegrade. They thus tend to persist in the environment and may bioaccumulate in humans, plants, and animals, posing ecological and human health hazards. In order to better understand the ecological hazards associated with PFAS, high quality ecotoxicological testing data are required. However, the characteristics of available data have not been assessed in terms of PFAS that have been tested, the types of tests (acute vs chronic), and the adherence of these tests to standardized toxicity testing protocols. The goal of this study was to examine the types and quality of data available from PFAS aquatic toxicity tests. A literature search of the ECOTOX Knowledgebase (US EPA – United States Environmental Protection Agency) was conducted to identify relevant published data under the broad category of PFAS and aquatic toxicity testing and a critical review of the testing methods was conducted to determine the strengths and weaknesses of available data. In this review, we have summarized methods from 80 papers on PFAS aquatic toxicology from the years 2000 to 2019. An initial summary of information suggests that

current literature on aquatic toxicity tests on PFAS fall gravely short in providing data that adheres to toxicity testing data quality guidelines. The most fundamental problem was the lack of verification of exposure dose. Well over half of the studies did not measure PFAS exposure concentrations in the test water and even fewer measured PFAS in control or dilution water. Other issues included lack of chemical identification (i.e., not providing a CAS (Chemical Abstract Service) number), not defining or adhering to standard protocols, and lack of reporting of standard environmental physical and chemical characteristics and testing water source. These shortcomings can have a significant impact on the quality of data available for the determination of aquatic ecological hazards related to PFAS exposure.

4.09.04

An Approach to Derive Mean Measured Concentrations From Aquatic Toxicity Tests With Unstable Test Substances

B. Erzgraeber, BASF SE / Agricultural Solutions - Global Environmental Fate Modelling; F. Groezinger, BASF SE / Agricultural Solutions - Global Ecotoxicology

The EFSA technical report on recurring issues in ecotoxicology (EFSA, 2019) has proposed a decision scheme to assess the acceptability of aquatic toxicity tests with non-constant exposure. In this scheme it is suggested that studies are not suitable for Tier 1 risk assessment when the exposure was < LOQ during / at the end of the test and interim analytical measurements were not available or not usable. The reason for discarding such studies is therefore often due to uncertainty about actual exposure. In the case that more than 20% degradation of the test substance is expected until the end of the exposure time, it is therefore recommended to perform studies with flow-through or static-renewal design. However, this might not always be feasible (or desired), e.g. for algae studies, where such designs are not established, or for very fast degrading compounds, where it would still be challenging or even impossible to meet the requirements laid down in the EFSA recurring issues document. If concentrations are expected to decline rather quickly in the chosen study system, more frequent concentration measurements enable a better monitoring of the actual exposure. However, the mean measured exposure is still often based on geometric mean considering only the distinct measurements. Here, an advanced approach for describing the exposure and calculating mean measured concentrations is proposed, especially suitable for substances or test systems, where significant decline is expected during the study period. If concentration analysis is conducted with sufficient sampling frequency, a kinetic degradation model can be fitted to the measured concentrations, following guidance established in the area of environmental fate (e.g. FOCUS Kinetics). In case of a successful fit of the degradation model, exposure in the study can be continuously described and the model enables for both inter- and extrapolation. The estimated kinetic parameters can be used to calculate mean measured concentrations as time-weighted average over the exposure period. The approach will be illustrated based on an example data set for the case where degradation follows single-first order (SFO) kinetics. Using this approach, valuable additional information can be gained even from studies with no recovery at the end of the test. This may be useful not only in Tier 1 studies, but also for higher tier assessments (e.g. pulsed-dose exposure designs) or even for subsequent effect modelling.

4.09.05

Using the Hydrocarbon Block Method and COSMOtherm to Predict Partition Coefficients in Heavy Fuel Oils

A. Celsie, Trent University / Chemistry, CEMC; J. Parnis, Trent University / Cemistry; D. Mackay, Trent University / Chemistry

Exposure and risk assessments are conducted as part of regulatory schemes to protect humans and the environment from potential adverse effects of chemicals.[1-3] Mechanistic mass balance exposure models have been developed to estimate human exposure to chemicals. A key challenge in improving exposure estimates for constituents of mixtures for any existing mass balance exposure model is to account for changes in mass transport (diffusion, volatilization) due to mixture effects. There are several estimation methods for predicting mixture properties, of which the most commonly applied tools include UNIFAC[4] and COSMOtherm.[5] Both of these estimation methods perform calculations based on equilibrium criteria, however COSMOtherm has outperformed UNIFAC in several comparative studies, especially with increasing molecular complexity.[6-8] COSMOtherm has also been applied to model the partitioning of chemicals involving complex phases or those with poorly characterized composition, including binary and ternary mixtures,[8]polymers,[9-10] and UVCBs.[11] King et al.[12] introduced the 'hydrocarbon block method' where hydrocarbon component groups or 'blocks' were defined and this method has successfully applied to predict the fate of gasoline components, for example by Foster et al.[13] Heavy fuel oil (HFO) is the leftover product after all other products have been distilled off of light crude oils, and it is often used as marine fuel. In this study we apply the hydrocarbon block method to HFO and use COSMOtherm to predict partition ratios in a HFO/water binary system. References:1) Stockholm Convention on Persistent Organic Pollutants (POPs), U.N.E.P. 2001.2) Regulation (EC) No 1907/2006, in L 136. 2007, European Commission, OJ. p. 3-280.3) Rowbotham & Gibson. Food Chem. Toxicol., 2011. 49(8): p1661-73.4) Fredenslund *et al*. AIChE J., 1975. 21(6): p1086-99.5) Klamt, J. Phys. Chem., 1995. 99(7): p2224-35.6) Marsh *et al*., Atmos. Chem. Phys., 2017. 17(9): p. 5583-99.7) Turchi *et al*., Chem. Eng. Sci., 2019. 197: p. 150-158.8) Liu *et al*., Int. J. Chem. Eng. Appl., 2017. 8(2): p. 82-86.9) Goss, Analytical Chemistry, 2011. 83(13): p. 5304-08.10) Parnis & Mackay. Environ Sci Process Impacts, 2017. 19(3): p. 270-275.11) Niederer & Goss. Environ. Sci. Technol., 2007. 41(10): p3646-52.12) King *et al.* CONCAWE Report no. 96/52.13) Foster *et al.*, Environ. Sci. Technol., 2005. 39(8), 2711-18.

4.09.08

Ecotoxicity Testing of UVCBs: Compartmentalised Hazard Assessment for Natural Complex Extracted Substances (Type 2) CHANCES2

P. Thomas, P. Bicherel, KREATiS; N. DELPIT, Laboratoires des Pyrénées et des Landes; S. Antoniotti, Université de Nice; A. Lapczynski, RIFM / Environmental Science

Testing of UVCBs poses a regulatory challenge and especially so when the accent is on the "U" for Unknown as well as "V" for Variable as is often the case for botanical extractions. Certain categories of Natural Complex Substances present multiple complexities for regulatory dossier submission: Only a relatively small fraction (typically < 20% of the constituents) have been identified. Constituent concentrations may vary according to batch and quality. They may have high viscosity with a thick honey-like to waxy structure, have volatile, low solubility and/or hydrophobic constituents making them difficult to test and contain hundreds of constituents making them difficult to analyse. They may have recognised toxicity, but it is unknown which constituents are responsible and how these properties can be extrapolated to other compartments. The CHANCES2 project started in October 2020 and aims to develop a new method of hazard assessment for NCS2 with a fractionation approach. Substances are tested whole and are split into three fractions: volatile "terpenic"; non-volatile & soluble; "inert". Ecotoxicity tests are performed on the whole substance and each of the fractions separately when possible. Targeted analysis will be performed on the volatile and soluble fractions. Long terms studies on daphnids will be performed to verify the inert nature of the remaining fraction. In silico approaches using the iSafeRat® WAF calculation will be used to verify if the toxicity of the fractions is predictable. Indeed, this high-accuracy method, based on Phase Equilibrium Thermodynamics, has already effectively been used to predict WAF toxicity of essential oil dossiers under REACH. If necessary, adaptations and new algorithms will be appended to the existing calculation method in order to account for the block approach for NCS type 2 substances. Laboratory studies are ongoing and the poster will discuss experimental results obtained in the light of the in silico predictions.

4.09.09

Predicting ENvironmental Toxicity of Petroleum Substances: PetroTox Implementation in KNIME

Y. Verhaegen, CONCAWE; L. Camenzuli, ExxonMobil; A. Martin-Aparicio, European Petroleum Refiners Association / REMG; C. Prosser, A.D. Redman, Exxon Mobil; D.M. Saunders, Shell International / Toxicology; S.A. Villalobos, BP / Global Product Stewardship - Applied Sciences; N. Wang, TOTAL France; D. Lyon, CONCAWE

Petroleum substances (PS) contain an indeterminably large and variable amount of constituents of different sizes and classes. For the purposes of Classification and Labelling and Chemical Safety Assessment, a large amount of ecotoxicity testing would be necessary to adequately cover the compositional complexity and variability of PS. In addition, achieving adequate effective and lethal test concentrations is a challenge and sometimes not technically feasible, due to the very low water solubility, and/or the high volatility of PS constituents. The PetroTox model can be used to conservatively predict, based on composition, acute and chronic lethal and effect concentrations usually obtained via standardized laboratory ecotoxicity testing methods for fish, invertebrates, algae and WWTP micro-organisms. PetroTox applies the Hydrocarbon Block Method (HBM) to extrapolate available compositional information to concentrations of up to 1512 representative constituents. For each constituent, the dissolved

concentration in the test system is predicted by a mass balance model based on Raoult's law. The Target Lipid Model (TLM) is then used to estimate the constituent specific LC50, EC10 and NOEC, based on K_{ow} or on the target lipid-water partition ratio (K_{TLW}). The dissolved concentration is subsequently divided by the LC50, EC10 or NOEC to obtain the acute or chronic Toxic Unit (TU) ratios. As the principal adverse effect of hydrocarbons is additive narcosis, the constituent's TUs can be summed to represent the full substance ∑TU. PetroTox applies an iterative process to identify the substance LL50, EC10 or NOEL, which equates to loading concentrations that lead to ∑TU = 1. PetroTox v4.0 is an implementation of the model in the KNIME analytics platform. Compared to the previous Microsoft Excel-VBA environment, KNIME provides a more visual, traceable, and extendable environment, which allows for the model to run in batch mode and which facilitates the analysis of intermediary data, the communication of results to stakeholders, and debugging. More options are also provided for assigning missing compositional mass, for limiting the choice of hydrocarbon classes, and for the HC5-based calculation of the constituent PNECs. In addition, estimation of acute and chronic toxicity to multiple soil and sediment species has been integrated based on TLM and Equilibrium Partitioning (EqP).

Interactions between Global Climate Change and Contaminants: the Need for a New Environmental Assessment Paradigm

4.10.05
A Modelling Framework to Project the Future Vulnerability of Aquatic Invertebrates to Chemicals With Different Mode of Action
F. Polazzo, IMDEA Water Institute / Aquatic Ecotoxicology; S. Domisch, Institute of Freshwater Ecology and Inland Fisheries (IGB); M. Flörke, Ruhr-University Bochum; S. van den Berg, Wageningen University & Research / Environmental Risk Assessment; P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences; A. Rico, IMDEA Water Institute
Climate change has already modified several meteorological and hydrological parameters. However, the effects of the changing climate are expected to become more severe in the near future (2050). Changes in water discharge, increasing number of days with low flow and increased water temperature will force many aquatic taxa to change their actual distribution to find suitable habitats. In the present framework, we use hydrological models able to project changes in several hydrological parameters to predict changes in aquatic invertebrates' distribution at the European level. In order to simulate present-day natural flow regimes and future flow regimes under climate change, the global hydrology model WaterGAP3 is applied. All calculations for current and future conditions (2050s) are carried out on a 5' ×5' European grid. Calculations of future hydrological conditions are based on the Intergovernmental Panel on Climate Change (IPCC)'s Fifth Assessment scenarios, and predictions of future water availability are generated using the WaterGAP3 modelling framework. This modelling approach is subsequentially exploited to derive projections on how the predicted changes in hydrological conditions are likely to shift present-day distribution of aquatic invertebrates, as a consequence of a modification of their habitat in terms of hydrology and climatology. Finally, we will use the projected future species distribution to study how the vulnerability of aquatic invertebrates to chemicals with different modes of action is likely to change by 2050. To do that, we combine two modelling approaches, both using species traits as common currency to calculate species sensitivity and vulnerability (based on population recovery capacity) to chemicals with different mode of action. Overall, the framework we are presenting, is composed by several steps and combined numerous modelling approaches. It brings together experts form different disciplines and it proposes, for the first time, a new tool able to give indications on how the sensitivity to climate change is likely to shift the relative portion of vulnerable aquatic invertebrates to chemicals with different modes of action.

4.10.08
The Effect of Elevated Temperature on Triclosan-Induced Changes in Earthworm Eisenia Fetida Life Cycle and Oxidative Stress Levels
D. Miškelytė, Vytautas Magnus University / Department of Environmental Sciences; J. Zaltauskaite, Vytautas Magnus University / Department of Enviromental Sciences
Antimicrobials are essential for the maintenance of public health and life quality. Numerous different compounds are used in larger quantities every year. Triclosan (2,4,4′-trichloro-2′- hydroxy diphenyl ether, 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 (EOCs). Earthworms Eisenia fetida are directly exposed to soil pollutants with their thin epithelium and their feeding, thus, they are highly relevant for the assessment of the toxicity of inorganic and organic contaminants to soil biota. Climate change is an increasingly urgent problem with potentially far-reaching consequences for life on earth. There is a growing awareness of the importance of anticipating the interactions between natural and chemical stressors, and the way they affect organisms and their performance. While an increasing body of knowledge has demonstrated the physiological and biochemical effects on soil biota induced by TCS, however, changes in ecotoxicity of TCS due to climate change have not been investigated in terrestrial organisms. The objective of the study is to evaluate the influence of elevated temperature on triclosan-induced changes in earthworm Eisenia fetida life cycle and oxidative stress levels. Triclosan-contaminated soil studies with Eisenia fetida were performed under different temperatures (20°C and 25°C). Triclosan at 5 different concentrations (10 - 750 mg TCS kg-1) in soil was applied. Earthworm lifecycle indicators (weight growth rate, survival rate, reproduction), biochemical indicators (the activity of enzymes) and the damage of oxidative stress (lipid peroxidation) were detected.

4.10.10
Application of Green Toxicology in Early Development Stages of Bio-Hybrid Fuels
K.E. Braun, Institute for Environmental Research, RWTH Aachen University / Institute for Environmental Research; S. Heger, RWTH Aachen University / Institute for Environmental Research; H. Hollert, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology; M. Roß-Nickoll, M. Du, RWTH Aachen University / Institute for Environmental Research
Increasing industrialization and population growth are leading to an increase in global energy and fuel demand, particularly in the transportation sector. Moreover, the use of petroleum-derived diesel and gasoline fuels also poses a significant risk for the environment and the global climate. Therefore, research on developing alternative and renewable fuels is increasing rapidly. Bio-hybrid fuels represent promising alternatives to fossil fuels. They are generally considered to be more environmentally friendly and more sustainable than fossil fuels and contribute less towards global climate change, especially when produced with excess renewable energy. However, due to the focus on engineering and technical enabling, an important factor, the toxicity of these novel fuels, is often not adequately considered. Toxicity investigation of biofuel candidates should be integrated in the fuel development process and can provide important information on potentially hazardous biofuels. With regard to the large amount of fuels required by the transportation sector and the consequential contamination of the environment by fuel spills and leakage, the introduction of sustainable, non-toxic fuels provides an opportunity to avoid harmful effects on the environment and human health. Following the continuous implementation of the previously proposed framework for a "Green Toxicology" approach we apply in silico and in vivo tools to investigate individual substance and mixture toxicity of bio-hybrid fuel candidates and blends thereof. Through early implementation of the aforementioned framework we participate in the early development stages of future fuels and are able to optimize them with regards to their environmental impact. This enables our partners, representing the fields of chemistry, process and combustion engineering, to select the least toxic substances while still maintaining a blend with the desired chemical properties. The focus of this investigation is a five-component blend which has been identified as a possibly superior fuel for combustion engines regarding combustion performance and emission. The mentioned framework is being applied during the ongoing development to identify the components which are mostly contributing to the toxic potency of the blend.
This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany´s Excellence Strategy – Exzellenzcluster 2186 „The Fuel Science Center".

4.10.11
Safe and Sustainable-By-Design Chemicals: A Computer Based Approach to Improve Biodegradability
J. van Dijk, Copernicus Institute of Sustainable Development, Utrecht University / PRAS unit; H. Flerlage, University of Amsterdam; S. Beijer, C. Slootweg, Van 't Hoff Institute for Molecular Sciences, University of Amsterdam; A. van Wezel, University of Amsterdam/IBED

Institute
Chemicals have a fundamental role in our daily life. Over the last years, the worldwide consumption of chemicals has increased not only in volume but also in diversity. Due to their wide-spread use, chemicals are continuously released and can subsequently harm environmental health. Many well-known historical chemical pollution problems are the result of the release of highly persistent chemicals and hence persistency is seen as a major cause of concern [1]. The aim of this work was to explore opportunities to prevent environmental pollution early in the chemical life-cycle by redesigning structures for biodegradability whilst the initial function of the chemical is preserved. For our case-study, the NORMAN database was screened and triisobutyl phosphate (TiBP) was selected for redesign. TiBP is used as a flame-retardant on textiles and is persistent in sediments [2]. Alternatives for TiBP were generated with an *in silico* approach, creating a large virtual library with circa 8.2 million chemical structures. Relevant properties for these structures (including LogKow) were predicted with QSAR models. A multi-criteria selection process was developed by assigning a 'desirability score' to the generated structures. The scoring system revealed chemical alternatives with an improved profile of predicted environmental properties. From the top 500, one alternative structure was selected and synthesized based on expert judgement. Properties of the new chemical are currently tested. The developed approach can identify chemical structures with improved (environmental) properties, and is in line with current policy goals as defined under e.g. the EU Green Deal. Safe and sustainable-by-design chemicals can play a pivotal role in enabling a circular economy by 2050 when aligned with concepts of circular chemistry [3]. [1] Cousins, I. T., Ng, C. A., Wang, Z., & Scheringer, M. (2019). Why is high persistence alone a major cause of concern?. Environmental Science: Processes & Impacts, 21(5), 781-792. [2] Alygizakis, N. A., Oswald, P., Thomaidis, N. S., Schymanski, E. L., Aalizadeh, R., Schulze, T., ... & Slobodnik, J. (2019). NORMAN digital sample freezing platform: A European virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in "digitally frozen" environmental samples. TrAC Trends in Analytical Chemistry, 115, 129-137. [3] Keijer, T., Bakker, V., & Slootweg, J. C. (2019). Circular chemistry to enable a circular economy. Nature chemistry, 11(3), 190-195.

Marine and Freshwater Pelagic and Benthic Harmful Algal Blooms: Toxins Production, Detection, Fate, Effects, Monitoring and Management.

4.11.04
Development and Evaluation of a Sensitive, Diffusive Gradients in Thin-Films (DGT) Method for Determining microcystin-LR Concentrations in Freshwater and Seawater
E. D'Angelo, University of Kentucky / Plant and Soil Sciences
A Diffusive Gradients in Thin-Films (DGT) passive sampling technique was developed for microcystin-LR (MCLR), one of the most common and toxic microcystins. Three types of resins (HP20, SP700, and XAD18) were evaluated for MC-LR uptake kinetics, capacities, and extraction efficiencies and simple procedures were developed for determining MC-LR concentration in binding disc extracts by Adda-ELISA (U.S. EPA Method 546). The XAD18-DGT/Adda-ELISA method had a 7-d deployment time detection limit of ≈0.05 µg/L and capacity of > 250 µg/L of MC-LR in water samples which encompass U.S. EPA and WHO advisory concentrations for drinking and recreational waters. The XAD18-DGT/Adda-ELISA method determined time-averaged MC-LR concentrations in waters with wide ranging pH (4.9–8.3) and ionic strength (0.04–0.8 M) under well-stirred and quiescent conditions with 90–101% accuracy. In addition to high sensitivity and accuracy, the method is simple, inexpensive, and applicable for determining MC-LR and related MCs concentrations in waterbodies with wide ranging chemical characteristics and hydrodynamic conditions.

4.11.07
Cylindrospermopsin and Glyphosate Accumulation in Lettuce (Lactuca sativa) Simultaneously Exposed to Both Toxicants in Hydroponic and Soil Systems
S. Sengupta, School of Bio Sciences and Technology Vellore Institute of Technology, Vellore- 632 014, Tamil Nadu, India; M.M. Freitas, School of Health, Polytechnic Institute of Porto; E. Pinto, I. Ferreira, LAQV/REQUIMTE, Departament of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal; F. Oliveira, J. Azevedo, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450–208 Porto; A. Prieto, L. Diez-Quijada, A. Jos, A.M. Cameán, Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, Profesor García González n◦2, 41012 Seville, Spain / Toxicology; A. Campos, Interdisciplinary Centre of Marine and Environmental Sciences, CIIMAR; V. Vasconcelos, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450–208 Porto
In nature, the simultaneous occurrence of multiple emergent contaminants such as cyanotoxins (e.g., cylindrospermopsin (CYN)) and herbicides (e.g., glyphosate (GLY)), is highly expectable and it can be anticipated, mainly in the aquatic and terrestrial environments. The use of contaminated water for irrigation can be hazardous to the agricultural sector and some studies have reported that, individually, these contaminants can be accumulated in the edible tissues exerting a negative influence on crop plants safety and ultimately in human health. Furthermore, recent studies have suggested that some cyanotoxins (e.g., microcystins) can change the membrane permeability of roots, resulting in changes in the accumulation rates of other contaminants in plants. Since edible plants are exposed to a wide variety of substances through irrigation water, there is increasing concern in the potential adverse effects of the interactions between those substances when present simultaneously, especially when this can have potential public health consequences. The aim of this study was to determine the accumulation of CYN in *Lactuca sativa* simultaneously exposed to GLY at environmentally relevant concentrations. Lettuce plants were exposed for 15 days to 50 µg/L or kg of CYN-containing crude extract (*Chrysosporum ovalisporum* culture - LEGE X-001) and 750 µg/L or kg of GLY, in hydroponic and soil systems, respectively. The concentration of CYN and GLY in lettuce plants (roots and leaves) was determined by LC/MS-MS. The results show that, at the described conditions, CYN was accumulated in roots (0.06-7.62 µg CYN/g Dw) and leaves (0.13-1.1 µg CYN/g Dw) of lettuce, especially when plants were exposed in hydroponic system. However, interestingly, when lettuce plants were exposed simultaneously to both toxicants the concentration of CYN assimilated by lettuce plants (roots and leaves) was respectively, 1.5-fold and 1-3-2.2-fold lower than in the exposure to isolated CYN. Conversely, the plants exposed to the mixture in soil system, showed that the concentration of GLY incorporated by lettuce (roots and leaves) was higher than in the exposure to the isolated compound (0.04 - 0.21 µg GLY/g and < LOQ - 0.84 µg GLY/g, respectively). This finding highlights the potential for the enhancement of GLY accumulation in lettuce plants due to their co-occurrence with CYN, and it underlines the importance of further research regarding the mechanism involved. **Keywords:** Bioaccumulation, cylindrospermopsin, *Lactuca sativa*, glyphosate. **Acknowledgements:** This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 823860.

4.11.08
Effects of Cylindrospermopsin and Glyphosate at Environmentally Relevant Concentrations on Growth and Mineral Content of Beetroot PLANTS (Beta vulgaris)
V. Priya, Aquaculture Biotechnology Laboratory, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore- 632 014, Tamil Nadu, India; M.M. Freitas, School of Health, Polytechnic Institute of Porto; E. Pinto, A. Almeida, LAQV/REQUIMTE, Departament of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal; F. Oliveira, J. Azevedo, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450–208 Porto; A. Campos, Interdisciplinary Centre of Marine and Environmental Sciences, CIIMAR; R. Sudhakaran, Aquaculture Biotechnology Laboratory, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore- 632 014, Tamil Nadu, India; V. Vasconcelos, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450–208 Porto
Natural toxins produced by freshwater cyanobacteria, such as cylindrospermopsin (CYN), have been regarded as an emergent environmental threat. Cyanotoxins can be applied directly to soil by using contaminated water for agricultural irrigation. Despite the risks for food safety, the impact of cyanotoxins in agriculture is not yet fully understood. Furthermore, in soil-plant system the

simultaneous occurrence of cyanotoxins and pesticides can be highly expectable. Pesticides, especially residues of glyphosate (GLY), have been frequently detected in soils and have been recognized to contribute to soil toxicity. Beetroot (*Beta vulgaris*) are root vegetables, extensively consumed worldwide with great importance for human nourishment and economy. It is, therefore, important to evaluate the effects of using water contaminated with CYN and GLY on beetroot cultivation. This study aimed to assess the effects of environmentally relevant concentrations of CYN, GLY and a mixture of both on growth and mineral content of beetroot (roots and leaves) cultivated in soil system. Plants were exposed in controlled conditions to CYN-crude extracts (50µg/kg) (*Chrysosporum ovalisporum* culture - LEGE X-001), isolated and in mixture with GLY (750 µg/kg) for 4 months. Beetroot growth was assessed by determining its fresh (Fw) and dry weight (Dw). The determination of mineral content was made by inductively coupled plasma-mass spectrometry (ICP-MS), after sample mineralization by microwave-assisted acid digestion. The results denote that Fw and Dw of beetroot (leaves and roots, respectively) were significantly changed (<i>P

4.11.09

Risk Assessment of Cyanotoxins in Agriculture - an Overview of the Effects in Plant Performance, Yield and Contamination

A. Campos, Interdisciplinary Centre of Marine and Environmental Sciences, CIIMAR; M. Freitas, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research; B. Oudra, Faculty of Sciences Semlalia Marrakech; V. Vasconcelos, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450–208 Porto

Fresh water resources in many countries are vulnerable due to their biogeographical and climatic characteristics. Moreover higher water consumption and higher human impacts in the downstream water bodies is leading to a higher eutrophication with increased incidence and intensity of cyanobacteria blooms and their toxins. The scarcity of clean water resources leads to the compulsory use of water containing cyanobacteria and their toxins in agriculture. Research carried out in particular in the last decade, has showed that Microcystins (MCs), the most prevalent group of cyanotoxins in freshwaters in the world, can induce several adverse effects in plants (terrestrial and aquatic) including agricultural species. Among the effects reported are the inhibition in germination and growth, morphological alterations in roots and aerial organs, impairment of photosynthesis, and reduced yield. Nevertheless, the impacts in crops depend on many factors, including genetic. Other plant stress factors can also enhance the toxicity of cyanotoxins. In this work, a review is carried on the toxic effects of cyanotoxins in agricultural plants, focusing in particular those linked to environmental realistic scenarios. This analysis highlights that MCs at 1 µg/l, are not harmful to plants thereby, can be acceptable in irrigation waters. Moreover, most crops may tolerate MCs to a concentration up to 10 µg/l. Few morphological and physiological impairments were linked to exposure to this range of concentrations. Moreover, the toxicity of the water with 10 µg/l MCs, can be ameliorated for instance by promoting the growth of crop species that are more tolerant to MCs or, by withholding the use of contaminated water during germination and seedling development, since the sensitivity of crops increase considerably in these stages of development. These procedures will also help avoid the accumulation of MCs in plants and edible tissues and avoid food safety issues.In conclusion, this range of concentrations can be considered in the domains of water management and water treatment for agricultural purposes; and also in terms of future regulation development, concerning the maximum levels of cyanotoxins (MCs) admitted in irrigation waters. **Keywords:** Cyanotoxins, Microcystins, Crops, Phytotoxicty, Food safety.
Acknowledgements: This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 823860.

4.11.10

Generating Ecotoxicity Information on Cyanotoxins and Prymnesins Using Extracts of Cultures and Surface Water Samples

J.M. Lazorchak, U.S. Environmental Protection Agency / Office of Research and Development Cincinnati; H. Haring, Formerly Pegasus currently SVL Analytical; W. Thoeny, Pegasus co US EPA Cincinnati / Office of Research of Development Cincinnati; M. Ghorab, Formely Pegansus Inc c/o U.S. EPA currently US.EPA OCSPP D.C. / OCSPP; J. Jones, Formerly Pegasus Technical Services, inc. currently U.S. EPA Cincinnati / Office of Research & Development Cincinnati; N. Dugan, U.S. EPA Cincinnati / Office of Research and Development; J. Allen, U.S. Environmental Protection Agency / Office of Research and Development Cincinnati; T. Sanan, U.S. EPA Office of Research and Development Cincinnati / CESER-WID-CMTB; C.T. Nietch, U.S. Environmental Protection Agency / ORD/CEMM/WECD/WMD; D. Tettenhorst, U.S. EPA Office of Research and Development / Office of Research and Development Cincinnati

There is a lack of information to estimate safe levels for aquatic life concerning the toxicity of natural toxins produced by cyanobacteria and the freshwater invasive microalgae *Prymnesium parvum*. Given the uncertainty of standards for toxins, the cost of using them to conduct acute and chronic toxicity tests and their potential impurities, a new approach is proposed using cultures and ambient samples. In this study we have used laboratory cultures of a toxin producing unicellular *Microcystis aeruginosa* and *P. parvum* and non-toxin producing filamentous *Anabaena flos-aquae.* Each culture was centrifuged to remove each species from its culture media, then resuspended in moderately hard water. The *M. aeruginosa* culture was then frozen/thawed three times at -20 °C following procedures used for ELISA analyses. A similar lysing procedure was also used on lake water samples. Acute 48-hr tests and 7-day short term chronic tests were conducted with a cell density of 3.73×10^6 (2017), 4.42×10^6 (2018), 4.16×10^6 (2019) and 1.75×10^6 (2020) cell/ml (average microcystin concentrations 73, 52, 37, and 855 ug/L) with *Ceriodaphnia dubia*, *Neocloeon triangulifer*, *Hyalella azteca* and larval *Pimephales promelas*. A stock concentration of 1746 ug/L was prepared for 100% exposure. For all studies, exposures were conducted at 0, 6.25, 12.5, 25, 50 and 100% of the stock concentration. Microcystin concentrations in the lysed samples as high as 74 ug/L did not cause any acute toxicity greater than the lab water controls to any of the 4-test species. *A. flos-aquae* caused mortality greater than the controls to *N. triangulifer.* For chronic tests, 80 L of the culture *M. aeruginosa* were used. No treatment levels exhibited any adverse survival effects in *C. dubia*, *P. promelas* or *H. azteca* tests. The only adverse effects noted in these tests were the sub-lethal point estimate endpoints for reproduction inhibition (IC_{25}=30.96% [1.37×10^6 cells/ml]) in the *C. dubia* bioassay. August 2017 Lake Harsha sample (total # of cells 300,000 cells/ml) was not acutely toxic to any of the 4-test species. For chronic toxicity lake water sample, the long term lethal concentration (LC_{50})% was 7.81, 15.33, and 7.59 in *C. dubia*, *P. promelas* and *N. triangulifer* tests, and IC_{25} were 1.03, 8.36, and 1.14 %, respectively. For *P. parvum* LC_{50}s were 493,850 cells/ml for *C. dubia* and 121,761 cells/ml *N. triangulifer*. One observation that was made is that cultures of similar cells/ml did not produce the same concentration of intracellular toxin.

4.11.11

Toxicity of microalgae to non-target organisms is related to species, fraction and culturing

Y. Sakka, University of Bremen / General and Theoretical Ecology; A. Gavalás-Olea, Bremerhaven University of Applied Sciences / EcoMaterials: Sustainability.Raw Materials.Processes.; J. Köser, University of Bremen / Center for Environmental Research and Sustainable Technology; N. Nentwig, University of Bremen / Center for Environmental Research and Sustainable Technology UFT; T. Hauser, I. Lang, Bremerhaven University of Applied Sciences / EcoMaterials: Sustainability.Raw Materials.Processes.; J. Filser, A. Siol, University of Bremen / Center for Environmental Research and Sustainable Technology UFT

Exopolymers (EPS) of microalgae are promising candidates as additives for lubricants. The EPS producing red algae *Dixoniella grisea* is not described as toxin producing species in the literature while some strains of the also EPS producing cyanobacterium *Cyanothece* sp. are. In addition, culture conditions may affect quality and quantity of both EPS and toxins. For using microalgae as sustainable suppliers of raw materials, negative effects to different non-target organisms need to be excluded under a broad set of algal culture conditions. For this purpose, a rapid screening using a set of non-target organisms representing three different exposure pathways, namely *Daphnia magna*, *Folsomia candida* and *Enchytraeus crypticus*, was developed. It was used to test the culture, parts of the culture, the algal material and EPS for their potential to induce acute toxicity in any of these species. Each culture was tested singly to take into account the culture conditions in the evaluation. Overall, EPS most frequently caused toxic effects to any of the non-target organisms. Of all test species, *E. crypticus* was most often affected. The culture conditions had different impact for the different algae strains tested, so that analytical results of the test substance composition were compared to identify possible drivers of ecotoxicity. All kinds of samples were analysed for their monosaccharide, fatty acid, and amino acid composition, and

compared according to sample type, age of the algae, and culture conditions used. Up to now, no single substance or combination of substances could be related to a toxic effect. Still, the research described here is part of an ongoing project, so that additional results will be added to the poster.

4.11.12

Cylindrospermopsin Induces Alterations on mRNA Expression of Selected Genes in a Human Kidney Cell Line

L. Diez-Quijada, Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, Profesor García González n◦2, 41012 Seville, Spain / Toxicology; M. Puerto, D. Gutierrez-Praena, University of Sevilla / Toxicology; A.M. Cameán, A. Jos, Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, Profesor García González n◦2, 41012 Seville, Spain / Toxicology

Cylindrospermopsin (CYN) is a cyanotoxin with a worldwide distribution that can be in contact with humans by different routes, and among them the oral intake of contaminated water and food is the main one. CYN induces toxic effects in different organs including the kidney, its excretion pathway due to its hydrophilic nature. But its toxicity and the responsible mechanisms are not yet fully elucidated. In this work, the influence of CYN in the expression of selected genes involved in metabolism (*CYP1A1, CYP1A2*), DNA damage responsive (*TP53, CDKN1A)*, oxidative stress (*SOD1*, *GPX1, CAT*) and cell death (*BAX, BCL2*) has been evaluated in Hek293 cell line from human kidney. For this purpose, cells were exposed to 0.5 and 5 µg/mL CYN for 4 and 24 h and the gene expression was evaluated by Quantitative Real-time Polymerase Chain Reaction (qPCR). Results obtained demonstrated that CYN caused alterations (up-regulation) mainly at the higher concentration evaluated (5 µg/mL) and the longer exposure time considered (24h). This is the case of *CYP1A1, CYP1A2, TP53, CDKN1A, GPX1, CAT* and *BCL2.* The only genes altered after 4h were *CYP1A1* and *SOD1.* Moreover, no changes in the expression of *BAX* in Hek293 cells were observed under the conditions evaluated. The results obtained indicate that CYN produces changes in the mRNA expression of genes in Hek293 cells, although further studies are required to get a full picture of the molecular mechanisms entailed in CYN toxicity.

Acknowledgments: Spanish Ministerio de Economía y Competitividad for financing the project (AGL2015-64558-R, MINECO/FEDER, UE), Spanish Ministerio de Ciencia e Innovación for the project PID2019-104890RB-I00 MICINN and for the grant number BES-2016-078773 awarded to Leticia Diez-Quijada Jiménez.

4.11.13

Genotoxic Effects of the Cyanobacterial Hepatotoxin Cylindrospermopsin in an Advanced In Vitro Hepatic Model System

K. Hercog, M. Štampar, National Institute of Biology / Department of Genetic Toxicology and Cancer Biology; A. Štern, National Institute of Biology / Genetic toxicology and cancer biology; M. Filipic, B. Zegura, National Institute of Biology / Department of Genetic Toxicology and Cancer Biology

The cyanotoxin cylindrospermopsin (CYN) is considered as an emerging health threat worldwide as the hepatotoxin was shown to exert numerous harmful effects *in vitro* and *in vivo* and was shown to be genotoxic. Toxic cyanobacterial blooms are increasing globally and the distribution of CYN producing cyanobacteria is expanding into temperate zones, due to climate change and water eutrophication. Furthermore, as CYN is highly water soluble, very stable and persistent in aquatic environments, and is found predominantly extracellularly, human exposure to CYN is more likely compared to other cyanotoxins. I*n vitro* studies describing the genotoxic activity of CYN in metabolic competent test systems are accumulating. However, the traditional *in vitro* test systems lack several phenotype specifics of the corresponding *in vivo* cell types and hence have low predictive value for the human health hazard assessment. Therefore, the cyto-/genotoxic activity of CYN was evaluated in an advanced 3D cell model developed from the human hepatocellular carcinoma cell line, HepG2. HepG2 spheroids were formed by a forced floating method and were cultured under static conditions for three days prior to the exposure to CYN (0.125, 0.25 and 0.5 µg/mL) for 72h. CYN influence on spheroid growth was measured daily. The cell viability was determined by the MTS assay and by microscopic evaluation using live/dead staining with confocal micrograph image analysis. The influence on cell proliferation, cell cycle alterations and induction of DNA damage (gH2AX foci) was determined using flow cytometry. The results revealed that CYN dose-dependently reduced the size of spheroids and affected cell division by arresting HepG2 cells in the G1 phase of the cell cycle. No increase in the induction of DNA double strand breaks was determined at the applied conditions. The advanced 3D HepG2 cell model, due to its more complex structure and improved cellular interactions, provides more physiologically relevant information and more predictive data for adverse effects in humans, and can thus contribute to more reliable genotoxicity assessment of chemicals including cyanobacterial toxins.

4.11.14

Assessment of the Toxic Effects Induced by Cylindrospermopsin in a 28-Days Oral Toxicity Study in Rats

L. Diez-Quijada, Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, Profesor García González n◦2, 41012 Seville, Spain / Toxicology; R. Moyano, V. Molina-Hernández, University of Córdoba / Department of Anatomy and Comparative Pathology and Toxicology; A. Jos, A.M. Cameán, Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, Profesor García González n◦2, 41012 Seville, Spain / Toxicology

Cylindrospermopsin (CYN) is a cyanotoxin that stands out among cyanobacterial toxins due to its worldwide distribution and its potential health risks. Humans can be in contact with this cyanotoxin by the oral route through the consumption of contaminated water and food. Thus, the aim of this work was to assess the toxicity of pure CYN in a repeated dose 28-days oral toxicity study in rats following the OECD guideline 407. CYN was administered to male and female Sprague-Dawley rats at dose levels of 18.75, 37.5 and 75 µg CYN/kg body weight. No mortality and no changes in body weight, body weight gain, food consumption and feed conversion ratio were detected. However, differences in water intake were reported in males exposed to the medium dose in the third week, without toxicological significance. Furthermore, small not dose-dependent alterations in some haematological parameters were observed in females. Also, some biochemical parameters such as glucose levels, urea and cholesterol concentrations were affected in females after exposure to CYN. The histopathology study carried out reported not significant differences in rats exposed to the highest dose in comparison to the control group. Results show that females seems to be more sensitive to CYN than males based on biochemical parameters alterations.

Acknowledgments: Spanish Ministerio de Economía y Competitividad for financing the project (AGL2015-64558-R, MINECO/FEDER, UE), Spanish Ministerio de Ciencia e Innovación for the project PID2019-104890RB-I00 MICINN and for the grant number BES-2016-078773 awarded to Leticia Diez-Quijada Jiménez.

4.11.16

Selected Cyanobacterial Toxins (Microcystins, Nodularin and Cylindrospermopsin) DO Not Directly Interact With Human Toll-Like Receptors

J.D. Hansen, U.S. Geological Survey / Western Fisheries Research Center; K.A. Loftin, Z.R. Laughrey, U.S. Geological Survey / Kansas Water Science Center; O. Adamovsky, Masaryk University, RECETOX / RECETOX

Mass occurrences of cyanobacteria are frequently reported in numerous freshwater lakes, that serve as direct sources of drinking water. Currently, the effects of cyanobacterial toxins on disease resistance in mammals is a largely unexplored area of research. However, aside from classic toxic effects including hepatotoxicity and tumor promotion, cyanotoxins are also known to modulate the immune system. Recent studies have suggested that freshwater cyanotoxins can elicit immunomodulation through interaction with specific components of innate immunity, thus potentially altering disease susceptibility parameters for fish, wildlife, and human health owing to the conserved nature of the vertebrate immune system. In our study, we investigated the effects of three microcystin congeners (LR, LA, and RR), nodularin-R, and cylindrospermopsin for their ability to directly interact with nine different human Toll-like receptors—key pathogen recognition receptors for innate immunity. Toxin concentrations were verified by LC/MS/MS prior to use. Using an established HEK293-hTLR NF-κB reporter assay, we concluded that none of the tested toxins directly interacted with human TLRs in either an agonistic or antagonistic manner. These results suggest that earlier reports of cyanotoxin-induced NF-κB responses likely occur through different surface receptors to mediate inflammation.

MixTox (I): Field Evaluation of Real-World Mixtures

4.12.10

Bioanalytical Assessment of Organic Micropollutants Collected With Use of Long-Term Passive Sampling During the Joint Danube Survey 4 (JDS4) and Setting a Baseline for Future Monitoring in the Danube River

Z. Tousova, Masaryk University, Faculty of Science, RECETOX; P. Sauer, University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters / Laboratory of Aquatic Toxicology and Ichtyopathology; B. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; B. Vrana, Masaryk University, Faculty of Science, RECETOX / RECETOX; M. Koenig, R. Schlichting, Helmholtz centre for environmental research - UFZ / Cell Toxicology; P. Mikusova, M. Smutna, J. Novak, R. Prokes, J. Sobotka, P. Fialova, Masaryk University, Faculty of Science / RECETOX; R. Grabic, University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters / Laboratory of Environmental Chemistry and Biochemistry; K. Hilscherova, Masaryk University, Faculty of Science, RECETOX / RECETOX

The Joint Danube Survey (JDS) is a regular monitoring campaign of the Danube River based on comprehensive characterization of well-defined sampling sites along the river course performed every six years. Existing data on (bio)analytical assessment reflect only the actual situation during short sampling periods carried out in the previous campaigns. However, deeper insight into the pollution and effect profiles of the Danube River sites using long-term integrative samples, which mitigate the impact of random extreme conditions at some time points, is crucial for better understanding the overall river contamination. The main objective of the present study was to establish a baseline for future (bio)analytical assessments of water quality in the Danube River by means of long-term passive sampling carried out within the Joint Danube Survey 4 (JDS4) combined with a battery of *in vitro* bioassays and comprehensive chemical analyses. Passive samplers of two types (silicone rubber sheets for hydrophobic compounds and Affinisep HLB disks for hydrophilic compounds) were deployed at nine sampling sites in surface water along the Danube River for 101 – 105 days from end of May to beginning of September in 2019. Sample extracts were tested in a battery of *in vitro* bioassays to assess anti-/androgenicity, dioxin-like activity, retinoid-like activity, anti-/thyroid activity, estrogenicity, anti-/glucocorticoid activity, activation of peroxisome proliferator-activated receptor and adaptive stress response to oxidative stress. Cytotoxicity was tested in parallel with each of the listed specific endpoints. Dioxin-like activity appeared in most samples. PPARɣ activity occurred frequently in the samplers for hydrophilic compounds and ranged between 0.20 to 11 ng/L rosiglitazone equivalents. Estrogenicity was detected at two sampling sites at concentrations ranging from 0.06 to 0.12 ng/L estradiol equivalents. Our on-going work will complete the data for all listed bioassays and the resulting effect profiles of the Danube River sites combined with the results of multiresidue chemical analysis will serve as a baseline for future (bio)analytical assessments of water quality using longer-term integrative samples. The contribution of the wide spectra of detected compounds to the observed effects will be assessed. Our work also aims to prioritize the effect drivers of selected observed biological activities. The work is supported by the Czech Science Foundation Grant No. 20-04676X.

4.12.11

Temporal Variation of Ecotoxicological Risks in Agricultural Ditches Assessed Using Passive Sampling, Bioassays, and Chemical Analysis

H. Boonstra, Wetterskip Fryslân; M.L. de Baat, KWR Water Research Institute / Chemical Water Quality & Health; F.v. Meer, Wetterskip Fryslân; I. Roessink, Wageningen Environmental Research / Environmental Risk Assessment; M. Kraak, University of Amsterdam / IBED-FAME

Traditional chemical monitoring of water bodies fails to accurately assess and explain ecotoxicological effects, because it does not incorporate the combined action of the present-day complex mixtures of chemicals present in the aquatic environment. Therefore, there has been an increasing interest in the combination of passive sampling and effect-based methods (bioassays) to identify environmental risks. However, since pollution loads substantially fluctuate over time, the toxic pressure in the receiving water bodies may also be subject to temporal variation, but this remains virtually unknown. Therefore, the aim of this study was to elucidate the temporal variation of ecotoxicological risks in agricultural ditches using passive sampling and bioassays. Four locations in the arable area of the province of Friesland, The Netherlands, were sampled during four consecutive sampling periods (May to October, 2017). Silicone rubbers and polar organic chemical integrative samplers (POCIS) were exposed to the surface water for the passive sampling of hydrophobic and hydrophilic organic compounds, respectively. Subsequently, an *in-situ* daphnid test, 3 *in-vivo* and 16 *in-vitro* bioassays were subjected to extracts of the passive samplers. Bioassay responses were compared to effect-based trigger (EBT) values to identify potential ecotoxicological risks. Responses were detected in 18 out of the 20 bioassays. EBT values were exceeded in seven of the applied assays and allowed ranking of locations and sampling periods based on ecotoxicological risks. Ecotoxicological risks in the ditches fluctuated over time, with most frequent EBT exceedances in spring, and largest differences in EBT exceedances in autumn. To identify the potential drivers of the observed effects, passive sampler extracts and surface water grab samples were chemically analysed. This revealed that a broad and changing spectrum of chemicals that exceeded environmental quality standards throughout the season was presumably responsible for the observed effects. This study illustrates that ecotoxicological risks in agricultural ditches fluctuate substantially over time, and depend on the pollutant loads at monitoring sites. Hence, timing of (passive) sampling in future monitoring programs must be carefully considered, as it has considerable influence on the detection of ecotoxicological risks and the subsequent chemical water quality assessment.

MixTox (II): Regulatory Approaches to Real-World Mixtures

4.13.04

Endocrine Disruption Levels of Surface Waters in Wallonia: Use of In Vitro Bioassays YES/YAS for the Screening of Estrogenic and Androgenic Activities

Y. Marneffe, Inst. Scientific de Service Public / Ecotoxicology; C. Chalon, D. Leroy, ISSeP / Ecotoxicology

Nowadays, the issue of endocrine disruptors (ED) potentially altering the functions of the endocrine system at very low concentrations is a priority. The recent introduction of the hormones 17β-estradiol (E2) and 17-α-ethinylestradiol (EE2) in the Watch list of Directive 2013/39/UE requires Member States to monitor these hormones on a limited number of stations. This monitoring is however difficult and bioassays could be very useful and be used as a screening tool before performing ?, if necessary, costlier chemical analyses. The aim of the present study was to carry out a characterization campaign of the level of endocrine disruption of a representative selection of surface waterbodies. In particular, it aimed at presenting the (anti-)estrogenic and (anti-)androgenic activities of the waterbodies samples using genetically modified yeast bioassays (YES and YAS tests). These activities are compared with the results of chemical analyzes carried out in parallel in order (i) to investigate possible correlations between chemical concentrations and ED activities and (ii) to assess the interest of these bioassays, which are more sensitive and generally less costly than some chemical analyzes, as screening tool for the quality of surface waters. For this purpose, 31 river sampling points from the regional monitoring network (WFD surveillance control) were sampled during 2019. Estrogenic activity was detected and quantified in 77 % of the samples and could reach 13.8 ng E2eq/l (mean: 3.01 ± 3.40 ng E2eq/l). It was detected in 94 % of the samples. Activities were higher in the Scheldt hydrographic district than in the Meuse one. Androgenic activity was never detected. On the other hand, estrogenic and androgenic antagonist activities were detected in 23 % and 19 % of the samples, respectively. Results are compared with former results.

4.13.06

Case Studies on ENvironmental Risk Assessment of Groups of Chemicals Referring to WHO/IPCS Framework on Risk Assessment of Combined Exposure to Multiple Chemicals"

K. Yamazaki, Ministry of the Environment / Environmental Health

The goal of our study on mixtures assessment is to develop the way to introduce “assessment of combined exposure to multiple chemicals” into environmental risk assessment practices for regulatory objectives, such as selecting high-priority chemicals, setting criteria/standards, or identifying chemicals to be regulated. The WHO/IPCS Framework on risk assessment (RA) of combined exposure to multiple chemicals would be useful for regulatory approach. We have been considering how we should adapt the framework to our RA practices, from screening-level to comprehensive ones, through case studies on environmental RA for some groups of chemicals, such as acrylates and phthalates. After Tier 1 level assessment for the two groups concluded that “the respective group of chemicals may cause significant risk to the environment,” Tier 2 assessment is being implemented. The Framework, designed for human health RA, cannot be applied to regulatory environmental RA as it is. Tiers in the Framework can be correlated with respective stages of the existing regulatory RA practices, where PNEC values have been derived referring to ecotoxicological studies. Characterization of common effects might not necessarily be

practical from ecotoxicity data, where knowledge on mode of action cannot always be available or identifiable. Detailed hazard assessment has been attempted for the two groups to see whether we should be satisfied with derivation of relative potency factors (RPFs) by just knowing similarities/tendencies of ecotoxicity values. Progress of the attempt to Tier 2 environmental risk assessment as case studies will be presented at the Meeting. .

4.13.07
Predicting Antagonism of the Androgen Receptor by Complex Mixtures Using Generalized Concentration Addition
J.J. Schlezinger, W. Heiger-Bernays, Boston University School of Public Health / Dept. Environmental Health; T.F. Webster, Boston University / Dept Environmental Health
Nuclear receptors are ligand-activated transcription factors and are the mechanism by which lipophilic hormones and hormone-like molecules regulate gene expression. Thus, they are critical targets of endocrine disrupting chemicals. The androgen receptor (AR) is a nuclear receptor that binds ligands including hormones (e.g., testosterone), pharmaceuticals (e.g., flutamide) and environmental endocrine disrupting chemicals (e.g., cyprodinil, vinclozolin, procymidone). The vast majority of environmental AR ligands are antagonists.We developed Generalized Concentration Addition (GCA) to be able to predict the effect of mixtures of ligands with varying efficacies. AR acts according to the classic homodimer activation model: each AR protein in the cytoplasm binds ligand, undergoes a conformational change that relieves inhibition of dimerization, and binds to DNA response elements as a dimer. We previouslydeveloped a pharmacodynamic model for this system that meets the mathematical requirements for GCA and showed that it predicts the activation of AR by binary ligand mixtures. Here, we tested the efficacy of GCA in predicting the effect of complex mixtures of environmental ligands on AR activation. We generated individual dose-response data for BPA, BPC, DDE, HPTE, methoxychlor, procymidone and vinclozolin using the MDA-kb2 AR reporter cell line. Because these ligands are competitive antagonists, dose responses were determined in the presence of the full agonist BMS564929, and binding affinities were estimated using a Schild analysis. A mixture was designed with equipotent concentrations of the chemicals, and we used the reporter assay to assess AR activation by the mixture using a ray design. Empirical results were well predicted by GCA. We currently are investigating the ability of GCA to predict the effect of mixtures on endogenous target gene expression (*PSA*) in a human prostate cancer model (VCap cells).

4.13.11
Multivariate Analysis of the Effects of Chemical Pollution and Further Stressors on Freshwater Organisms
N. Markert, North Rhine-Westphalia Office of Nature, Environment and Consumer Protection (LANUV) / Ecotoxicology; M. Palt, University Duisburg-Essen; B. Guhl, North Rhine-Westphalian State Agency for Nature, Environment and Consumer Protection (LANUV NRW) / FB 32; C.K. Feld, University Duisburg-Essen
The ecological status of surface water bodies is influenced by a variety of anthropogenic stressors, such as elevated nutrient or metal concentrations and hydrological or morphological changes. The adverse effects of chemical pollution (e.g. pesticides, pharmaceuticals) have increasingly come into focus, however, to date have been rarely considered in the joint analysis of multiple stressors. For the development of effective management measures to improve the ecological status it is important to identify both the individual and the combined effects of chemical pollution in concert with other stressors. We present the conceptual approach of a project that aims at identifying and quantifying effects of multiple stressors on selected river biological quality elements (benthic invertebrates, benthic algae, fish) as defined by the EU Water Framework Directive (WFD). Chemical pollution will be analysed together with impacts of hydrological and morphological alteration and human land use. For this purpose, a multi-stressor data set will be compiled based on WFD monitoring schemes and supplemented by extended chemical monitoring programs of local water boards. By using multivariate techniques, the effects of these stressors on the biological quality elements will be empirically analysed and quantified for single stressor as well as combined stressors effects. Interactions between stressors will be analysed using regression models including generalized linear mixed models. Moreover, “tipping points” of the stressors are attempted to be identified using gradient analyses in combination with indicator type analyses. The results will give an indication of the relative importance of the different stressor groups considered, which will inform a general risk assessment system. Additionally, the results can help to tailor programs of management measures under the influence of multiple stressors, including chemical pollution.

4.13.14
A Holistic Approach to the Assessment and Management of Environmental Chemical Mixtures in Canada
A. Gosselin, Environment and Climate Change Canada; M. Hewitt, Environment and Climate Change Canada / Water Science and Technology Directorate; G. Tardif, Environment and Climate Change Canada / Science and Risk Assessment Directorate; R.J. Letcher, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health Division; T. Harner, Environment and Climate Change Canada / Air Quality Processes Research Section; D. McGoldrick, Environment and Climate Change Canada / Water Science and Technology Directorate; S. Smyth, Environment and Climate Change Canada / Science and Risk Assessment Directorate; L. Dornan, M. Guy, L. Maddison, Environment and Climate Change Canada / Industrial Sectors and Chemicals Directorate; K. Hartin, Envrionment and Climate Change Canada / Industrial Sectors and Chemicals Directorate; V. Balakrishnan, Environment and Climate Change Canada / Aquatic Contaminants Research Division; M.A. Bonnell, Environment and Climate Change Canada / Ecological Assessment Division; A. Baig, K. Nyarko, Environment and Climate Change Canada / Science and Risk Assessment Directorate
Exposure to chemicals in the environment involves multiple chemicals, which may or may not interact, creating complex environmental chemical mixtures scenarios. These real-world mixtures exert effects in the Canadian and global environments. It is becoming increasingly evident that the substance-by-substance approach to chemicals management, which has been the main regulatory approach used so far in Canada and internationally, has limitations when trying to address exposure to environmental mixtures. The analytical and bioanalytical tools and techniques for tackling complex mixtures of chemicals in our environment and associated cumulative effects are evolving rapidly. An important challenge remains to apply these tools holistically in a regulatory context in order to identify, assess and manage chemicals exerting their effects as part of environmental mixtures. To start tackling this challenge, a holistic approach is being developed in the context of Canada’s Chemicals Management Plan, with the long-term goal of having environmental mixtures identified, prioritized, assessed (i.e., investigation of causes of observed harmful effects) and managed (i.e., development of abatement options) to address harmful effects observed in the environment. The approach will aim to unify work across environmental media (water, sediment, air, soil and biota) and integrate with human health. This poster will present the conceptual design for this holistic approach, with the goal of seeking feedback from the international community.

Pharmaceuticals in the Environment - a Global Challenge

4.14.03
A Challenge for Legislation: Closing the Gaps on ENvironmental Data of Active Pharmaceutical Substances
I. Rönnefahrt, S. Schmitz, I. Ebert, German Environment Agency UBA / Section IV 2.2 Pharmaceuticals
The EU Strategic Approach to Pharmaceuticals in the Environment, which was published in 2019, sets out six areas for action. Two of them are: Improve the environmental risk assessment (ERA) and its review (including a public access to main ERA results) Fill knowledge gaps (with a special focus on legacy products which were not yet subject to ERA)A detailed analysis of the functioning of the EU legislation on medicinal products in respect to environmental safety reveals shortcomings in the following areas: Ineffective authorisation procedures and lack of surveillance in the post-market phase Knowledge gaps and lack of publicly available data Inadequate linking of medicinal products legislation and other regulatory areas The main reason for knowledge gaps is the fact, that a review program of legacy products, which were approved before the ERA requirement was set, has not been implemented into the EU legislation. Until now, environmental data of about 350 active pharmaceutical substances became available as result of ERA as part of the marketing authorisation. However, the number of active substances of potential concern on the German market is about 1300. 15 years after implementing the ERA into the EU legislation on medicinal products this is a quite unsatisfactory situation.Data on environmental fate and effects are relevant for many stakeholders. Nevertheless, they are nearly not publicly available. The EU medicinal products legislation provides only very limited options for risk management because the access to pharmaceuticals for human patients and animals

should not be restricted. Therefore, measures along the whole lifecycle of a medicinal product are necessary. However, ERA data obtained within the authorization are protected and must not be used for other regulatory areas as e.g. the Water framework directive. A paradigm shift towards a substance based ERA and a collection of environmental information in substance files (monographs) is the key measure to ensure the environmental safety of medicinal products. This was also recognized by the new EU legislation for VMPs (2019/6). The overall aim of such a monograph system is to generate a comprehensive set of valid environmental fate and effects data which will enable a shared use by different applicants and for different purposes. For example, Monographs on active substances could also provide data to be used for risk management in other regulatory frameworks, as e.g. the EU water policy.

4.14.04
Advantages of a Monograph System for Environmental Risk Management of Veterinary Medicines Products
I. de la Casa-Resino, AEMPS-Agencia Española de Medicamentos y Productos Sanitarios / Veterinary medicines; A. Haro Castuera, R. Casimiro Elena, C. Rubio Montejano, R. Carapeto-García, Spanish Agency of Medicines and Medical Devices / Veterinary medicines
The impact of pharmaceuticals in the environment is a growing concern that has led to the development of several initiatives to tackle the issue in the last years. In this sense, Regulation (EU) 2019/6 ('new veterinary regulation' [NVR]), which is applicable from 2022 and repeals Directive 2001/82/EC, reinforces the environmental protection and enacts certain measures accordingly. Indeed, Article 156 of the NVR does provide the opportunity to investigate the feasibility of the future performance of the environmental risk assessments (ERAs) based on the properties of the active substance ('monographs') or any other potential alternatives. The development of a monograh system might constitute an opportunity to cover some shortcomings identified in the legislation and to thus obtain a more protective, proportional and consistent regulation of veterinary medicines products (VMPs). A monograph that included a complete risk assessment, and risk mitigation measures would improve the ERA of VMPs in terms of harmonization as its conclusions would apply to all VMP containing the substance. In addition, this would allow the risk managers to prioritize and classify which subtances suposses the highest environmental concerns in tems of risk to aquatic organisms, terrestrial organisms and groundwater and to identify PBT substances. Besides, the risk of the spread of AMR in the environment could be more effectively addressed, as soon as a specific methodoly becames available. Moreover, once a substance is identified as a concern, it could be susceptible of specific monitoring programs in water, soil or sediment. This information could be used for other regulatory frameworks to manage the risk identified (if any). For instance, the conclusions of the monograph would be useful for other regulatory frameworks with shared active substances, like human medicines (Directive 2001/83/EC), the Water Framework Directive (Directive 2000/60/EC) or biocides regulation (Regulation [EU] 528/2012). Indeed, it would be highly recommended to share the information and the mitigation measures applied to align all the different regulatory frameworks. Last, but not least it should be remarked that the availability of VMPs is a major concern that is also tackled by the NVR. In consequence, the monograph system should be designed in a way that doesn't imply a unjustified reduction on the availability of VMPs, e.g. by prioritisation schemes, public-private partnerships or taylored ERAs.

4.14.05
Approach to ENvironmental Risk of PPCPs in Japanese Ambient Water
K. Yamazaki, Ministry of the Environment / Environmental Health
While the responsible ministry issued "Guidance on assessment of effects to the environment in development of pharmaceutical products" in March 2016, as for now Japanese legislation on pharmaceuticals does not require industries to submit data on environmental effects. Stakeholders might have been paid little attention to pharmaceuticals in the environment.Ministry of the Environment, Japan (MOEJ) has continued an internal project to address pharmaceuticals and personal care products (PPCPs) in the environment for decades. Collecting information on both hazard (toxicity to aquatic organisms) and exposure (existence in the aquatic environment), we have been trying to prioritize PPCPs of which environmental risk assessment should be conducted. The project has been expanding its activities to collect relevant information, in concert with MOEJ's existing programs. "Environmental Survey and Monitoring of Chemicals" is an MOEJ's program to measure and detect anthropogenic chemicals in the ambient environment. PPCPs have been addressed in this program from 2013; measurement of the first batch of PPCPs in Japanese ambient water was conducted in 2014 and the result was publicized in 2015, in which pharmaceuticals such as macrolide and sulfonamide antibiotics were detected at many of the sampling sites. Additional batches of PPCPs have been subjected to measurement and some of them were detected in the ambient water. "Initial Environmental Risk Assessment of Chemicals" is an MOEJ's program on screening-level environmental risk assessment to identify candidate chemicals for further investigation. Since 2016 PPCPs have been addressed in this program, where most of the target substances had been selected from among industrial chemicals. Initial environmental risk assessment of clarithromycin was publicized in 2017, in which the substance was concluded as "candidate for further work". Additional PPCPs has been subjected to this program and some of them may be concluded similarly.Aquatic toxicity testing of chemicals was traditionally conducted by MOEJ to fill data gap of industrial chemicals, when the national government had not been authorized to require manufacturers to submit relevant data. After amendment of Chemical Substances Control Law in 2003, the tests have been conducted for only limited number of industrial chemicals. MOEJ obtained financial resource for aquatic toxicity testing of PPCPs from 2019 and contracted with laboratories for testing several pharmaceuticals. Updated situation of MOEJ's approach to PPCPs in the environment will be presented at the Meeting.

4.14.07
Exposure, Hazard and Risk Assessment of Psychopharmaceuticals and Illicit Drugs in Surface Waters
C. Davey, M. Kraak, University of Amsterdam / IBED-FAME; A. Praetorius, University of Amsterdam/IBED Institute / Department of Environmental Science and Analytical Chemistry ACES; T.L. ter Laak, KWR / Chemical Water Quality & Health; A. van Wezel, University of Amsterdam/IBED Institute
Pharmaceuticals are a vital backbone of society and, currently, there are around 4,000 active pharmaceutical ingredients (*APIs*) administered worldwide. APIs also include psychoactive pharmaceutical ingredients (*PAPIs, or psychopharmaceuticals*) of which over 600 have been registered worldwide.
Psychopharmaceuticals are primarily used to treat mental disorders and illnesses, as well as other conditions relating to the nervous system, and the use of psychopharmaceuticals has been increasing around the world due to factors such as increased access to medicine, increased range of medicine, loss of social stigma, aging populations and growing populations. After use, pharmaceutical residues including psychopharmaceuticals and psychoactive metabolites are emitted into the sewage system and eventually enter surface waters. Psychopharmaceuticals are designed to interact with the nervous system of patients, and consequently these substances affect organisms at very low, environmentally relevant, concentrations. Yet, it remains virtually unknown if the presence of these psychopharmaceuticals in surface waters present an ecological risk for aquatic non-target organisms. Illicit drug use can also be a source of anthropogenic psychoactive substances, since the structure of many illicit drugs and psychopharmaceuticals do not differ to a large degree, and therefore must also be considered in the aquatic environment. Therefore, the current study aimed to provide vital insights into the risk posed by psychopharmaceuticals and to identify the gaps in the current understanding of these drugs in the aquatic environment, specifically surface waters. First, the availability and quality of data on the concentrations of psychopharmaceuticals in surface waters (*exposure*) and on the toxicity to aquatic organisms (*hazard*) were reviewed. Public databases and literature were both used in obtaining this data. If both exposure and toxicity data were available, risk quotients (*risk*) were calculated. In cases where enough toxicity data was available, a species sensitivity distribution (*SSD*) was constructed, from which the hazardous concentration for 5% of the species (HC_5) was derived. 708 drugs could be categorised as psychopharmaceuticals based on a combination of all anatomical therapeutic class (*ATC*) 'N' drugs and a list of illicit drugs according to the Dutch Opium Act. Of these, only for 48 compounds enough data were publicly available to calculate risk quotients (*RQs*), and for fewer still enough toxicity data was available to create SSDs. The results from this study broadens the understanding of the risk of psychopharmaceuticals in the environment by highlighting exactly which compounds are of concern, predicted to be of concern and which compounds lack the data for any calculation of risk.

4.14.08
Human Health Risk Assessment of Pharmaceuticals in Environmental Waters of India

A. SENGAR; A. Vijayanandan, Indian Institute of Technology Delhi

Pharmaceuticals are getting detected in surface and groundwater around the world with advancements in detection technologies. Since pharmaceuticals are biologically active compounds, they might cause risk even at trace concentrations to human health. Despite the fact that India is a major production hub and consumer of pharmaceuticals, studies are limited on the occurrence and risk assessment of pharmaceuticals in India. In this paper, we have compiled the occurrence data of pharmaceuticals in environmental waters and conducted a human health risk assessment for Indian scenario. Risk quotient (RQ) was calculated separately for surface and groundwater by dividing the maximum detected concentration of a pharmaceutical by its drinking water equivalent level (DWEL). DWEL values were calculated for different age groups by using the acceptable daily intake (ADI) values of the pharmaceuticals. The main obstacle in conducting human health risk assessment studies is the unavailability of the ADI values for all the pharmaceuticals. In this study, we calculated the ADI values from the lowest therapeutic doses by applying suitable safety factors. The occurrence of 50 pharmaceuticals has been documented in surface and groundwater in India. In surface water, 11 pharmaceuticals were found to exhibit possible risks to human health, whereas in groundwaters, only 2 pharmaceuticals were found to exhibit risks. RQ values calculated for few pharmaceuticals in surface water were found to be very high. In decreasing order, compounds with high RQ values are- cetirizine (1185), ciprofloxacin (286), fluconazole (173), citalopram (47), and ofloxacin (20). These high RQ values correspond to the extremely high concentration of the pharmaceuticals detected in and around Hyderabad city. Hyderabad area has clusters of drugs producing industries of international reputation which produces drugs in bulk for the international market. The mismanagement of the effluent from these industries and inadequacy of the treatment scheme have resulted in the contamination of ground and surface waters of Hyderabad. Other pharmaceuticals having RQ >1 for surface water are difloxacin, enrofloxacin, norfloxacin, enoxacin, clarithromycin, and glibenclamide. In the case of groundwater, cetirizine and pitavastatin were found to exhibit possible risks to human health. The present study has prioritized the pharmaceuticals based on the occurrence levels and risk quotients.

4.14.09

Environmental Risk Assessment for the Cephalosporin Antibiotic Ceftriaxone in European Surface Waters

A. Häner, F. Hoffmann-La Roche Ltd / Group Safety, Security, Health & Environmental Protection (SHE)

An Environmental Risk Assessment (ERA) was performed for the third-generation cephalosporin antibiotic ceftriaxone for Europe. Ceftriaxone, synthesised and isolated 1978 for the first time, is listed in the WHO Essential Medicines List (EML). Ceftriaxone is marketed in the Roche product ROCEPHIN. So far, only a limited dataset describing biodegradability, environmental fate or ecotoxicity for ceftriaxone and no ERA have been available. The present ERA is based on old environmental data and on new tests, all performed in compliance with GLP quality assurance, for physicochemical characteristics, partitioning, environmental fate, biodegradability and acute and chronic toxicity and on sales amounts for the products containing ceftriaxone in Europe.Both a new and an older simulation study in activated sludge are used to discuss the bioelimination of ceftriaxone in sewage treatment. A predicted environmental concentration (PEC) in Europe from all products containing ceftriaxone was calculated based on compound actual use data from IQVIA MIDAS per annum and country, incorporating population data from Eurostat, for the years 2013–2017. A crude initial PEC was derived based on standard ERA assumptions of no removal in sewage treatment or surface waters. The crude PEC was refined by incorporating predicted sewage works removal, based on new biodegradability data and data from the literature, and by country-specific dilution factors. The lowest of the no observed effect concentrations from chronic tests with cyanobacteria, algae and *Daphnia* was divided by an assessment factor of 10 to derive the chronic-based predicted no effect concentration (PNEC). Potential risk for surface waters was then quantified by dividing the PECs by the PNEC. Potential risk from ceftriaxone was also assessed for sewage works and for the emergence of antimicrobial resistance (AMR). In addition, ceftriaxone is not expected to bioaccumulate nor to adsorb to sewage sludge or to sediment to a significant extent. Conclusions on potential risks of ceftriaxone are given in the poster.

4.14.10

The Global Database "Pharmaceuticals in the Environment" - New Analysis From the 2020 Update

A. Hein, German Environment Agency (UBA) / Section Pharmaceuticals; S. Graumnitz, D. Jungmann, Dresden University of Technology / Institute of Hydrobiology; G. Maack, German Environment Agency / Ecotoxicological Assessment of Pharmaceuticals

Over the past decades, human consumption of pharmaceuticals has steadily increased. The influence of pharmaceuticals on the environment is an increasing concern. It is known that their growing use is leading to detectable levels in all environmental compartments, conceivably causing harm to ecosystems. Worldwide environmental concentrations (MECs) of human and veterinary pharmaceutical residues have been measured in 75 countries in all UN regions. More than 770 active substances, their metabolites or transformation products were reported and nearly 600 for the European Union. To organize the huge amount of information caused by the global environmental exposure situation, the German Environment Agency (UBA) initiated this project to collect all these data within one publicly available database. The data were transferred from the publications, reports and other data sources into the database (MEC database). You are invited to browse in the publicly available database for download as Microsoft Excel© or as Microsoft Access© file free of charge and without registration from the UBA website (www.uba.de/db-pharm). In an ongoing review and comprehensive analysis, the updated database version 3 will include all relevant publications until end of 2020. The key results of this update and new features will be presented.

4.14.12

The ENvironmental Risk Assessment of Antimicrobial Resistance Related to the Use Veterinary Medicinal Products Under the New Veterinary Regulation

R.v. Herwijnen, RIVM / VSP-MSP; R. Carapeto-García, Spanish Agency of Medicines and Medical Devices / Veterinary medicines; C. Schwarz, Federal Office of Consumer Protection and Food Safety (BVL); S. Hickmann, German Environment Agency (UBA); K. Stapleton, Veterinary Medicines Directorate; J. Weeks, IEH Consulting Ltd.; M. Montforts, RIVM / Centre for Safety of Substances and Products; D. Bouchard, French Agency for Food, Environmental and Occupational Health & Safety; M. Empl, European Medicines Agency

In the first half of 2021, the EU CVMP (Committee for Medicinal Products for Veterinary Use) is expected to publish a reflection paper, on the question of the potential impact of using antimicrobial veterinary medicinal products (VMPs) on antimicrobial resistance (AMR) in the environment. This reflection paper has been written by an interdisciplinary team comprising experts from the Antimicrobials Working Party (AWP) and the Environmental Risk Assessment Working Party (ERAWP) of the CVMP. The paper identifies the major environmental exposure pathways from using VMPs and further examines the potential release scenarios that may be significant. Subsequent to this, the likely accumulation and mobility of antimicrobial residues (ARs) and antimicrobial resistance genes (ARGs), excreted from animals treated with VMPs, is discussed in context to the naturally occurring levels of ARGs, already present in the environment. Consideration is also given to the potential consequences of AMR in the environment on animal and human health. The key findings from this paper highlight significant knowledge gaps around specific mechanisms and pathways of AMR. Further, there is little information on the potential impacts that AMR, resulting from VMP use, can have on the functioning of the ecosystem and its species. In addition, it is contemplated whether the risk assessment of VMPs in EU member states, should be amended to evaluate the risk(s) arising from AMR in the environment, following use of VMPs. Although the reflection paper concludes that, currently, the environmental risk assessment (ERA) for VMPs cannot yet be amended to consider the risks posed by the accumulation of ARs and ARGs in the environment from the use of VMPs, Article 8(2)(a) of the new veterinary regulation (Regulation [EU] 2019/6), requires that '[...] documentation on the direct or indirect risks to public or animal health or to the environment of use of the antimicrobial veterinary medicinal product in animals [...]' to be assessed starting in 2022. Therefore, further work is needed to develop guidance to implement these new requirements. Since the draft reflection paper was completed at the end of 2017, new knowledge on the issue has become available. With these latest scientific developments in mind, the aim of the present poster is to discuss potential approaches that could be included as part of the ERA process for VMPs, to evaluate AMR in the environment. Experts are invited to share their knowledge on this topic.

4.14.14

OECD TG 201 Study Results From Clindamycin, Linezolid, Flucloxacillin and Metronidazole Tests

R. Wess, Innovative Environmental Services (IES) Ltd. / Regulatory Consulting; M. Loetscher, Université de Lorraine - UdL; S. Hoger, Innovative Environmental Services (IES) Ltd; T. Schmidt, IES Ltd / Ecotoxicology; A. Dupont, Innovative Environmental Services IES Ltd

Pharmaceutical residues in freshwater is the subject a 2019 issue of the OECD studies on water series. It states that mis- and over-use of antibiotics is an important contributing factor of the emergence and spread of antimicrobial resistance - a global health crisis with the potential for enormous health, food security and economic consequences. Another specific concern is that antibiotic modes of actions potentially affect the photosynthetic activity of primary producers and subsequently primary biomass production and carbon dioxide fixation. In 2018, the European Medicines Agency (EMA) has published in a new draft guideline intending to implement a new tailored environmental risk assessment scheme for antibiotics. Documented threshold effect levels to three fixed representative species of green algae (*Raphidocelis subcapitata*) and cyanobacteria (*Anabaena flos-aquae* and *Synechococcus leopoliensis*) will be required. This poster presents OECD TG 201 study results from clindamycin, linezolid, flucloxacillin and metronidazole, which complement the literature data to achieve full datasets as demanded by the EMA draft. We discuss a case of a remarkably flat dose-response relation, significant differences to published literature data and varying 72 h and 96 h results.

4.14.15

Synthesizing and Collating Evidence for the Behavioural Effects of Pharmaceuticals on Aquatic Wildlife - a Systematic Map

J.M. Martin, Monash University / School of Biological Sciences; M.G. Bertram, Monash University / Biological Sciences; P. Blanchfield, Fisheries and Oceans Canada; J.A. Brand, Monash University / School of Biological Sciences; T. Brodin, Swedish University of Agricultural Sciences (SLU) / Department of Wildlife, Fish, and Environmental Studies; B.W. Brooks, Baylor University / Department of Environmental Science; D. Cerveny, Swedish University of Agricultural Sciences / Department of Wildlife, Fish, and Environmental Studies; M. Lagisz, University of New South Wales; I.Y. Ligocki, Millersville University / Department of Biology; M. Michelangeli, Swedish University of Agricultural Sciences (SLU); S. Nakagawa, University of New South Wales / School of Biological, Earth and Environmental Sciences; J. Orford, Monash Univeristy; J. Sundin, Swedish University of Agricultural Sciences (SLU); H. Tan, B.B. Wong, Monash University / School of Biological Sciences; E. McCallum, Swedish University of Agricultural Sciences

Globally, there is growing concern regarding the impact of pharmaceutical pollution on aquatic wildlife. Recently, a wealth of research has focused on if and how exposure to pharmaceuticals alters animal behaviour—a key determinant of animal reproduction and survival. Scientists, environmental managers, and policy-makers alike, are increasingly interested in using behavioural endpoints in chemical regulation. With pharmaceutical pollution recognised as an emerging environmental concern, and an international spotlight placed on their management, synthesizing the data currently available is an incredibly timely and necessary step. We will present a protocol and preliminary data for a systematic map of evidence that identifies, catalogues, and synthesizes all studies on the effects of human and veterinary pharmaceuticals on aquatic wildlife behaviour, with a particular focus on pharmaceuticals that are psychoactive, psychiatric or behaviour-modifying. Our map follows PRISMA and CEE guidelines for systematic mapping. The literature search will cover Web of Science and Scopus, and the search string has been developed using a "PECO" framework, to capture all eligible studies which used an aquatic organism (P, population), to test the impacts of a pharmaceutical (E, exposure) on behaviour (O, outcome). Eligible studies must also have a control group (C, comparison). We are screening studies in two stages, title and abstract screening, followed by full-text screening and meta data extraction. Decision trees have been designed *a priori* to appraise studies for inclusion at both stages of screening. Both stages will be completed by two independent reviewers who must agree on the inclusion criteria. Our findings will explore what compounds, species, and behavioural endpoints are being most studied, identifying gaps in the literature for future focus. Systematic maps are an under-utilized tool in ecotoxicology, but are an effective way to collate evidence in an unbiased manner for broad research topics. As such, systematic mapping can improve chemical risk assessment and decision-making by providing a comprehensive and unbiased summary of literature relevant for future policy.

4.14.16

Sublethal Effects of Psychotropics on Aquatic Species, Populations and Ecosystems, How Relevant Are Subtle Effects for Real-World Ecosystems?

E. Versteegen, Wageningen Environmental Research - WUR / Aquatic Ecology and Water Quality Management; P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences

Antidepressants and their metabolites are frequently detected in municipal sewage effluent and surface waters. The current rise in the prescription and usage of psychotropic drugs is expected to continue, which may result in an increase in the concentrations of psychotropics in freshwater environments. Current environmental risk assessment of psychotropic drugs fails to reflect on the subtitle effects of low chemical concentrations. Due to the biologically active nature of psychotropics, their interaction with the nervous system and their resemblance to infochemicals, the drugs might affect organism behavior even at low environmental concentrations. At these low concentrations, effects on the individual survival might seem absent, but small changes in behavior may result in changes at population and ecosystem level. To account for these subtitle effects in the current environmental risk assessment, there is a need for new endpoints to access effects of single pharmaceuticals, mixtures of pharmaceuticals and treated sewage effluent across different levels of biological organisation (i.e. individual, population, community and ecosystem). My poster will present and visualize my proposal for my PhD project, where I will explore new endpoints for determining psychopharmaceutical toxicity and aim to predict the effects of single and mixtures of psychotropics with different modes of action at sublethal concentrations. The project has the following research objectives: (1) Exploring and evaluating new endpoints for determining toxicity of understudied psychotropic drugs at individual, population, community and ecosystem level. (2) Predicting and assessing the toxicity of mixtures of psychotropic drugs with different modes of action, and discern the main drivers of mixture toxicity using a mixture toxicity model. (3) Determining the efficiency of different removal methods for sewage treatment by assessing the effects of sewage effluent on populations, communities and ecosystems. (4) Translating obtained results into proposals for adapting the current environmental risk assessment regulations. The effects at different levels of biological organization will be evaluated in single species, indoor microcosm and outdoor mesocosm setups. I expect this project to establish new endpoints for toxicity assessment and expand the current knowledge on mixture toxicity, and hence contribute to the current environmental risk assessment of psychotropics.

4.14.17

Supported Ionic Liquids As New Strategies to Reduce Toxicity of Cyclophosphamide in Aqueous Solutions

R. Francisco, University of Aveiro / CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro; B. Monteiro, University of Aveiro / Department of Biology; C. Venancio, University of Coimbra / Centre for Functional Ecology CFE; M. Santos, F.A. e Silva, M.C. Neves, A. Sousa, M.G. Freire, University of Aveiro / Department of Chemistry & CICECO; I. Lopes, University of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies

Cancer prevalence increased rapidly over the last decades, with over 18 million new cancer cases in 2018. Consequently, the consumption of anti-cancer drugs such as cytostatics also increased. Given the expected escalation in cancer cases, and as a consequence in the consumption of cytostatics, it is expected that the levels of these highly toxic compounds in the urban water cycle will rise. Therefore, it is necessary to develop effective treatment/removal strategies in order to prevent the contamination of aquatic environment. In this line, supported ionic liquids (SILs) can be considered as suitable materials to remove cytostatics from aqueous samples. In this work, several SILs using silica as the support material were synthesized and characterized. Their adsorption capacity for the model cytostatic cyclophosphamide was evaluated by the determination of adsorption kinetics and isotherms. Based on the potential of the prepared SILs to be used as alternative adsorbents for the removal of cyclophosphamide, the performance of the best SIL to decrease the toxicity of a cyclophosphamide rich aqueous sample was also evaluated. Overall, the results demonstrate that the modification of the ionic liquid cation structure significantly improves the removal of cyclophosphamide from aqueous samples.

4.14.18

Assessing the Impacts of Cytostatic Drugs on

Freshwater Biota

B.R. Monteiro, Department of Biology, University of Aveiro / Department of Biology, University of Aveiro; C. Venancio, University of Coimbra / Centre for Functional Ecology CFE; R. Francisco, University of Aveiro / CICECO- Aveiro Institute of Materials, Department of Chemistry, University of Aveiro; A. Sousa, University of Aveiro / Department of Chemistry & CICECO; I. Lopes, University of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies

Cytostatic drugs are a class of pharmaceuticals that have been increasingly used for cancer treatment. After being metabolized these drugs are excreted mainly via urine, reaching wastewater treatment plants, that do not always possess the proper means to effectively eliminate them, meaning they continuously enter the environment, possibly reaching surface and drinking waters. Since most anticancer drugs possess carcinogenic, teratogenic, genotoxic, and mutagenic properties, they pose a potential risk to environmental and human health. In this context, the present work aimed at assessing the ecotoxicity of three commonly used cytostatic drugs (cyclophosphamide-CYP, mycophenolate mofetil-MMF, mycophenolic acid-MPA) on freshwater species representing different trophic levels and functional groups: the microalga *Raphidocelis subcapitata*, the rotifer *Brachionus calyciflorus*, and the fish *Danio rerio*. The following endpoints were monitored for each species: yield and population growth rate for the alga after an exposure of 72h; mortality for the rotifer after an exposure of 24h; and mortality, malformations, and hatching for the fish after an exposure of 96h. Overall, MMF and MPA proved to be the most toxic compounds, with LC_{50} values of 0.046 and 1.41 mg/L for zebrafish assays, respectively, against an LC_{50} of 1305.6 mg/L for CYP. Similar results were obtained for *R. subcapitata*. For the rotifer, LC_{50} values could not be computed for MMF and MPA, since at the highest tested concentration (40 and 30 mg/L, respectively (close to the solubility limit of the compounds)) no mortality was observed. Though an LC_{50} of 6397 mg/L for CYP was determined. Based on these results, the predicted no-effect concentrations (PNEC) were derived for each compound to calculate the risk quotient. The environmental predicted or measured concentrations in superficial waters were retrieved from the literature. In general, CYP revealed a low risk for freshwater biota (RQ = 0.003), while MMF and MPA presented RQ values above 1 (RQ=3.0 and 4.1, respectively), indicating a high risk to freshwater organisms.

4.14.20

Effects of Pharmaceutical Wastewater on Antioxidant Markers in the African Clawed Frog (Xenopus laevis)

N. Basopo, National University of Science & Technology / Applied Biology and Biochemistry; D. Ndebele, S. Ngwenya, National University of Science and Technology / Applied Biology and Biochemistry

Pharmaceuticals are substances used in the diagnosis, treatment or prevention of diseases thereby improving the health of humans and livestock. Traces of various chemicals both synthetic and natural chemicals used in the production of pharmaceuticals end up in waste effluent. Unfortunately some of the pharmaceutical effluent finds its way into freshwater bodies where it affects aquatic organisms. We investigated the effects of pharmaceutical wastewater on antioxidant markers in the frog *Xenopus laevis*. The physiochemical parameters of the pharmaceutical wastewater was determined. Groups of frogs were exposed to 0.01% v/v of pharmaceutical wastewater for up to 14 days. On days 1, 5, 10 and 14 two frogs were subsampled from each exposure tank and the frogs were sacrificed. Liver samples were homogenized and the post mitochondrial fraction (PMF) used for analyzing antioxidant enzyme activity as well as levels of malondiadehyde. Increases in superoxide dismutase and glutathione peroxidase were observed in all the frogs exposed to pharmaceutical wastewater. Malondialdehyde levels were significantly increased ($p< 0.05$) in all the frogs exposed to pharmaceutical wastewater when compared to malondialdehyde levels in the controls. The findings of the study showed that pharmaceutical wastewater cause oxidative stress to the frogs an indicator that such effluent affects the normal wellbeing of aquatic organisms and may be toxic to other non-target aquatic organisms. Pharmaceutical wastewaters should be sufficiently pre-treated to safeguard the health of aquatic ecosystems

4.14.22

Ecological Impacts of Pharmaceutical Products on Sediment Microbial Communities of Three Hydrographic Basins Receiving Wastewaters

I. Beltran de Heredia, Plentzia Marine Station of Basque Country University (EHU/UPV); J. Urra, University of the Basque country (UPV/EHU) / Chemical and Environmental Engineering; B. Gonzalez-Gaya, ESTACIÓN MARINA DE PLENTZIA. UPV/EHU / Analytical Chemistry; C. Garbisu, NEIKER; N. Etxebarria, University of the Basque country (UPV/EHU) / Analytical Chemistry; E. Ruiz Romera, University of the Basque country (UPV/EHU) / Chemical and Environmental Engineering

Pharmaceutical products are currently considered emerging contaminants whose production and consumption have escalated in recent years. Most active pharmaceutical ingredients (APIs) are complex molecules that get only partially degraded within the human body and therefore, enter the sewage system as biologically active parent compounds and metabolites. Furthermore, a high number of these pharmaceutical residues are not fully removed by wastewater treatment processes and consequently are discharged into water bodies. Among pharmaceuticals, antibiotics have received great attention as their presence in the environment drives changes in the structure and functionality of natural microbial communities. Particularly, there is a growing concern about the influence of antibiotic sublethal concentrations in the acquisition of antimicrobial resistance (AMR) and the dissemination of antibiotic resistance genes (ARG). Moreover, bacteria coexist with a wide variety of pollutants, among which metals can promote a co-selection between metal resistance genes (MRG) and ARGs. The aim of this work was to assess the impacts of urban, industrial, and hospital wastewater treatment plants (WWTP) on the ecological status as well as on the presence and abundance of resistance genes of sediment microbial communities of three hydrographic basins in the Basque Country (Spain). The temporal and spatial dynamics of pharmaceutical products and metals were monitored through the physicochemical characterization of both soluble and particulate phases. To do so, water and sediment samples were taken upstream and downstream of the WWTPs discharge sites, and hydrological criteria were considered regarding sampling frequency. In addition, the ecological effects of these contaminants on the functionality of sediment bacterial communities were also evaluated through different microbiological indicators. Finally, the relative and absolute abundances of different ARGs, MRGs and genes associated with mobile genetic elements were determined by q-PCR. Preliminary results of the ongoing study show that WWTPs can play a role in the appearance and propagation of AMR, the latter probably due to the exposure of bacteria to high levels of contaminants and high cell densities during wastewater treatments. These findings suggest that not only is there a constant release of antimicrobial pharmaceuticals but also that ARGs and resistant bacteria can reach aquatic systems through the effluents of these facilities.

ACKNOWLEDGEMETS: This work was financially supported by the Spanish Ministry of Science and Innovation through the RTI2018-093989-B-I00 project.

4.14.23

Antifoulants, Veterinary Medicinal Products and Organic Material Can Affect Marine Sediment Organisms, but to What Extent? - ANTIVENOM

S. Rodriguez, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; K. Borgå, University of Oslo / Department of Biosciences; T. Gomez, NIVA / Ecotoxicology and Risk Assessment; A. Lillicrap, NIVA / Section of Ecotoxicology and Risk Assessment; K. Petersen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; A. Ruus, NIVA / Environmental Contaminants; A. Macken, NIVA / marine pollution

The ANTIVENOM project will investigate whether the use of chemicals in aquaculture have a different impact on the environment than previously considered, and if existing guidance on the assessment of their environmental risks is sufficient to cover specifically acting chemicals or their combined effects. We aspire to gain a greater understanding of the hazards and risks of current chemical use in aquaculture. The generated knowledge will serve as a basis for recommendations to improve effect and environmental risk assessments (ERAs) of veterinary medicines and antifoulants used in aquaculture to support future sustainable practices within the industry. The project will focus on assessing the single and combined effects of chemicals, in mixture with the contribution of organic matter, on non-target sediment organisms, using non-standard hazard assessment strategies. These strategies will be based on data gathered through the quantification (monitoring) and modelling of these chemicals to ensure realistic exposure scenarios. The science delivered through the ANTIVENOM project will support and influence policy makers on the changes necessary to improve ERA of chemicals used in aquaculture, and to ensure better protection and mitigation of the impacts to the marine environment from aquaculture practices. Specific outcomes will be to improve the

regulatory frameworks and guidance documents for veterinary medicines and antifoulants, by proposing new sediment toxicity tests and testing requirements that are most relevant for the protection of northern European marine waters.

4.14.24

Effects of Antibiotics on Greenhouse Gas Emissions: A Case Study Assessing Methane Productivity of Freshwater Sediments

E. Bollinger, University of Koblenz-Landau, iES Landau, Institute for Environmental Sciences / iES, Institute for Environmental Sciences; J.P. Zubrod, University of Koblenz-Landau / Institute for Environmental Sciences; S. Filker, University of Technology Kaiserslautern, / Department of Molecular Ecology; A. Lorke, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment

Methane (CH_4) is after carbon dioxide (CO_2) the second most important greenhouse gas and is *inter alia* produced in natural freshwater ecosystems. Given the rise of CH_4 emissions from natural sources, researchers are investigating environmental factors and climate change feedbacks to explain this increment. Despite being omnipresent in freshwaters, knowledge on the influence of chemical stressors of anthropogenic origin (e.g. antibiotics) on methanogenesis is not present to date. To address this knowledge gap, we incubated freshwater sediment for 42 days under anaerobe conditions in presence of a five-component antibiotic mixture at four levels (from 0 to 5000 µg/L). Weekly measurements of CH_4 and CO_2 in the headspace, as well as their compound-specific $\delta^{13}C$, showed that the CH_4 production rate can be increased by up to 94% at 5000 µg/L and up to 29% at field-relevant concentrations (i.e., 50 µg/L). A better mechanistic understanding is achieved through sediment community analyses involving next-generation sequencing targeting the archaeal and bacterial communities. Despite the complications of transferring experimental CH_4 production rates to realistic field relevant CH_4 emissions, the study suggests that chemical stressors contribute to the emissions of greenhouse gases by affecting the methanogenesis in freshwaters.

4.14.27

Fate of Organic Micropollutants in a Large-Scale Horizontal Flow Constructed Wetland : AZHUREV

N. Maurice, M. Pons, N. Adouani, CNRS / Laboratoire Réactions et Génie des Procédés; C. Pochet, Grand Reims

The AZHUREV constructed wetland (CW) is a large-scale pilot (6 ha) CW built at the outlet of the Grand Reims wastewater treatment plant (WWTP) (250,000 equiv. inh.). This CW is composed of three basins (2 ha/basin), supplied in parallel by treated wastewater, each with different types and amount of initial planted vegetation (*Phragmite australis*, *Scirpus lacustris* and *Glyceria maxima*). *P. australis* is the only planted species still present in the basins; the others were eliminated (coypu diet). Despite this, the three basins are vegetated due to the natural development of opportunistic species (*Ceratophyllum sp.* and *Lemna minor*). The main objective of this CW is to further improve the quality of part of the treated water (250 m^3/h) from WWTP Grand Reims and to treat urban runoff. The second objective is to create a reservoir for biodiversity. Since August 2019, sampling campaigns are conducted with the aim of collecting water, at the inlet and outlet of the CW, and sediments/plants, in the basins, to study the fate of organic micropollutants (OµMs) (80 pharmaceuticals, PFOS and AMPA). Up to now, only analyzes of OµMs in water have been performed. The extractions and analyzes of OµMs in plants and sediments are planned at the beginning of 2021. Among the 80 pharmaceuticals, only 13 (ketoprofen, diclofenac, atenolol, metoprolol, propranolol, sotalol, tramadol, oxazepam, carbamazepin, gabapentin, irbesartan, sulfamethoxazole, metformin) are detected at the inlet and outlet. The OµMs are removed to a different degree according to the seasons and by different processes in this CW : (i) ketoprofen, diclofenac, atenolol, metoprolol and propranolol are easily removed (>60%) throughout the year potentially due to photodegradation or sediment adsorption; (ii) tramadol, sotalol, oxazepam, carbamazepin, gabapentin, irbesartan, sulfamethoxazole and metformin are removed (20-35%) mainly during the summer due hypothetically to biodegradation or plant uptake; and (iii) AMPA and PFOS are barely removed (< 15 %). There are no significant differences between the three basins for the removal of these compounds.

4.14.28

High Summer: Water Temperature Affects the Biotransformation and Accumulation of a Psychoactive Pharmaceutical and Its Biologically Active Metabolite in Aquatic Organisms

D. Cerveny, Swedish University of Agricultural Science / Department of Wildlife, Fish, and Environmental Studies; J. Fick, Umea University / Department of Chemistry; J. Klaminder, Umea University / Ecology and Environmental Science; E. McCallum, Swedish University of Agricultural Sciences; M.G. Bertram, Swedish University of Agricultural Sciences / Department of Wildlife, Fish, and Environmental Studies; N. Castillo, Florida International University / Earth and Environment; T. Brodin, Swedish University of Agricultural Sciences (SLU) / Department of Wildlife, Fish, and Environmental Studies

Despite its seasonal fluctuation in natural systems, the effect of temperature on toxicokinetics of pharmaceutically active compounds is poorly understood. The aim of the present work was to evaluate bioconcentration and biotransformation of a psychoactive human pharmaceutical in fish and macroinvertebrates under various temperature regimes. Young-of-the-year perch (*Perca fluviatilis*) and dragonfly larvae (Sympetrum spp.) were exposed to 2 or 5 µg L^{-1} of temazepam (a benzodiazepine drug), respectively, at a low (10 °C) or high (20 °C) temperature for 8 days. After exposure, the parent compound and its biologically active metabolite, oxazepam, were determined in fish muscle and brain tissue, and in the whole body of dragonflies. The toxicokinetics of temazepam were affected by temperature to a great extent in fish. Twice as much oxazepam (312 ng g^{-1}, brain tissue, n = 5) was produced and accumulated via the metabolic pathway in perch exposed to temazepam at 20 °C compared to individuals from the 10 °C temperature treatment. Moreover, concentrations of the metabolite oxazepam significantly exceeded those of the parent compound temazepam in perch maintained at 20 °C, which was not seen in fish exposed at 10 °C. Bioconcentration of the parent compound was also affected by temperature, but in a different manner, with significantly higher concentrations of temazepam being measured in fish from the 10 °C treatment (238 ng g^{-1}, brain tissue, n = 5, BCF = 88) compared to individuals exposed at 20 °C (149 ng g^{-1}, brain tissue, n = 5, BCF = 62). This was most likely a result of slower metabolic transformation of temazepam into oxazepam at the lower temperature. Unlike in fish, low bioconcentration potential, no biotransformation, and no effect of temperature was seen on the toxicokinetics of temazepam in dragonfly larvae, indicating species-specific effects. The mean whole-body temazepam concentration (n = 15) was 2.8 and 2.4 ng g^{-1} in the low and high temperature treatments, respectively (BCF < 0.5 L kg^{-1}). As both temazepam and oxazepam are psychoactive pharmaceuticals belonging to same class, the possibility of combinatory (e.g. additive) effects in non-target organisms needs to be taken in account. The sum of the parent drug and its metabolite concentrations in brain did not differ significantly between temperature treatments (only their ratio), but did differ greatly from the parent compound concentration, especially at the higher temperature. Hence, our results clearly demonstrate that the common practice in ecotoxicology of evaluating bioconcentration/effects of single compounds is likely to underestimate risks that might occur when biologically active metabolites and temperature effects are not considered.

4.14.29

Evaluation of the Oxidative Effects of 2 Non-Steroidal Analgesics in Aquatic Organisms

R. Moreno Gutiérrez, Doctoral Program in Biological and Health Sciences. UAM-Iztapalapa; S. Sarma, Facultad de Estudios Superiores Iztacala, UNAM / Laboratorio de Zoología Acuática II, Unidad de Morfología y Función; A. Sobrino-Figueroa, Universidad Autonoma Metropolitana / Hidrobiology

Pain drugs are freely sold products and its are the substances most frequently released into aquatic systems. These compounds can cause harmful effects on aquatic organisms as they are designed to have a physiological effect at very low concentrations. The objective of this work is to evaluate the toxicity of 2 non-steroidal analgesics: Diclofenac and Ibuprofen, in organisms of different trophic levels: the microalgae *Monoraphidium pusillum*, the cladoceran *Cerodaphnia dubia* and the zebrafish *Danio rerio*. Static bioassays were performed with a duration of 48 (cladocerans) and 96 (microalgae and fishes) hours, where 5 concen-trations of the drugs were tested, to determine the LC50. Subsequently, a sublethal test with a duration of 10 days was carried out where the organisms were exposed to 3 sublethal concentrations (LC50/10, LC50/100 and LC50/1000) to evaluate the following responses: microalgae: production of chlorophylls, carotenes and degree of Lipoperoxidation and in cladocerans and fishes the degree of lipoperoxidation and antioxidant enzyme activity (SOD, CAT, GPX). The results obtained showed that the most toxic drug was Diclofenac. In the sublethal tests, it was evident that the drug with the greatest oxidative effect was Diclofenac for microalgae and fish and

Ibuprofen for cladocerans. In the bioassays with microalgae, a decrease in the chlorophyll concentration was observed at the LC50/1000 concentration and an increase in the production of carotenes in all cases. In cladocerans, an increase in the activity of antioxidant enzymes was observed in organisms exposed to analgesics. And in fish a decrease in the activity of SOD and CAT was observed in the tests with Ibuprofen. According to the results, we can conclude that both drugs affect micro-algae, cladocerans and fish in sublethal concentrations and can cause harmful effects to populations that are exposed to these drugs.

4.14.33
Environmentally Relevant Concentrations of Anticancer Agents Affect Daphnia - the Studies Linking Molecular and Individual Levels
M. Grzesiuk-Bieniek, Warsaw University of Life Sciences (SGGW); E. Grzesiuk, Institute of Biochemistry and Biophysics Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland; D. Garbicz, Institute of Biochemistry and Biophysics Polish Academy of Sciences; D. Mielecki, Institute of Biochemistry and Biophysics Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland
Pharmaceuticals are used in medical treatment on a large scale and as a waste contaminate freshwater ecosystems. Chemotherapeutics used in cancer treatment are found in freshwaters at low concentrations (in the range of ng L^{-1}) and can be toxic or mutagenic to aquatic organisms. The aim of the study was to examine the impact of the alkylating anticancer agents, cyclophosphamide and cisplatin, on three *Daphnia magna* clones. We evaluated the life history parameters, protein and transcriptome profiles of crustacean exposed for five generations to environmentally relevant cyclophosphamide (CP) and cisplatin (CDDP) concentrations. Long time contact with pharmaceuticals results in delayed reproduction and lower egg number. Animals from one of tested clones cultured with CDDP died out before first reproduction during the generation. Moreover, we observed changes in proteome and transcriptome profiles of daphnids exposed to chemotherapeutics. To conclude the contaminating environment with anticancer agents are relevant contaminants that reduce *Daphnia* fitness. This work was supported by the National Science Centre, NCN Poland, UMO-2016/21/B/NZ8/01542

Tire Wear and Microrubber Particles - from Problems to Solutions

4.15.02
Characterization of Tire Wear Particle Using Non-Target and Target Screening
Y. Jeong, KICT / Department of Land, Water and Environment Research
Microplastics in the environment has been both social and environmental problems due to their significant and increasing amounts and hazardous effects. One of major sources of microplastics is tire wear particle. Considering the traffic loads in South Korea, the quantification of tire wear particle would contribute to establishment of management plan targeting microplastics in environment. This study aims to investigate the feasibility of using tracer in quantification of microplastics from tire wear particles. The quantification method of tire wear particle was developed by selecting tracer compounds and optimizing their analytical methods. Tire wear particles used in this study were generated by tire wear particle generator, mobile sampling system in the laboratory. First, Zn was demonstrated as tracer compound and its pretreatment and analytical method with ICP-MS was developed. By using non-target and target screening with a high-resolution mass spectrometer, benzothiazole and 2-hydroxy benzothiazole, a derivative of benzothiazole, were selected as tracer compounds. The linear correlation between the concentration of all tracer compounds and tire wear particles was confirmed. Furthermore, the effect of tread wear factor on the released concentration of benzothiazole group was found. To better track tire wear particle released on the road, further analysis of other derivatives of benzothiazoles is currently in progress.Acknowledgments: This study is financially supported by Korea Institute of Civil Engineering and Building Technology (KICT) (project number: 20190158-001)

4.15.05
Toxicity of Tyre Wear Particles and Their Leachates to Freshwater Organisms
U. Rozman, University of Ljubljana; G. Kalčíková, University of Ljubljana / Faculty of Chemistry and Chemical Technology
Tyre wear particles (TWP) are considered as one of the most widespread microplastic particles in the environment. They are generated while driving cars due to the abrasion of tyres. Consequently, they can be emitted into the air or are deposited on the road or at the roadside. They are then found in the terrestrial environment or transported by runoff to the aquatic ecosystems. In this context, the aim of our study was to evaluate the toxicity of TWP (47.4 ± 22.2 µm) and their leachates on two freshwater organisms; waterflea *Daphnia magna* and duckweed *Lemna minor.* TWP were a mixture of old tyres and were generated when tyre tread was cut off from a tyre. Particles were sieved before the experiments to obtain only the smaller fraction (< 150 µm). The concentration of particles in tests was 100 mg/L. Leachates were obtained after the incubation of TWP in test medium for 48h and 7 days for *Daphnia magna* and *Lemna minor,* respectively, at the same conditions as toxicity tests with particles were performed. TWP and their leachates did not have a significant impact on *Daphnia magna*. Furthermore, TWP and their leachates also did not have a negative impact on duckweed's growth rate and chlorophyll *a* content, however TWP did significantly reduced duckweed's root length compared to control (37 ± 6%). As the negative impact on root length was not observed when duckweed was exposed to the leachates, it can be concluded that the negative effect on the root length was not due to chemical leaching, but it was due to mechanical abrasion of TWP, as they floated on the water surface with duckweed.

4.15.06
Toxicity of Tire Rubber Microplastics to Freshwater Sediment Organisms
V. Carrasco Navarro, University of Eastern Finland / ENVIRONMENTAL AND BIOLOGICAL SCIENCES; A. Nuutinen, University of Eastern Finland / Department of environmental and biological sciences; J.V. Kukkonen, University of Eastern Finland / ENVIRONMENTAL AND BIOLOGICAL SCIENCES
Tire rubber microparticles (called microrubber) may not literally be considered as microplastics by many researchers. However, their anthropogenic origin, massive production and unintentional discharge to the environment make them a relevant subject of study in ecotoxicology. Global emissions of microrubber are up to six million tonnes y^{-1}, of which 0.1 and 10% may reach surface waters. Studies on the toxicity of microrubber to freshwater organisms are scarce and thus more efforts are needed to decipher the consequences of such pollution in inland ecosystems. In our study, the toxicity of microrubber in two different sizes was assessed using the non-biting midge *Chironomus riparius* as model organism. It is a widely used organism in toxicity tests and an important prey of fish, birds and even terrestrial organisms. The microrubber sizes were a fine fraction (2.5 to 260µm) and a fraction obtained from sport turf fields (approx. 0.5 to 3mm). Microrubber was spiked to natural reference sediment from Lake Höytiäinen at three concentrations (1, 3 and 10% of sediment dry weight). Chironomids were added as first instar larvae and growth (10d) and emergence (28d) tests were performed. Monitored endpoints were mortality, length and head capsule length and width (growth tests) and time of emergence, emergence rate, sex ratio and reproduction (emergence tests). In addition, we used the freshwater oligochaete *Lumbriculus variegatus* as a model animal to test the toxicity of the microrubber fine fraction in tributyltin (TBT)-contaminated sediments. The sediment contaminated with TBT was sampled from Huruslahti bay in Varkaus, Finland. Lake Höytiäinen sediment was used additionally as a reference. Microrubber was spiked at 1, 5 and 10% of sediment dw and additional controls, without microrubber were set for both sediments. Tests (28d) follow existing guidelines and endpoints to be monitored are reproduction (number of worms) and biomass (dry weight). The results obtained so far indicated that there are not differences between controls and microrubber- exposed *C. riparius* in any of the two sizes used. In still ongoing tests, microrubber does not appear to affect *L. variegatus* despite the presence of TBT. Previous literature stated that microrubber does not affect *L. variegatus* but information about the joint toxicity with TBT is not available. The results will be of great help to ascertain how microrubber may affect animals present in naturally contaminated sediments.

Using Ecosystem Services to Frame and Assess Protection Goals: Taking a Holistic Approach

4.16.05
Challenges of the Ecosystem Service Approach in the Context of Pesticide Regulation and Biodiversity Protection
S.E. Egerer, Umweltbundesamt / Plant Protection Products; S. Matezki, German Environment Agency UBA / Plant Protection Products; S. Pieper, German Federal Environment Agency (UBA) / Plant Protection Products; K. Swarowsky, J. Wogram, German Environment Agency UBA / Plant Protection Products
During the last two decades, the concept of ecosystem services (ES) became increasingly popular as a principle for ecosystem assessment

and management. Several typologies for ES with partly different focus have been developed [e.g. 1, 2, 3]. While all these approaches have been developed in the effort of protecting biodiversity, they are foremost anthropocentric, i.e. value nature by its contribution to human wellbeing. The debate on how ES relate to biodiversity and the condition of the ecosystem is on-going. Generally, biodiversity is considered to found the basis for functioning of the ecosystem and the delivery of ecosystems services. Protecting biodiversity itself by operationalizing the ES concept is therefore challenging and has limitations. In the context of European pesticide regulation, EFSA adopted the ES concept as a tool to derive and communicate Specific Protection Goals (SPG) for non-target organisms that are affected by direct or indirect effects of pesticides [e.g. 4] to facilitate the operationalization of the general environmental protection goals laid down in regulation EU No. 1007/2009. With our contribution, we highlight the challenges in implementing the ES concept in the risk assessment of pesticides arising from the uncertainties regarding the linkage between ES and biodiversity. We show that knowledge gaps, e.g. regarding the specific functioning of species and community interactions, limit the approach e.g. for the protection of structural biodiversity and thus bear the potential for missing protection goals for biodiversity across regulations. We provide proposals how to identify SPG options by using the ES approach in a flexible way, allowing the integration of aspects that are currently not properly addressed, and to streamline the proposals with the protection goals of other EU environmental legislations. We think that a truly holistic approach is needed, especially in the light of the European Commission's Green Deal.
References: [1] Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC. [2] TEEB, 2010. The Economics of Ecosystems and Biodiversity: Mainstreaming the Economics of Nature: A Synthesis of the Approach, Conclusions and Recommendations of TEEB. [3] Haines-Young R, Potschin M, 2013. CICES V4.3-Report. 441EEA/IEA/09/003. [4] EFSA Scientific Committee, 2016. EFSA Journal 2016;14(6):4499.

Extended submission 4 - Ecological risk assessment and human health risk assessment of chemicals, mixtures and stressors and risk mitigation strategies

4.17.01
Study on Species Sensitivity Distribution of 35 Pesticides With Different Distribution Models
P. Huang, Tongji University, Shanhghai - China
Pesticides are often detected in soil and water environment because of their widespread use and persistent pollution characteristics. Considering the potential hazards and wide detection range of pesticides, it is particularly important to evaluate the ecological risk of pesticides. However, a major objective of ecological risk assessment is to determine the safety threshold for a chemical, that is, to obtain the predicted no effects concentration (PNEC) of the chemical. The most accepted method for derivation of PNEC is the species sensitivity distribution (SSD) method. The SSD method is more and more used in the derivation of water quality criteria. However, there is no authoritative research to prove that SSD curve belongs to a specific curve distribution. In the field of ecotoxicology, the selection method of SSD fitting model has no specific principles. In this paper, the aquatic ecotoxicity data of 35 pesticides were collected and 9 two-parameter models were used to fit the SSD curve. The optimal fitting model is determined by the principle of minimum root-mean-square error with a cumulative probability of less than 50%. In terms of the fitting effect, the arctangent function appeared the most frequently in the optimal model, 15 times in total, accounting for 42.9%; the Gompertz function appeared 7 times, accounting for 20%. The optimal model was used to calculate HC_5 (hazard concentration of 5% organisms) and HC_{10} (hazard concentration of 10% organisms) of 35 pesticides, and we found that HC_5 and HC_{10} have a power function relationship. Through extrapolation of 35 pesticide PNEC and recalculation of evaluation factors, it was found that the $PNEC_5$ derived by HC_5 was consistent with the $PNEC_{10}$ derived by HC_{10}. Through SSD studies on 35 pesticides, this paper provides reference for the selection of SSD models, and the feasibility analysis of deriving PNEC by HC_{10} was discussed, which provided new ideas and basic data support for ecological risk assessment.

4.17.02
Metabolomic Approach for the Biological Response of Procambarus Clarkii Against Pharmacologically Active Compounds by Combined Organic Mass Spectrometry Techniques
G.R. Moro, Universidad de Huelva / Deparment of Chemistry; S. Ramírez Acosta, Research Center for Natural Resources, Health and the Environment (RENSMA). University of Huelva / Chemistry. Faculty of Experimental Sciences; J. Kazakova, University of Seville; J. Gómez Ariza, University of Huelva; M. Bello López, R. Fernandez-Torres, University of Seville; N.P. Abril, University of Córdoba, Campus de Rabanales, Edificio Severo Ochoa / Department of Biochemistry and Molecular Biology; T. García Barrera, University of Huelva
The problem of environmental pollution due to the occurrence of pharmacologically active compounds (PACs), organic compounds or heavy metals in aquatic ecosystems has led to a growing interest about the study and use of biomarkers [1]. In this sense, aquatic invertebrates are very good bioindicators that result particularly useful for assessing the quality of aquatic ecosystems. In addition, they present a relationship between the content of contaminants in their tissues and the concentration of them in the environment. In the present study, we investigated the metabolic response in digestive gland tissue of the crayfish *Procambarus clarkii* after exposure of diclofenac, cadmium and arsenic at environmentally relevant concentration for 28 days and the metabolic response compared against a control. In this way, it is very important the evaluation of the physiological status of the organisms by monitoring the digestive gland, which is the main centre for metabolic regulation, participating in the mechanisms of immune defense and homeostatic regulation of the internal, as well as in the processes of detoxification and elimination of xenobiotics. In this context, the omics are now the new tool to assess the global biological response to pollution, in particular the presence and modifications suffered by metallobiomolecules and the metabolites, used as biomarkers of environmental stress. To this end, a metabolomic platform based on the use of mass spectrometry with ultra-high performance liquid chromatography (UPLC) and gas chromatography (GC) in the digestive gland of *Procambarus clarkii* has been applied to establish alterations in metabolic pathways, and the defence mechanism that is triggered by exposure to these compounds. Partial least square discriminant analysis (PLS-DA) was employed to determine differences between the groups and shows a clear classification of studied groups, indicating the existence of altered metabolites. The results show the potential of mass spectrometry as a key tool to elucidate the mechanism of action of drugs and metals; and understand the metabolic response of free-living aquatic organisms to environmental problems.

4.17.03
Commercial Preparations of Pesticides Cause Adverse Effects on Earthworm (Eisenia andrei)
C. Lackmann, Goethe Universität Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology; A. Šimić, S. Ečimović, Josip Juraj Strossmayer University of Osijek / Department of Biology; T. Seiler, Hygiene-Institut des Ruhrgebiets / Directorate; H. Hollert, Goethe University Frankfurt / Department of Evolutionary Ecology and Environmental Toxicology; M. Velki, Josip Juraj Strossmayer University in Osijek, Department of Biology
Adverse effects of pesticide exposures have been observed for decades worldwide. These effects have resulted in biodiversity declines in various ecosystems worldwide. Commercial preparations of pesticides often get applied directly in soil ecosystem through agricultural practices, even though only the effects of the active substance are included in common EU regulations. Thus, the toxicity of commercial pesticide preparations should be investigated in more detail to better understand their effects on e.g. earthworms that are known to play a key role in soil fertility and quality and are thus often used as model organism in soil ecotoxicology. In this study, the effects of four commercial preparations of pesticides on earthworm (*Eisenia andrei*) exposed for 48 h in soil are assessed: namely, insecticides Calypso (active ingredient: thiacloprid) and Sumialfa (active ingredient: esfenvalerate), and herbicides Filon (active ingredient: prosulfocarb) and Frontier (active ingredient: dimethenamid-p). The acute toxicity for all four pesticide preparations could be determined, namely, the median lethal concentration (LC_{50}) values were 558.37 mg/kg for Filon, 249.80 mg/kg for Frontier, 10.14 mg/kg for Sumialfa and 102.03 mg/kg for Calypso, active substances on the contrary showed a lower toxicity for all four investigated pesticides. Sublethal concentrations of the commercial preparations were chosen and a comprehensive test-battery, including avoidance behaviour, different enzymatic biomarkers (catalase, glutathione S-transferase, glutathione reductase, acetylcholinesterase, carboxylesterase) and other endpoints (multixenobiotic resistence activity, oxidative

stress), applied. Effects on subcellular endpoints could be observed for each pesticide. Furthermore, avoidance behaviour could be observed after exposures to Filon, Frontier and Sumialfa. The observed effects show the potential negative effects of the investigated pesticides on earthworms present in the environment and the need to include considerations about the possibly different toxicity of the commercial preparation into the classical risk assessment.

4.17.04

Caenorhabditis elegans Reproduction As a Bioanalytical Tool for Fast Screening of a Wide Range of Chemicals and Chemical Mixtures

M. Castro, University of Copenhagen / PLEN - Department of Plant and Environmental Sciences; I. KARADIMOU, University of Copenhagen; C. Henriksen, H. Bayat, E. Bak, N. Cedergreen, University of Copenhagen / PLEN - Department of Plant and Environmental Sciences

Caenorhabditis elegans is a bactivorous nematode widely found in soil ecosystems. Their small size (adults reach about 1 mm in length) makes culturing easy and their short-life makes chronic toxicity studies (e.g. growth and reproduction) feasible, making this an exciting invertebrate animal testing model at reduced time and expense. In addition, *C. elegans* is a widely studied model in biomedicine (e.g. in neuronal development studies), while it is largely ignored in the field of ecotoxicology. In this study, we aimed at investigating the potential of *C. elegans* being used as a fast bioanalytical tool for early toxicity screening and describing the effects of different stressors in this model organism. We tested the effect of abiotic (temperature, salt), biotic (food) and chemical stressors in the reproduction of *C. elegans*. The chemical stressors were comprised of chemical mixtures or single chemicals such as natural toxins, surfactants, lipid metabolism and endocrine disruptors, carcinogens, pesticides, pharmaceuticals and neuroactive chemicals. While the traditional endpoint recorded is the offspring number produced by a single adult (ISO 10872:2010), we additionally investigated offspring size and total biomass produced and their respective interactions as possibly more informative endpoints. *C. elegans* showed high sensitivity towards temperature increase, food decrease, solvents such as methanol and ethyl acetate, while for several pesticides and a range of industrial chemicals no effects were observed. A daily pulse of 2h at 30°C (control temperature = 20°C) was enough to decrease the production of offspring by 50%. For methanol and ethyl acetate, the acceptable concentrations were 0.45 and 0.46% (%solvent in media), respectively, as it resulted in an average of 10% decrease in offpsring production for the adult animals. For some of the chemicals tested no significant effects were observed even up to their water solubility limit in the mg/L range, which indicates that the chronic reproduction toxicity test with *C. elegans* might be less sensitive compared to e.g. the widely used *Daphnia* sp. acute immobilization test (OECD 202). In this communication, we will present the highlights of the our findings so far and a short comparison to the widely used model organism *Daphnia magna*.

4.17.05

Understanding and Mitigating the Subtle Effects of Psychoactive Drugs on Aquatic Ecosystem Functioning

A. Dubey, Netherlands Institute of Ecology (NIOO-KNAW) / Aquatic Ecology; E. van Donk, Netherlands Institute of Ecology NIOOKNAW; M. Lurling, Wageningen University / Aquatic Ecology Water Quality; L. de Senerpont Domis, Netherlands Institute of Ecology NIOOKNAW / Aquatic Ecology

Use of psychoactive drugs such as antidepressants and antipsychotics is on the rise, with about 270 million consumers worldwide. These pharmaceuticals can end up in water bodies through human excretion or flushing of unused medicines. Since signaling pathways are highly conserved between vertebrate and invertebrate taxa, these psychoactive drugs can affect non-target aquatic organisms. These effects have been observed at very low and environmentally relevant concentrations. Currently, there is inadequate information regarding the environmental risks of psychoactive drugs and the standard test protocols do not account for subtle effects. However, psychoactive drugs can contribute to subtle effects by influencing natural communication along the aquatic food chain (infodisruption) or by causing other behavioral changes, which could potentially disrupt ecosystem functioning. To study these unforeseen impacts, first, we will conduct a literature review. Second, based on the findings of the review we will conduct small-scale lab experiments with chronic low dose exposure. Third, to test the ecological relevance of the laboratory results we will conduct large-scale outdoor mesocosm experiments. In a companion PhD project, the most promising wastewater treatment measures for removing psychoactive drugs will be tested. Based on these results, we will expose near-realistic foodwebs to (diluted) effluent, and test for ecosystem level effects of environmentally relevant discharge scenario of psychoactive drugs relative to non-exposed treatments. The combined results from the small-scale and large-scale experiments will be used to incorporate subtle non-lethal effects in environmental risk assessment frameworks.

4.17.06

Selenium Protective Effects Against Oxidative Stress in Tuna

N. Serra, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; F. Sánchez Soberón, Universitat Rovira i Virgili / Chemical Engineering; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering; V. Linares, University Rovira i Virgili / Laboratory of Toxicology and Environmental Health; M. Bellés, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health

The importance of minerals in fish to satisfy micronutrient requirements in the human diet is well known. Selenium (Se) is one of the most interesting micronutrients and its presence in tuna is essential because plays a critical role in multiple metabolic pathways, including those involved in antioxidant defense. Se is a component of antioxidant enzymes, such as glutathione peroxidase (GPx). Acting in conjunction with GPx, are superoxide dismutase (SOD), catalase (CAT), glutathione oxide (GSSG) and reduced glutathione (GSH). The action of these antioxidant systems against free radicals generated by heavy metals, such as methylmercury (MeHg), is essential to reduce oxidative stress and therefore the toxicity of these pollutants. We determined the levels of Se, SOD, CAT, GSSG, GSH and GSH/GSSG ratio in farmed tuna (*Thunnus thynnus*), both red and dark muscle. The determinations were made on both raw and cooked tuna. A total of 43 tuna samples delivered by Balfegó & Balfegó from l'Ametlla de Mar (Spain North-east Mediterranean area), were analyzed. In raw tuna, the results showed that in red muscle the levels were 0.647 mg Se/kg, 0.049 mmol CAT/min/gr, 129.80 nmol GSSG/min/gr, 58.73 nmol GSH/min/gr, 0.461 GSH/GSSG ratio and 385.8 U SOD/gr. In dark muscle, the results were 15.38 mg Se/kg, 0.351 mmol CAT/min/gr, 9.97 nmol GSSG/min/gr, 1.14 nmol GSH/min/gr, 0.187 GSH/GSSG ratio and 2550 U SOD/gr. In cooked tuna, the results in red muscle were 0.862 mg Se/kg, 0.014 mmol CAT/min/gr, 15.73 nmol GSSG/min/gr, 6.12 nmol GSH/min/gr, 0.407 GSH/GSSG ratio and 264.5 U SOD/gr. However, in dark muscle the levels were 17.04 mg Se/kg, 0.015 mmol CAT/min/gr, 5.13 nmol GSSG/min/gr, 6.83 nmol GSH/min/gr, 1.48 GSH/GSSG ratio and 975.3 U SOD/ gr. The results showed that in red muscle the oxidative stress was neutralized through the glutathione pathway (GSSG and GSH). However, in dark muscle the pathway used was the activation of CAT. It was also observed that in cooked tuna all enzyme levels were lower, probably because a part of these enzymes were degraded by the effect of cooking. In conclusion, Se exerted protective effects against oxidative stress induced by the MeHg content in tuna.

4.17.07

Mechanism of Action of Indoors Particulate Matter and Its Effects at the Respiratory Level

N. Serra, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; F. Sánchez Soberón, Universitat Rovira i Virgili / Chemical Engineering; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering; V. Linares, University Rovira i Virgili / Laboratory of Toxicology and Environmental Health; M. Bellés, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health

Indoor air pollution is considered one of the most important preventable causes of death. Among indoor air pollutants, particulate matter (PM) less than 2.5 μm ($PM_{2.5}$) is considered the most harmful due to its smaller size that can reach deeper parts of the respiratory system. On the other hand, children are a particular vulnerable population group because they have higher particles inhalation rates and because their immune systems are not yet fully developed. Our objective was to study, in human alveolar cells, the mechanisms of action involved in the toxicity induced by indoors PM. To achieve this, two PM fractions ($PM_{2.5\text{-}1}$ and $PM_{1\text{-}0.25}$) were collected in three schools located in areas influenced by different PM-emitting human activities: urban, petrochemical and chemical. We studied apoptosis markers of type II alveolar lung cells (A-549) at 0, 6, 24, 48 and 72 h after exposure to PM. In the three areas studied and at 6 h post-exposure, the results did not show differences in the apoptosis markers levels analyzed (Bad, Bax-bcl2, Bcl-xl, Bim and Mcl-1) between the two PM sizes collected. However, at 24 h post-exposure and in the urban and petrochemical areas, all the analyzed markers significantly increased in $PM_{1\text{-}0.25}$ fraction compared to $PM_{2.5\text{-}1}$ fraction. Moreover,

in the suburban area the highest values of apoptosis were observed in $PM_{2.5-1}$. These differences were significant 24-48 h after exposure and increased until reaching the maximum value at 72 h post-exposure. The results of this study will contribute to a better understanding of the mechanism of action of PM and its effects at the respiratory level in children exposed to PM indoors.

Ex-ante Life Cycle Assessment of Emerging Technologies

5.01.02
Consistent Incorporation of Multiple Background Scenarios Into One Background Database: Proposition of a New Approach
C. Harpprecht, Leiden University / Institute of Environmental Sciences (CML); B. Fuchs, German Aerospace Center / Institute of Networked Energy Systems; B. Steubing, CML Leiden University
When analyzing future impacts of emerging technologies, one has to account for future developments in the background system to ensure temporal consistency between the foreground and background system. This can be achieved by incorporating scenarios into a background database, such as ecoinvent. Combining multiple scenarios can cause conflicts if several scenarios adapt the same process, and can result in an intransparent generation of scenario databases. We propose an approach which enables a transparent and reproducible incorporation of multiple scenarios into a single background database. It builds on and extends already existing brightway libraries, such as wurst and the superstructure. The recently developed brightway library wurst allows to systematically incorporate electricity scenarios from one source, i.e., the integrated assessment model of IMAGE. Incorporating additional scenarios, e.g., with higher regional resolution or for more sectors, such as greener steel production, extends the scope and accuracy of future background databases. An example for regional conflicts would be the incorporation of both average electricity scenarios for Europe and specifically for Germany. Our approach builds on the superstructure principle which produces one scenario database combining the background database with the processes and flows required for the scenarios. Secondly, it generates excel sheets which specify the values of flows for all scenarios. The scenario database and scenario excel sheets can be imported into the activity-browser, the graphical user interface for Brightway. The activity-browser enables a scenario-based LCA calculation and interpretation, which is easy-to-use also for non-pythonic LCA practitioners. Moreover, the generated superstructure database along with the scenario excel sheets can be easily shared. Our approach provides an extension to the superstructure principle to resolve conflicts caused by different scenario sources. We aim at creating a reliable and reproducible workflow to transparently generate a superstructure database which consistently combines scenarios from multiple sources. The goal is to make this approach available to the LCA-community as an open-source tool. Thus, our proposed approach contributes to the usability of background scenarios, and facilitates the cooperation as well as the exchange of scenarios between LCA practitioners.

5.01.06
Transformation of the Transport Sector for Achieving Climate Targets - a Methodological Approach to Assess the ENvironmental Impacts of Emerging Means of Transport Change Options
S. Hanesch, TECHNISCHE UNIVERSITÄT DARMSTADTIWAR / IWAR; L. Schebek, Technische Universitaet Darmstadt / Institute IWAR Material Flow Management and Resource Economy Germany
In order to achieve the climate targets in the transport sector, a large number of new technologies are currently developed in the field of electromobility. These must be evaluated as quickly as possible regarding their contribution to achieve the goals. However, a clear methodological approach is lacking (European Commission 2020) to improve the comparability of transport change options (fuel change, powertrain change, complete vehicle change) (BDI/BCG/Prognos 2018), since cross-study comparability is difficult to achieve due to different Technology Readiness Level (TRL) and the corresponding data availability and uncertainty constraints (Moni et al. 2019). This new method is designed to consider only that part of the production chain that is affected by technological change and the changed energy sources use of the means of transport. The traditional LCA modelling is modified in a step-by-step and prioritising application. Initially, available data for modelling the current state of the emerging transport technology will be integrated. Uncertain parameters, which result in a performance difference between technology alternatives, must be identified. Then differences between TRL of conventional and new technology must be classified and considered by integrating changes after the actual state modelling, e.g. scenarios for the future electricity mix. The goal is to develop a model that will simplify and clearly classify the LCA of transport transformation options. Since the greatest environmental impacts occur in the use phase and the emissions from energy consumption and combustion - accounted to the direct transport-related GHG emissions (UBA 2016) - have a direct effect on climate target improvements in the transport sector, the modelling of the use phase is given top priority. Subsequently, the upstream processes of energy supply, vehicles and required infrastructure must be considered. For the exemplary application of the method to technology changes on a pilot scale, first results concerning the evaluation process of overhead line trucks in the system environment eHighway are presented. The approach shall help to consider the actual and expected performance of emerging transport technology towards climate targets and to correctly classify uncertainties from the TRL. It is important to point out possible changes and to show these assumptions transparently so that the final results can be communicated to decision makers in a clear and comprehensible way.

5.01.10
Life Cycle Environmental and Techno-Economic Impacts of Carbon-Based Perovskite Solar Cells
N. Rajagopalan, Flemish Institute for Technological Research (VITO) / Partner in EnergyVille, Belgium; A. Martulli, S. Lizin, Hasselt University / Partner in EnergyVille and Solliance, Belgium; T. Meyer, Solaronix S.A; S. Claes, C. Spirinckx, Flemish Institute for Technological Research VITO / Partner in EnergyVille, Belgium; F. Gota, U. Paetzold, Karlsruhe Institute of Technology (KIT) / Institute of Microstructure Technology- Light Technology Institute; B. Vermang, imec-Hasselt University / Partner in EnergyVille and Solliance, Belgium
Perovskite solar cells (PSC) have shown great promise as a photovoltaic technology as power conversion efficiency increases, jumping from 3,8% to 25,2% in a few years [1]. A PSC is a perovskite structured compound with a hybrid organic-inorganic light harvesting active layer . Despite the rapid growth of this technology, key challenges still exist for commercializing technology: (1) long term stability, (2) scalability, and (3) toxicity of some materials [2]. Carbon materials used in PSCs have shown to improve the stability of the cells [3]. In this study, a carbon-based PSC (CPSC) is evaluated using a life cycle assessment (LCA), a techno-economic assessment (TEA) approach and a model providing the relevant energy yield information. A cradle to grave approach is applied for the LCA analysis with pilot-scale data on CPSC manufacturing and a lab-scale recycling process data obtained from the manufacturer. The TEA focuses on a gate-to-gate approach of the CPSC manufacturing. The LCA study is conducted for a functional unit of 1kWh. The preliminary results for the LCA analysis highlight that the glass substrate has high impacts in all impact categories except resource use, minerals and metals using the Environmental Footprint method 3,0. The silver contacts contribute to high impacts in that category. Three end of life scenarios- reuse, recycle and landfill are modelled. The reuse scenario is modelled based on a patent application from the manufacturer while the other two scenarios are based on literature data. An energy payback time (EPBT) calculated for the CPSC shows a low payback time of 3 months comparable with thin film PV technology. The TEA analysis consists of four steps: (1) market study, (2) process flow diagram and mass and energy balance definition, (3) economic viability assessment, and (4) sensitivity analysis. The preliminary assessment of the CPSC using TEA shows that the majority of module production costs are attributed to the operational expenditures (OPEX), in particular the glass is the material with the highest associated cost. This analysis highlights the potential of CPSCs in improving solar cell performance. There is potential for improvement in environmental performance and costs. There is potential to partially offset impacts with a recycle/reuse plan in place. This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement Nº 850937. [1] N. NREL, "Best research-cell efficiencies." National Renewable Energy Laboratory: Golden, Colorado, 2019. [2] D. Wang, M. Wright, N. K. Elumalai, and A. Uddin, "Stability of perovskite solar cells," *Sol. Energy Mater. Sol. Cells*, vol. 147, pp. 255–275, 2016. [3] M. Hadadian, J.-H. Smått, and J.-P. Correa-Baena, "The role of carbon-based materials in enhancing the stability of perovskite solar cells," *Energy Environ. Sci.*, vol. 13, no. 5, pp. 1377–1407, 2020, doi: 10.1039/C9EE04030G.

5.01.12
Application of Ex-Ante LCA Strategies to Construct an LCI of REDIFUEL, a Novel Biofuel
A.E. van den Oever, VUB / ETEC; D.M. Costa,

M. Messagie, Vrije Universiteit Brussel (VUB) / ETEC / Mobi / Evergi
An enormous challenge of ex-ante Life Cycle Assessment (LCA) is to make assumptions of what the future will look like. It requires a more philosophical and constructivist discipline than conventional LCA and the development of this discipline calls for more application in real case studies. This work attempts to do just that by discussing the application of ex-ante LCA strategies to compile a Life Cycle Inventory (LCI) of a novel EN590 compliant biofuel with a high alcohol share, REDIFUEL. The production of this fuel is under development in the likewise named project REDIFUEL(Robust and Efficient processes and technologies for drop-in renewable FUELs for road transport), funded by European Union's Horizon 2020 research and innovation programme under the Grant Agreement nº 817612. To guide the future developments of the process concept from an early stage on, a well-to-wheel LCA of REDIFUEL is ongoing. The basis for constructing the LCI is a storyline on the future techno-economic system in 2030, when REDIFUEL is assumed to be introduced to the market. Foreground data on biomass transformation processes are generated within the project based on experimental results on a pilot-scale and process simulation software for upscaling to a commercial-scale plant. Data on feedstocks are derived from woody biomass availability projections for different European regions in 2030, according to the scientific literature and experts. When it comes to the background energy system, the current policies and market trends indicate the evolution over the next decade. Two Shared Socioeconomic Pathways (SSP2 and SSP2-45) representing a world in which social, economic, and technological trends do not change markedly were selected to model the future energy system with spatial differentiation. Where possible, uncertainty distributions were constructed using experimental data, expert opinion or the Pedigree matrix. Our main finding is that a novel biofuel can lead to many different scenarios and it is important to investigate the effect of different fuel blends, feedstocks, geographic locations, process set-ups, and assumptions regarding the future and the combination of each of these. This may create an extra challenge during the life cycle interpretation stage, mainly when several indicators are being investigated. The next step of our work will be to tackle this challenge and find efficient and effective ways to interpret and communicate the results of the LCA.

5.01.13
Algae System Modeling Approaches: Critical Review and Improvements
L. Braud, K. McDonnell, F. Murphy, University College Dublin / School of Biosystems and Food Engineering
Life Cycle Assessment (LCA) has been used for more than 20 years to assess environmental impacts of algae systems. The variety of LCA results is explained by a large algae-based product portfolio combined with the diversity of cultivation and processing techniques. The integration of algae systems into a concept of biorefinery increases their complexity by considering local waste streams, co-products, and recycling loops. Although harmonisation methods have been developed, LCA studies with distinct algae systems remain difficult to compare. This study introduces a method to improve algae systems modeling approaches and Life Cycle Inventory (LCI) data interoperability and transparency. First, computational tools such as Natural Language Processing and Text and Data Mining were used to extract relevant information from a large number of LCA studies in order to identify common modeling and LCI approaches. Based on the limitations identified, a modular LCI framework was developed to adapt current methodologies to algal biorefineries. The literature review results highlight the need to include seasonality in LCI of algae systems as it changes biomass content and productivity. In addition, water, electricity, and nutrient sources influence LCA results. The flexible LCI framework builds on Brightway2 and uses additional Python packages to integrate seasonal variations into LCI, facilitate mass balances, and enhance LCA results visualisation. A dynamic algae growth model generates seasonal data for an open raceway pond cultivation system. Foreground and background scenario development allows the assessment of various algae-based products with different biogenic carbon flow dynamics. The novel LCI framework guides LCA practitioner through modeling choices and simplifies the linking of foreground and background systems. By facilitating algae system modeling, it improves the transparency and interoperability of LCI data and models. In addition, its flexibility facilitates prospective LCA studies and allows comparison of various foreground and background system combinations. The algorithm was tested using data collected in a pilot scale *Spirulina* cultivation facility in the frame of the European SpiralG project (BBI-H2020). Since the algae dynamic growth model can be adapted to any micro- or macro-algae system, the framework supports the development of any algae-based product from laboratory to industrial scale.

5.01.17
Low-Carbon Hydrogen Production: Life Cycle Assessment of the Photocatalytic Route
M. Rumayor, J. Corredor, M. Rivero, I. Ortiz, University of Cantabria / Dept. of Chemical and Biomolecular Engineering
The increasing concern about the energy crisis and environmental pollution resulting from the extreme use of fossil fuels has encouraged scientific community to search for sustainable and clean energy sources that should be implemented in a near future to meet Paris Agreement's goal of a climate-neutral society by 2050. In this context, the hydrogen economy has arisen as a proposed solution where hydrogen (H_2) is produced and used extensively as the primary energy carrier contributing to the decarbonization of the energy system. Several emerging technologies for the sustainable H_2 production have been demonstrated in the latest decades. Among them, photocatalytic H_2 generation from organic solution wastes (e.g. crude glycerol from biodiesel) is a promising and environmentally friendly alternative to the conventional fossil-process such as the steam reforming route. Moreover, it can make a sound contribution to the circular economy. However, some challenges are still inherent to the technology that need to be overtaken, especially the quite modest H_2 production rates that depend on the catalyst performance. In this study, a life cycle assessment (LCA) will be used to evaluate the challenges and opportunities determining the best technological performance to assist future developments that make this technology feasible from an environmental perspective. Two impact categories will be considered in the study: (i) Global warming potential (GWP) representative of the carbon footprint and, (ii) Abiotic depletion potential (ADP elements and fossil) that is representative of the natural resource consumption. The results obtained indicate that values of GWP in the rage of 30 kg of CO2eq (per kg of H_2 produced) can be reached in medium/long term developments. Energy consumption by LEDs to illuminate the photoreactor is the major impact on GWP followed by the impact of the catalyst fabrication that could contribute up to 30% to the overall value of GWP if noble metals are present. This comparison allowed us to determine the figures of merits for H_2 production by photocatalysis that can guide the medium/long term developments. Furthermore, the results obtained will be compared with those values related to other sustainable processes to situate this technology within the portfolio of sustainable H_2 production technologies.

5.01.18
Carbon Footprint Assessment of the Implementation of Carbon Capture and Utilization in a Hard-To-Abate Sector
M. Rumayor, University of Cantabria / Dept. of Chemical and Biomolecular Engineering; A. Dominguez-Ramos, A. Irabien, University of Cantabria / Department of Chemical and Biomolecular Engineering
To achieve the Paris climate agreement goal of limiting global warming below 2°C it is essential to transform energy and industrial systems towards net-zero CO_2 emissions. Nowadays, the decarbonization of some sectors such as transportation, building or energy production are better understood and developed since their markets are less open to international competition. However, the opportunities for decarbonizing industry, especially heavy or basic manufacturing (i.e. those based on intensive-energy processes), are still less obvious and largely absent from the climate change mitigation proposals. The decarbonization strategies proposed for these hard-to-abate processes include: (i) the use of biomass as fuel or feedstock; (ii) the transition to Hydrogen as intermediate energy vector; (iii) the integration of carbon capture and utilization (CCU); and (iv) the electrification of heat. Among them, CCU has been stated as a potential solution because it can lead to positive revenues as well as positive social-political images. In this case of study, we aim to analyse the global warming impact of the integration of a CCU plant that produces CO_2-based methanol (CO_2-MeOH) within the hard-to-abate synthetic sodium carbonate (soda ash) production sector. The CCU scheme was based on a combination of technologies at different TRL: (i) the use of monoethanolamine (MEA) for CO_2 capture; and (ii) the novel production of CO_2-MeOH by the electrochemical reduction (ER) of CO_2 which has been demonstrated at lab-scale to be an environmentally-friendly production alternative to the fossil-dependant route. A power-to-heat (PtH) unit was included in the integrated plant to provide a fossil-free source of heat. With the emphasis on the rigorous assessment of the net CO_2 emissions, the Life cycle assessment (LCA) was used to determine the overall carbon footprint (CF) of the integrated process. Several scenarios have been assessed considering the

future innovation of the CCU technology. The results obtained in this study show that it is possible to reduce the CF of the synthetic soda ash production up to 75% thanks to medium/long term developments. The insights gained in this study may be of assistance in the sustainable implementation of CCU in energy-intensive manufacturing processes.

5.01.19
Comparative Life Cycle Assessment of Direct Air Capture Technologies
K. Madhu, University of Freiburg; S. Pauliuk, University of Freiburg / Industrial Ecology Research Group; S. Dhatri, University of Freiburg; F. Creutzig, Mercator Research Institute on Global Commons and Climate Change
The role of carbon removal technologies to meet the climate targets, as stipulated by the Paris Agreement, has been highlighted by the International Panel on Climate Change (IPCC). Based on technological potential to be deployed at a large scale, direct air capture (DAC) of atmospheric carbon dioxide (CO_2) has emerged as one of the main technological contenders. However, there is a lack of knowledge on the comparative environmental impact of the current prototypes of the DAC technologies and the environmental implication of their large scale deployment. The goal of this study is to provide a systematic comparative evaluation of environmental impacts of current prototypes of the two DAC technologies, namely, Temperature Swing Adsorption (TSA) and High Temperature Aqueous Solution (HT-Aq) DAC, under various scenarios encompassing different technical parameters. Moreover, the study also aims to investigate the changes in the impacts when these technologies are deployed at a large scale i.e. 1Mt CO_2 captured per year. For the analysis, Life Cycle Assessment methodology is used and the technologies are evaluated under three dimensions: climate change, metal depletion and particulate matter formation. Our results show that in all the three dimensions, the life cycle environmental impact of TSA DAC is 2-3 times smaller than HT-Aq DAC, mainly due to lesser energy requirement in the use phase. Under different technological parameters, the Carbon Capture Efficiency (CCE), i.e. net life cycle CO_2-eq emissions per ton CO_2 captured, of the prototypes of TSA DAC ranges from 30% to 80% while that of HT-Aq DAC ranges from -50% to 40%. Moreover, the CCE of TSA DAC increases to 85% and HT-Aq to 70% when the DACs were supplied with low-carbon energy. The maximum impact for the impact categories metal depletion and particulate matter is due to construction and demolition of the DAC plant for both the technologies. Due to the modular size of the TSA DAC, a linear scale-up and a fast technology learning can be expected with negligible CCE increase apropos its current prototype. The scale-up scenario of HT-Aq DAC reports CCE of 60% with current Canadian energy mix, due decrease of material intensity per ton CO_2 captured. Our study concludes that utilizing low carbon energy source in the use phase of the DACs and increasing the lifetime of the sorbents can substantially decrease the overall environmental impact of the DACs.

5.01.20
Prospective LCA for Development and Manufacturing of Pharmaceuticals - Environmental Load Reduction Effect by Transgenic Silkworms -
H. Nakamura, Tokyo City University; H. Sezutsu, National Agriculture and Food Research Organization; N. Itsubo, Tokyo City University / Graduate School of Environmental and Information Studies
In this study, although the environmental load per product unit is large in the experimental scale, Prospective LCA, which is expected to reduce the environmental load in the production scale, was applied to the manufacture of pharmaceuticals. In order to resolve the problem of heavy environmental impact by using selected raw materials and special equipment for manufacturing pharmaceuticals, GHG emissions of climate change on the production of antibody drug substances for pharmaceutical materials using transgenic silkworms with high protein productivity is targeted. The evaluation boundary was artificial feed, solution raw materials / breeding containers, materials and electric power, further until the breeding residue, waste liquid / cocoon, breeding container, and materials discharged from the system boundary were disposed of via waste transportation. The functional unit was 0.88 [L] of an antibody drug solution containing 20 [g] of an antibody made from the cocoon of a transgenic silkworm. The calculation was processed using primary data from Immuno-Biological Laboratories Co., Ltd., the inventory database is IDEA2 (Inventory Database for Lifecycle Analysis) and 2011 version WIO (Waste Input-Output table) developed by Waseda University, and the impact assessment method is LIME2 (Life-cycle Impact Assessment Method based on Endpoint modeling). From this calculation, it was found that the electric power consumption of the clean room to prevent the contamination of viruses and bacteria has a large influence in the extraction / purification process due to the process of extracting and purifying the antibody drug substance. Subsequently Prospective LCA was carried out after scenario planning of future technology and energy system from various technical information in addition to the conventional LCA data. In contrast to the current experimental scale, at the pilot scale, the floor area of the existing clean room was effectively used, and the manufacturing capacity per unit time has been improved by the system of multiple equipment. In addition, at the production scale, a new chromatography process, the latest continuous processing equipment and an energy-saving clean room were introduced. As a result, it was confirmed that GHG emissions, including raw materials, transportation, and disposal, could be reduced to about 1/5 in the pilot scale and about 1/10 in the production scale compared to the experimental scale in the extraction / purification process.

5.01.21
Carbon Footprint Comparison of Vapor Compression and Phase Change Cold Storage Systems for Refrigerated Vehicle
J. Wu, University of Padova / Industrial Engineering; A. Manzardo, University of Padova / Department of Industrial Engineering; G. Liu, Guangzhou University / School of Management; A. Scipioni, University of Padova / Department of Industrial Engineering
Refrigeration transportation requires energy to maintain the cold chain activities, which leads to a large amount of greenhouse gas (GHG) emissions (A. Rai et al, 2017). The vast majority of refrigerated vehicles employ vapor compression refrigeration systems (VCRS) driven by a diesel engine. Such technology is recognized as relatively expensive, noisy and its efficiency is only 35 – 40% (Liu M. et al, 2012). Ongoing research is currently developing new technologies based on phase-change refrigeration systems (PCCSS) that use electric energy for refrigeration. This new solution is promising and is considered to become a good alternative to traditional VCRS systems in the future (Liu G. et al, 2019). The goal of the study is to assess and compare the GHG emissions of VCRS and PCCSS Life Cycle Assessment (LCA) according to ISO 14040-44 was used as a reference methodology with a focus on GHG emissions. The functional units are defined as a volume of 16,8 m3 refrigerated at the temperature of 0° over ten years lifetime when the outside temperature is + 30°C. This is the operating condition that can be found in Guangzhou (China) where the research was carried out. The model considered a life cycle cradle to grave perspective including production, installation, transport, use, repair, and recycling on greenhouse gas emissions expressed as the CO_2 equivalent emissions in total. Inventory data on the two different refrigeration systems were collected adopting the same data quality parameters. Primary data on energy use and refrigerant use of the two systems were collected. Results showed that the PCCSS understudy due to its high energy use resulted to have slightly higher GHG emission (228.030 kgCO2 eq) if compared to VCRS (219.640 kgCO2 eq) in a life cycle perspective. The reason for these results depended on the storage capacity design of the PCCSS understudy that resulted to have an unreasonable cold storage capacity. The study proved that, in order to reduce GHG emissions of PCCSS, the cold storage capacity should be reduced from the original 443 kg to 150 kg. As a consequence, the carbon footprint efficiency of the PCCSS can be increased by 31.2%. Another issue that was identified is the electricity use profile of PCCSS. This aspect should be more investigated especially at night when energy use is not productive. Further research is needed. PCCSS has better potential performances related to climate change regarding the trough electricity of the entire region or city.
Keywords: carbon footprint; refrigerated vehicle; life cycle assessment; refrigeration system

How Life Cycle Assessment (LCA) Can Serve Effectively Environmental Foot-Printing and Policy Making: Challenges and Opportunities

5.02.06
Techno-economic analysis and life cycle assessment of hydrogenated vegetable oil and biodiesel from waste valorisation in Spain
G. Puig-Samper Naranjo, IMDEA Energy / Systems Analysis Unit; D. Bolonio, S. Jiménez-Oyola, M.F. Ortega, M. García-Martínez, L. Canoira, Universidad Politecnica de Madrid / Energy and Fuels
Purpose: The goal of this study was to assess and compare life cycle costs and environmental impacts of Hydrogenated Vegetable Oil (HVO) and biodiesel produced from animal fat and Waste Cooking Oil (WCO). For the latter, the study also aimed to evaluate two collection

systems based on Madrid and Bilbao models. Biofuels were compared with traditional fossil fuels and new fuels as R33 and B7. The ultimate goal was to provide a reliable work that helps to decide future policies in transportation. **Methods:** End-of-life process for animal fat and cooking oil valorisation was evaluated and analysed using Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) methodologies. The inventory was completely regionalised to Spain, thoroughly analysing the waste collection and transport stages. Environmental impacts were characterised employing the Environmental Footprint 3.0 method, focusing on the Climate Change impact category. Different allocation methods and system expansion were applied to evaluate the robustness of the results. **Results and discussion:** The results shown that the production of HVO and biodiesel from animal fat generates much lower Green House-effect Gases (GHG) emissions than their manufacturing from WCO (GWP: HVO_{af}=13.40, $Biodiesel_{af}$=20.40, HVO_{WCO}=82.20, $Biodiesel_{WCO}$=81.40 gCO_2-eq/MJ). The large difference between the two wastes is manly caused by the collection and transport of the WCO. In this sense, it was found that the WCO collection through fixed collection points (Bilbao) presents 10 times higher collection rates (4.2 kg/person) and reduces GHG emissions (46-55%) relative to Madrid's hybrid model. The new fuels considered, R33 and B7, present GHG emissions comparable to those generated by HVO and biodiesel from WCO. In terms of total cost of ownership, biofuels from animal fat have the lowest cost and HVO from animal fat represented almost half the cost of biodiesel (0.592 €/kg HVO). **Conclusions:** It was concluded that HVO produced from animal fat performs the best in terms of GHG emissions and cost of ownership. The work demonstrated that transport is the major contributor to the environmental impacts of HVO and biodiesel from WCO, highlighting the significance of the collection system. From the biofuels assessed, only HVO and biodiesel from animal fat or their blends produced from a mix of animal fat and WCO, comply with the 60% GHG emissions reduction stated by the Directive (EU) 2018/2001 for 2020.

5.02.07

The Current Status of Food Waste Recycling in Korea and the Direction of Management Considering Greenhouse Gas

s. lee, J. Park, Korea Institute of Civil Engineering and Building Technology (KICT) / Department of Land, Water and Environment Research; C. Ahn, Korea Institute of Civil Engineering and Building Technology / Department of Land, Water and Environment Research

According to the Ministry of Environment (2019) in Korea, domestic food waste disposal is treated and recycled using feedstock (55.8%), composting (23.4%), bio gasification (13.0%), and other methods (7.8%). While most countries rely on landfill and incineration, the recycling rate (95% above) of food waste in Korea is leading. These days, research on bio gasification has been expanding along with Korea's New Deal policy. However, greenhouse gases (CO2, CH4, N2O, etc.) generated from food waste disposal processes are overlooked. In recent studies, an institutional and methodical approach to food waste disposal is being taken in terms of reducing the generation of GHG (greenhouse gas). Typically, the EC (European Commission) is making efforts to reduce GHG by building a system that takes into account the overall process from composting to CO2 uptake of plants. Besides, research on monitoring greenhouse gases is underway in the process of composting, and Mak et al (2020) emphasized the importance of periodic bio-economic policies on food waste. In line with recent studies, it is believed that it is time to discuss food processing in Korea considering the issue of climate change and the net environmental system. In this study, we would like to present a system that improves compost, a product of food waste, so that it can be used elsewhere than in farmland. For example, making part of a riparian zone, technology for reducing urban non-point sources, soil loss.Acknowledge: This research was funded by the Major Project of the Korea Institute of Civil Engineering and Building Technology, grant number 2020-0648.

5.02.08

Life Cycle Assessment of Oyster Production From Aquaculture in Ria De Aveiro, Portugal

T.P. Costa, University of Aveiro / Department of Environment and Planning; P. Quinteiro, University of Aveiro / Environment and Planning; A. Dias, University of Aveiro / Centre for Environmental and Marine Studies (CESAM) - Department of Environment and Planning

Seafood production has a substantial role in food security and global supply. Oysters have progressively increased their role in the aquaculture sector in Portugal and still have great potential for growth. However, they are consumed mostly in France, opposing the current policies to reduce the distance between producer and consumer in order to mitigate environmental impacts. Therefore, the aim of this work is to apply life cycle assessment (LCA) to evaluate the environmental impacts associated with the oysters produced from aquaculture in Ria de Aveiro (Portugal) and consumed in France to identify hotspots and support decision making. Oyster fattening is performed through an extensive system where the feed is exclusively natural. The functional unit is the production of 1 t of oyster delivered to depuration in France. System boundaries include the transport of juveniles from France, the fattening stage and transport of the oysters to depuration. The carbon sequestration due to oyster shells growth was also included. The hatchery and nursing stage in France are excluded from the system boundaries, as well as the consumption and end-of-life. The characterisation factors used in this study are those suggested for conducting a Product Environmental Footprint (PEF). The fattening stage was particularly relevant for the impact categories of human health (both cancer and non-cancer effects), freshwater eutrophication and resource use (mineral and metals). The impacts in this stage resulted mainly from the end-of-life at landfill of several materials used in the infrastructure, such as oyster iron racks, steel hooks and rubber anchor bands. Therefore, policies that encourage alternative end-of-life management (e.g. reuse or recycling) could improve the sustainability of oyster production. The transport to depuration is crucial for impact categories such as climate change, photochemical ozone formation and acidification, emphasizing the importance of policies that encourage short supply chains in food production. Therefore, LCA showed its potential for policy-making purposes in the seafood sector, since it identifies opportunities to improve the processes and can support the different policy makers involved in the value chains of seafood production to opt for the most environmentally friendly option.

5.02.11

Life Cycle Assessment of Generic City Districts: Decision Support for Resource Efficient Refurbishment Strategies

B. Koch, Technische Universitaet Darmstadt / Institue IWAR Chair of Material Flow Management and Resource Economy; L. Schebek, Technische Universitaet Darmstadt / Institute IWAR Material Flow Management and Resource Economy Germany

Urban planning processes are increasingly taking place at the district level, which is an economically and technologically interesting level for energetic planning. The currently high dynamic of distract planning due to changing requirements for living and working in urban centres offers the opportunity to integrate measures of resource efficiency, in terms of climate mitigation in the use phase, but also energy and resources incorporated in the building materials, into these planning processes. Life cycle assessment (LCA) is the suitable tool to identify the most resource efficient strategies and support decisions of planners. Although LCA has been applied widely in the building sector, the district level has rarely been addressed: LCA studies focus either on the single building or on full cities, analysing resource consumption or greenhouse gases (GHG). In the case of buildings often so-called generic buildings are used, since LCA results are not only valid for one individual building, but for types of buildings. The same is desirable for LCA of city districts, however up to now only few case studies of real districts have been performed and no generic outline of districts has been specified. In this study, a framework for LCA for investigation of resource efficiency of city districts is developed, including use phase as well as production of materials. The framework is based on generic districts (GD) developed within a district typology for Germany. The LCA framework consists of three parts: First, the in total 36 GD are characterizes based on their energy and material related parameters and the useful energy demand is calculated as a basic parameter for the assessment of the use phase. Second, an LCA for each of the 36 GD is performed. The corresponding LCIA results display the baseline against which refurbishment measures can be compared to. In the third part of the framework, the reduction of LCIA indicators, notably as to GHG emissions and resource consumption, from selected refurbishment measures are evaluated. These refurbishment measures may include active or passive measures for the individual buildings within the districts as well as active measures for the district as a whole. In order to assess resource efficiency, in a hot-spot analysis key indicators for the analysed refurbishment measures are identified.

5.02.13

Nutrient Recovery and Zero Liquid Discharge Concepts: Technological Screening Through Life Cycle Assessment and Methodological Challenges in the

Quantification

J. Senan-Salinas, IMDEA Water (G84912732) / Membrane Technology; J. Landaburu-Aguirre, C. Tamarit, S. Molina, IMDEA-Water Institute; E. García-Calvo, UNIVERSTY OF ALCALA

Nutrient recovery (NR) and Zero Liquid Discharge (ZLD) are two of the most trending concepts around Circular Economy in water sector. Both are focused on different recovery degrees of substances present in wastewaters and brines. Although NR technologies focuses uniquely on bringing back nutrients, ZLD schemes are more exhaustive and their goal usually is the maximization of the water reusing and recovery of salts, nutrients or other components to avoid any liquid discharge into the medium. This work aims to analyse the potential use of Life Cycle Assessment (LCA) in the technological screening under environmental criteria. With this aim, LCA studies (n=34) of NR technologies were analysed and harmonised around the functional unit of 1 m^3 of wastewater treated with consequential modelling and system expansion of the products recovered. Regarding ZLD schemes, the low number of LCA studies (n=3) was solved with the development of Life Cycle Inventories (LCI) from techno-economic analysis (n= 14) found in the literature. An index was developed to analyse the degree of the reliability and detail of the system boundaries and LCIs. This index was based on other works related to wastewater treatment plants. OpenLCA v1.10 with Ecoinvent v3.4 database were used for modelling. ILCD impact method categories were used for the Life Cycle Impact Assessment. First, the preliminary impact results allowed the identification of the most important environmental trades-off between the impact of the technologies used and the environmental accreditation of products recovery. Preliminary results indicate that ZLD technologies have a higher energy use than NR schemes. In many cases, the high energy use impact is not compensated by the environmental accreditation of the salts recovered. Therefore, the overall balance is a net impact. Secondly, a factor analysis of mixed data will be applied to analyse the influence of i) the technological scheme, ii) the water quality treated, iii) different LCI parameters as energy use; and iv) the system boundaries and inventory detail in the results with the cited index described above. The present study also points the need of some type of standardization and a methodological framework herein proposed. Nonetheless, the present work evidenced important technological trends as well as methodological gaps of LCA in the application over those type of end-pipe technologies.

How to Evaluate the Sustainability of the Transition to a Circular Economy? The Role of Life Cycle and Circularity as well as Risk Assessment Methods

5.03.04

Integrating Circularity Indicators Into an LCA Tool: A Review on Options Using a Case Study

A. Koyamparambath, CyVi - ISM - University of Bordeaux / ISM-CyVi; G. Sonnemann, University of Bordeaux - ISM / ISM-CyVi; S.B. Young, University of Waterloo / SEED

Resource depletion, excess waste and emissions to the environment are amongst the few reasons encouraging organizations to shift towards a circular economy (CE). Indicators to measure circularity are developed by several research organizations aiming to quantify the transition towards a circular economy, while other few indicators measure the effects of this transition. Integrating these indicators along with life cycle indicators for sustainability assessment is a complex task to achieve; this contribution aims at reviewing the existing circularity indicators for their ability to be implemented into a Life Cycle Assessment (LCA) tool using a case study. Indicators evaluating the degree of circularity such as the Material Circularity Indicator (MCI) (Ellen MacArthur Foundation, 2015) are reviewed to verify its ability to adapt to the characterization model of LCA. The review is focused on the input parameters, level of assessment (Micro, Meso, or Macro), additional data inputs required, and sub-indicators. Five out of the twenty indicators reviewed are found suitable to be integrated into an LCA tool as independent methods. The selected indicators are compared using a case study where each indicator is presented as a characterization model similar to that of LCA indicators. The flexibility in the empirical calculation of these indicators is one of the main aspects to be considered. Although circularity indicators (CI) require additional data for its evaluation, our results demonstrate that the chosen indicators are more viable and adaptable to integrate into an LCA tool. The integration of these indicators will make it a more complete sustainability assessment tool with regard to the Area of Protection Natural Resources in LCA.

5.03.09

Prospective LCA of Mixed Plastic Waste Pyrolysis and Gasification

M. van der Hulst, Radboud University / Environmental Science; A. Ottenbros, Radboud University / Department of Environmental Science; . Ferjan, Utrecht University / Copernicus Institute of Sustainable Development; A. Schwarz, M. Hauck, TNO / Department of Climate, Air and Sustainability; M. Huijbregts, Radboud University Nijmegen / Department of Environmental Sciences; E. Worrell, Utrecht University / Copernicus Institute of Sustainable Development; B. van der Drift, Synova; T. van Harmelen, TNO / Department of Climate, Air and Sustainability

Emerging plastic waste treatment technologies may reduce environmental impacts that are associated with the increasing demand for plastic products. A transition from incineration and landfilling to more circular treatment alternatives for end-of-life plastics can contribute to a reduction in global warming, fossil resource depletion, and plastic littering. Ideally, plastic waste is processed to new plastic products, thereby contributing to a circular economy. However, due to the composition and quality of available plastic waste streams, direct use of plastic waste as feedstock for plastic production is not always possible. For contaminated, mixed plastic waste streams, pyrolysis and gasification may be preferred solutions for plastic recycling to reach full circularity. These technologies decompose plastic at moderate (pyrolysis) to high (gasification) temperatures in absence of oxygen. A variety of compounds can be obtained, depending both on waste composition and processing parameters. These compounds can be used as feedstock for the production of plastics, but can also be used as is, or transformed to other chemical products. Several pyrolysis and gasification systems have reached the pilot (TRL 6) or demonstration (TRL 7-8) plant level, yet commercial application has not been achieved yet. The goal of this work is to forecast environmental impacts of both technologies at an industrial scale (TRL 9). For this, prospective LCA is used. Expected technological advancements are assessed, as well as projected changes in feedstock availability and quality. Furthermore, the optimal use case scenarios for the obtained compounds are evaluated. The total GHG emission reduction potential for the Netherlands is assessed by considering the projected available volume of mixed plastic waste and the demand for products that can be derived from these waste streams. Pyrolysis is found to be outperformed by gasification, because its more stringent processing parameters inhibit the processing of part of the mixed plastic waste. Lessons learned from performing this case study are generalized for applications to prospective LCAs of other end-of-life waste treatment systems.

5.03.10

Valorisation of All Fractions of CDW, a Route to Circular Economy: LCA of Leroy Merlin Project

S. Groslambert, A. Léonard, Liège Université / Chemical Engineering; S. Gerbinet, Université de Liège / Chemical Engineering

From resource prospective, the building and construction sector is responsible for more than one third of global resource consumption, and its generation of solid waste is estimated to be 40% of the total waste volume. A very significant part of construction and demolition waste (CDW) is not recycled today, due to heterogeneity and dispersion of waste flows, which decrease efficiency and economic viability of recycling. To address this issue, the VALDEM project aims to overcome barriers to increase up-cycling applications. In this context, we had the opportunity to evaluate the environmental benefits of a project of Leroy-Merlin Company. It has adopted a circular economy approach by recycling a part of the demolition waste of the Douai store in the form of recycled aggregates (RA) integrated into the concrete slab of their new Tourcoing store (4-20 mm fraction). The remaining 4-20 mm aggregates are recovered and valorised in concrete for other (building) projects by Eqiom, and the fines (0-4 mm) are valorised in resin slabs by EtNISI, through Recynov (waste management company). The first LCA concerns the 4-20 mm fraction and it compares the production and transport of natural and recycled aggregates, and their respective use in the production of concrete. If we consider the production of 1 m^3 of concrete, with only NA or substituting 20% for RA, the difference is not significant, since most of the impacts come from cement. On the other hand, if we focus only on the aggregates, this recycling of the "waste" from the Douai demolition site as a substitute for NA allows an environmental gain compared to the use of NA alone. This benefit is all the more important as the location of their use is close to the demolition site, and is less than the supply distance for NA. The second LCA concerns the valorisation of the fine fraction (0-4 mm) in slabs called Wasterial. These tiles can be substituted for ceramic tiles, which require a large amount of (fossil) energy

for firing. The production of Wasterial tiles emits less than half as much CO2 as ceramic tiles. In conclusion, the recycling of the demolition waste from the Leroy Merlin site in Douai provides a significant environmental gain especially for the GWP impact category.
Acknowledgements - The authors would like to thank the Valdem project (Convention n° 1.1.57 - Interreg FWVL European Fund), partly financed by the European Regional Development Funds, and the Walloon Region.

5.03.12
Recyclability Definition and Niche Identification Through Life Cycle Thinking in End-Of-Life Reverse Osmosis Direct Recycling
J. Senan-Salinas, IMDEA Water (G84912732) / Membrane Technology; J. Landaburu-Aguirre, IMDEA-Water Institute; R. García-Pacheco, Lequia Universitat De Girona; E. García-Calvo, UNIVERSTY OF ALCALA
By 2030, around 3 million reverse osmosis modules will be discarded. Their reuse and recycling into nanofiltration and ultrafiltration has been studied from techno economic and environmental point of view. However, previous studies have been constrained to quantify through life cycle assessment the environmental outcome of the substitution of new membrane products. Those studies also include a substitutability ratio for comparing the performances between the recycled and the new produced membranes. The present work aims to overcome the previous studies integrating the potential impact differences during the operation as a potential major energy consumption due to the lower permeability of recycled membranes compared to the new produced counterparts. In order to analyse the recyclability potential, different factors have been analysed: i) the theoretical energy demand of commercial and recycled membranes related to the water quality and pressure; ii) the introduction of energy reduction strategies in the design of pressure tubes; and iii) the sensitivity of the background electricity production processes (current mixes versus removable future scenarios). The energy modelling and water quality was performed with R v3.5. Results were validated with the results of the commercial ROSE software. Life Cycle Assessment was performed with OpenLCA v1.10 and the ILCD impact method v1.10 categories were used. Results point the high contribution of the operation stage and the permeability in the energy consumption in the overall impact. Nonetheless the design of longer pressure tubes (with more modules) and second stages reduce importantly the energy use of recycled membranes maintaining the conversion factor (ratio of recovered water from the original flow) and maintaining quality of product water. Otherwise, the use of different energy sources toward renewal ones can reduce the overall impact and the contribution of the operation stage contribution as in climate change category.

5.03.13
Wood As a Sustainable Building Material - the ENvironmental Implications of Circular Economy of Wood
N. Emmanouil, The University of Southern Denmark / SDU Life Cycle Engineering, Department of Green Technology (IGT); S.C. Andersen, Danish Technological Institute / SDU Life Cycle Engineering, Department of Green Technology (IGT); M. Birkved, The University of Southern Denmark / SDU Life Cycle Engineering, Department of Green Technology (IGT)
Considerable focus is on finding solutions that facilitate the sustainability transformation of the built environment, thus finally achieving the total sector's decarbonation, meeting the 2050 Paris Agreement goals. The industry focus on energy consumption reduction has led to contemporary buildings inducing fewer environmental impacts during their operational stage than the impacts generated from manufacturing and disposal/recycling of materials. It has thus become urgent to shift onto material specific circular metabolisms, such as e.g. circular economy of wood. Structural properties of wood are appealing when substituting carbon intensive mineral materials in beams or slabs. Life Cycle Assessment (LCA) is considered to be the most suited method to quantify the global warming potential for buildings. Comparative LCAs regarding wood construction are of major interest and the results indicate high potentials regarding carbon mitigation. Through different future scenarios, this paper will illustrate an innovative approach in quantifying wood stock while also forecasting the to-be generated embodied carbon emissions caused by the increased wood consumption. The direct investigative approach is applying three different methodologies in order to assess wood's sustainability potential. The calibration of a prospective model for different building material consumptions will clarify the connection between the emissions and the marginal coefficients related to the materials. The work will identify key drivers that influence the performance of the emissions generated in the building sector and predict how this performance changes under alternative future scenarios. Furthermore, a crucial parameter to support future policies is analysis of the substitution benefits derived from wood cascading. In this context of resource efficiency, the method proposed is using a quantitative tool from economy, the Computable General Equilibrium (CGE) model. This dynamic assessment analyzes how different inputs are used to manufacture a commodity or service and define how the substitution of different intermediate inputs and outputs changes in response to a change in relative prices within the economy, by introducing substitution elasticities. Finally, the combination of Material Flow Analysis (MFA) and LCA leads to a hybrid MFA-LCA scenario-based methodology suitable for covering upstream supply chain activities and capable of identifying hotspots with high environmental impact.

5.03.15
Lifecycle Assessment of Monosodium Glutamate Made From Non-Edible Biomass
K. NAKAMURA, Ajinomoto Co., Inc. / Research & Business planning Dept; N. Itsubo, Tokyo City University / Graduate School of Environmental and Information Studies
The open burning of agricultural residues derived from rice and corn is a serious social issue in Thailand because of the smoke air pollution. One way to prevent smoke air pollution is to pulverize glycosylated non-edible biomass and convert it to monosodium glutamate (MSG). This study assessed MSG produced by fermentation of pulverizing and glycosylating non-edible biomass and compared the environmental performance of MSG produced by conventional processes using tapioca starch. This study compares the environmental footprints of MSG by two types of raw materials. The scope of this study is the cultivation of raw materials, processing of raw materials, and the production of MSG. The adopted impact categories are carbon, water, and air pollution. The primary data are the average unit input and fuel consumption of MSG annual production. The secondary data are Ecoinvent3, the Water Footprint Network inventory, and the EMEP/EEA air pollutant emission inventory. We evaluated the carbon footprint using ISO14067 as carbon dioxide (CO_2) emission intensity, the water scarcity footprint using ISO14046 as an impact assessment, and air pollution footprint using PM2.5 intensity. We also conducted an impact assessment for health impacts and weighting across several impact categories using the lifecycle impact assessment method based on endpoint modeling 3 (LIME3). The carbon footprints of MSG from non-edible biomass and tapioca starch are 4.34 kg CO_2/kg-MSG and 4.35 kg CO_2/kg-MSG, respectively. Non-edible biomass and tapioca starch are similar but non-edible biomass requires electricity to pulverize cellulose. Water scarcity footprints of MSG from non-edible biomass and tapioca starch are 556 L/kg-MSG and 1,768 L/kg-MSG, respectively. The values differ significantly because rice straw as non-edible biomass was not allocated to the water scarcity footprint because, in Thailand, it has no market value. Air pollution footprints of MSG from non-edible biomass and tapioca starch are 6.45E-6 DALYs/kg-MSG and 2.25E-5 DALYs/kg-MSG, respectively. The values differ significantly because rice straw open burning is avoided. The human health impact assessments of MSG from non-edible biomass and tapioca starch are 1.22E-5 DALYs/kg-MSG and 3.67E-5 DALYs/kg-MSG, respectively. The 2.45E-5 DALYs/kg-MSG difference is because of declining water scarcity and air pollution footprints. We found that using rice straw as non-edible biomass prevents water scarcity and smoke air pollution caused by open burning.

LCA and Beyond - Integrating Sustainability and/or Other Dimensions in order to Improve Decision Support

5.04.03
Responsibility of Consuming Countries for Mining Capacity - Decomposition Analysis of Scarcity-Weighted Metal Footprints in the Case of Japan -
R. Yokoi, National Institute of Advanced Industrial Science and Technology (AIST); K. Nansai, K. Nakajima, T. Watari, National Institute for Environmental Studies (NIES); M. Motoshita, National Institute of Advanced Industrial Science and Technology (AIST)
Metals are indispensable for our life and thus metal scarcity is an important issue. Many consuming countries depend on metal mining in other countries, imposing various mining-related issues in producing countries including pressure on mining capacity, which increases the potential risks to local sustainability in mining. In this study, to represent the responsibility of consuming countries for pressure on mining capacity in producing

countries, a scarcity-weighted metal footprint (S-MF) concept is adopted. The S-MFs are calculated by incorporating metal scarcity indicators expressed by the ratio of mine production to availability with metal footprints. For international coordination and resource governance towards sustainable metal use, it is important for consuming countries to understand metal-specific status about their responsibility for mining capacity and identify key factors for efficient improvement. However, such analysis has not been conducted. This study aims to evaluate the responsibility for pressure on mining capacity of producing countries associated with the final demand of Japan and to identify key factors for consuming countries to reduce their responsibility. As proxy indicators for responsibility for mining capacity, S-MFs of Japan are calculated for 2005 and 2011. Then, by using decomposition analysis, S-MFs are decomposed to identify factors which cause the changes of the S-MFs between 2005 and 2011. The analysis is conducted for iron, copper, and nickel, which are vital to economy and consumed in large quantities. The results indicate that the dependency of Japan on regions of metal supply is different among metals. Furthermore, the significance of regions in terms of the S-MF and non-weighted metal footprint is different. The decomposition analysis reveals that the changes of trade partner of Japan increase the SMFs for copper and nickel. On the other hand, changes of the scarcity in producing countries contribute to the changes of the S-MFs more than the changes of induced mine production and trade partner of Japan for all target metals. These results suggest that the responsibility of consuming countries can vary depending on the factors which are not directly related with actions of consuming countries. Our analysis will support the discussion about the effective options for reducing the responsibility of consuming countries in terms of pressure on mining capacity.

5.04.06

Eco-Design Tool for Shopping Bags Within the Product Environmental Footprint-Based "Made Green in Italy" Scheme

A. Marson, University of Padua; A. Fedele, F. Zuliani, F. Aguiari, University of Padua / CESQA - Department of Industrial Engineering; A. Manzardo, University of Padova / Department of Industrial Engineering

In 2018, the Italian Ministry of the Environment issued the regulation for the implementation of the voluntary national scheme for the assessment and communication of the Environmental Footprint of products, called “Made Green in Italy”. This scheme is derived from the Product Environmental Footprint (PEF) and classifies products based on their environmental impact with respect to the market benchmark. This evaluation is carried out considering the Single Score, obtained by aggregating the weighted results of the three most relevant impact categories. The tool became operational only in February 2019, with the publication of the first Product Category Rules for reusable polyethylene (PE) bags. This document defines the rules and assumptions for conducting the study, as well as the benchmark and the thresholds of the performance classes. The most relevant impact categories, defined in accordance with the PEF methodology, for the specific product category are: Resource Use – Fossil, Climate Change and Particulate Matter. In this context, producers of reusable PE bags need a simple and immediate tool for a screening evaluation of the Single Score right from the design stage. An in-depth analysis of different models of bags allowed us to identify the variables that most influence the result: geometric characteristics of the bag (weight / volume ratio), material (LDPE / HDPE), recycled content, yield and energy consumption for the production process (from extrusion of the granule to printing and cutting) and quantity of inks. This set of variables was subsequently screened and compared with the variability of the products on the market, identifying the parameters to be implemented in the eco-design tool. These variables were then combined to obtain an equation for the preventive evaluation of the Single Score. This tool is also re-proposed in graphic form, through the creation of an eco-design diagram. Finally, a comparison between the results obtained with the eco-design tool and the complete studies is proposed, in order to discuss the effectiveness and limits of the preliminary assessment. The instrument has proved capable of predicting, with reasonable accuracy, the Single Score, thus allowing the design of PE bags suitable for falling into the highest performance class envisaged by the "Made Green in Italy" scheme.

5.04.09

Life Cycle Dashboards to Focus Rebound Effects in Environmental Management and Industrial Policy Planning: A Case Study in the Packaging Sector

D. Camana, S. Toniolo, A. Manzardo, F. Zuliani, A. Scipioni, University of Padova / Department of Industrial Engineering

The dashboards of sustainability are suitable to summarise multiple aspects in a single visualised scheme and to support decision making process among shareholders. The purpose of the study is to assess if the dashboards may be advantageous for highlighting rebound effects and environmental backfires in different scenarios. Moreover, the aim is to graphically evaluate the effects of theoretical sustainability assumptions in the preferability of alternatives. A baseline case study of packaging, including thermal recovery of waste, is conducted, comparing two different scenarios. The effect of changing functional unit is shown, by using a modified dashboard of sustainability, with the purpose of displaying differences in the two competing packaging options. When the focus is the mass of material used for packaging, the first packaging seems environmentally preferable. However, is the functional unit chosen is the energy of the system, the second scenario appears to be better from an environmental point of view, considering avoided burdens of energy recovery. The choice of the functional unit depends on the limiting factor in the system boundaries. In fact, in some regions the local burden is the energy available while in other provinces the materials are scarce. Therefore, in local studies, functional unit may depend on stocks accessible, leading to different environmental preferences. Secondly, the effects of different weights of environmental impacts are assessed. The minimisation of the Global Warming Potential Impact is considered as a starting point. Subsequently, other environmental impacts are added and summed using different priority weights. Dashboards of sustainability are used for showing the effects of multicriteria decisions. Moreover, other aspects of sustainability are added in the dashboard, as social concerns, or economic gains, and graphical changes are assessed. Results achieved suggest the possibility of using the dashboards of sustainability to picture and analyse the meaning of priorities in sustainability studies, by highlighting backfires effects in functional unit, impact assessment and theoretical choices. Furthermore, dashboards appear to be useful to communicate results and weight of choices to non-technical people involved in the stakeholders’ debate. Some weak points of the methodology are also provided. Future studies are desirable to verify uses, pros, and cons in more complex scenarios at various local scales.

5.04.10

Are Bio-Based Synthetics a Viable Substitute for the Conventional Polyester Fiber in Moving Towards a Sustainable Textile Industry?

T. Ivanovic, EMPA Technology & Society Lab; R. Hischier, EMPA / Technology and Society Lab; C. Som, Empa - Swiss Federal Laboratories for Materials Science and Technology / Technology and Society Lab

Numerous studies have shown that the textile sector has a high environmental footprint incl. fossil-fuel dependency [1]. Currently, fossil-based polyester made out of polyethylene terephthalate (PET) polymer makes up about a half of the industry's fiber production while volumes are expected to increase. In the effort to break from the fossil fuels, the industry has a possibility to do bio-sourcing - source part or all carbon for the products' backbone from the bio-feedstock. Polyester can be substituted by 3 bio-based alternatives: bio-based PET; polytrimethylene terephthalate (PTT) and polylactic acid (PLA) fibers. In spite of the existing political will to prioritize bio-based products [2], the environmental effects of feedstock substitution have not been studied abundantly [3]. This paper therefore performs a comparative cradle-to-gate LCA of polyester fiber and 3 substitutes, taking into account the state-of-the-art productions. The inventories were modelled for 6 scenarios for bio-based PET, 2 for bio-based PTT and 1 for PLA, which differ in the feedstock and respective monomer production processes. The impact assessment was performed with the European Environmental Footprint method. It was found that all 3 bio-based fibers are produced from the first-generation feedstock (crops). Currently, only the partially bio-based PET and PTT polymers are commercialized. Bio-sourcing for polyester is found to offer a limited improvement in a small number of impacts with negligible contribution to the overall score while it causes substantial environmental burdens elsewhere. None of the bio-based alternatives performs better than the conventional polyester in the overall score. Eutrophication, acidification, water scarcity impacts and land use rise with fiber's bio-content and largely stem from the agricultural practices. The environmental performance of the bio-based fibers may be increased through improved agricultural practices or with an alternative feedstock option [4]. [1] Nathani et al. (2019). Environmental hotspots in the supply chain of Swiss companies, BAFU, 29.04.2019 [2] European Commission, The European Green New Deal, https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en, last

04.2020 [3] Walker, S., & Rothman, R. (2020). Life cycle assessment of bio-based and fossil-based plastic: A review. Journal of Cleaner Production, 261, 1–15 [4] Ivanović T., Schmutz M., Hischier R. & Som, C.* (2020)., In prep.

5.04.11

Variability of the ENvironmental Impact of a Passenger Car Tire Assessed Using a Life Cycle Assessment (LCA)

T. Hennequin, Radboud University Nijmegen / Department of Environmental Science; M. Huijbregts, Radboud University Nijmegen / Department of Environmental Sciences; R. van Zelm, Radboud University / Environmental Science

When assessing the environmental impact of a product, variability is rarely explicitly modelled. Here, we present a stochastic approach to model the environmental impact occurring during a tire's life cycle. The goal of this paper is to demonstrate that such a model can help determine the mitigation potential of a tire. This study can also help bridge an information gap on tire's impact assessment. Indeed, industry assessments are closely guarded and scientific literature is scarce. To achieve this goal, several parameters were selected to vary, such as the rolling resistance, the load, or the lifetime of the tire. This input variability was then propagated through the model using Monte-Carlo simulations and converted to environmental impact using life cycle assessment (LCA). The LCA was conducted using cradle to grave system boundaries and a life cycle inventory (LCI) based on literature. The distributions of the input parameters were built to represent an average passenger car European radial tire. The links connecting the parameters and the LCI were modelled either theoretically or based on empirical observations. Finally, the influence of the various parameters on the results was assessed using rank correlation. Preliminary results show that there is indeed a large potential of mitigation for a tire's environmental impact. The main means to achieve such a reduction differ depending on the impact category considered. For example, in the case of global warming, the most efficient mitigation can be achieved by reducing the tire's load. For land use, however, the tire's lifetime and radius are the most influential parameters. It can also be observed that the largest impacts occur during the use and production of the tire. During the use phase, the main hotspots are the fuel consumption allocated to the tire and the associated exhaust emissions as well as tire wear particles. In the case of the tire's production, the materials with the highest contribution are the steel wires, the natural rubber, and the carbon black filler.

5.04.12

Life Cycle Assessment of Air Conditioners Using HEMS Data and Artificial Intelligence

G. Sugiyama, Tokyo City University; T. Honda, AIST Tsukuba West; N. Itsubo, Tokyo City University / Graduate School of Environmental and Information Studies

Although Japan's final energy consumption has been on a declining trend in recent years, energy consumption in the household sector has increased 1.9 times since 1973, and the Global Warming Prevention Plan, approved by the Cabinet in 2016, sets a goal of reducing greenhouse gas emissions from the household sector by about 40%. In particular, the electricity consumption of air conditioners fluctuates depending on the time of year, with air conditioners accounting for 7.4% of household energy consumption throughout the year, but it has been reported that the ratio of air conditioners to household energy consumption exceeds 50% during the summer and winter peak periods. The LCA for air conditioners shows that about 90% of the environmental impact is occupied by the use stage of the air conditioner, and the calculation results differ greatly depending on local climate, user attributes, product function, and usage conditions. In addition, the JIS standard, which is used as the standard for the usage time in conventional calculations, assumes 18 hours of usage time per day, and this numerical value is not realistic for calculation, and the methods for grasping the actual usage are also different and have their own merits and demerits, so the existing surveys have not been able to calculate the greenhouse gas emissions. It is stated that the collection of real data for calculation was difficult. In this study, we use HEMS data to consider regional characteristics and residential attributes, and the energy consumption that matches the actual usage of each region that has not been fully reflected in the past is considered. In order to calculate the amount of greenhouse gas emissions and improve the accuracy of LCA for air conditioners, we aim to build an optimization model for electricity consumption and greenhouse gas emissions by creating a model that estimates electricity consumption based on the regional and residential attributes of HEMS data, utilizing artificial intelligence. The HEMS data includes time stamps for 569 households, electricity consumption, air conditioner type, insulation performance, number of floors in the building, total floor area, and other data, and contains three years of data collected every hour, or approximately 50 million data.

5.04.13

Life Cycle Evaluation for Green Bond Certified Office Buildings

T. KITAMI, Tokyo City University; K. Ito, SHIMIZU CORPORATION; N. Itsubo, Tokyo City University / Graduate School of Environmental and Information Studies

The Paris Agreement adopted at COP21 in 2015, set a target of lowering the global average temperature by 2℃. The Paris Agreement has increased the interest among companies to take environmentally friendly actions, with a shift to renewable energy and a reduction of greenhouse gases (GHG) emissions. The Task Force on Climate-related Financial Disclosures (TCFD), established by the Financial Stability Board, allows companies and organizations to optionally support a framework for disclosing information on climate change risks and opportunities. Environmental, social and corporate governance (ESG) investments are increasingly used to support and promote corporate environmental businesses. Global assets under management in ESG strategies has grown to $40.5 trillion in 2020, reaching levels prior to the economic crisis of 2008. An ESG investment, called Green Bond, is a bond that is issued by allocating funds to a project certified by a third-party organization. After the funds have been allocated, the third party assesses the environmental impact of the project and makes a public announcement. Current literature analyzing ESG investment in Japan and investment in environmental projects has suggested that it is difficult to reduce the environmental impact of a business. This is because there is limited information that measures the impact of environmental data disclosed by companies other than the amount of carbon dioxide emissions. Similarly, little data is showing various environmental impacts other than global warming. In addition, the use of funds appropriated through ESG investments is left to the company's discretion, and the financial institutions that allocate the funds use traditional fundamental analysis. Therefore, it was raised that it is not an investment process based on ESG evaluation of companies, which is the original purpose. To analyze whether investments could reduce environmental impacts, it was thought that it would be more comprehensive to quantify the environmental impact as an amount of damage. In order to measure the environmental impact reduction effect related to investments, life cycle assessment (LCA) was used to evaluate business sectors that received green bonds. LIME method was used to evaluate the cost-effectiveness of the investments.

Life Cycle Impact Assessment Modeling and Application

5.05.06

Optimizing an Agricultural Wood Production Supply Chain Within Planetary Boundaries: Exploring a "Cradle to Grave" Life Cycle Assessment of a Short Rotation Coppice Case Study

E. Perdomo E., University of Natural Resources and Life Sciences Vienna / Department of Economics and Social Sciences; F. Hesser, Wood K plus / Market Analysis and Innovation Research

Besides the efforts of several life-cycle assessment (LCA) studies, the environmental implications of agricultural wood production from short rotation coppice (SRC) value chains continue to be debated. As discussed in previous literature, the overall outcomes of SRC LCA's depend on methodological choices, such as time-dependent boundaries and environmental impact categories selected. These factors can influence the overall results and their interpretation. Notably, only few studies have explored SRC plantations' long-term impacts after the cultivation period. For instance, land use impacts on soil organic carbon (SOC) stocks vary during different life cycle phases ("cradle to grave"). This issue can lead to misleading conclusions on the environmental impacts of SRC projects. The purpose of this case study is to close this gap by assessing the absolute environmental impacts (AEI) of an SRC value chain. To reach this goal, we apply a dynamic LCA combined with the planetary boundaries (PB) to understand the AEI of SRC value chains. A scenario analysis will address the AEI results, seeking to provide the optimal SRC configuration that minimizes the impacts on the PB's on a short and long-term scale. As the current study is in progress, only preliminary results are available. The first findings depict the negative impact of stool removal on SOC stocks. Thus, highlighting the relevance of including the SRC end of life, as this can substantially affect climate change and land use impact categories. Further results will focus on the impacts on biodiversity, eutrophication, and acidification. Overall, this contribution expects to develop a framework that optimizes agricultural wood production supply chains, and

through this, provide advances in LCA methodology connected to the PB's.

5.05.07
Multiple Climate Tipping Points Indicators for Improved Sustainability Assessment of Products and Services
S. Fabbri, Technical University of Denmark / DTU Management, Session for Quantitative Sustainability Assessment; M.Z. Hauschild, Technical University of Denmark / Division for Sustainability, Department of Technology, Management and Economics; T.M. Lenton, University of Exeter; M. Owsianiak, Technical University of Denmark
Mounting evidence indicates that climate tipping points can have large, potentially irreversible, impacts on the Earth System and human societies. Yet, climate change indicators applied in current sustainability assessment methods generally do not consider these tipping points, using arbitrarily determined time horizons and often assuming that the climate impact of a product or service is independent of emission timing. Here, we propose a new method for calculating climate tipping indicators of greenhouse gases (here, carbon dioxide, methane and nitrous oxide). It covers 13 projected tipping points, incorporates the effect that the crossing of a given tipping point has on accelerating the crossing of other tipping points, and addresses uncertainties in the temperature thresholds that trigger the tipping points. To demonstrate the added value of the new indicators, we apply them to marginal greenhouse gas emissions stemming from end-of-life of plastic polymers and compare them with commonly used metrics. This highlights the need to consider climate tipping in sustainability assessment of products and services.

5.05.08
Impacts of Chemicals in Consumer Products on Human Health and Ecosystems Using the USEtox Nearfield Model
O. Jolliet, L. Huang, University of Michigan / Environmental Health Sciences, School of Public Health; N. Aurisano, DTU (Technical University of Denmark) / Quantitative Sustainability Assessment, Department of Technology, Management and Economics; P. Fantke, Technical University of Denmark / Quantitative Sustainability Assessment, Department of Technology, Management and Economics
Though being product focused, most LCA studies have paradoxically neglected chemical intake during the product use-stage. However, exposures resulting from indoor or near-field chemicals can often be substantially higher than exposures resulting from environmental far-field emissions. Therefore, this project aims to extent the USEtox model to enable user to calculate characterization factors for exposure to chemicals during product use for LCA. High throughput quantitative exposure assessment is performed according to the Product Intake Fraction (PiF) framework and its implementation within the USEtox model. We applied complementary model to chemicals in articles, toys (613 product-chemical combinations), building products (624 combinations), paints (1098 combinations & 160 chemicals in 90 solvents), food and beverage packaging (4492 combinations), cleaning and home maintenance products (1604 combinations), personal care and other skin applied products (2769 combinations), e) near-person, indoor and outdoor air. product specific exposure pathway were added using mass-balanced based models: a dermal uptake model for skin uptake and skin contact with various materials and dust, gaseous dermal uptake sor SVOCs, as well as a mouthing models for toys, showing good predictive powers for a large range of chemical-material combinations, as illustrated for the predicted against experimental migration rates from mouthing (R^2=0.86, without any parameter adjustment). Among the 20 household products with the highest impacts on users, home maintenance products such as adhesive remover, paint stripper, concrete cleaner and home applied pesticides have the highest substantial impacts of the order of magnitude of 1000 µDALY/user/d or 500 minutes of healthy life lost per day. Personal care products and cleaning products are also found among the 23 most impacting chemicals on users, but rather in the order of a 100 µDALY/user/d or 50 minutes of healthy life lost per day. 14 main chemicals of concern include tetrachloroethylene, dichloro-methane, furfuryl alcohol, D-limonene, formaldehyde, butyl benzyl phthalate, as well as diazinon and 1,4 dichlorobenzene for use of pesticides at home. The developed USEtox enables to screen and produce mass-balanced based characterization factors for near-field human health impacts of tens of thousands of chemical-product combinations, operationalizing the inclusion in LCA of the substantial impacts associated with chemicals in products.

5.05.09
Identification of Dissipative Emissions for Improved Assessment of Abiotic Resources in LCA
M. Owsianiak, Technical University of Denmark; M.Z. Hauschild, Technical University of Denmark / Division for Sustainability, Department of Technology, Management and Economics
There is a growing understanding that for resource use impact assessment the issue is continued accessibility of raw materials, rather that conservation of natural resources containing them. Hence, focus in life cycle assessment (LCA) and environmental footprinting should be on dissipative losses from product systems, rather than resource inputs to them. This work addresses operationalization of environmental dissipation (i.e., the type of impact occurring solely through emissions to the environment), as an impact category in LCA. Characterization factors for environmental dissipation have recently been made available for a large number of metallic elements, but their application in LCA requires that truly dissipative emissions are differentiated from just environmental pollution (that is, simple flow through of trace elements). We present a new method that allows for this differentiation to be made. It determines (1) whether an emission flow reported in an inventory actually contributes to loss of accessibility of a given element when environmental fate mechanisms are considered, and (2) whether the element comes from a source that would be considered as a mineral resource for any generation living between the present and the time frame of assessment. To demonstrate the added value of the new method, we apply it to three different life cycle emission inventories, and characterize the resulting list of truly dissipative emissions using long-term environmental dissipation potentials (EDP) as indicators. Impact scores are reduced by up to a factor of 3 when the new method is applied, when compared to the scores calculated for the unprocessed emission inventory where all emission are assumed dissipative. This highlights the need to carefully differentiate dissipative emissions from just environmental pollution, simple flow through of trace elements, in resource use impact assessment.

5.05.11
Life Cycle Assessment of Anaerobic Digestion Biofertiliser Value Chains - How Methodological Choices Impact LCA Outcomes
M.J. Black, J. Simaitis, H. Lin, A. Borrion, University College London / Department of Civil Engineering & Geomatic Engineering
Anaerobic Digestion (AD) is increasingly employed as a closed-loop circular solution to manage organic waste from animal, crop and food waste, as well as municipal and industrial effluents. The AD process yields biogas, which can be used in bioenergy applications and 'raw digestate', composed of the undigested fraction of the original feedstock. The digestate co-product is often promoted as being a valuable source of nutrients for application to land however, limitations to application are increasingly a concern (due to soil nutrient saturation and digestate contamination). New opportunities for the valorisation of digestate may offer better environmental solutions to digestate management, as limitations for digestate application to land are reached. Life Cycle Assessment (LCA) is an invaluable methodology used to assess the environmental impact of processes and products. In this presentation we introduce a novel mobile digestate treatment design (Novel Organic recovery using Mobile ADvanced technology (NOMAD)) and examine the environmental impacts of applying a treated, nutrient rich digestate to land, using LCA. Methodological approaches to LCA can lead to significantly different outcomes, depending on the approach take. In this presentation, we explore the outcomes of LCA methodological choices on the environmental impacts of digestate management. We examine how attributional and consequential LCA approaches can lead to different outcomes in the LCA study, using a UK based AD system and the NOMAD digestate treatment system as an example.

5.05.12
Modelling the Environmental Impacts of Organic Agriculture: Critical Aspects of the Goal, Scope and Life Cycle Inventory in LCA
E. Montemayor, IRTA Institute of Agrifood Research and Technology / ORGANIC WASTE INTEGRAL MANAGEMENT; A. Bonmatí, IRTA / ORGANIC WASTE INTEGRAL MANAGEMENT; A. Assumpcio, IRTA / GIRO-Integral Organic Waste Management Program
Agriculture is a significant contributor to global emissions of greenhouse gases, nitrates and pesticides, as well as a large contributing factor in soil and biodiversity loss. Currently, organic agriculture (OA) has gained popularity due to its view as a more sustainable method of farming. Nevertheless, OA and conventional agriculture can be found to have similar or varying environmental performance using tools such as life cycle assessment (LCA). However, the current state of LCA methodology does not seem to accurately reflect the effects of certain

OA practices, thus could not be fairly compared to conventional agriculture. This article presents for the first time, specific critical aspects of the goal and scope and life cycle inventory stages of LCA, in application to OA through an analysis of state-of-the-art organic crop life cycle inventories (LCIs) from current and recommended LCA databases, and detailed ways to improve upon them. The manufacturing of plant protection products (PPP) and organic fertilizers were found to be the main limitations in background processes, due to the unrepresentative inputs used. Many PPP and fertilizers listed in organic LCIs used pesticide or mineral fertilizer proxies, which may indirectly contain OA prohibited chemicals. The effect of using these proxies can contribute between 4 – 78% to resource and energy-related impact categories. Additionally, a lack of manufacturing datasets for many common natural PPPs and organic fertilizer pre-treatments was found. These critical aspects can be transferred to respective LCAs that use this data, potentially yielding unrepresentative results for some categories. Recommendations such as creating new OA-specific manufacturing proxies for PPPs and fertilizers could help improve accuracy. Nevertheless, LCA can be an appropriate methodology to perform environmental assessments due to its comprehensive and system-based scope but it should be improved to better reflect OA practices.

5.05.13
Dataset Fit Accuracy - a Methodology for a Systematic LCI/A Dataset Selection for Power Generation Systems and Power PLANTS
N. Scholliers, TU Darmstadt / Chair of Material Flow Management and Resource Economy; L. Schebek, Technische Universitaet Darmstadt / Institute IWAR Material Flow Management and Resource Economy Germany; C. Geyer, TU Darmstadt / Institute IWAR Chair of Material Flow Management and Resource Economy
We propose a method that allows to semi-quantitatively evaluate existing life cycle inventory (LCI) and life cycle assessment (LCA) datasets with regard to their accuracy of fit on an examined power generation system or power plant. Research questions dealing with the choice of suitable datasets for electricity consumption vary broadly. Energy is a main driver for environmental impacts and the change of country specific electricity mixes is very dynamic. Therefore the selection of datasets influences not only the results of power generation systems giving electricity as an output but also the results of any LCA using electricity as a process input. Choosing datasets which suit the specific underlying research case best is non-trivial and a “wrong” selection may be detrimental for the quality of the study results. Therefore, we developed a standardized dataset selection for finding best fitting LCI/A datasets for various research cases which deal with electricity as process output or input. This improves the objectivity and transparency of the dataset selection. Furthermore, it guides the practitioner through the process of finding accurately fitting data form a large number of datasets which speeds up the whole process. In order to evaluate existing datasets with regard to their fit towards a reference object (e.g. power generation system or power plant) we introduce the term of *data fit accuracy.* The semi-quantitative evaluation approach, based on pedigree-matrix, uses 6 criteria. Five are context depending criteria (*geographical, temporal, technological, manufacturing location, energy carrier location, methodological actuality*) and one is an inherent criterion (*data quality*). For examining the overall data fit accuracy we developed a grading scheme that ranges from 1 to 5 but also allows to set not applicable criteria to zero. The final calculation of the overall data fit accuracy is based on a modified arithmetic mean equation. In the end this term indicates how well a LCI/A datasets fits to the assessed reference object. The so generated grade for each dataset makes it simple to compare different datasets among each other and to select the best fitting one for each underlying research case. Furthermore, research gaps can be revealed, where no appropriate dataset is available.

5.05.14
The Effect of Price Variance on the Uncertainty of Footprints in Hybrid-Life-Cycle-Assessment
A. Jakobs, Uni-Freiburg (DE) / Industrial Ecology; S. Pauliuk, University of Freiburg / Industrial Ecology Research Group
Hybrid Life Cycle Assessment (HLCA) methods attempt to address the limitations of more traditional Process- and Input-Output Life Cycle Assessment (PLCA, IOLCA). Most commonly, HLCA is seen as a way to mitigate the truncation errors in PLCA that lead to underestimation of the environmental impacts, by using environmentally-extended (multi - regional) input-output (EE-MRIO) data to account for missing flows in the PLCA system. However, it is argued in the literature that the inclusion of low-resolution EE-MRIO data in the high-resolution PLCA supply chains can lead to a loss of precision due to the high aggregation level in IO models. Furthermore, due to the use of different units, HLCA methods have to rely on price information of the products in the system to convert the physical units used in process inventories to the monetary units commonly used in EE-MRIO tables. Given the multitude of buyer-seller relationships in today’s global supply chains, prices for the same commodity can vary significantly between different supply chains, or even between various levels in the same supply chain. Because of the linear relation between the price of the reference product of a process to be hybridised and the inputs from the Input-Output industries into this process, variance in the price of the reference product, leads to added uncertainty in the hybrid part of the footprints. However, while literature has shown the influence of theoretical price uncertainty in a system-wide hybridisation [1], no studies using data for the price variance are known to us at the time of writing. Here we use international trading statistics from BACI to model the price variances of commodity prices, and use these in an integrated hybrid model of the process database ecoinvent with the EE-MRIO database Exiobase. With this model we then analyse the effect of price variance on the footprint of Swiss household consumption. We find that the variance of the IO part of the hybrid footprint has a 95% confidence interval of (-20%,+81%) relative to the median. Although the variance is calculated for a specific case, the magnitude highlights the importance of taking price variance into account when performing hybrid-LCA. [1] Yu, M. and Wiedmann, T. (2018). Implementing hybrid LCA routines in an input–output virtual laboratory. Journal of Economic Structures 7, 33. doi:10.1186/s40008-018-0131-1

Life Cycle Sustainability Assessment: Integrating the Environmental, Economic and/or Social Pillars of Sustainability

5.06.07
Economic Indicators for Life Cycle Sustainability Assessment: Going Beyond Life Cycle Costing
V. Arulnathan, University of British Columbia / Biology; M. Heidari, University of British Columbia; M. Doyon, Laval University; E. Li, N. Pelletier, University of British Columbia
Life cycle thinking and derivative assessment methods have become a cornerstone of contemporary sustainability measurement and management, globally. Life Cycle Sustainability Assessment (LCSA) consists of three parallel methods – Environmental Life Cycle Assessment (LCA), Life Cycle Costing (LCC), and Social Life Cycle Assessment (SLCA) - to represent and assess the environmental, economic and social dimensions of sustainability. LCC is universally accepted as the method of choice for economic assessment in LCSA due to its conformance with the life-cycle approach, steady-state analysis, and ability to represent costs relative to the inventory flows utilized in environmental LCA. However, it also has significant weaknesses such as a disconnect from the larger economic system and lack of impact assessment. The singular focus on costs is also ineffective in truly representing the multi-faceted problems of economic sustainability. This review is intended to identify possible indicators to complement LCC in economic assessments in LCSA. The review identified 44 economic indicators, of which 21 were selected for detailed analysis using criteria reflecting general characteristics, indicator methodology, indicator use, and suitability for LCSA. The indicators generally performed well with respect to methodology and use – signifying their potential suitability as economic sustainability indicators. However, most are unsuitable for direct integration into the LCC/LCSA framework due to the inability to aggregate across life cycles and lack of granular data. The indicators were grouped into six economic impact categories – profitability, productivity, innovation, stability, customers, and autonomy – each of which represents a significant aspect of economic sustainability. On this basis, a conceptual framework is proposed that maintains the utility of LCC while also integrating additional indicators to enable more holistic economic sustainability assessments in LCSA.

5.06.10
What Is the Economic and Environmental Potential of Heat Recovery in Whisky Distilling?
I. Schestak, Bangor University; J. Spriet, Trinity College Dublin; K. Black, Arbikie Distillery, Arbikie Highland Estate, Inverkeilor, UK; D. Styles, University of Limerick; A. Williams, Bangor University
Introduction: Whisky distilleries are hotspots for water and energy consumption due to heating and cooling requirements for mashing and distilling. About 90% of the heat is still generated by burning fossil fuels such as natural

gas and oil, as a study on Scottish whisky production has shown, responsible for considerable GHG emissions [1]. At the same time, the distillery operations allow for heat recovery from process and waste streams, reducing both water and fuel use. Objective: The objective of this study is the assessment of heat recovery options in a distillery as energy and water efficiency measures and to identify barriers and drivers for their application by assessing their economic and environmental potential. Materials and methods: Eco-efficiency is a standardised methodology [2] for the integration of economic and environmental aspects. In this study, environmental burdens of heat recovery are determined by life-cycle assessment of the required equipment, while the economic assessment considers capital and labour costs for installing the heat recovery system, as well as operational savings through recovered energy and water. Three options for heat recovery are considered and compared, based on process data from the Scottish distillery Arbikie: Heat recovery from: a) mashing b) pot still and column distillation and c) the waste streams pot ale and spent lees. Results: The three eco-efficiencies are compared in order to determine the most economically and environmentally favourable option. Environmental impacts through heat recovery are compared to the overall impacts of whisky production determined in a previous study by the authors. Furthermore, financial and environmental payback times are showcased and the possibility of increasing economic attractiveness of the measures through carbon pricing will be explored. **1. 2. 3.** *Table 1 could look like this* Conclusions: The results will inform policy making which aims at incentivising low carbon measures in distilleries and are expected to encourage the uptake of heat recovery in industry. **References** [1] R. Sibille, “Scotch whisky pathway to net zero. Report for Scotch Whisky Association.,” Ricardo Energy & Environment, Glasgow, UK, 2020. [2] ISO, “ISO 14045:2012 - Environmental management — Eco-efficiency assessment of product systems — Principles, requirements and guidelines,” *International Organisation for Standardisation (ISO)*, 2019. [Online]. Available: https://www.iso.org/standard/43262.html. [Accessed: 14-May-2020].

Extended submission 5 - Life Cycle Assessment and foot-printing

5.07.01
Reduction of Greenhouse Gas Emission by Adopting Food Waste Biofuel
Y. Jeong, KICT / Department of Land, Water and Environment Research; Y. Lee, D. Shin, K. Ahn, J. Jung, I. Kim, Korea Institute of Civil Engineering and Building Technology (KICT)
Food waste has been considered as promising biomass due to the increasing amount and high carbon content. Attempts to convert food waste into co-firing material with coal in a thermoelectric power plant have been made. Food wastes processed by pyrolysis and demineralization have shown acceptable fuel qualities including calorific value, chlorine, and mercury content. However, some doubts about co-combusting food waste biochar with coal still exist. The overall process from raw food waste to food waste biochar needs to be evaluated in a life cycle perspective to ensure that this is an effective and practical solution to treat food waste and provide alternative energy resources. Therefore, this study aims to estimate the environmental impacts of food waste biochar. Considering the current development stage, available data set and uncertainties are characterized. The reduction of greenhouse gases (GHG) of food waste biochar and other existing fuels are assessed and compared. This work will assist policymakers and other stakeholders in decision making of food waste treatment. This research was funded by the Korea Institute of Civil Engineering and Building Technology (KICT), grant number 20210105-001.

Creating a Sustainable Future for the Marine Environment

6.01.02
Quality Assurance of the Measurement of Chemicals in the Marine Environment- Past, Present and Future of the Quasimeme Proficiency Testing Scheme
W. Cofino, Wageningen Environmental Research / Environmental Risk Assessment; S. Crum, Wageningen Environmental Research - WUR / Environmental Risk Assessment; A. Asjes, Wageningen UR / Wageningen Marine Research; A. Sneekes, QUASIMEME/Wageningen Marine Research; T. Bult, Wageningen UR / Wageningen Marine Research
Marine ecosystems provide a range of invaluable services to humans. Consequently, actions and policies to protect marine environment are of paramount importance. Such polices are based on research, monitoring and assessment carried out in an international context. The information base underpinning these activities must be reliable for environmental management to be effective. In the past, monitoring and assessment was dominated by the investigation of contamination of the marine environment by chemicals and radioactive substances. Nowadays, an integrated approach is taken that takes pressures from different types of human activities and different issues into consideration. Monitoring and assessment of contaminants remains an important issue. Society is dynamic, “old” chemicals are phased out, innovations in materials, processes and changes in use give rise to new classes of compounds that can harm ecosystems and may require regulation. In addition, new analytical methodologies are developed enabling us to monitor the environment in a different manner, non-target screening being an example. Such dynamics pose challenges that marine monitoring needs to address. Between 1980 and 1989, international collaboration in a series of interlaboratory studies conducted under auspices of ICES and through international assessments of the environmental quality of the North Sea and Baltic Sea revealed time and again that laboratory results were poorly comparable and that the monitoring programs needed to be improved. The North Sea task Force was created to increase scientific knowledge and to make *inter alia* a monitoring master plan for the North Sea. The Quasimeme proficiency testing scheme was started as a spin-off of the NSTF in 1989 funded by the EU. It was designed as an holistic quality assurance programme for marine environmental monitoring information in Europe. Quasimeme has developed into a European network that supports globally the large majority of measurands in marine environmental programmes for both monitoring and research purposes. Recent examples of new interlaboratory studies include ocean acidification, PFOS and microplastics. Information on the operation of the Quasimeme network will be provided in relation to the path the programme takes to respond to the challenges that monitoring of the contamination of the marine environment faces with a view to pursue a high level of support to the quality of marine information in this area.

6.01.03
Estimating Domestic Wastewater Emissions to Marine Environments Using a Spatial Extrapolation Approach
C.M. Holmes, E. Tandy, Applied Analysis Solutions, LLC; Z. Schleicher, Southern Illinois University Edwardsville / Laboratory for Applied Spatial Analysis; J. Kilgallon, Unilever; J. Hodges, Unilever / SEAC
Domestic wastewater discharged to the environment may contain chemical substances that are the result of everyday human activity such as personal care, home cleaning and using medications. While most discharge is to freshwater rivers and lakes, some facilities in coastal areas discharge into the marine environment. As population densities continue to grow in coastal areas, understanding where these marine emissions occur is increasingly important for assessing both marine and freshwater ecological risk. Making this type of evaluation across large spatial scales is challenging though because information on wastewater treatment plant (WWTP) location and discharge type is often lacking. To address this data gap, we developed a global-scale dataset focused on marine emissions with the goal of understanding what fraction of the population in coastal areas may be discharging to a marine environment via a WWTP. Information on WWTP location, population served, and effluent flow were collected for 44,000 WWTPs across 34 countries serving one billion people. Using these training data, we developed a spatial extrapolation method utilizing urban and rural populations, their likelihood of purchasing products based on economic productivity, and how far they live from the coast. Using global spatial datasets, extrapolation was possible from our training data (e.g., an entire country or only a selected portion thereof) to any target country that lacks source data for WWTPs. The extrapolation results in aggregated estimates (e.g., administrative units, hydrologic basins) of the fraction of population connected to WWTPs discharging directly to the marine environment. This poster will present information on the source WWTP data collected, global spatial data utilized, extrapolation methods examined with results, along with an evaluation of results using data outside the training set. A spatial understanding of the WWTP effluent fraction discharged to the marine environment not only increases our ability to assess and protect near shore marine habitat, it also refines emission estimates for freshwater ecological risk scenarios used in regulatory assessments.

6.01.06
Exposure to Low ENvironmental Copper Concentrations Does Not Affect Survival and Development in Atlantic Cod (Gadus morhua) Early Life Stages

J. Farkas, SINTEF Materials and Chemistry / Environment and New Resources; L. Svendheim, Nord University; T. Jager, DEBtox Research; T.M. Ciesielski, Norwegian University of Science and Technology / Department of Biology; T. Nordtug, SINTEF Ocean / Climate and Environment; B. Kvæstad, B. Hansen, SINTEF Ocean / Environment and New Resources; T. Kristensen, Nord University; P.A. Olsvik, Nord University / Faculty of Biosciences and Aquaculture
As most heavy metals, Cu is a natural constituent in the marine environment, with background concentrations in estuarine and coastal waters in the ng to low µg/L range. However, human activities can significantly increase environmental Cu concentrations, with some of the major anthropogenic sources to the marine environment being mining activities, which are expected to increase over the coming years. Many of the environmental challenges related to mining activity are associated with the large amounts of tailings typically generated. In Norway, and a few other countries, several mines and processing plants have permissions to place tailings in the marine environment. Depending on the ore, mine tailings may contain considerable amounts of metals. Historic and present mining disposal sites could thus be relevant local sources of Cu to the coastal marine environment. For several fish species, including commercially important fish such as Atlantic cod (*Gadus morhua*), Norwegian fjords are important spawning and nursing grounds. In fish, Cu is of concern because it is easily accumulated and may disturb biological mechanisms even at relatively low exposure concentrations. In this study, we investigated potential impacts of Cu exposure at low, environmentally relevant, concentrations on early live stages of Atlantic cod. Cod embryos and larvae were exposed to 0.5 µg/L (low), 2 µg/L (medium), and 6 µg/L (high) Cu from 4 days post fertilisation (dpf) until 17 dpf. Hatching success, mortality, oxygen consumption, biometric traits, and malformations were determined. A dynamic energy budget (DEB) model was applied to identify potential impacts on bioenergetics by simultaneously analysing a range of traits. A positive correlation was found between Cu exposure concentrations and Cu body burden in eggs, but not in larvae. The tested concentrations did not increase mortality in either embryos or larvae, or larval deformations. Further, the DEB model did not indicate effects of the tested Cu concentrations. In conclusion, we documented that Cu accumulates in eggs, but the applied exposure concentrations (< 10 µg/L) did not impact survival or development in cod early-life stages. The DEB model provided an accurate explanation for the unaffected life history of the yolk-feeding stages, and is thus a potentially useful tool for analysing and predicting toxic effect in this context.

6.01.07
Impacts of Salmon Lice Treatment (H2O2) on Coastal Shrimp Populations: A Probabilistic Risk Assessment Approach
J. Moe, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; D. Hjermann, Norwegian Institute for Water Research (NIVA); O. Nøst, G.H. Refseth, Akvaplan-niva; T. Jager, DEBtox Research; M. Norling, Norwegian Institute for Water Research (NIVA); R.K. Bechmann, NORCE Norwegian Research Centre / IRIS
Our study addresses the impacts of salmon lice biocides on populations of the Northern shrimp (Pandalus borealis), which is an economically and ecologically important species in Norwegian fjords. Hydrogen peroxide (H2O2) is a commonly used biocide in salmon farms, with the treatment water discharged to the sea. Laboratory experiments have demonstrated that even short pulses of exposure to H2O2 in concentrations well below the prescribed treatment concentrations can result in mortality of shrimp. Our previous presentations from this study system have focused on the combination of different modelling approaches for extrapolating results from laboratory experiments to population-level effects: (1) an oceanographic model for the distribution of H2O2 in shrimp fields, (2) a mechanistic effect model for individual survival probability (General Unified Threshold model of Survival), and (3) an age-structured population model for extrapolation to population-level effects. Here, we focus on how this model chain can be used to improve environmental risk assessments for coastal shrimp populations. Environmental risk assessment is traditionally based on a PNEC (predicted no-effect concentration), and to which degree the PNEC for a given species or community is exceeded by measured or predicted environmental concentration (PEC). In the case of shrimp populations exposed to H2O2, both PNEC and PEC values are often associated with high uncertainty, which is not straight-forward to account for in a traditional risk assessment. We propose an alternative approach to risk assessment based on the Relative Risk Model framework, which has been used for probabilistic environmental risk assessment for many regions and ecosystems worldwide. A probabilistic causal network model (Bayesian network - BN) is developed for integrating the input and output of the mentioned individual models as well as the uncertainty associated with each model, by conditional probability distributions. The fully quantified BN model will incorporate environmental and seasonal factors in four coastal study regions. The model can be applied to predict risk in terms of the probability of shrimp population decline under different management scenarios, such as the timing and the frequency of H2O2 treatment.

6.01.08
Nanoparticle As Functional Haloperoxidase Analogues to Prevent Biofouling
N. Keltsch, Bundesanstalt für Gewaesserkunde / Water Chemistry - G2; C. Dietrich, A. Wick, Federal Institute of Hydrology / G2 -Water Chemistry; T.A. Ternes, Federal Institute of Hydrology
Biofouling is a major problem in different areas like aquafarming, filters for water treatment or shipping industry [1]. To prevent biofouling, tributyltin-containing paint was used in the past, but was recognized as ecotoxicological hazard for marine organisms. Nowadays biocides like elementary copper, copper oxide and organic copper compounds are used to prevent biofouling in the shipping industry. Nevertheless, these antifouling agents are still toxic for some organisms and thus the development of more sustainable and environmentally friendly alternatives is ongoing [2]. As one possible, solution cerium dioxide nanoparticles as functional haloperoxidase analogues have been developed to prevent biofouling [1]. First experiments have shown antifouling properties of nanoparticle coated material and it was suspected that catalytically formed hypobromite could influence the cell-cell communication and prevent the initial step in biofilm formation [1]. However, the mechanism is not well understood and research is needed about the influence from cerium dioxide nanoparticles on the cell-cell communication and the biomimetic against this mechanism [1]. In this study different signaling molecules have been examined, which are involved in cell-cell communication of gram-negative bacteria. Observed reaction kinetics revealed a complete degradation of some compounds in the presence of hypohalides, whereas others did not react at all. Evolving transformation products (TPs) have been determined by high-resolution mass spectrometry coupled with liquid chromatography (LC-Orbitrap-MS, LC-QTOF-MS), whereas literature known TPs could be confirmed and previously unknown have been identified. First experiments with nanoparticles and haloperoxidase enzymes (vanadium-haloperoxidase) yielded identical TPs and substantiated the experimental approach to be representative. The proposed transformation pathways are based on an electrophilic reaction mechanism of the hypohalides with electron density rich molecule moiety's. Some of the transformation products exhibited a reduction in cell-cell communication activity as compared to the activity of the corresponding natural analogues [3]. [1] Herget et al., Adv. Mater. 2017, 29, 1603823. [2] Thomas et al., Biofouling 2010, 26, 73-88. [3] Syrpas et al., Beilstein J. Org. Chem. 2014, 10, 2539-2549.

6.01.09
Are REE Contaminants of Concern in the Marine Environment? An Insight to the Elementary Project
S. Piarulli, SINTEF Ocean; T.M. Ciesielski, Norwegian University of Science and Technology / Department of Biology; B. Hansen, SINTEF Ocean / Environment and New Resources; A. Malzahn, SINTEF Ocean; B. Jenssen, Norwegian University of Science and Technology / Biology; P.A. Olsvik, Nord University / Faculty of Biosciences and Aquaculture; C. Sonne, Aarhus University / Department of Arctic Environment; A. Zocher, M. Bau, Jacobs University; A. Booth, SINTEF Ocean / Environmental Technology; J. Farkas, SINTEF Materials and Chemistry / Environment and New Resources
Rare earth elements comprise a total of 16 elements: the 15 lanthanides and yttrium (REY). Despite their name, they are widespread in the earth`s crust. Due to their unique physical-chemical properties, REY are essential for a wide range of human applications including new and traditional industries such as renewable energy, automotive industries, and metallurgy), agriculture and medical diagnostic. Rising REY production and use can lead to an increased release into the environment and thus represents a potential environmental concern. Current knowledge on anthropogenic REY contamination in the marine environment, potential release hot-spots in coastal areas and consequences at organismal and ecosystem levels are scarce. The aims of the Elementary project are to investigate i) whether anthropogenic activities causes REY contamination in coastal areas ii) determine which REY are most widely released and at which concentrations and iii) whether REY

(anthropogenic and geogenic) are taken up and affect key marine organisms. To determine potential hot-spots of anthropogenically released REY and their bioavailability, seawater, sediments and a range of marine organisms (macroalgae, benthic invertebrates and fish) with different ecology and/or feeding modes are sampled in coastal areas in Norway and analysed with ICP-MS. Furthermore, potential impacts are studied on different life stages of relevant fish species, including REY uptake, organ distribution, as well as physiological and molecular responses. Results obtained from this project are expected to provide knowledge on the spatial distribution patterns of REY in Norwegian coastal areas, as well as on potential biological and ecological risk associated to anthropogenically released REY. Such knowledge is essential for future regulatory frameworks.

6.01.10
Sediment Contamination or Dietary Exposure, Which One Plays the Greater Role in Intertidal Species Mercury Accumulation?
H. Vieira, M.D. Bordalo, University of Aveiro; A.M. Soares, Universidade de Aveiro / CESAM & Department of Biology; J. Rendón-von Osten, Autonomous University of Campeche / EPOMEX Institute; S. Abreu, F. Morgado, University of Aveiro / CESAM & Department of Biology
Mercury (Hg) is classified as a priority substance by Directive 2013/39/EU of the European Parliament and by the Conventions for the Protection of the Marine environment of the North-East Atlantic (OSPAR Conventions). Due to its toxic effects, Hg contamination is a serious problem for human's health and wildlife. Coastal areas are among the most vulnerable environments to Hg contamination as they are located on the transition from the terrestrial to the oceanic environments. In the aquatic environment, Hg has a great affinity for the fine particles of the sediment, binding or just adsorbing by different processes. Thus, sediments play an important role in the retention of Hg and at the same time they act as a source of Hg for aquatic food webs. This study evaluated Hg concentrations in coastal sediments sites and species representing three different groups (Chlorophyta: *Ulva lactuca*, *Ulva intestinalis* and *Ulva lactuca*; Rhodophyta: *Chondrus crispus*, *Gracilaria multipartita*, *Procamium cartilagineum*, *Corallina officinalis*, *Ceramium virgatum*, *Asparagopsis armata*, *Pterocladiella capilacea*, *Gelidium pulchellum* and *Chondracanthus teedei*; Mollusca: *Mytilus galloprovincialis*, *Nucella napillus* and *Gibbula umbilicalis*) from eight sampling sites along the northwest (NW) coast of Portugal. Sediments from all sampling sites were classified as "uncontaminated" according to the Portuguese law's guidelines for sediment quality. The sediment samples present [Hg] below the European Union Water Framework Directive (EU-WFD) reference value and also bellow of the maximum established by OSPAR Convention through the Ecotoxicological Assessment Criteria (EACs). Nevertheless, sites located further south presented higher [Hg], possibly due to the transport of particles with higher Hg load (from the Hg contamination focus Aveiro lagoon) in the prevailing coastal sea current towards south. Despite this, no correlation was found between the [Hg] found in the sediment and in the biota. On the other hand, Hg levels found in species from the different sampling sites suggest an increment in the [Hg] from lower trophic levels (producers) to higher trophic levels (consumers) as well as a potential Hg transfer from prey for the predator. Moreover, the Biota-sediment accumulation factor (BSAF) < 1 for all species indicate that Hg levels in the biota do not depend on the concentration found in the sediments but rather on the Hg present in their food sources.

Environmental Impact Assessment and Socioeconomic Analysis for Sustainable Risk Management of Chemicals

6.02.01
Environmental Assessment of Actives Substances of Plant Protection Products: A Question of Interpretation?
B. Smith, BVL / Department for plant protection products; G. Czub, Federal Office of Consumer Protection and Food Safety BVL / Plant Protection Products; A. Gathmann, BVL / Department for plant protection products
The approval of active substances and the authorisation of plant protection products have to be in accordance with the level of protection laid down in Commission Regulation (EU) 1107/2009 in combination with the uniform principles (Regulation (EU) 546/2011). It could be concluded that therewith the question should be answered what an acceptable effect is. No substantial room for interpretations how the stipulated protection goals can be achieved should exist. But the reality of the zonal authorisations demonstrates that in member states different interpretations of protection goals exist which leads to national items. In consequence these national items result in non-harmonised decisions. In this poster an analysis of these "national items" in the environmental assessment from a German perspective is presented. Additionally, the interference with other sector specific regulations like drinking water protection bring additional protection goals on the board which are not covered in the plant protection regulations. It is presented why zero exposure is not a realistic expectation. Explanations are given which factors influence residues in adjacent areas or in groundwater. Also low level residues are seen as problematic for organic farming, baby food production, drinking water qualities and in the perception of residents. An outlook is given which ongoing activities exist to improve the situation of harmonisation of protection goals and monitoring of residues in the environment.

6.02.02
Can Pesticides With a High Risk-Potential Be Substituted? Results From a Coupled Environmental-Risk and Agronomic Analysis
M. Korkaric, I. Hanke, D. Grossar, R. Neuweiler, B. Christ, J. Wirth, Agroscope; M. Hochstrasser, Strickhof / Fachstelle Pflanzenschutz; P. Dubuis, T. Kuster, S. Breitenmoser, B. Egger, S.M. Perren, S. Schürch, A. Aldrich, T. Poiger, O. Daniel, Agroscope
Environmental impact of farming can be reduced by relating the use of plant protection products (PPPs) to cross-compliance payments. However, trade-offs between restricting PPP-use and limiting plant protection options have to be analyzed. To this end, an interdisciplinary project had been launched in Switzerland, in which risk-scores for PPP active substances authorized in 2019 were determined in a simplified and standardized comparative risk assessment for groundwater, surface waters and bees. Subsequently, an agronomic evaluation was carried out for a possible substitution of the 29 active substances with the highest risk-scores. It was examined to which extent alternative active substances with lower risk-scores are available in important arable and vegetable crops, fruit and berry production and viticulture, taking into account efficacy and anticipated problems in resistance management. Results indicate that for most of the top-ranking herbicides and fungicides, with high risk potentials mainly for the groundwater, sufficient chemical alternatives exist, with the exception for several vegetable crops. Restricting the use of insecticides with the highest risk potentials, mainly for surface waters and bees, would severely hinder plant protection for several major arable and vegetable crops. In fruit and berry production and viticulture, there are currently several effective chemical alternatives available. The two step analysis, combing environmental risk assessment with an agronomic analysis, allows to identify PPPs with a high risk-potential and opportunities for their substitution. We present possibilities to illustrate risk-scores in violin plots to ease the communication of uncertainties to the risk manager. A further option is to use sales data to weight risk potentials in the light of PPP use. Overall, coupling evaluations of ecological risks and impacts on agricultural productivity can provide an important basis for a cost-benefit analysis by decision makers and enables them to choose the optimal management options.

6.02.05
Global Expansion of the Environmental Risk Based Assessment Tools: Strategy, Milestones and Results
O. Barduk, O. Birketveit, M. Stipanicev, M-I SWACO, a Schlumberger company
Industries and companies see the need for proper environmental stewardship and implement environmental management systems because it is crucial for their reputation, their license to operate and return on investment of stakeholders. Environmental care has become a part of proper business strategies.Oil companies disclose their environmental efforts and initiatives in their annual reports. The response is often strategic and operational, but it is rarely quantified, because there is no a clear unified framework for assessing environmental activities globally. Reductions in pollution, waste, carbon emissions or energy used are described in terms of initiatives but almost never in terms of environmental risk. Performance targets and forward-looking commitments are even rarer seen in these reports.In Scandinavia, open and productive communication between business and authorities helped to develop an efficient framework on how to measure and report environmental risks related to contaminants in water discharges – the Environmental Impact Factor tool. This approach is very pragmatic and offers a concrete way for oil companies to identify an environmental concern and document improvements. Rule of law for oil companies, to annually assess and report their environmental performance in terms of EIF, has pushed local operations to become more innovative in produced water management. This includes implementation of advanced equipment

for produced water clarification and chemicals with better environmental properties.Raising awareness about risks assessment models and how they can be applied to measure and report produced waters discharges is a powerful way to include local environmental considerations in the assessment and to strive to a more harmonized application of available tools and techniques around the world. Strong globally unified regulatory standards for environmental risk assessment would protect both marine organisms and competitive companies from risks caused by less developed regulations. This approach would also foster constructive dialogue with Regulators and other stakeholders on a global level – that is especially prevalent in a highly regulated oil industry. The strategy, potential milestones and forecasted results of global extension of risk-based approach for produced water discharges is defined in this presentation.

6.02.08
High-Throughput Screening of Near-Field Human Health Impacts for Organic Chemicals in Building Materials
L. Huang, O. Jolliet, University of Michigan / Environmental Health Sciences, School of Public Health; P. Fantke, Technical University of Denmark / Quantitative Sustainability Assessment, Department of Technology, Management and Economics
Chemicals used in building materials can be a major passive emission source in the indoor environment, which has been associated with the deterioration of indoor air quality. Historically, studies have focused on evaluating particular chemicals, but do not cover the broad range of chemical substances found in building materials. The present study thus aims to screen the various chemicals used in building materials for potential near-field human exposures and related health impacts, and identify chemicals and products of concern to inform risk reduction efforts. We developed a mass balance-based, parsimonious and high-throughput suited model for predicting the chemical emissions from building materials with the consideration of indoor sorption. Using this model, we performed a high-throughput screening-level exposure assessment for chemicals in building materials, starting from product chemical composition data reported in the Pharos Building Products Database. Health risks were then assessed by combining the exposure estimates and toxicity measures, which can be further extended to arrive in health impacts in DALYs. Exposure was estimated for 315 and 624 chemical-product combinations from the Pharos Common Products Database and Individual Products Database, respectively, of which 238 (94) and 478 (141) had non-cancer (cancer) toxicity data available. A list of chemical-product combinations of concern was identified using criteria of hazard index > 100 or lifetime cancer risk > 10^{-4}. A total of 47 chemicals was identified as being of concern, including for example, two diisocyanates, triethanolamine and formaldehyde, with some of which yielding hazard index > 1000 or cancer risk > 10^{-3}. The identified chemicals of concern call for more refined investigations and more human and eco-friendly alternatives. However, the risks, especially carcinogenic risks, cannot be evaluated for a significant amount of chemicals due to lack of toxicity data or estimates, calling for more widely applicable toxicity estimation methods. Maximum allowable concentrations in different building products were calculated for chemicals with toxicity data available. The presented approach can be used to quickly screen many chemical-building product combinations to identify chemicals/products of concern to be targeted for risk reduction, or to aid alternative selection when developing more sustainable building materials.

6.02.09
The Role of Ecotoxicology in the Health Impact Assessment: An Innovative Ecosystem Approach for the Protection of Human Health in Italy
M. Carere, Italian Institute of Health; I. Lacchetti, Italian Institute of Health (ISS) / Department of Environment and Health; m. soggiu, Istituto Superiore di Sanità / Deparment of Environment and Health; L. Mancini, Italian Institute of Health / Department of Environment and Health
Human health depends ultimately upon ecosystem products and services (such as availability of fresh water, food, pharmaceuticals) and significant direct and indirects human health impacts can occur if ecosystem services are no longer adequate to meet social needs, this concept is embedded in the new European chemical strategy for sustainability that has been launched by the European Commission in October 2020. The Health Impact Assessment (HIA) is a procedure aimed at protecting the health of the populations exposed to the impacts caused by the emissions and releases for example of large industrial plants. The Italian Legislative Decree 104/2017 implemented the European Directive 2014/52/EU on the Environmental Impact Assessment (EIA). The Decree prescribes, for new plants or significant modifications of existing plants belonging to the specific category (e.g., large combustion plants, refineries), to carry out a HIA to protect the populations from the significant impacts caused by these plants. The Italian guidelines elaborated by the National Institute of Health describes an approach based on the five stages of the HIA procedure: screening, scoping, assessment, monitoring and reporting. The introduction of chemical mixture of contaminants into ecosystems due to anthropogenic activities can cause adverse health effects that are still often unknown and innovative monitoring tools are needed to protect human health. In this context, an ecotoxicological approach has been included for the first time in the HIA procedure with the aim to detect toxic effects in the ecosystems components (water, soil, air, biota) affected by the potential emissions, discharges and releases of large industrial plants. These assessments (with effect based methods) play an important role in the scoping and monitoring stages of the HIA procedure. A special emphasis has been given to the detection of genotoxicity effects because this mode of action is highly linked to the human health aspects. The inclusion of the ecotoxicological assessment in the Italian HIA procedure will play an important role as early warning and screening system and will contribute to apply the appropriate precautionary measures needed to prevent or mitigate adverse health effects caused by the potential exposure of population to the environmental impacts of industrial emissions.

6.02.10
Socioeconomic Impacts of Fragranced Consumer Products
U. Klaschka, University of Applied Sciences; A. Steinemann, The University of Melbourne
A recent representative survey showed that a fifth of the German population report one or more types of adverse health effects from exposure to fragranced products, such as cleaning supplies and perfumes. Nearly every fourth fragrance-sensitive person reports to have become sick from fragranced product exposure in the workplace. Serious health effects result in personal costs due to lost workdays. Economic personal costs due to fragrance exposure in the workplace are estimated to be 14.8 Billion Euros per year in Germany. This study integrates information on self-reported exposure and experienced health, economic, and societal effects due to fragranced products. The potential positive and negative implications are described. Based on a preliminary analysis, the most straightforward risk management option for fragranced products is the non-use scenario.

How Can Ecotoxicology Research Be Improved to Increase Utility in Regulatory Decision Making?

6.03.01
Review of OECD Test Guidelines for the ENvironmental Risk Assessment of Chemicals Regarding Their State of the Art in Science and Technology
C. Schlechtriem, Fraunhofer IME / Department Bioaccumulation and Animal Metabolism; S. Walter-Rohde, German Federal Environment Agency (UBA); S. Hahn, Fraunhofer ITEM / Chemical Safety and Toxicology; J. Schoenfeld, Federal Environment Agency; M. Schopel, Ramboll Deutschland GmbH; A. Polcher, Ramboll
The OECD test guidelines for the testing of chemicals are a special instrument for evaluating potential effects of chemicals on human health and the environment. These internationally standardized test guidelines for the testing of chemicals are used and accepted all over the world by industry, science and the authorities involved in the testing and evaluation of chemicals (industrial chemicals, pesticides, biocides, pharmaceuticals, etc.). As part of the OECD Test Guidelines Program (TGP), the OECD Test Guidelines are developed and updated by the OECD Working Group of National Coordinators for the OECD Test Guidelines Program (WNT). In order to ensure that the OECD test guidelines reflect the state of the art in science and technology and meet the regulatory requirements of the member states, the OECD test guidelines should be continuously expanded and updated. As a regular review of the OECD test guidelines is not required by default, it is the responsibility of the OECD member states to identify, propose and carry out the projects necessary to update the test guidelines. As a result, the focus is often on guidelines for which member countries have a particular interest and sufficient resources are available to initiate a revision process. Test guidelines that are perhaps not used that often or which are of limited interest are rather neglected with this approach, although a revision might be required. In autumn 2020, the German Federal Environment Agency (UBA) has initiated a project that aims to identify the OECD test guidelines that do not correspond to the state of the art. Only OECD test guidelines that focus on

the effects of chemicals on biotic systems, on the behavior and fate of chemicals in the environment or on their physico-chemical properties if used for environmental risk assessment are considered. Following prioritization of the OECD test guidelines, a survey based on a detailed questionnaire will be conducted to ascertain the necessary revision requirements. The survey is open to all interested parties from industry, science and the authorities. Upon conclusion of the survey the results will be discussed in three thematic workshops which will take place at the end of 2021. Recommendations for the revision of OECD test guidelines will be developed and prioritized, and finally presented to the members of the WNT. The poster will inform on the status of the project, the further course of the test guideline evaluation process and on how experts can get involved in this process.

6.03.03
Keeping up With Biocides and Chemical Regulations After Brexit
N. Keeble, Pentlands Science Park
The EU Biocidal Products Regulation (BPR) and REACH regulations have been transposed into UK law but, as time passes, the two regulations may move in different directions. It is no secret that Brexit has caused tremendous stress and strain on the chemical industry, but now that the Brexit process is completed, keeping up with regulatory compliance for these two evolving regulations will be challenging. This poster will compare the UK and EU regimes with respect to the BPR and REACH regulations. What the impact of Brexit has meant to companies, the measures already undertaken and the measures they must take to maintain regulatory compliance under UK & EU REACH and biocide regulations. Areas of divergence will be highlighted, together with the impact on companies supplying chemicals to both the UK and EU.

6.03.07
Development of a Higher-Tier, Indoor Microcosm Study Using Chironomus riparius
Z.L. Jones, Cambridge Environmental Assessments (CEA) / CEA; M. Brown, H. Schuster, Cambridge Environmental Assessments (CEA) / Ecotoxicology; J. Smith, Cambridge Environmental Asessments (CEA) / Ecotoxicology; N. Taylor, Cambridge Environmental Assessments (CEA) / Ecotoxicology; S. Taylor, G. Kraetzig, ADAMA Deutschland GmbH
The larval stages of *Chironomus riparius* reside in the sediment of freshwater habitats such as ponds, rivers and lakes. They are considered as a key indicator species for ecosystem health and commonly utilised as a test species in standard, regulatory ecotoxicology studies for effect assessment of plant protection products (PPPs) and their active substances. Standard (Tier I and Tier II) ecotoxicity tests are typically conducted under worst case conditions of exposure using axenic populations of determinate age. It is generally accepted that endpoints from these studies are conservative and lack a degree of environmental realism, and therefore context is required when extrapolating to real-world situations. For example, standard ecotoxicity studies do not consider species interactions, lifecycles and an organism's ability to recover and/or recolonise following a perturbation to their environment. Higher tier ecotoxicological testing and bespoke study designs are able to take into account these factors, enabling refinement of effect endpoints to be used in environmental risk assessment. Here, we present our findings from the design and conduct of an indoor microcosm study with *Chironomus riparius*. The main objective of this work was to develop a method to evaluate the effects of an insecticide on multiple life-stages of *C. riparius* and to derive effects endpoints based on mortality, emergence and reproduction, including an assessment of recovery following short-term exposure. The study was designed to incorporate several life-stages in an attempt to determine baseline mortality and emergence data in a microcosm setting. A representative insecticide was used to test the feasibility of the experimental design in a 28 day single pulsed exposure, focusing on the rate of emergence over time for this mixed-age-group population. Based on our results, the design of the study was successful in determining a baseline level of mortality and emergence within the system and was eminently practical for determining organism recovery and assessment of population effects. As such, the system could be applicable for use with other sediment dwelling species and may prove to be an acceptable approach for higher tier ecotoxicity testing. Further work is needed using other representative toxicants and species.

6.03.08
Use of Mesocosm Studies to Test the Effects of Plant Protection Products (PPP's) on Sediment and Benthic Organisms
M. Brown, H. Schuster, Cambridge Environmental Assessments (CEA) / Ecotoxicology; Z.L. Jones, Cambridge Environmental Assessments (CEA) / CEA; J. Smith, Cambridge Environmental Asessments (CEA) / Ecotoxicology; N. Taylor, Cambridge Environmental Assessments (CEA) / Ecotoxicology; A.C. Brooks, Cambridge Environmental Assessments / Ecotoxicology Risk Assessment; A. Abu, Cambridge Environmental Assessments
Sediment has an integral role in the functioning of aquatic ecosystems. It provides a habitat and refuge for a range of sediment dwelling and benthic organisms, acts as a substrate and nutrient source for aquatic plants, and is an essential part of the ecosystem structure and function. The sediment can also act as a sink for hydrophobic organic chemicals, it is therefore vulnerable to contamination, and sediment organisms are at risk of exposure. Current data requirements relating to impacts on sediment organisms comprise of tier 1 studies (*Chironomus* sp. or *Myriophyllum* sp.). Concern has been raised that these tests may not be fully representative of the risks of PPPs to the community of sediment organisms and plants, and that sediment is currently under-represented in environmental risk assessment. In addition, in repaired FOCUS scenarios, accumulation in sediment is expected to occur which could cause an increase in the number of sediment toxicity tests that are triggered. In response to such concerns, in 2015, the EFSA Panel on Plant Protections Products and their Residues (PPR) published an opinion on the risk assessment for benthic organisms and in 2016, the European Chemicals Agency (ECHA) updated the sediment section of the "Guidance on Information Requirements & Chemical Safety Assessment". These publications advocate the use of sediment-spiked toxicity data and micro/mesocosm tests in a tiered approach in order to improve the link between exposure and effects in sediment risk assessments. One of the benefits of using sediment spiked micro/mesocosm studies is the ability to assess long-term effects at the population or community level under more realistic exposure conditions. However, these studies are rarely used due to the lack of guidance on how to design, conduct and interpret such studies. In this poster we will highlight considerations when developing methods for the collection of data on sediment organisms in higher tier studies, including the realistic worst-case exposure scenario, the measurement of sediment concentrations, and accounting for accumulation of PPP in sediments. We will offer suggestions and make recommendations for the practical methods and study design of sediment mesocosms that aim to achieve reliable and robust population and community level endpoints. We will also discuss the potential issues with these studies in the context of risk assessment and regulatory acceptability and offer some potential solutions.

New Standardized Methods for Nanomaterial Testing: Knowledge Transfer from Research to Regulation and Vice Versa

6.04.02
Paths Forward to Meet Challenges in Environmental Risk Assessment of Nanomaterials for Regulatory Applicability Under REACH and CLP
D. Volker, German Environment Agency; K. Schwirn, W. Galert, German Environment Agency - UBA; J.T. Quik, RIVM / DMG; L. Tietjen, German Environment Agency - UBA
Within the European regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH, EC No 1907/2006) specific provisions for nanomaterials were included, which have become effective on 1 January 2020. To support registration of nanomaterials, the European Chemicals Agency (ECHA) provided nanospecific annexes to its guidance on information requirements and chemical safety assessment under REACH. Besides that, there are activities underway at the level of the Organisation for Economic Co-operation and Development (OECD) to provide nanospecific test guidelines and guidance that will improve reliability of data for an assessment of nanomaterials. Despite these activities, and although knowledge on the peculiarities of testing and assessing release, fate and effects of nanomaterials in the environment strongly increased in the last years, uncertainties about how to perform a reliable and robust environmental risk assessment for nanomaterials still remain and thus, robust PNECs and PECs for nanomaterials are still not available to a great extent yet. These uncertainties are of special relevance in a regulatory context, challenging both industry and regulators. We will present the current challenges in regulatory environmental hazard and exposure assessment under REACH and CLP and will make proposals to address them. The poster will put forward how to account for the uncertainties in hazard testing for nanomaterials to support achieving more valid, reproducible, and comparable data for hazard

assessment in a regulatory context in a reasonable and pragmatic manner. In order to improve regulatory exposure assessment for nanomaterials, the poster will critically question the appropriateness of current release factors and focus on needs for scientific advances in modeling exposure that need to be pursued and adapted for regulatory use. The analysis presented aims to promote the discussion on the demands of regulatory risk assessment of nanomaterials with respect to improving data quality and in the same time dealing with uncertainty. Even though the poster will emphasize the challenges within REACH and CLP, the nanospecific considerations made are expected to also be relevant for environmental risk assessment approaches in other regulations of chemical safety.

6.04.06

Assessing Particle Toxicity With the RTgill-W1 Cell Line Assay

N. Martin, T. Lammel, University of Gothenburg / Department of Biological and Environmental Sciences

Tests based on the permanent fish cell line RTgill-W1 established from rainbow trout gill represent a promising alternative to conventional *in vivo* tests in whole fish to assess fish acute toxicity of chemicals. Toxicity is tested exposing RTgill-W1 cell monolayer cultures in multiwell plates to serial dilutions of the test chemical for 24 h followed by measurement of cell viability using fluorescence-based biochemical assays. Commonly, reagents with different excitation and emission maxima, such as alamarBlue™, 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) and neutral red, are used as these can be applied simultaneously/consecutively to the same set of cells. The fluorescence intensity measured in the treated wells are corrected for unspecific background fluorescence (blank) and then expressed as percentage of the fluorescence intensity values measured in the untreated wells (unexposed cells). The relative cell viability is plotted against the chemical concentration, and the data fitted using sigmoid functions and or analysed using statistical tests to derive toxicological dose descriptors such as EC_x, NOEC and LOEC. The RTgill-W1 cell line assay is easy to implement, straightforward in the experimental realization, and yields robust results, which for the majority of (soluble) chemicals correlate well with *in vivo* fish acute toxicity data. A standard test procedure was published by the International Organization of Standardization in 2019, and an application for approval of a new Test Guideline by the OECD is in preparation. Thus, it can be expected that the RTgill-W1 cell line assay will increasingly be accepted into regulatory frameworks in the future, and be used for environmental safety assessment of a large number of chemical substances, including substances of particulate nature such as manufactured nanomaterials. We have extensively used the RTgill-W1 cell line assay for toxicity testing of manufactured nanomaterials in our research and in this contribution would like to share our insights regarding some of the encountered challenges and possible approaches to address these challenges. At the example of recent work with TiO_2 NPs we illustrate how modifications in the test procedure can help to uncover, reduce and quantitatively estimate particle-caused interference with fluorescence-based assays, and thus help to avoid effect over-/underestimation, and misinterpretation regarding the relative cytotoxicity of included treatments.

6.04.07

Joint Citotoxicity of ZnO Nanoparticles and Chlorpyrifos in Fish-Derived Cells

P. Sanchez-Arguello, INIA - National Institute for Agricultural and Food Research and Technology / Environment; D. Franco, INIA-National Institute for Agricultural and Food Research and Technology / Laboratory for Ecotoxicology. Deparment of Environment and Agronomy; C. GarciaGomez, INIA / Dpto. Environment and Agronomy; M. Fernández, INIA-National Institute for Agricultural and Food Research and Technology / Laboratory for Ecotoxicology. Department of Environment and Agronomy

ZnO nanoparticles (ZnO NPs) have many technological applications in energy and agriculture and can be included in consumer products such as pharmaceutics and cosmetics, among others. As a result of their extended use, residues of manufactured products that contain ZnO NPs can reach the aquatic environment. Although toxicity of ZnO NPs to aquatic organisms has been extensively studied, scarce information has been provided about the effects associated with their interaction with other pollutants. The objective of the present study is to determine the *in vitro* effects of ZnO NPs and chlorpyrifos (CPF) co-exposure on fish. In line with this aim we selected a combination of binary mixtures covering effect concentrations for each compound which have been reported in the RTG-2 cellular line. Cytotoxicity of ZnO NPs in RTG-2 was previously observed in our laboratory by measuring the following battery of cellular endpoints: Alamar Blue/CFDA-AM to test cell viability and plasma membrane integrity, ROS to test intracellular oxidation, MTT to test mitochondrial function and the neutral red uptake assay. These same endpoints and the acetylchlorinesterase (AChE) activity, as specific cellular biomarker for testing CPF effects, have been measured in binary combinations covering a range of both CPF (1.25 - 100 µg/L) and ZnO NPs concentrations (10 - 100 µg/L). This study allows us to draw some conclusions about the complex cellular responses produced by mixtures with NPs, such as CPF cytototoxicity modulation due to presence of ZnO NPs, the influence of CPF/ZnO NPs concentrations for this modulation and the importance of endpoint selection when assessing mixture joint effects. This work was supported by the Community of Madrid project S2018/BAA-4330.

6.04.09

Examining Bioavailable Ce3+ to Explain the Differences in Toxicity of Nano Cerium Oxide Between Two Soil Invertebrates

J. Velicogna, Environment and Climate Change Canada / Environment and Climate Change Canada; H. Chen, Environment and Climate Change Canada / Biological Assessment and Standardization Section; D. Schwertfeger, Environment and Climate Change Canada / Biological Assessment and Standarization Section; R.P. Scroggins, Environment and Climate Change Canada / Biological Assessment and Standardization Section; J. Princz, Environment and Climate Change Canada / Biological Assessment and Standarization Section

Nano cerium oxide ($nCeO_2$) is expected to enter into the soil system via land application of biosolids, landfill leachate, deposition of airborne material (e.g., via vehicle exhaust) and, soil remediation. The fate and effects of engineered nanomaterials (ENM) in the soil environment are dependent on the characteristics of the ENM and soil physical-chemical properties (e.g., humic acid, organic matter, pH and ionic strength). In this study, the fate and toxicity of $nCeO_2$ (uncoated powder 10-30 nm) was evaluated in a field soil and compared to a readily soluble form of Ce (i.e., $CeNO_3$) in order to determine the influence of the dissolved free metal (i.e., Ce^{3+}) on toxicity. The effects of $nCeO_2$ on the survival and reproduction of two soil invertebrates (i.e., *Eisenia andrei* and *Folsomia candida*) were evaluated, along with measures of total and 0.01M $CaCl_2$ extractable (i.e., bioavailable) Ce forms. Effects on reproduction demonstrated IC50s of 150 (91-280) and 466 (328-672) mg/kg for *F. candida* exposed to $nCeO_2$ and $CeNO_3$, respectively. In contrast, *E. andrei* reproduction was insensitive to $nCeO_2$ (IC50 > 902 mg/kg), but was affected by $CeNO_3$, with an IC50 of 294 (147-630) mg/kg, all based on total Ce. The resultant chemistry data demonstrated that the concentration of the bioavailable fraction of Ce in the soil was less in treatments with $nCeO_2$ compared to the $CeNO_3$, and less in *F. candida* tests compared to *E. andrei* tests. Hence, bioavailable Ce^{3+} could not explain the differences in toxicity, suggestive of a potential nano-specific effect. Single particle ICP-MS will be conducted on soil extracts to evaluate the presence of particulate Ce. The results of this study demonstrate relatively low toxic risk from Ce, but suggest that current risk assessment models based on the solubilized metal form may not be applicable for $nCeO_2$.

6.04.12

ENTRANS: Investigating the ENvironmental Impacts of TRANSformed Engineered Nanomaterials Released From Wastewater Treatment Plants

A. Georgantzopoulou, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; A. Booth, SINTEF Ocean / Environmental Technology; J. Farkas, SINTEF Materials and Chemistry / Environment and New Resources; C. Coutris, Bioforsk / Dpt. for Soil Quality and Climate; R. Kaegi, Eawag - Swiss federal Institute of Aquatic Science and Technology / Process Engineering; M. Rehkamper, Imperial College London; A. Macken, NIVA / marine pollution; K. Ndungu, NIVA; P.A. Carvalho, SINTEF Materials and Chemistry; S. Kuehr, University of Siegen; S. Samanipour, University of Amsterdam; K. Thomas, The University of Queensland / Queensland Alliance for Environmental Health Sciences (QAEHS); K. Petersen, NIVA - Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; T. Gomes, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; M.T. Hultman, Norwegian Institute for Water Research / Section of Ecotoxicology and Risk Assessment; A. Lillicrap, NIVA / Section of Ecotoxicology and Risk Assessment

The production, diversity and use of engineered nanomaterials (ENMs) increases globally as the market and number of applications for ENM expands. Silver (Ag), zinc (Zn) and titanium dioxide (TiO2) ENMs are among the most widely used in industrial processes and

consumer products leading to increased releases to wastewater treatment plants (WWTP) from domestic and industrial sources. Material flow analyses suggest that landfills or agricultural soils and sediments are the main receiving compartments for ENM, depending on the application and ENM type. However, knowledge on the fate and transformation of ENMs in WWTP biosolids following their use as fertilizer on agricultural land, their impacts on soil and sediment ecosystems released through run-off after land-application are only poorly understood. ENTRANS aims to improve the understanding of the behavior and physicochemical transformation processes impacting ENM in different environmental media (wastewater, biosolids, soil, sediment) and how this transformation influences ENM bioavailability, bioaccumulation and toxicity in organisms from receiving environments considered to be the final sinks for ENMs, soil and sediments. The ENTRANS project will follow and characterize the physicochemical transformation of ENM in WWTP and environmental compartments. Using isotopically labelled Ag, Zn and TiO2 ENMs, the transformation and further impact of these particles, including bioavailability, bioaccumulation, biodistribution and toxicity, will be tracked and studied using relevant in vitro and in vivo models to provide a better understanding of the link between transformation, uptake and observed toxicity. Existing guidelines will be improved to incorporate environmentally relevant exposures and toxicity endpoints of regulatory relevance and novel bioassays will be developed focusing on immune and stress responses. The transformation processes, exposure and uptake, biodistribution and toxicity data will be carefully generated so that the obtained results can be integrated into computational fate and exposure models and a risk assessment can be performed.

6.04.13

Safer-By-Design Layered Double Hydroxides: Bridging the Gap Between Ecotoxicity and Chemical Compositions

D. Carneiro, University of Aveiro / Department of Biology; T. Oliveira, F. Maia, Smallmatek - Small Materials and Technologies, Lda.; S. Loureiro, Universidade de Aveiro / Biology; R. Martins, Department of Biology, University of Aveiro / CESAM & Department of Biology

Layered double hydroxides (LDH) are inorganic engineered nanomaterials used as drug carriers, additives, agro-products or adsorbents for environmental remediation, among other applications. These anionic nanoclays are structurally characterized by positively charged (e.g. Zn^{2+}, Mg^{2+}, Al^{3+}) layers stabilized by an inner layer containing water molecules and anions (e.g. nitrates (NO_3^-)) that can be exchanged upon specific triggers. Different compositions of LDHs have been explored aiming at developing a low toxic nanoadditive with the capacity of controlling the release of corrosion inhibitors (1,2,3-benzotriazolate (BTA^-) and nitrites (NO_2^-)). LDH were chemically modified according to the divalent cation (Zn^{2+}, Mg^{2+}) and intercalated anion (NO_3^-, NO_2^-, BTA^-). Despite the numerous toxicological studies, the effects of LDH exposure in aquatic organisms are still scarcely studied. Therefore, this study aims at presenting an integrative toxicity assessment of six compositions of LDH using a total of 10 species representative of decomposers (bacteria), producers (microalgae) and consumers (invertebrates). Exposure tests included growth inhibition tests using bacteria (*Idiomarina seosinensis, Halobacillus locisalis;* 24 h of exposure; n=4) and microalgae (*Isochrysis galbana, Tetraselmis chuii, Phaeodactylum tricornutum*; 72 h, n=4, ISO 10253), and acute tests using molluscs (*Scrobicularia plana, Mytilus galloprovincialis*, *Gibbula umbilicalis, Monodonta lineata*; 96 h, n= 5, ASTM E724-98) and crustaceans (*Artemia salina;* 48 h, n=3, ASTM E1440-91). Exposure concentrations ranged from 1 to 100 mg/L (five treatments plus a negative control of artificial saltwater). For each species, the concentration causing 50% mortality (LC_{50}), effects or inhibition (E/IC_{50}) was calculated through non-linear regression models. No observed effect concentrations (NOEC) were derived from one-way ANOVA followed by the Dunnett test, whenever significant differences between treatments and control were noted ($p< 0.05$).Overall, based on the NOEC values, the toxicity of LDHs can be simplified as follows: Mg-Al LDH-NO_2 (*less toxic*) < Zn-Al LDH-NO_2 < Mg-Al LDH-BTA < Mg-Al LDH-BTA < Mg-Al LDH-NO_3 < Zn-Al LDH-NO_3 (*more toxic*). The results suggest that Mg-Al LDH-NO_2 is a promising anti-corrosion nanomaterial (NOEC: < 1, 1–10 and >100 mg/L in the case of bacteria, microalgae and invertebrates tested species, respectively) featuring a low environmental hazard.

6.04.14

Harmonising Methodologies in Nano-Ecotoxicology: Disentangle Exposures to Accurately Derive Effects of an Innovative Nanoclay to a Freshwater Microalga

F. Campos, Universidade de Aveiro / CESAM e Departamento de Biologia; R. Martins, Department of Biology, University of Aveiro / CESAM & Department of Biology; S. Loureiro, Universidade de Aveiro / Biology

The nanotechnology field has witnessed an unprecedented growth marked by the development of new innovative nanomaterials with unique physicochemical characteristics for a wide range of applications. Cu-Al layered-double hydroxides (LDH) are hexagonal nanoclays with 20 to 40 nm height standing as an example of an innovative nanomaterial (NM) that can act as a carrier of active compounds and exhibit antifouling properties. These characteristics also lead to issues while developing strategies to accurately assess nanohazards. Standard guidelines to evaluate the ecotoxicity of substances are available but do not account for the specificities of nanomaterials, leading sometimes to inaccuracies and increase in uncertainty. Herein, the present study aimed to assess the impact of two different exposure methodologies (serial dilutions of the stock dispersion vs. direct addition of NM powder to each concentration) on the toxicological profile of different powder grain sizes of Cu-Al LDH (bulk, < 25, 25-63, 63-125, 125-250 and >250 µm) in the population growth of the freshwater microalgae *Raphidocelis subcapitata*. For the exposure methodology, the < 25 µm grain size was selected. In the direct addition of NM powder methodology, the nanomaterial was weighted individually for all target concentrations (1, 2, 3, 5, 7, 9 mg/L). In the serial dilutions methodology, a stock dispersion was made and used as the highest tested concentration, with the remaining concentrations being achieved by serial dilution. Results revealed that both methodologies were suitable to be used in further tests, as no difference was observed between both. For experimental execution and reproducibility purposes, the serial dilutions methodology revealed less variability and was selected as the preferred methodology to carry out the remaining growth inhibition tests with the other sizes. Regarding the different grain size toxicity assessment, a size-dependent response was observed. Powders with low grain size caused high effects on microalgae growth. Although no differences were reported between exposure methodologies, further research involving other nanomaterials and key species have to be carried out to achieve standardization and validation of inter-laboratory comparison. Moreover, Cu-Al LDH revealed to be less toxic to the algae's growth compared to other available Cu forms (e.g. nano CuO). However, more studies have to be performed for proper risk assessment.

6.04.15

Effect of Sample Preparation Technic on the Surface Charge and Bioactivity of Silica Coated Magnetic Nanoparticles

T. Marochko, L. Bondarenko, Moscow Aviation Institute; V. Terekhova, Lomonosov Moscow State University / Lab of Ecotoxicological Soil Analysis; L. Kovalevskaya, M. Sharapova, Moscow Aviation Institute (National Research University); P. Uchanov, Severtsov Institute of Ecology and Evolution; K. Kydralieva, Moscow Aviation Institute

This study was aimed to estimate of the effect of hydrodynamic diameter and surface charge on bioactivity of Fe_3O_4 NPs modified by 3-aminopropyltriethoxysilane, APTES and synthesized by various condition (air and argon atmosphere). The toxicity of various Fe_3O_4 NPs functionalized with APTES in three fractions: initial suspension, supernatant and fitrate of this initial suspension were investigated. Behaviors of NPs were investigated in the aspects of their toxicity to *Sinapis alba* and *Paramecium caudatum.* Fe_3O_4 NPs were prepared by a chemical *coprecipitation in situ* from $FeCl_2$ and $FeCl_3$ in the presence of NH_4OH. The NPs formulated by sol-gel method were Fe_3O_4-APTES (Ar) and Fe_3O_4-APTES (air) (various formulation conditions: in argon or air atmosphere, drying in at 70 °C in vacuum or 150 °C). After synthesis, the nanoparticles were fractionated in a centrifuge and a white ribbon filter. The zeta potential and hydrodynamic diameter data were determined by dynamic light scattering. According data of FTIR spectroscopy, there are free amino-groups onto the Fe_3O_4-APTES NPs surface. With HA increasing, the isoelectric point (IEP, pH at $\xi=0$) for Fe_3O_4 shifts to the acid region. The EC_{20} parameter for NPs with positive charge (Fe_3O_4, Fe_3O_4-APTES (air) and Fe_3O_4-APTES (Ar), $\xi=$ +10 mV and +17 mV, respectively) higher then EC_{20} for NPs with negative charge (HA, $\xi= -37$ mV). It means that NPs with positive charge surface are less toxic towards infusorians in compare with ones for negative charge surface. A correlation between zeta potential and biological activity for samples after filtration and centrifugation was found. It has been shown that nanoparticles with zeta potential near IEP have greater toxicity. The primary amines on the surface of the Fe_3O_4-APTES give rise to the large positive surface charge and have previously been reported to cause a toxic effect. Higher plants turned out to be less sensitive to

the selected nanoparticles at all range of concentrations, while the nanoparticles showed toxic effects in relation to the ciliates at concentrations of more than 0.1%.
Acknowledgement. *This research has been financed by the Russian Foundation for Basic Research (19-33-90149).*

6.04.16
Anti-Fouling Efficacy Assessment of Smart Additives Developed for Sensing Corrosion on Aquatic Structures
C.O. Nelson, AquaBioTech Group / Research & Development; I.C. Pimparel, S. Larroze, AquaBioTech Group / ABTL; A. Sushkova, M. Wilhelm, J. Tedim, University of Aveiro / Department of Materials and Ceramic Engineering & CICECO
The MarTERA project "SMARTAQUA" (ERA-NET cofund scheme of H2020) is developing an eco-friendly low-cost early corrosion detection tool which can be applied directly on maritime steel structures. Hexacyanoferrate ions $[(Fe(CN)_6]^{3-}$, which can coordinate with Fe^{3+} ions resulting from the dissolution of steel giving a blue colour, were incorporated in layered double hydroxides (Mg-Al LDH). Phenolphthalein (PhPh), which can respond to pH changes linked with the start of corrosion process giving a pink colour was encapsulated in silica mesoporous nanocapsules (SiNC). Entrapment of active compounds in nanocarriers renders controlled release under conditions relevant for corrosion processes. Magnetic nanoparticles (MNP), which can detect the change in magnetic field in the presence of electrical corrosion currents, were prepared to be used underwater, where colorimetric detection is not possible. The anti-fouling efficacy of these innovative materials was evaluated using the marine diatom *Phaeodactylum tricornutum* and the mussel *Brachidontes pharaonis*. Cultures of *Phaeodactylum tricornutum* were maintained in an illuminated incubator at 20 ± 1 °C and a photoperiod of 12 h light: 12h dark. Medium (F/2+ Si Guillard) was prepared with filtered (0.45 µm) and autoclaved seawater. Growth inhibition following exposure tests was evaluated following the OECD 201 (2011) with some modifications. *Brachidontes pharaonis* were collected from the shore of Malta and were acclimatised over a 72h period in the dark, with continuous aeration and under ambient temperature. Byssus thread formation and mortality were accounted for daily during inhibition testing using 6-well microplates, under constant darkness at 24 h, 48 h and 72 h. The anti-microfouling efficacy of the materials developed was quantified by defining the half maximal inhibitory concentration (IC_{50}) on diatom *P. tricornutum*. SiNC, SiNC-PhPh, $[Fe(CN)_6]^{3-}$ and Mg-AI LDH displayed the greatest anti-microfouling efficacy with an IC_{50} < 21 mg/L. When performing tests on the diatom with dark coloured substances such as MNP, shading was observed causing growth restriction due to low light availability. The anti-macrofouling efficacy was quantified by defining the half maximal effective concentration (EC_{50}) on mussels. None of the compound tested displayed anti-macrofouling efficacy against *B. pharaonis*, with all EC_{50} >100 mg/L.

Rethinking Science Communication: Storytelling in the Web 2.0 Era, New Tools and New Public

6.05.02
Scientific Comic
J. Bedrossiantz, IDAEA CSIC / Environmental Toxicology
Comics can be, among other divulgations, an original way to transmit scientific information. Its use can be very versatile: to promote the celebration of a special day in Sciences, to tell a great discovery in the History of Sciences or to explain a fact, a result, a technique... the Comic is a format perfectly adjustable to the content and it is always attractive for the public. From the area of communication, it is a project that can be carried out alone or in collaboration, by students, teachers, researchers and technicians. You don't need to have Da Vinci's talent (although it can always be an advantage) to produce a good scientific comic. It is simply a question of having a little imagination and astuteness when manipulating the tools at your disposal: drawings, texts, supports, softwares... even photography can be part of the conception and realization of a comic. There are no limits to productivity except your own patience and disposition to transmit knowledge. The creation of a comic also means respecting a series of codes such as author's rights, data protection, or even the right to privacy of the people concerned and their own image. It is therefore a good publishing exercise for any scientist who will need it in his professional career. It is clear that the main objective of the comic is to explain the scientific context, or related to the sciences in some way. The purpose of the comic is to inform and educate the public. The degree of precision and the adequacy of the language will make that we address to a general or specialized public. Finally, the diffusion of the comic or another illustrated work has to be done through the medium that is considered more appropriate and more convincing. We always look for it to reach the majority, as quickly as possible and with the greatest impact.

6.05.03
Getting Plentzia Marine Station to the People: "PiEnette" the Research Vanette
O. Diaz de cerio, University of the Basque Country; I. Cancio, Research Centre for Experimental Marine Biology and Biotechnology Plentzia Marine Station PIE Areatza z/g, E-48620 Plentzia, Basque Country (Spain); U. IZAGIRRE, UNIV BASQUE COUNTRY / Dept. Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU); M. Zarragoitia, Research Centre for Experimental Marine Biology and Biotechnology Plentzia Marine Station PIE Areatza z/g, E-48620 Plentzia, Basque Country (Spain); M. Soto, University of the Basque Country / Zoology and Animal Cell Biology; B. Zaldibar, E. Bilbao, Research Centre for Experimental Marine Biology and Biotechnology Plentzia Marine Station PIE Areatza z/g, E-48620 Plentzia, Basque Country (Spain)
The Research Centre for Experimental Marine Biology & Biotechnology (Plentzia Marine Station PiE-UPV/EHU; www.ehu.eus/PIE/) of the University of the Basque Country is a reference institution for the research and postgraduate education in Marine Sciences. PiE-UPV/EHU is part of the European network EMBRC and high education in three Erasmus Mundus masters related with marine environment and pollution effects. In addition, one of its main purposes is to develop science dissemination activities because eight years after opening doors in 2012, the marine station is still unknown among the wide public, and if science wants to be sustainable it needs to reach the public Thus, with the aim of disseminating our research work and getting closer to citizens, researchers at PiE-UPV/EHU have developed an itinerant show. Volunteer researchers participate in different science fairs and/or trade exhibitions of sea products (i.e. fish markets) to explain their own projects and results in an accessible and comprehensible way using our mobile laboratory, the we usually use to get samples. This Pi*Enette* is fully endowed with laboratory equipment (centrifuges, microscopes, stereoscopes….etc.), fishing nets and sampling gears. We show different marine organisms, histological slides of an assorted variety of organisms.....and we invite people on board!!!. We have participated among others, in the annual fish market exhibition of Tuna World Capital of Bermeo (https://www.bermeotunaworldcapital.org/)or during the Science Week events in Bilbao (http://zientzia-astea.org/es/) where with the help of microscopes and stereoscopes we show how pollutants interact with organisms in marine environments. We show tissue alterations provoked by pollutants (i.e. tumors, gill malformations…etc) or/and the presence of microplastics in mussel tissues. Therefore, people learn about effects of pollutants in marine biota driving their interest towards environmental conservation. In addition, we make kids feel science through gamification, using for instance a family card game based on the developmental stage of organisms. If they are lucky, depends on the season, they can even see some of them *in vivo*! Overall, the show is active and people gets interested making questions to real researchers.

6.05.04
Scientific Validation of a Citizen Science Protocol Targeting Soil Quality
S.S. Oliveira, Center of Marine and Environmental Research CIIMAR; D. Kumar, D. Martins, FCUP – Faculty of Sciences – University of Porto; J. Pereira, University of Aveiro / Department of Biology & CESAM; P. Santos, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research; FCUP – Faculty of Sciences – University of Porto / Biology; R. Pereira, Faculdade de Ciências da Universidade do Porto / Biology
Despite being a valuable non-renewable resource that provides food and habitat for thousands of species, soil is becoming increasingly degraded due to human activities. Agricultural practices that involve the application of fertilizers and plant protection products are just one example of how human societies are compromising soil's structure and function. In order to address the threats that soil currently faces, it is important to engage the citizens in its conservation. In fact, public participation is widely regarded as an essential component of successful Nature conservation initiatives. Being a participative approach, citizen science is a great strategy to engage stakeholders in this process, while also collecting relevant data for soil monitoring. Currently, there are several examples of citizen science projects targeting soil and although

there is literature highlighting their scientific value, most do not actually report how the data collected by the public directly compares with the ones obtained by applying standardized protocols. Therefore, the goal of the current work was to address this knowledge gap by assessing how soil quality data collected by non-experts compares to the results of earthworm avoidance tests. For this purpose, a national citizen science project was launched in Portugal with the aim of involving schools and the general public in soil quality monitoring. While taking part, citizen scientists performed simple tasks to measure biological, physical, and chemical proprieties of the soil. For example, they measured soil pH with pH stripes and qualitatively evaluated soil water holding capacity by squeezing a soil sample and checking how humid it was. In the end, by attributing scores to each assessed parameter, volunteers were able to classify the quality of the studied soil as either bad, reasonable, or good. Earthworm avoidance tests are primarily used to discriminate contaminated soils; however previous studies have shown that these organisms also respond to the physical-chemical properties of soils during these short-term assays. Hence, these were used to evaluate the scientific accuracy of the data collected and analysed by citizens. The final results of our study will be presented at the conference.

6.05.06
DaNa4.0 - Providing Information on the Safety of Advanced Materials Via a Web-Based Knowledge Base
D. Kühnel, Helmholtz-Centre for Environmental Research / Bioanalytical Ecotoxicology; A. Mattern, Helmholtz Centre for Environmental Research UFZ / Bioanalytical Ecotoxicology; C. Steinbach, DECHEMA; N. Bohmer, N. Möller, Society for Chemical Engineering and Biotechnology e.V. (DECHEMA); H.K. Krug, NanoCASE GmbH; C. Marquardt, K. Nau, Karlsruhe Institute of Technology KIT / Institute for Automation and Applied Informatics
A topic that has attracted much attention by the public and scientific community alike is nanotechnology and related research on the safety of nanomaterials. The tininess of nanoparticles as well as their anticipated (even though not always realistic) usages e.g. in science-fiction like nano robots, makes it hard for people to capture their benefits and weight potential risks at the same time. Here, 10 years ago, the DaNa project stepped in and has since then taken on the task of providing information on the safety of nanomaterials and communicating to interested people. This is done via a web-based knowledge base (www.nanoobjects.info). The content of the knowledge base is based on a synthesis of the latest research results obtained from relevant research projects and the scientific literature. In order to ensure accuracy and reliability of the provided information a structured approach for generating the contents of the knowledge base has been developed. This content is tailored to an audience coming from different backgrounds (consumers, journalists, citizens, scientists from diverse fields). This is achieved by presenting the content in three levels of complexity. By this, the interdisciplinary DaNa team has established a comprehensive knowledge base on the safety of currently 26 different nanomaterials, considering their potential effects on humans as well as the environment. But, as a team of scientists, we also had to learn to adapt to our audience's perception and accept that dry texts are not very appealing, even if the facts are correct. Therefore, over the years we have implemented a number of measures to make the content more attractive. These include social network activities, which facilitate interaction with our audience and help us to promote new website content. In 2020, the DaNa team took up a new challenge by broadening the scope of materials presented in the knowledge base from nanomaterials to advanced materials. These materials represent an even more diverse group of engineered materials with a multitude of applications such as lightweight plastics with fibre reinforcement or hybrid materials used in 3D printing. This novel class of materials encompasses nanomaterials as a subgroup. Here we will show what we learnt on science communication from nanomaterials so far and will reflect on our experiences on how we, as scientists, present scientific facts about advanced materials in a way that is understandable for everyone.

Extended submission 6 - Environmental policy, risk management, and science communication

6.06.01
How to Integrate Sediment Remediation and Ecosystem Recovery? (Hint: They Should Go Hand in Hand)
R. Johnston, Applied Ecological Solutions; D.W. Moore, U.S. Army Corps of Engineers / Engineer Research & Development Center (ERDC); K.M. Payne, Enthalpy Analytical / Aquatic Toxicology; A. Kascak, University of Louisiana, Lafayette / Biology; B. Henry, Anchor QEA, LLC
Cleanup at many contaminated sediment sites can be very costly, time consuming, and ineffective if larger scale pressures and ecological processes are not adequately addressed by the remedial design. Ideally, a holistic approach is desired for remedies that brings together remediation and reuse (restoration) by exploiting synergies that minimize costs and environmental impacts and achieves whole-system sustainability benefits. Sustainable remediation strategies should be informed not only by considerations of regulatory compliance but also by stakeholder goals, values and expectations. Over the last few decades, remedies selected at contaminated sediment sites have focused on mass removal or the reduction of exposure to sediment-associated contamination to manage human health and ecological risks. At many contaminated sediment sites, assessing contaminant-focused remedy effectiveness measures has been confounded by the complexity of ecosystem processes, the lack of comprehensive monitoring data sets, and the need to align remediation objectives with ecosystem recovery goals for restoring ecosystem processes that support a wide range of ecosystem services and functions that are necessary to sustain ecosystem recovery. Additionally, sediments (whether contaminated or not) are linked to processes occurring within the watershed making them susceptible to, and potential drivers of, chemical, physical and biological stressors from larger scale social and economic pressures (land use change, hydrology perturbations, new and emerging chemicals), climate change (sea level rise, extreme events, invasive species), and other disturbances associated with the Anthropocene. Drawing on the results of case studies, key concepts and lessons learned to better integrate sediment cleanup and ecosystem recovery goals were discussed during a Session sponsored by the Sediment Interest Group at the recent SciCon2 conference and you are cordially invited to join the discussion.

COVID-19 Pandemic Consequences, Detection and Surveillance Through the Lenses of Environmental Toxicology and Chemistry Scientists

7.01.04
Lessons From Chemical Risk Assessment for a Pandemic: Questions to Ask Before Trusting a Model
P. Thorbek, BASF SE / Agricultural Solutions; A.S. Johnston, Cranfield University / School of Water, Energy and Environment; H. Thulke, Helmholtz Centre for Environmental Research-UFZ / Department of Ecological Modelling; V. Forbes, University of Minnesota / Ecology, Evolution & Behavior; V. Grimm, Helmholtz centre for environmental research - UFZ / Department of Ecological Modeling
The world has faced major disruption and uncertainties during the COVID-19 pandemic while governments have scrambled to manage the outbreak. Models represented the best tools for planning interventions that balanced the risks of widespread infection, the economy and social disruption until an effective treatment is established. However, there were contradictory outputs from different models with widely differing assumptions and structures. While the differences in models were not the only cause, countries have chosen very different strategies ranging from letting it spread, over managed “herd immunity” to rigorously enforced lock-down to eradicate COVID-19 from the population. Because the strategies chosen have far reaching consequences, it is crucial that decision makers choose models that are fit for purpose. But how can they explore which models are fit for purpose? Protocols for good modelling practice have been established in many fields, but transparent and clear model communication is also critical to support decision makers when choosing models to support real world problems. Here we present lessons from the chemical risk assessment where comprehensive guidance exists for how to use models for decision making. In order to help in situations when time is severely limited, we distilled the advice from different sources on good modelling practice down to three critical questions decision makers should always ask before using any model for decision support. To help decision makers choose models that are fit for purpose we formulated three essential questions: Question 1: what is the model’s purpose? Question 2: how is the model organized? Question 3: is there evidence the model works? Model purposes fall into three main categories: demonstration, understanding, and prediction. Given these different purposes, models also reflect different scopes. While all three categories have their rightful place in science, the consequences can be detrimental if the models are used for purposes for which they

are not suitable. Modellers tend to present their models so they look right with the claim of being realistic enough for their purpose. However, looking right, has many dimensions, and we suggest the three screening questions presented here as a simple approach for decision makers to disentangle them. Chemical risk assessment faced the same issues with placing trust in models to predict complex ecosystem dynamics, but in this area the different stakeholder groups in SETAC have worked together to develop a common language to discuss model design, assessment, application and interpretation. While models should form a nexus between science and policy, communication often holds us back. Lessons can be learnt from the mistrust of models during the Covid-19 pandemic, but regulators, industry, and academia need to work towards this common goal.

7.01.05
Environmental Management of Professional Association Football Team
K. Takahashi, Tokyo City University; N. Itsubo, Tokyo City University / Graduate School of Environmental and Information Studies; S. Sakuma, Ventfore Kofu; H. Sawada, Green Sports Alliance
Japanese soccer was temporarily suspended and forced to play without spectators due to measures to prevent the spread of the 2020 coronavirus. In Japan's professional soccer league, the J-League, it has been reported that some club teams are losing money or going insolvent due to a decrease in admission fees and other revenue as a result of these measures against the new coronavirus. Several LCA case studies of sports events such as marathon, golf tournaments and triathlon have already been carried out by authors. Carbon footprint of Tokyo Olympic Game also been calculated to realize carbon free event. But there are few examples of environmental footprint for sports professional team in Japan. In addition, there are still few papers that evaluate the environmental impact of a pandemic. In this study, we evaluated carbon footprint for Ventfore Kofu, professional football team. We covered the venue, athletes, management, visitors, sponsors, promotional items in the analysis. Through the analysis, we found the key processes to reduce the carbon emissions efficiently. The results are also shared with various stakeholders using mass media. Due to the spread of COVID-19 and measures for avoidance of infection, the number of visitors has dropped and the activities of team also completely changed. We estimated the carbon footprint of this year to compare the differences from that of the last year.

7.01.12
An Improved Method for Detection and Quantification of SARS-CoV-2 RNA in Wastewater
B. Peinado, L. Martínez-García, F. Martínez, L. Nozal, M. Sánchez, IMDEA Water Institute
For long time the sewage, a complex matrix with highly variable composition, has been considered only waste. However, the analysis of wastewater has been shown that can be used as a method for early detection of viral diseases like norovirus or hepatitis A virus, as early warning before symptoms show up on patients. Since the beginning of Covid-19 pandemic, scientists have developed different methods not only to detect but also to quantify the presence of SARS-CoV-2 RNA in wastewater with the objective to use it to predict the appearance of clinical cases. However, there is not any approved method and these procedures encounter different problems, such as the volume and temperature to store samples, how to concentrate the sample, the genes used for detection and the internal controls among others. After a review of the bibliography available, three different RNA concentration procedures were selected to optimize conditions for quantification and identification of SARS-CoV-2 virus in wastewater: ultrafiltration (centricon), polyethylene-glycol (PEG) and aluminum hydroxide precipitation methods. For RNA extraction a commercial kit was used. Subsequently, two regions of nucleocapsid gene (N1 and N2) have been targeted using RT-qPCR as described by Centers for Disease Control and Prevention (CDC) for SARS-CoV-2 virus. Samples from a wastewater treatment plant (WWTP) in Madrid (Spain) were provided weekly during several months to stablish the optimal conditions. After several assays, our results showed that the volume of sample analyzed and the reduction of organic matter used in the PEG method, before the concentration by ultrafiltration, improve the recovery percentage of virus and therefore the detection and quantification of SARS-CoV-2 RNA.

Environmental Quality Affecting Agro-Food Systems and Water Resources across the Mediterranean Area

7.02.01
Challenges and Opportunities of Sustainability in Productive Agriculture: From Sustainability Goals to Meaningful Practices
C. Vaj, Corteva agriscience / The Agriculture Division of DowDuPont; A. Alix, Corteva Agrisciences / Risk Management; M. Bradascio, Corteva agriscience / The Agriculture Division of DowDuPont; E. Dereudre, Corteva agriscience; P. Marchesini, Corteva agriscience / The Agriculture Division of DowDuPont; A. Vermazeren, Corteva agriscience
The European “Green Deal” presents the European Commission’s plan towards a more sustainable economy. Within this plan, the Farm to Fork (F2F) policy aims to put in place a fair, healthy and environmentally friendly food production system. F2F is seeking to bring more sustainability and resiliency to the European food production system, impacting on food production itself but also, food supply and food consumption by European Countries. These new European policies are aligned with some of the FAO global goals for sustainable development. A successful implementation of these new policies in European farms will operate through an evolution of practices in the farming system, and require the involvement of all stakeholders involved in the food production chain. This poster will illustrate the challenges and opportunities offered by the F2F policy from the perspective of an agriculture company, especially focusing on the solutions that can serve multiple - and sometimes perceived as conflicting - objectives of maintaining productive agricultural systems in a sustainable use of the land alongside ensuring a high level of human and environmental protection. Using the example of Southern Europe, where a variety of crops are cultivated under a range of cultivation systems, the poster will present the goals defined for each of the 4 main pillars: farmers, consumers, environment and operations, and illustrate how these can be connected to concrete actions and practices responding to the F2F policy objectives, and how these embed into national policies and sustainability action plans. A focus will be made on goals related to environmental quality, i.e. soil health, water stewardship and biodiversity strategy. The poster will finally propose to discuss criteria regarding sustainability of plant protection products in relation to the F2F policy and their role in crop protection systems, including integrated pest/disease management.

7.02.06
Identifying the Presence of Antibiotic Resistant Bacteria and Multidrug Resistant Bacteria During Unplanned Water Reuse for Crop Irrigation
M. Sánchez, L. Martínez-García, B. Peinado, A. de Santiago Martín, R. Meffe, G. Teijón, V. Martínez Hernández, IMDEA Water Institute
There is an increasing scarcity of water worldwide, affecting mainly countries in the Mediterranean area, such as Spain. This has increased the interest in reusing water and its regulation. However, there is little information about effects of the unintentional use of surface water highly impacted by wastewater treatment plants (WWTPs) effluents, which is defined as unplanned water reuse. The discharge of WWTP effluents introduces in the environment different chemical and biological contaminants of emerging concern (CECs), including antibiotic, antibiotic resistant microorganisms and resistance genes. These microorganisms include some of those present in the human gut microbiome, such as Enterobacteriaceae and *Clostridium perfringens.* This research aimed to evaluate the presence of such biological CECs in the water-soil system, as a result of the unplanned water reuse for crop irrigation. To this aim, the presence of these microorganisms was analyzed in irrigation water, infiltrating water and agricultural soil throughout the maize growth period (3 months). Results showed no temporal changes in the number of target microorganisms. Then, a group of isolated Enterobacteriaceae (coliforms) were selected to study their antibiotic resistance phenotype. In the case of antibiotic resistant phenotype, isolates were classified as resistant or susceptible following the guide of Clinical and Laboratory Standards Institute (CLSI, 2018). Most of the isolated, both in water and soil, were susceptible to the eight antibiotics analyzed and only few showed clinical resistance. Also, few of the isolates showed resistance to three or more antibiotics, known as multidrug resistant (MDR) (6% in irrigation water, 11% in infiltrating water and 2.1 % in soil). Sometimes, this MDR phenotype could be associated with the presence of plasmids (mobile genetic elements). This possibility was studied in three MDR isolates, with resistance to five and eight antibiotics. Data showed the presence, in all isolates, of plasmids which were capable of increasing the resistance to several antibiotics when they were introduced into susceptible bacteria.

7.02.07
Bioavailability of 7-Hydroxy-6-Methoxycoumarin in Mediterranean Agricultural Soils: Effect of Soil Type

J.A. Galán, Instituto de Recursos NaturalesCSIC / Agroquímica Ambiental; B. Gámiz, R. Celis, Instituto de Recursos Naturales y Agrobiología de Sevilla. IRNAS-CSIC
Coumarins are a family of naturally-occurring compounds that, because of their fungicidal, bactericidal, insecticidal, nematicidal, and herbicidal properties, have been proposed as potential biopesticides. Screening of coumarins for phytotoxicity has shown that a C7 hydroxyl group seems to contribute significantly to their herbicidal activity. Umbelliferone, esculetin, and scopoletin are the most widespread 7-hydroxycoumarins in nature. The phytotoxicity of scopoletin (7-hydroxy-6-methoxycoumarin), described as the most common coumarin in higher plants, is well known, but its fate in soils is poorly understood. In this work, we conducted batch sorption and incubation experiments to characterize the sorption and dissipation of scopoletin in different Mediterranean agricultural soils, and then designed bioassay experiments to compare its phytotoxicity towards a model plant species (*Eruca vesicaria*) under soil-less and soil-pot conditions, to address the possible role of soils with different characteristics in the expression of its phytotoxic activity. The results showed that the expression of the phytotoxicity of scopoletin depended on soil type because of the influence that soil characteristics exerted on its sorption and persistence. Reversible hydrophobic interactions with soil organic matter controlled sorption in acid soils, and the role of mineral constituents (phyllosilicates and Fe/Al oxides) became more relevant in alkaline soils with high clay contents. Irreversible binding mechanisms, such as surface complexation reactions, appeared to contribute to the sorption of scopoletin by soil minerals. The degradation of scopoletin in soils was microbial-mediated and occurred more slowly in acid soils than in alkaline soils. As a result, the phytotoxic activity that scopoletin displayed in soilless experiments was expressed in acid soil but not in alkaline soil. Overall, our results illustrate how the bioactivity of hydroxycoumarins can be highly dependent on their sorption and persistence in the soil environment and show that knowledge of the environmental fate of natural pesticides can be of fundamental importance to understand biochemical-mediated interactions in ecosystems and the real potential of applying such interactions for pest control.
Acknowledgments: Funded by FEDER/Spanish Ministry of Science, Innovation, and Universities (Grants AGL2017-82141-R and PRE2018-083293). **Keywords:** bioavailability, biodegradation, biopesticides, sorption

7.02.08
Matrix Encapsulation of Chitosan Polymers and Essential Oil Alginate From Satureja Montana and Ruta Graveolens
D. Castillo, Universidad del Valle / Department of Chemistry; M.I. Paez, Universidad del Valle / Universidad del Valle
Essential oils are chemical compounds that are extracted from plants, which are characterized by being hydrophobic and highly volatile. They have been widely used in the pharmaceutical, food, and agriculture industries as flavorings, antioxidants, and biopesticides. Ruda (Ruta graveolens) and Satureja Montana essential oils have been shown to have nematicidal activity against Meloidogyne spp nematode. However, the biological activity of this kind of extracts can be decreased due to the volatilization, thermodegradation or photodegradation of their components, which difficult its application. In order to have a possible application in agriculture, Ruta graveolens and Satureja Montana essential oils were encapsulated in chitosan and alginate polymeric matrices. The encapsulation technique allows to have a tangible and more lasting nematicidal effect caused by the slow release of their active ingredients and thus it is expected to reduce the constant application as well as the needing of higher doses. To achieve this, the main components of each oil were first identified, employing the GC-MS technique. Then the essential oils were encapsulated in the polymeric matrices. The encapsulates were characterized by infrared spectroscopy, particle size, and zeta potential; in addition, the encapsulation efficiency and the pesticide loading were determined.

Global Plastic Contamination: a Journey towards Scientifically Informed Policies and Solutions

7.03.01
Risk Posed by Microplastics: Scientific Evidence and Public Perception
A. Catarino, Flanders Marine Institute / Oceans and Human Health; J. Kramm, C. Völker, ISOE - Institute for Social-Ecological Research; T. Henry, Heriot-Watt University / Institute of Life and Earth Sciences (ILES); G. Everaert, Flanders Marine Institute / Ocean and Human Health
Microplastic pollution has sparked interest from researchers, public, industries and regulators, due to concerns on their effects and to increasing reports on microplastics presence in the environment, household dust, drinking water and food items. However, so far only few studies have reported on microplastic risk for biota, and currently there has been no risk assessment for humans. Even though current evidence indicates that microplastic presents a low risk to biota, the public perception that microplastics are a serious environmental and health risk has motivated political action. The discrepancy between scientific evidence and public risk perception has generated debate among researchers within natural and social sciences. In this work, we reviewed the evidence on the risks of microplastics to ecosystem and human health, and considered the relation between evidence and public perception of microplastics risk. Despite the fact that risk assessment remains incomplete, scientific attention and the societal relevance of plastic pollution have led to a high public awareness of the topic (including on plastic pollution in general). To have enough weight of evidence to conclude on the risk of microplastics to the environment and to humans, there is a need to improve the quality and confidence on effects assessments. To be able to inform the public, regulators and industries on the risk of microplastics to humans there is a need to assess human exposure and effects, which are currently mostly unknown. Science communications should contextualize scientific findings in order to inform and not alarm the public. Research has shown that communicating uncertainty does not reduce trust in the communicator or “elicits psychological reactance”, thus “people can handle the truth”. We should not delay, however, precautionary measures to combat microplastic pollution until science is ready and reaches a consensus on the toxicity of microplastics for biota and humans. While, it has been argued that microplastics distract attention from other more important environmental issues, in the context of areas such as Cradle to Cradle, Safe by Design or Green Chemistry, which aim to develop more sustainable chemicals and materials, environmental issues do not compete with each other but are jointly addressed. This is also a matter of science communication.

7.03.04
Plastic Flux for Innovation and Business Opportunities in Flanders (PLUXIN)
L. Devriese, Flanders Marine Institute VLIZ; E. Toorman, Katholieke Universiteit Leuven; C. Janssen, J. Asselman, Ghent University / Blue Growth Research Lab; S. Sterckx, Flemish Institute for Technological Research VITO; E. Knaeps, Flemish Institute for Technological Research VITO / Remote Sensing; B. Teunkens, S. Van Damme, Universiteit Antwerpen; G. Everaert, Flanders Marine Institute / Ocean and Human Health
Plastics are globally dispersed and reported at increasing concentrations in marine ecosystems. Due to their persistence in aquatic environments the global plastic problem will last for decennia. Removal of plastic at the source prior to reaching the marine environment is instrumental. Hence, plastic detection methods and plastic remediation measures are urgently needed and may become obligatory in the future. A first prerequisite to take effective plastic remediation measures is to know where and when action should be taken. However, to date there is a critical knowledge gap about the whereabouts of plastics and about their flux towards the marine environment. This information is crucial to fast track cost-efficient plastic remediation measures. A central objective in the Flanders Innovation & Entrepreneurship (VLAIO) via the Blue Cluster funded project PLUXIN is to develop a two dimensional-horizontal (2DH) plastic dispersal model of the estuarine and harbor environments in Flanders. The model will be calibrated and validated with vertical movement experiments and field sampling data. Plastics will be identified from remote sensing reflectance data through image recognition algorithms (`Machine Learning'), hence resulting in an automated plastic detection method. This information in combination with in situ sampling will validate the 2DH-model. Flanders is a top region in terms of hydrologic and hydraulic expertise, and hosts key international companies active in offshore and coastal engineering and the circular economy. Flemish knowledge institutes are internationally recognized for their plastics-related expertise. This holistic project will integrate this knowhow and take essential steps to further our expertise. Based on the transdisciplinary PLUXIN research initiative a comprehensive and systematic picture of distribution of plastic in the aquatic environment will be obtained. Remote sensing and in-situ observations in combination with numerical models will contribute to our understanding of the sources, circulation patterns and fate of plastic in the aquatic environment.

Extended submission 7 - Think-outside-the-box (fundamentally new

concepts, innovative/controversial ideas, interdisciplinary issues)

7.04.01
Honey Bee Safety Evaluation of Insecticidal Compounds Employing Novel Molecular Approaches
D. Boaventura, Bayer Ag / Early Enviromental Safety; J. Haas, Rheinische Friedrich-Wilhelms-University of Bonn; Bayer AG; T. Leicher, R. Nauen, Bayer CropScience
Honey bee (*Apis mellifera*) early safety screening of novel chemical entities for crop protection largely relies on standardized laboratory toxicity bioassays. This framework is time/resource demanding and season-limited in temperate zones. With honey bee safety being an increasingly important topic in early phases of insecticide discovery there is an urgent need to develop tools that may be used year-round in early screens to support the discovery of novel lead compounds with a favorable safety profile towards bee pollinators. Here, we present an overview of some state-of-the-art methodologies which potentially could be implemented in an early toxicological screening framework to predict honey bee toxicity. Recent findings on the molecular determinants of insecticide and acaricide selectivity support the crucial role of cytochrome P450 monooxygenases (P450s) within the honey bee's defense system against xenobiotics. Therefore, our P450-centered approach aims to identify compounds which are readily detoxified by honey bees, thus providing tolerance and reducing adverse effects. A fluorescence-based high-throughput *in vitro* assay with recombinantly expressed honey bee P450 enzymes, for instance, has shown high potential of predicting and identifying pesticide selectivity. Moreover, the generation of transgenic *Drosophila melanogaster* lines ectopically expressing bee pollinator *CYP* genes such as honey bee *CYP9Q3* allowed functional studies on insecticide metabolization *in vivo*. The fly lines are publicly available and demonstrated potential to predict the P450-mediated detoxification capacity in *A. mellifera*. The novel toxicogenomics-based approaches complement and support pesticide risk assessment. Their implementation in early discovery is likely to complement or even partially replace the inventory used to select compounds with improved toxicological profiles to bee pollinators.

From Ecological Concepts to Ecological Scenarios for Mechanistic Effect Model Applications in Risk Assessment and Management

8.02.14
Toxicokinetic and Dynamic Modelling of Interacting Pesticide Mixtures in Daphnia magna and Chironomus riparius
N. Cedergreen, University of Copenhagen / PLEN - Department of Plant and Environmental Sciences; D. Rudnicka, University of Copenhagen; Y. Cao, University of Copenhagen / Department of Plant and Environmental Sciences
Chemical synergies is an area of general concern, because it devours the models we use to assess the risk of chemical mixtures. Understanding the mechanisms behind chemical interactions, and ideally being able to predict them, is therefore paramount in being able to identify synergists and evaluate to what degree they can cause synergistic interactions in different species. In this study we present the results of 10-12 mixtures of azole fungicides and a pyrethroid insecticide and 2-3 additional mixtures of organophosphates and pyrethroid insecticides conducted using a ray design in the two aquatic species *D. magna* and *C. riparius.* The azoles are proposed to act as synergists through their inhibitory effect on P450 monooxygenases responsible for biotransforming pyrethroids, and the organophosphates are known to inhibit esterases likewise involved in the biotransformation of pyrethroids. The mixtures cause different degrees of synergy both within and between species, and earlier comparisons between the degree of inhibition of enzymes responsible for biotransformation and the degree of synergy have not proven particular strong correlations. As toxicity is a dynamic process, so are interactions on toxic responses. In this study we revisit old data, add new data and re-analyse all data using a toxicokinetics and dynamic approach including both the effect of the synergists on biotransformation (toxicokinetics) of the pyrethroid, and the toxic effect of the synergists themselves to the organisms. The purpose is to predict the synergistic interactions based on *in vivo* measurements of the effects of the synergists on biotransformation.

Keyword Index

Accumulation. 1.02.11,1.02.13,1.04.05,1.10.01,2.05.04,2.05.08,2.09.16,2.11.12,2.12.18,3.07.09,3.07.12,3.11.11,3.12.20,3.12.30,3.13.01,3.13.08,3.15.06,3.15.15,3.15.20,3.15.27,3.15.30,3.15.33,3.16.09,3.16.14,4.01.04,4.03.23,4.04.04,4.04.15,4.08.08,4.11.07,4.14.28,4.17.02,6.01.10,7.02.03,8.01.01,8.01.02,8.01.03,8.01.04,8.02.01,8.02.02,8.02.03,8.02.04,8.02.05,8.02.06,8.02.07,8.02.08,8.02.09,8.02.10,8.02.11,8.02.12,8.02.13,8.03.01,8.03.02,8.03.03,8.03.04,8.03.05,8.04.02,8.04.03,8.04.04,8.04.05,8.04.06,8.04.07,8.05.01,8.05.02,8.05.03,8.05.04,8.05.05,8.05.06,8.06.01,8.06.02,8.06.03

Acute toxicity. 1.01.12,1.03.09,1.04.14,1.05.17,1.07.03,1.07.04,1.07.20,1.09.08,1.10.03,1.10.06,2.01.15,2.01.16,2.01.17,2.01.22,2.02.05,2.02.18,2.04.01,2.04.04,2.05.29,2.08.09,2.08.10,2.08.15,2.08.16,2.10.06,2.11.11,2.12.07,2.13.06,3.05.05,3.09.08,3.09.15,3.10.25,3.12.01,3.12.05,3.12.07,3.12.09,3.15.18,4.01.08,4.01.12,4.02.03,4.05.04,4.06.02,4.07.02,4.07.03,4.07.06,4.07.09,4.08.02,4.09.02,4.09.10,4.11.10,4.11.11,4.12.05,4.13.04,4.14.17,4.15.05,4.17.07,6.04.06

Adsorption. 3.04.06,3.11.21,3.15.16

Aquatic toxicity. 1.01.01,1.01.02,1.01.05,1.01.07,1.01.11,1.01.15,1.02.02,1.02.04,1.02.08,1.02.15,1.02.16,1.03.02,1.03.03,1.03.04,1.04.01,1.04.03,1.04.12,1.04.14,1.05.04,1.05.06,1.05.09,1.05.12,1.05.14,1.05.15,1.05.16,1.05.22,1.06.01,1.06.09,1.07.01,1.07.02,1.07.03,1.07.06,1.07.09,1.07.11,1.07.12,1.07.15,1.07.16,1.07.17,1.07.18,1.08.01,1.08.04,1.08.05,1.08.07,1.08.10,1.08.13,1.09.07,1.09.08,1.09.09,1.09.10,1.09.11,1.09.13,1.09.14,1.10.07,2.01.05,2.01.12,2.01.13,2.01.20,2.02.01,2.02.03,2.02.05,2.02.09,2.02.10,2.02.11,2.02.13,2.02.14,2.02.16,2.02.18,2.02.19,2.03.10,2.04.03,2.04.06,2.04.07,2.04.09,2.04.14,2.04.15,2.05.15,2.05.29,2.06.02,2.06.03,2.06.06,2.06.07,2.06.08,2.06.09,2.06.12,2.08.01,2.08.02,2.08.03,2.08.04,2.08.05,2.08.06,2.08.07,2.08.11,2.08.12,2.08.13,2.08.14,2.08.16,2.08.17,2.09.21,2.11.11,2.13.06,2.13.07,3.03.19,3.08.25,3.09.13,3.09.15,3.09.16,3.12.02,3.12.03,3.12.05,3.12.06,3.12.07,3.12.08,3.12.10,3.12.12,3.12.15,3.12.19,3.12.31,3.13.28,3.15.18,3.15.19,3.15.31,3.16.16,4.01.02,4.01.03,4.01.08,4.01.09,4.05.02,4.05.06,4.05.11,4.06.01,4.06.02,4.06.11,4.06.22,4.06.23,4.06.25,4.06.26,4.06.28,4.06.30,4.07.02,4.07.03,4.07.06,4.07.07,4.07.08,4.09.03,4.09.04,4.09.07,4.09.10,4.10.05,4.10.10,4.10.11,4.11.02,4.11.09,4.11.10,4.12.02,4.13.02,4.13.06,4.13.10,4.14.05,4.14.06,4.14.14,4.14.18,4.14.19,4.14.22,4.14.23,4.14.24,4.14.26,4.14.29,4.14.31,4.14.32,4.14.34,4.15.04,4.15.05,4.15.06,4.15.07,4.16.02,4.17.04,4.17.05,6.01.07,6.01.09,6.03.04,6.03.07,6.03.08,6.04.04,6.04.07,6.04.14,6.04.16,6.05.01,6.05.03,7.01.06

Atrazine. 4.01.03

Behavior. 1.03.01,1.03.03,1.03.04,1.03.05,1.03.06,1.03.07,1.05.05,1.05.06,1.06.05,1.07.09,1.07.11,1.07.13,1.07.19,1.10.09,2.01.01,2.01.02,2.01.03,2.01.04,2.01.05,2.01.06,2.01.07,2.01.09,2.01.10,2.01.11,2.01.13,2.01.14,2.01.15,2.01.16,2.01.17,2.01.18,2.01.19,2.01.20,2.01.21,2.01.22,2.01.23,2.08.11,2.08.12,2.08.13,2.10.08,2.11.12,3.04.02,3.09.24,3.11.16,3.11.23,3.12.14,3.14.04,3.15.13,3.16.07,3.16.12,3.16.16,4.04.07,4.05.01,4.06.32,4.08.10,4.09.06,4.12.03,4.14.15,4.14.32,4.15.08,6.01.05,6.05.01

Bioaccumulation. 1.01.10,1.02.07,1.02.14,1.03.03,1.07.15,1.08.04,1.09.05,2.02.17,2.05.03,2.05.09,2.05.12,2.05.21,2.05.27,2.06.04,2.09.13,2.10.05,2.10.06,2.10.07,2.11.02,2.11.06,2.12.01,2.13.01,2.13.03,2.13.05,2.13.08,3.02.04,3.03.01,3.03.04,3.05.05,3.06.06,3.06.08,3.07.05,3.07.07,3.07.08,3.09.20,3.09.26,3.10.24,3.11.19,3.11.25,3.12.04,3.12.19,3.12.26,3.12.29,3.13.18,3.13.19,3.13.20,3.13.23,3.13.24,3.13.25,3.13.26,3.13.27,3.15.24,3.15.30,3.15.36,3.15.40,3.16.14,4.03.01,4.03.02,4.03.03,4.03.04,4.03.06,4.03.08,4.03.11,4.03.12,4.03.13,4.03.14,4.03.15,4.03.16,4.03.17,4.03.19,4.03.20,4.03.21,4.03.22,4.05.05,4.12.07,4.17.08,4.17.09,6.02.04,6.03.01

Bioavailability. 1.01.05,1.01.06,1.02.10,1.10.05,2.09.11,2.09.15,2.09.17,2.09.19,2.09.20,2.10.04,2.11.04,2.11.05,2.12.14,2.13.05,3.01.08,3.01.10,3.02.03,3.03.12,3.05.17,3.09.01,3.09.02,3.09.07,3.09.08,3.09.09,3.09.10,3.09.11,3.09.12,3.09.13,3.09.23,3.11.19,3.12.21,3.13.18,3.13.19,3.14.02,3.16.08,4.03.19,4.04.08,4.04.11,4.07.08,4.14.26,4.15.10,7.02.07,7.03.06

Bioconcentration. 1.01.03,1.10.06,2.01.09,2.05.15,2.06.01,2.09.16,3.05.11,3.05.12,3.07.10,3.11.25,4.03.07,4.03.08,4.03.13,4.12.08

Biodegradation. 2.10.14,2.10.15,2.10.16,2.10.17,3.01.01,3.01.02,3.01.04,3.01.07,3.01.08,3.01.10,3.01.12,3.01.17,3.01.21,3.03.13,3.04.03,3.04.04,3.04.05,3.04.06,3.04.08,3.04.11,3.06.01,3.11.24,3.12.19,3.13.25,3.14.01,3.15.25,3.15.26,3.16.01,4.04.01,4.04.03,4.04.06,4.04.07,4.04.08,4.04.23,4.04.24,4.07.10,4.10.11,5.03.16,7.02.02,7.02.07

Biomonitoring. 1.04.01,1.04.03,1.04.07,1.04.08,1.04.09,1.04.13,1.07.17,1.07.19,1.09.03,1.09.10,1.09.12,1.09.18,2.01.05,2.02.06,2.02.17,2.02.19,2.03.07,2.03.08,2.05.01,2.05.02,2.05.03,2.05.04,2.05.05,2.05.06,2.05.07,2.05.08,2.05.09,2.05.13,2.05.14,2.05.19,2.05.22,2.05.24,2.05.25,2.05.27,2.06.05,2.07.01,2.07.06,2.07.07,2.08.08,2.10.14,2.11.05,2.12.02,2.12.04,2.12.05,2.12.10,2.12.17,3.03.18,3.03.22,3.05.05,3.05.07,3.08.12,3.09.03,3.09.14,3.12.11,3.12.27,3.12.30,3.13.19,3.13.20,3.13.21,3.15.23,3.15.40,4.02.09,4.02.11,4.03.20,4.04.14,4.08.09,4.11.02,4.11.05,4.12.03,4.12.06,4.12.11,4.17.08,4.17.09,6.01.05

Bioremediation. 2.10.14,2.10.17,3.04.08,3.04.09,3.07.10,3.09.02,3.16.08,4.04.01,4.04.02,4.04.06,4.04.07,4.04.08,4.04.09,4.04.10,4.04.18,4.04.19,4.04.20,4.04.24,4.11.15

Biotransformation. 1.01.03,1.01.04,1.01.09,1.09.19,1.10.06,2.03.12,3.03.13,3.04.02,3.04.07,3.04.10,3.04.11,3.07.12,3.12.04,3.12.19,4.03.06,4.03.07,4.03.08,4.03.10,4.03.12,4.03.14,4.03.15,4.03.17,4.04.21,4.11.15,4.14.21,4.14.28,5.01.16

Case study. 1.04.12,1.08.12,1.09.07,2.02.02,2.02.16,2.05.28,2.10.01,2.11.10,3.02.08,3.02.11,3.06.01,3.06.02,3.06.05,3.08.12,3.08.20,3.09.26,3.11.12,3.11.23,3.11.27,3.12.24,3.12.34,3.13.16,3.13.18,3.13.26,3.15.02,4.02.07,4.02.09,4.03.15,4.03.16,4.03.22,4.05.10,4.06.01,4.06.13,4.06.14,4.06.16,4.07.01,4.07.04,4.08.08,4.11.03,4.11.05,4.14.24,4.16.02,5.01.04,5.01.06,5.01.07,5.01.09,5.01.10,5.01.12,5.01.13,5.01.14,5.01.17,5.01.18,5.01.19,5.01.20,5.02.03,5.02.05,5.02.06,5.03.03,5.03.05,5.03.09,5.03.10,5.03.11,5.04.03,5.04.09,5.05.01,5.05.06,5.05.10,5.06.03,5.06.04,5.06.10,5.07.01,6.01.05,6.04.04

Chemical signalling. 1.07.05,1.09.15,2.01.13,2.02.11,3.05.07,3.06.05,4.04.07,4.11.02,4.17.05

Chronic toxicity. 1.02.12,1.04.04,1.06.04,1.06.05,1.09.11,2.01.04,2.01.12,2.01.16,2.01.21,2.02.04,2.03.05,2.03.08,2.05.15,2.05.16,2.06.06,2.09.14,2.09.24,2.10.04,2.12.16,3.09.18,3.12.09,3.12.13,4.02.01,4.05.04,4.06.14,4.06.15,4.06.17,4.06.18,4.06.24,4.07.08,4.08.02,4.09.08,4.10.07,4.10.08,4.11.10,4.14.33,4.17.04,6.01.06,6.03.04,6.04.10

Climate. 2.04.07,2.04.08,2.07.01,2.08.04,2.08.05,2.08.06,2.08.07,2.08.11,2.08.12,2.09.12,2.10.08,2.10.09,2.10.10,2.11.01,2.11.07,3.02.09,3.15.22,3.16.04,4.04.05,4.10.01,4.10.02,4.10.03,4.10.04,4.10.05,4.10.06,4.10.07,4.10.08,4.10.09,4.14.19,5.01.06,5.01.10,5.01.13,5.01.18,5.01.19,5.01.20,5.01.21,5.02.02,5.02.11,5.03.03,5.03.07,5.03.08,5.03.15,5.04.05,5.04.15,5.05.03,5.05.07,5.05.11

Cytotoxicity. 1.01.02,1.01.05,1.02.03,1.02.04,1.02.05,1.02.07,1.02.16,1.03.11,1.03.12,1.04.02,1.07.05,1.08.11,1.09.04,3.02.01,3.02.05,3.05.04,3.07.06,3.10.02,3.11.30,3.12.33,4.11.13,4.17.07,6.04.06

Decision analysis. 3.16.05,4.01.05,5.01.05,5.01.09,5.02.03,5.02.11,5.03.12,5.04.07,5.05.02,5.05.06,5.06.02,5.06.04,6.01.04,6.02.03,7.01.01

Degradation. 1.06.08,1.09.13,1.09.14,2.12.04,3.01.03,3.01.06,3.01.09

,3.01.13,3.01.14,3.01.16,3.01.19,3.03.08,3.04.10,3.04.11,3.05.15,3.08.15,3.08.16,3.10.01,3.10.03,3.10.07,3.11.16,3.11.22,3.12.22,3.12.33,3.13.31,3.13.32,3.14.01,3.15.02,3.15.07,3.16.03,3.16.16,4.01.10,4.09.01,4.09.03,4.09.04,4.11.06,4.14.13,5.05.03,6.01.07,6.01.08,7.02.07

Depuration. 1.06.09,3.12.20,3.12.26,4.02.12,4.03.23,7.02.05

Desorption. 3.11.27,3.12.33

Development. 1.02.02,1.02.06,1.02.15,1.03.01,1.03.02,1.03.04,1.03.05,1.03.07,1.05.06,1.05.11,1.05.17,1.05.21,1.06.05,1.06.07,1.06.09,1.07.04,1.07.06,1.07.12,1.07.18,1.09.16,2.02.11,2.05.19,3.01.13,3.05.03,3.06.10,3.08.06,3.11.18,4.01.11,4.06.09,4.06.19,4.08.05,4.10.10,4.15.07,5.01.02,5.01.17,5.03.01,5.03.16,5.04.07,5.05.04,5.06.03,6.01.06,6.04.01,6.05.06,7.01.07,7.03.04

Dioxins. 2.09.07,2.09.19

Ecological risk assessment. 1.01.01,1.01.02,1.01.08,1.01.10,1.01.13,1.01.14,1.01.15,1.01.16,1.02.14,1.06.07,1.07.18,1.08.10,1.09.10,1.09.12,2.01.08,2.01.12,2.01.19,2.01.20,2.02.01,2.02.06,2.02.08,2.02.12,2.02.15,2.02.17,2.03.06,2.03.10,2.03.11,2.04.07,2.04.09,2.04.12,2.04.13,2.05.10,2.05.17,2.05.21,2.07.02,2.07.05,2.08.02,2.08.03,2.08.04,2.08.10,2.08.17,2.09.02,2.09.03,2.09.07,2.09.08,2.09.09,2.09.11,2.09.16,2.09.18,2.09.21,2.09.22,2.09.24,2.10.04,2.10.11,2.11.04,2.11.08,2.11.09,2.12.01,2.12.06,2.13.09,3.02.10,3.02.13,3.03.12,3.07.01,3.07.04,3.07.08,3.08.17,3.09.10,3.10.16,3.11.02,3.11.10,3.11.14,3.12.17,3.12.21,3.12.25,3.12.31,3.13.16,3.13.20,3.14.05,3.15.24,4.01.01,4.01.05,4.01.06,4.01.11,4.01.12,4.02.02,4.02.03,4.02.07,4.03.03,4.03.05,4.03.09,4.03.11,4.03.13,4.04.16,4.04.21,4.05.04,4.05.07,4.05.08,4.05.09,4.05.10,4.06.04,4.06.05,4.06.06,4.06.07,4.06.08,4.06.10,4.06.11,4.06.12,4.06.13,4.06.14,4.06.15,4.06.16,4.06.17,4.06.18,4.06.20,4.06.22,4.06.23,4.06.24,4.06.25,4.06.26,4.06.27,4.06.28,4.06.29,4.06.31,4.08.02,4.08.03,4.08.04,4.08.05,4.09.02,4.09.03,4.09.04,4.09.05,4.10.06,4.11.03,4.11.06,4.12.05,4.13.10,4.13.11,4.14.01,4.14.04,4.14.06,4.14.07,4.14.10,4.14.13,4.14.15,4.14.16,4.14.18,4.14.23,4.14.31,4.15.08,4.16.01,4.16.02,4.16.03,4.16.05,4.17.01,6.01.01,6.01.03,6.01.04,6.01.05,6.02.01,6.02.05,6.03.01,6.03.05,6.03.06,6.03.07,6.04.02,6.04.03,6.04.14,6.06.01,7.01.02

Ecotoxicology. 1.01.03,1.01.04,1.01.11,1.01.12,1.01.15,1.01.16,1.01.17,1.02.04,1.02.07,1.02.08,1.02.15,1.02.16,1.03.01,1.03.02,1.03.06,1.03.11,1.04.02,1.04.05,1.04.06,1.04.07,1.04.08,1.04.09,1.04.10,1.04.11,1.04.13,1.05.11,1.05.12,1.05.15,1.05.16,1.05.20,1.06.01,1.06.02,1.06.03,1.06.04,1.06.05,1.06.07,1.06.08,1.07.01,1.07.03,1.07.05,1.07.08,1.07.12,1.07.17,1.07.19,1.07.20,1.08.01,1.08.02,1.08.03,1.08.04,1.08.05,1.08.06,1.08.07,1.08.08,1.08.09,1.08.10,1.08.12,1.08.13,1.09.05,1.09.07,1.09.09,1.09.12,1.09.15,1.10.01,1.10.02,1.10.05,1.10.07,1.10.09,2.01.01,2.01.02,2.01.03,2.01.06,2.01.08,2.01.09,2.01.14,2.01.15,2.01.17,2.01.18,2.01.19,2.01.21,2.01.22,2.02.01,2.02.02,2.02.04,2.02.05,2.02.07,2.02.09,2.02.14,2.02.15,2.02.19,2.03.01,2.03.02,2.03.04,2.03.05,2.03.06,2.03.08,2.04.01,2.04.02,2.04.03,2.04.05,2.04.09,2.04.10,2.04.11,2.04.12,2.05.01,2.05.02,2.05.04,2.05.05,2.05.06,2.05.07,2.05.09,2.05.10,2.05.13,2.05.14,2.05.15,2.05.17,2.05.18,2.05.19,2.05.22,2.05.23,2.05.25,2.06.06,2.06.07,2.06.10,2.06.11,2.07.06,2.08.01,2.08.04,2.08.05,2.08.06,2.08.08,2.08.09,2.08.10,2.08.15,2.08.16,2.08.17,2.09.01,2.09.03,2.09.04,2.09.07,2.09.08,2.09.09,2.09.10,2.09.15,2.09.21,2.09.24,2.10.02,2.10.03,2.10.06,2.10.09,2.10.10,2.11.02,2.11.04,2.11.07,2.11.11,2.12.06,2.12.09,2.12.10,2.12.12,2.12.15,2.12.16,2.12.17,2.13.01,2.13.03,2.13.06,2.13.07,2.13.08,3.01.16,3.03.01,3.03.18,3.03.19,3.03.20,3.05.04,3.05.13,3.08.02,3.08.05,3.08.25,3.09.14,3.09.16,3.09.18,3.09.19,3.09.20,3.09.24,3.09.25,3.11.14,3.11.19,3.11.21,3.12.01,3.12.02,3.12.03,3.12.05,3.12.06,3.12.08,3.12.09,3.12.12,3.12.13,3.12.14,3.12.21,3.12.23,3.13.23,3.13.24,3.13.28,3.15.06,4.01.08,4.01.12,4.02.01,4.02.03,4.04.18,4.04.22,4.06.01,4.06.02,4.06.03,4.06.06,4.06.07,4.06.09,4.06.12,4.06.13,4.06.14,4.06.16,4.06.18,4.06.20,4.06.21,4.06.24,4.06.25,4.06.32,4.07.02,4.07.07,4.07.08,4.07.10,4.08.04,4.08.05,4.08.06,4.08.07,4.08.09,4.08.10,4.09.02,4.09.07,4.09.08,4.09.09,4.09.10,4.10.06,4.10.08,4.10.10,4.10.11,4.11.03,4.12.02,4.12.04,4.12.07,4.12.12,4.14.07,4.14.09,4.14.12,4.14.15,4.14.16,4.14.19,4.14.23,4.14.31,4.14.33,4.14.34,4.15.05,4.15.06,4.15.07,4.15.08,4.15.09,4.15.10,4.17.01,4.17.03,4.17.04,5.03.17,6.01.09,6.01.10,6.02.09,6.03.01,6.03.05,6.03.06,6.03.09,6.04.09,6.04.10,6.04.12,6.04.13,6.04.15,6.04.16,6.05.06,6.06.02,7.01.03,7.04.01,8.02.14

Elimination. 1.02.11,2.05.08,3.06.08,3.11.01,3.16.15,4.03.07,7.02.02

Endocrine disruption. 1.04.05,1.05.01,1.05.02,1.05.03,1.05.04,1.05.05,1.05.06,1.05.07,1.05.08,1.05.09,1.05.10,1.05.11,1.05.12,1.05.13,1.05.14,1.05.15,1.05.16,1.05.17,1.05.18,1.05.19,1.05.20,1.05.21,1.05.22,1.06.01,1.06.02,1.06.03,1.06.04,1.07.06,1.07.08,1.07.13,1.08.03,1.08.08,1.09.01,1.09.02,1.10.01,2.01.13,2.01.21,2.02.10,2.02.11,2.02.12,2.02.13,2.02.14,2.02.15,2.05.02,2.05.13,2.13.05,3.02.03,3.02.04,3.02.11,3.03.16,3.03.17,3.03.21,3.05.05,3.11.21,3.16.15,4.02.11,4.09.10,4.11.01,4.12.10,4.13.04,4.13.07,6.01.01,6.03.04

Genotoxicity. 1.01.17,1.02.07,1.04.01,1.04.03,1.06.06,1.07.02,1.08.06,1.10.03,2.06.12,2.07.06,2.09.09,2.09.10,2.10.07,2.11.09,3.01.18,3.03.17,3.03.21,3.10.02,4.04.04,4.11.13,4.12.01

Ground water. 1.09.13,2.09.06,3.03.02,3.09.04,3.10.05,3.10.06,3.10.13,3.10.18,3.10.27,3.13.10,3.15.21,4.03.24,4.04.10

Growth. 1.04.04,1.05.11,1.05.14,1.06.06,1.10.09,2.01.16,2.02.02,2.04.03,2.04.15,2.05.03,2.06.02,2.08.14,2.09.05,3.12.05,3.14.05,3.14.06,3.15.22,3.16.01,4.06.19,4.06.20,6.01.06

Herbicides. 1.09.06,1.09.13,2.01.11,2.04.07,2.04.12,2.04.13,2.05.07,2.05.14,3.03.17,3.05.10,3.10.05,3.10.20,3.10.22,3.15.10,3.16.16,4.06.04,4.06.22,4.10.04,4.11.07,4.11.08,4.17.03

Hormesis. 3.16.01,4.04.14

Human health. 1.03.12,1.05.01,1.05.04,1.05.20,1.07.04,1.07.13,1.09.04,1.09.07,1.10.03,2.03.10,2.03.11,2.03.12,2.05.12,3.02.01,3.02.04,3.02.05,3.02.06,3.02.07,3.02.10,3.02.13,3.03.07,3.03.21,3.07.09,3.08.05,3.11.09,3.11.25,3.12.16,3.12.28,3.12.34,3.13.11,3.13.13,3.13.22,3.13.29,3.15.11,3.16.06,3.16.13,3.16.14,4.08.08,4.11.07,4.11.12,4.11.13,4.13.08,4.17.06,4.17.07,5.03.15,5.04.14,5.05.08,5.07.02,6.02.08,6.02.10,7.01.02,7.01.04,7.01.08,7.01.09,7.01.13,7.02.04,7.03.01,7.03.05,7.03.06

Immunotoxicity. 1.01.17,1.02.01,1.02.02,1.02.03,1.02.05,1.04.06,1.04.11,2.01.23,2.07.06,2.11.09,3.12.08,4.11.16

In situ. 1.04.01,1.07.17,2.02.06,2.02.08,2.11.01,2.11.05,2.13.01,3.02.09,3.04.02,3.09.26,3.11.05,3.11.06,3.11.12,3.11.17,3.12.11,3.14.08,3.15.32,4.03.02,4.04.23,4.10.11,7.03.04

Insecticides. 1.03.02,1.03.06,1.03.10,1.07.14,1.08.03,1.08.06,1.08.08,1.08.09,1.10.02,2.02.04,2.02.12,2.02.20,2.05.20,2.06.01,2.06.07,2.06.08,2.07.01,2.07.04,2.08.06,2.09.22,3.16.08,4.06.30,4.08.06,4.10.06,4.10.07,4.12.03,4.16.03,4.17.03

Landscape. 1.04.02,2.01.07,2.01.19,2.01.20,2.04.13,2.06.04,2.07.01,2.07.02,2.07.03,2.07.04,2.07.05,3.03.15,3.10.13,3.10.14,3.10.15,3.10.16,3.10.17,3.10.19,4.01.06,4.05.09,4.05.10,4.06.30,4.06.31,4.08.06,5.02.03

Life-cycle assessment. 3.14.07,4.08.09,5.01.01,5.01.02,5.01.03,5.01.04,5.01.05,5.01.06,5.01.07,5.01.08,5.01.09,5.01.10,5.01.11,5.01.12,5.01.13,5.01.14,5.01.15,5.01.16,5.01.17,5.01.18,5.01.19,5.01.21,5.02.01,5.02.02,5.02.03,5.02.04,5.02.05,5.02.06,5.02.08,5.02.09,5.02.10,5.02.11,5.02.12,5.02.13,5.03.01,5.03.02,5.03.04,5.03.05,5.03.06,5.03.07,5.03.08,5.03.09,5.03.10,5.03.11,5.03.12,5.03.13,5.03.15,5.03.16,5.04.01,5.04.02,5.04.04,5.04.06,5.04.07,5.04.08,5.04.09,5.04.10,5.04.11,5.04.12,5.04.13,5.04.14,5.04.15,5.05.01,5.05.02,5.05.03,5.05.04,5.05.05,5.05.06,5.05.07,5.05.08,5.05.09,5.05.10,5.05.11,5.05.12,5.05.13,5.05.14,5.06.01,5.06.02,5.06.04,5.06.05,5.06.06,5.06.07,5.06.08,5.06.09,5.06.10,5.07.01,5.07.02,7.01.01,7.01.05

Mesocosm. 1.10.01,2.02.03,2.03.04,2.06.07,2.06.08,2.09.22,2.10.13,3.11.07,3.12.04,3.12.10,3.12.23,3.16.07,4.02.05,4.04.21,4.06.28,4.06.29,4.14.16,4.17.05,6.03.08

Metabolism. 1.01.04,1.01.09,1.04.10,1.05.18,1.05.19,1.06.01,1.06.03,1.07.14,1.08.13,1.09.01,1.09.03,1.10.02,2.01.18,2.01.23,2.02.09,2.02.10,2.03.05,2.03.12,2.04.15,2.05.16,2.06.09,2.08.14,2.08.15,2.12.16,2.13.02,2.13.03,2.13.08,3.04.08,3.06.06,3.07.07,3.07.11,3.12.15,4.01.04,4.03.08,4.05.05,4.14.21,4.14.30,4.14.32,4.17.02,7.01.10

Metalloids. 2.10.10,2.13.02,2.13.03,3.09.01,3.09.06,3.12.14,4.02.08,4.04.11,4.14.30

Metals. 1.01.15,1.02.12,1.02.13,1.04.06,1.04.07,1.04.08,1.04.09,1.04.

10,1.06.09,1.07.12,1.08.01,1.08.07,1.09.05,2.02.07,2.02.09,2.02.17,2.02.18,2.04.01,2.05.04,2.05.05,2.05.18,2.05.25,2.05.29,2.06.03,2.06.04,2.06.06,2.06.09,2.06.11,2.08.01,2.08.02,2.08.17,2.09.20,2.09.21,2.10.06,2.10.09,2.11.02,3.02.07,3.03.03,3.05.07,3.09.02,3.09.04,3.09.05,3.09.07,3.09.08,3.09.09,3.09.10,3.09.11,3.09.12,3.09.13,3.09.14,3.09.16,3.09.17,3.09.18,3.09.19,3.09.20,3.09.21,3.09.22,3.09.24,3.09.25,3.09.26,3.16.14,4.02.06,4.02.08,4.03.03,4.03.11,4.03.19,4.03.20,4.04.04,4.04.05,4.04.10,4.04.23,4.05.06,4.05.07,4.06.26,4.06.27,4.14.22,4.15.10,4.16.02,4.17.02,4.17.06,5.01.07,5.04.03,5.05.09,5.05.10,6.01.06,6.01.09,6.01.10,6.03.09,6.04.09,6.04.17

Microcosm. 1.09.15,2.04.08,2.06.03,2.08.07,2.10.05,2.10.12,3.04.04,3.09.17,3.14.04,4.04.10,4.04.20,4.05.06,4.14.25,6.03.07

Mixture toxicity. 1.01.16,1.02.08,1.02.17,1.03.11,1.04.12,1.07.08,1.09.01,1.09.06,2.01.12,2.02.04,2.02.10,2.02.16,2.04.01,2.04.04,2.05.28,2.06.03,2.08.09,2.08.16,2.09.07,2.10.03,2.13.07,3.01.01,3.01.03,3.02.07,3.03.16,3.08.25,3.09.05,3.09.07,3.09.17,3.09.19,3.12.03,3.12.09,3.12.15,3.12.33,4.01.03,4.02.02,4.05.06,4.06.21,4.06.25,4.09.05,4.09.08,4.10.10,4.11.01,4.11.08,4.11.11,4.12.01,4.12.03,4.12.04,4.12.05,4.12.10,4.12.11,4.12.12,4.13.02,4.13.06,4.13.07,4.13.08,4.13.10,4.13.11,4.13.12,4.13.14,4.13.15,4.14.30,4.16.04,4.17.01,6.04.07,8.02.14

Monitoring. 1.01.17,1.02.10,1.07.16,1.09.02,1.09.20,2.01.08,2.02.13,2.05.23,2.05.26,2.07.03,2.11.08,2.11.09,2.12.07,2.12.13,2.12.14,3.01.12,3.01.20,3.02.01,3.03.02,3.03.04,3.04.03,3.05.02,3.05.08,3.05.11,3.05.12,3.05.14,3.06.01,3.06.03,3.07.02,3.08.01,3.08.04,3.08.05,3.08.06,3.08.07,3.08.08,3.08.09,3.08.10,3.08.13,3.08.16,3.08.18,3.08.19,3.08.23,3.08.24,3.09.06,3.09.14,3.10.04,3.10.05,3.10.06,3.10.22,3.10.23,3.11.03,3.11.04,3.11.08,3.11.10,3.11.11,3.11.13,3.11.15,3.11.17,3.11.20,3.11.21,3.11.26,3.11.27,3.11.28,3.12.22,3.12.28,3.12.30,3.13.02,3.13.05,3.13.10,3.13.12,3.13.13,3.13.14,3.13.15,3.13.16,3.13.17,3.15.01,3.15.02,3.15.04,3.15.08,3.15.09,3.15.10,3.15.13,3.15.14,3.15.17,3.15.20,3.15.26,3.15.29,3.15.35,3.16.05,3.16.06,3.16.07,3.16.09,3.16.11,3.16.13,4.02.10,4.03.21,4.06.22,4.08.10,4.11.04,4.11.10,4.12.08,4.12.09,4.13.02,4.13.04,4.13.15,4.14.10,4.15.01,4.15.02,5.03.07,5.04.12,5.05.02,6.01.02,6.05.04,7.01.09,7.01.10,7.01.12,7.01.13,7.03.04,7.03.05

Multimedia. 3.01.19,3.01.21,3.02.13,3.06.04,3.08.13,3.15.05,3.15.24,3.15.33,3.15.37,4.03.01,4.13.14,4.15.02,6.02.07,6.05.02,6.05.05,6.05.07

Mutagenicity. 2.12.03,3.02.03,6.03.09

Nanomaterials. 1.02.01,1.02.02,1.02.03,1.02.04,1.02.05,1.02.06,1.02.08,1.02.09,1.02.10,1.02.11,1.02.12,1.02.13,1.02.14,1.02.15,1.02.16,1.02.17,1.02.19,1.08.07,1.09.05,1.09.06,2.03.01,2.03.02,2.04.02,2.08.13,2.09.13,2.09.14,2.10.02,2.10.03,2.10.04,2.10.05,2.10.12,2.10.13,2.11.11,2.11.12,2.13.08,3.02.02,3.02.05,3.08.02,3.08.06,3.08.24,3.09.07,3.11.14,3.11.16,3.11.24,3.11.29,3.12.14,3.12.16,3.12.26,3.12.29,3.12.31,3.14.03,3.16.12,4.01.10,4.01.11,4.01.12,4.03.04,4.04.11,4.04.22,4.07.06,6.01.08,6.04.01,6.04.02,6.04.04,6.04.05,6.04.06,6.04.07,6.04.08,6.04.09,6.04.10,6.04.11,6.04.12,6.04.13,6.04.14,6.04.15,6.04.16,6.04.17,6.05.06,7.03.07

Natural resource damage. 2.05.28,2.06.01,3.15.22,5.04.02,5.04.15

Nutrients. 2.04.08,2.09.12,2.10.02,2.10.13,2.10.17,2.13.02,3.01.12,4.02.12,4.03.24,4.04.19,4.04.20,4.11.08,5.02.13,5.05.05,5.05.11,5.06.03,6.04.03

Partitioning. 1.01.06,2.05.27,3.01.01,3.01.02,3.07.04,3.15.24,3.15.33,3.15.36,3.15.37,4.04.03,4.04.08,4.04.24,4.09.05,4.12.04,4.15.04,5.03.01,7.03.06

Passive sampling. 1.09.20,2.12.10,3.02.04,3.03.01,3.03.09,3.05.03,3.05.17,3.08.08,3.13.17,3.15.09,3.15.12,3.15.13,3.15.16,3.15.32,4.03.01,4.04.06,4.11.04,4.12.02,4.12.10,4.12.11,4.12.12

Persistent. 1.02.03,1.07.11,1.10.03,2.05.03,2.05.12,2.05.27,2.11.03,2.11.04,2.11.08,2.12.04,2.12.17,3.01.05,3.01.06,3.01.07,3.01.08,3.01.09,3.01.10,3.01.15,3.01.18,3.01.19,3.01.20,3.01.21,3.03.12,3.04.05,3.04.07,3.05.09,3.05.13,3.05.15,3.06.05,3.06.06,3.06.09,3.06.10,3.08.14,3.08.20,3.08.22,3.09.02,3.11.13,3.11.14,3.11.16,3.12.18,3.12.27,3.13.01,3.13.02,3.13.04,3.13.05,3.13.07,3.13.08,3.13.10,3.13.11,3.13.12,3.13.13,3.13.14,3.13.16,3.13.17,3.13.18,3.13.19,3.13.20,3.13.22,3.13.24,3.13.25,3.13.27,3.13.31,3.13.32,3.13.33,3.14.02,3.14.04,3.15.07,3.15.08,3.15.09,3.15.10,3.15.11,3.15.12,3.15.18,3.15.19,3.15.21,3.15.25,3.15.26,3.15.27,3.15.31,3.15.34,3.16.03,3.16.11,3.16.13,4.01.10,4.09.01,4.09.06,4.12.01,4.13.08,4.14.01,4.15.03,4.17.08,4.17.09,6.02.04,6.02.06,6.02.07,6.03.01,6.05.01

Personal care product. 1.02.17,1.09.03,2.05.02,3.01.01,3.01.02,3.03.11,3.03.22,3.04.10,3.07.01,3.12.04,3.12.32,3.15.16,4.05.09,4.07.07,4.14.05,4.14.06,5.05.08,6.01.03,6.02.10,6.04.11,7.01.08

Pesticide. 1.01.12,1.01.16,1.03.09,1.03.11,1.04.02,1.04.13,1.04.14,1.07.09,1.07.15,1.08.09,1.09.03,1.09.14,2.01.05,2.01.22,2.02.01,2.02.16,2.02.20,2.03.05,2.04.05,2.04.08,2.05.07,2.05.08,2.05.14,2.05.17,2.05.20,2.05.25,2.06.02,2.06.05,2.06.09,2.06.10,2.08.05,2.08.07,2.08.08,2.08.11,2.08.12,2.08.14,2.09.01,2.09.02,2.09.03,2.09.15,2.12.01,2.12.03,2.12.04,2.12.05,2.12.07,2.12.08,2.12.09,2.12.14,2.12.17,2.13.09,3.01.03,3.01.15,3.01.16,3.03.06,3.03.14,3.03.15,3.04.09,3.05.03,3.05.06,3.05.10,3.05.11,3.05.12,3.05.13,3.06.08,3.06.10,3.07.01,3.07.02,3.10.01,3.10.02,3.10.03,3.10.04,3.10.06,3.10.07,3.10.08,3.10.09,3.10.10,3.10.12,3.10.13,3.10.14,3.10.15,3.10.17,3.10.18,3.10.19,3.10.20,3.10.21,3.10.23,3.10.25,3.10.26,3.10.27,3.13.06,3.15.02,3.15.09,3.15.10,3.15.12,3.15.19,3.15.27,3.15.34,3.15.40,3.16.04,3.16.12,4.01.09,4.03.02,4.03.23,4.03.24,4.04.12,4.05.02,4.05.07,4.05.08,4.06.05,4.06.06,4.06.09,4.06.10,4.06.11,4.06.13,4.06.21,4.06.24,4.06.31,4.06.32,4.07.11,4.08.02,4.08.03,4.08.04,4.08.05,4.08.07,4.08.08,4.08.09,4.10.03,4.10.09,4.12.05,4.12.06,4.12.08,4.12.09,4.12.12,4.13.05,4.16.03,4.16.05,4.16.06,4.17.01,5.02.04,5.05.12,6.02.01,6.02.02,6.03.06,6.04.03,6.04.07,7.02.01,7.02.04,7.02.08,7.04.01

Pharmaceuticals. 1.01.02,1.03.03,1.07.02,1.07.07,1.07.15,1.08.13,1.09.11,1.09.14,1.09.16,1.09.18,1.10.05,1.10.06,2.01.04,2.01.06,2.01.09,2.01.10,2.01.14,2.02.14,2.03.04,2.03.11,2.05.06,2.05.19,2.06.12,2.12.12,2.12.13,2.13.05,3.01.14,3.03.01,3.03.08,3.03.11,3.03.23,3.04.06,3.04.10,3.05.04,3.06.08,3.06.10,3.07.01,3.07.02,3.07.04,3.07.05,3.07.07,3.07.08,3.07.09,3.07.10,3.07.11,3.07.12,3.12.06,3.13.06,3.15.06,3.15.21,3.15.23,3.16.06,4.02.04,4.03.07,4.03.09,4.03.12,4.04.13,4.10.02,4.10.03,4.14.01,4.14.02,4.14.03,4.14.05,4.14.06,4.14.07,4.14.08,4.14.09,4.14.10,4.14.11,4.14.12,4.14.13,4.14.14,4.14.15,4.14.16,4.14.17,4.14.18,4.14.19,4.14.20,4.14.22,4.14.24,4.14.25,4.14.26,4.14.27,4.14.28,4.14.29,4.14.30,4.14.32,4.14.33,4.14.34,4.17.02,4.17.05,5.01.20,6.01.01,7.01.06,7.01.08,7.01.10,7.01.11,7.02.02

Policy analysis. 4.07.01,5.02.01,5.02.02,5.02.04,5.02.07,5.02.08,5.02.09,5.03.01,5.04.01,5.04.09,5.07.01,6.01.01,6.02.06,6.02.09,6.06.01,7.01.04,7.03.04,7.03.07

Regulation. 1.01.07,1.01.08,1.01.10,1.02.14,1.03.05,2.01.01,2.01.02,2.05.24,2.05.26,2.09.04,2.09.06,2.12.03,2.12.07,2.12.08,2.12.11,2.12.15,2.13.09,3.01.08,3.01.10,3.01.20,3.03.05,3.03.14,3.05.08,3.09.08,3.09.12,3.10.05,3.10.15,3.10.18,3.13.02,3.15.20,3.16.04,3.16.05,4.01.01,4.02.06,4.03.13,4.05.11,4.06.08,4.07.13,4.08.03,4.13.10,4.14.04,4.14.12,4.14.14,4.16.05,6.02.01,6.02.03,6.02.04,6.02.05,6.02.06,6.02.07,6.03.02,6.03.03,6.03.04,6.04.01,6.04.02,6.04.03,6.04.11,6.04.14,7.02.01,7.03.05,7.03.07

Remediation. 1.02.12,1.09.06,3.03.02,3.06.01,3.09.23,3.10.09,3.13.01,3.13.03,4.04.03,4.04.04,4.04.05,4.04.11,4.04.14,4.04.16,4.04.22,4.12.09,4.14.17,6.02.03,6.06.01

Reproduction. 1.01.13,1.01.14,1.05.14,1.05.21,1.06.07,1.08.10,1.09.11,2.02.13,2.04.12,2.09.05,2.09.10,2.09.13,2.09.24,2.12.02,4.06.17,4.17.04

Risk assessment. 1.01.07,1.01.09,1.01.10,1.02.19,1.05.01,1.05.12,1.07.13,1.08.02,1.09.04,1.10.02,2.01.01,2.01.02,2.01.07,2.01.11,2.01.23,2.02.20,2.03.01,2.03.02,2.04.05,2.04.06,2.04.13,2.04.14,2.09.01,2.09.04,2.09.05,2.09.14,2.09.17,2.10.12,2.11.10,2.12.05,2.12.12,2.12.13,2.12.14,2.12.15,3.01.04,3.01.05,3.01.06,3.01.18,3.01.19,3.01.20,3.01.21,3.02.05,3.02.06,3.02.07,3.02.10,3.03.15,3.04.07,3.05.10,3.05.15,3.05.17,3.06.03,3.06.04,3.08.02,3.08.03,3.08.05,3.08.18,3.09.05,3.09.09,3.09.11,3.09.22,3.09.23,3.09.24,3.10.01,3.10.04,3.10.06,3.10.07,3.10.08,3.10.09,3.10.10,3.10.11,3.10.13,3.10.17,3.10.21,3.10.22,3.10.23,3.10.26,3.10.27,3.11.02,3.11.24,3.11.25,3.12.16,3.12.34,3.13.03,3.15.05,3.15.08,3.15.19,3.15.38,3.16.13,3.16.15,4.01.05,4.01.09,4.01.10,4.01.11,4.02.04,4.03.10,4.03.17,4.03.21,4.03.22,4.03.24,4.04.18,4.05.01,4.05.02,4.05.11,4.06.32,4.07.05,4.07.11,4.07.12,4.07.13,4.08.04,4.08.06,4.08.10,4.09.02,4.10.01,4.10.07,4.10.09,4.11.05,4.11.09,4.12.07,4.13.04,4.13.06,4.13.14,4.13.15,4.14.02,4.14.03,4.14.05,4.14.07,4.14.08,4.14.09,4.14.11,4.14.12,4.14.14,4.14.26,4.16.04,4.16.06,5.04.02,5.0

6.06,6.01.07,6.02.02,6.02.08,6.03.09,6.04.01,6.04.05,6.04.10,6.04.11,6.04.12,6.04.13,6.05.05,7.01.02,7.03.01,7.03.03,7.03.05,7.04.01

Risk management. 1.07.18,2.08.13,2.09.06,2.11.10,2.12.15,3.01.16,3.10.09,3.10.10,3.10.11,3.10.12,3.10.20,3.10.21,3.13.03,3.15.08,3.15.31,4.02.07,4.06.03,4.10.02,4.11.09,4.13.02,4.13.14,4.14.03,4.16.01,4.17.06,5.04.01,6.02.01,6.02.02,6.02.04,6.02.06,6.02.07,6.02.09,6.02.10,7.01.04,7.02.01,7.03.02

Roadway. 3.11.09,4.15.02

Sediment. 1.02.13,1.10.07,2.02.06,2.02.07,2.03.04,2.06.01,2.11.06,3.01.14,3.04.02,3.04.03,3.05.13,3.05.17,3.06.02,3.06.03,3.08.09,3.08.23,3.09.19,3.09.21,3.11.10,3.11.11,3.11.13,3.12.10,3.12.22,3.12.28,3.13.05,3.13.15,3.15.14,3.15.17,3.15.20,3.15.29,3.16.09,4.03.19,4.04.18,4.04.23,4.05.01,4.09.07,4.14.23,4.14.24,4.15.04,4.15.06,4.16.04,6.01.10,6.03.07,6.03.08,6.06.01

Soil. 1.08.02,1.08.06,2.03.02,2.03.11,2.04.05,2.09.01,2.09.03,2.09.04,2.09.05,2.09.08,2.09.09,2.09.10,2.09.12,2.09.13,2.09.14,2.09.15,2.09.16,2.09.17,2.09.18,2.09.20,2.09.22,2.09.23,2.10.01,2.10.02,2.10.03,2.10.08,2.10.09,2.10.10,2.10.12,2.10.13,2.10.14,2.10.15,2.10.16,2.10.17,2.11.08,2.13.09,3.01.03,3.01.07,3.01.15,3.03.11,3.03.12,3.07.07,3.07.12,3.09.03,3.09.04,3.09.06,3.09.23,3.10.08,3.11.13,3.12.08,3.13.01,3.13.02,3.13.05,3.13.25,3.13.27,3.14.01,3.14.02,3.14.03,3.14.04,3.14.05,3.14.06,3.15.25,3.15.26,3.15.27,3.15.34,3.16.01,3.16.03,3.16.07,3.16.08,4.02.08,4.04.01,4.04.05,4.04.06,4.04.14,4.04.16,4.04.19,4.14.01,4.14.13,4.14.25,4.15.09,4.16.03,4.17.03,5.02.07,5.05.03,5.05.09,6.03.02,6.04.09,6.05.04,7.01.03,7.02.06

Sorption. 2.09.17,3.01.17,3.13.08,3.13.27,3.15.14,3.15.36,3.16.03,4.04.12,4.04.13,7.02.07

Spatial. 2.01.07,2.05.20,2.07.02,2.07.05,2.09.12,3.08.12,3.10.14,3.10.16,3.10.18,3.10.19,3.11.09,3.11.10,3.13.15,3.13.17,3.13.26,3.15.05,3.15.25,4.05.01,4.05.07,4.05.08,4.06.30,5.02.09,5.05.04,6.01.03,6.01.04

Speciation. 1.01.05,1.10.08,2.03.12,2.13.02,3.06.02,3.09.01,3.09.03,3.12.17

Statistics. 1.01.08,1.01.13,1.01.14,1.08.11,1.08.12,2.04.11,2.05.20,2.09.02,3.03.09,3.06.04,3.06.05,3.08.16,3.08.18,3.15.36,3.15.38,3.16.05,4.01.01,4.06.02,4.06.04,4.06.08,4.13.11,5.04.12,6.03.02,6.03.06,6.06.02

Stormwater. 3.11.05,3.11.06,4.15.03

Surface water. 1.04.03,1.05.22,1.07.16,1.09.17,2.01.17,2.03.08,2.06.08,2.06.10,2.08.01,2.08.02,2.11.03,2.11.06,2.11.12,3.01.14,3.03.04,3.03.08,3.03.15,3.04.04,3.04.07,3.06.07,3.07.04,3.07.05,3.08.07,3.08.11,3.08.13,3.08.20,3.08.23,3.10.01,3.10.04,3.10.08,3.10.10,3.10.11,3.10.12,3.10.19,3.10.20,3.10.21,3.10.25,3.11.06,3.11.08,3.11.22,3.11.23,3.11.26,3.12.17,3.12.23,3.12.28,3.13.04,3.13.10,3.13.12,3.13.14,3.15.01,3.15.04,3.15.17,3.15.18,3.15.40,3.16.09,4.02.01,4.02.09,4.02.10,4.02.11,4.02.12,4.05.08,4.05.09,4.06.31,4.07.05,4.09.04,4.10.01,4.10.03,4.11.01,4.11.03,4.11.06,4.11.13,4.11.15,4.12.06,4.12.08,4.12.10,4.12.11,4.13.05,4.14.02,4.14.11,4.14.18,4.14.20,5.03.15,6.02.02,6.04.04,7.02.06

Sustainability. 2.05.28,2.09.11,2.10.01,2.10.15,3.07.06,3.11.01,4.10.02,4.11.11,5.01.02,5.01.05,5.01.07,5.01.10,5.01.11,5.01.16,5.01.17,5.01.18,5.01.20,5.02.01,5.02.02,5.02.05,5.02.06,5.02.07,5.02.08,5.02.09,5.03.04,5.03.06,5.03.09,5.03.10,5.03.11,5.03.13,5.03.16,5.03.17,5.04.01,5.04.02,5.04.03,5.04.04,5.04.06,5.04.09,5.04.14,5.05.01,5.05.02,5.05.06,5.05.12,5.06.01,5.06.02,5.06.03,5.06.04,5.06.05,5.06.07,5.06.08,5.06.09,5.07.01,6.01.04,6.01.08,6.02.05,6.02.08,6.04.13,6.05.01,6.05.04,6.05.05,7.01.01,7.01.02,7.02.01,7.02.05,7.03.02,7.03.07

Systems analysis. 1.08.08,1.09.02,3.08.13,3.08.14,3.08.21,3.11.28,3.14.01,4.13.05,5.01.02,5.01.04,5.01.09,5.01.11,5.01.13,5.02.01,5.03.07,5.04.07,5.04.08

Toxicity. 1.01.04,1.01.06,1.01.11,1.02.09,1.02.10,1.03.05,1.03.09,1.03.10,1.03.12,1.04.04,1.04.05,1.04.07,1.04.08,1.04.09,1.04.13,1.05.04,1.05.09,1.05.15,1.05.16,1.05.17,1.05.18,1.05.21,1.06.08,1.07.04,1.07.05,1.07.06,1.07.08,1.07.14,1.07.20,1.08.11,1.09.16,1.09.17,1.10.08,2.01.11,2.02.07,2.02.18,2.02.20,2.03.03,2.03.09,2.05.16,2.07.04,2.08.09,2.09.20,2.10.07,2.10.08,2.10.11,2.11.02,2.12.01,2.12.05,2.12.09,2.12.12,3.02.02,3.02.03,3.03.19,3.05.11,3.07.06,3.08.03,3.09.04,3.09.06,3.10.02,3.11.26,3.12.03,3.12.07,3.12.15,3.12.16,3.13.29,3.15.07,4.02.05,4.03.12,4.04.01,4.04.16,4.06.01,4.06.27,4.07.04,4.07.13,4.10.01,4.11.01,4.11.02,4.11.05,4.11.08,4.11.12,4.11.14,4.14.21,4.14.29,4.14.31,4.15.04,4.15.06,4.15.07,4.15.08,5.04.08,5.05.08,6.02.09,6.02.10,6.03.02,6.04.17,6.05.06,7.03.06

Toxicokinetics. 1.01.09,1.02.05,1.06.08,1.09.08,2.05.17,2.09.18,2.10.05,2.10.07,3.02.02,3.12.26,4.01.03,4.01.04,4.03.02,4.03.03,4.03.04,4.03.05,4.03.06,4.03.09,4.03.11,4.03.17,4.03.22,4.03.23,4.05.05,4.06.09,4.06.10,4.06.11,4.06.12,4.06.15,4.06.16,4.06.21,4.06.23,4.07.04,4.12.06,4.14.21,4.15.10,8.02.14

Uncertainty. 1.01.13,1.01.14,1.08.11,1.08.12,2.09.02,2.11.10,3.08.24,3.10.14,4.01.01,4.01.08,4.01.09,4.03.06,4.06.04,4.06.08,4.06.12,4.06.18,4.06.23,4.10.09,5.01.04,5.01.06,5.03.12,5.04.11,5.05.13,6.01.07,6.02.03,6.04.06,7.03.01,7.03.02

Urban. 2.05.01,2.07.05,2.12.03,3.02.08,3.02.12,3.08.12,3.11.05,3.11.09,3.13.13,3.13.22,3.14.06,3.15.04,3.15.11,4.02.06,4.04.03,5.02.07,5.02.11,5.04.05,6.05.03,7.01.09,7.01.11

Waste water. 1.02.19,1.05.22,1.07.19,1.08.01,1.08.02,1.09.16,1.09.18,1.09.19,1.09.20,2.03.01,3.01.13,3.01.17,3.03.02,3.03.03,3.03.04,3.03.07,3.03.09,3.03.11,3.03.13,3.03.14,3.03.16,3.03.17,3.03.18,3.03.19,3.03.22,3.03.23,3.04.06,3.04.08,3.05.04,3.05.07,3.05.08,3.05.09,3.06.07,3.07.08,3.07.09,3.08.10,3.08.17,3.08.20,3.08.23,3.11.03,3.11.11,3.11.24,3.12.18,3.12.32,3.13.06,3.15.28,3.16.06,4.01.07,4.02.01,4.02.02,4.02.03,4.02.04,4.02.06,4.02.07,4.02.08,4.02.09,4.02.11,4.02.12,4.04.13,4.04.21,4.04.24,4.07.05,4.14.11,4.14.17,4.14.20,4.14.22,4.14.27,5.02.13,5.06.10,6.01.03,6.04.12,7.01.06,7.01.07,7.01.08,7.01.09,7.01.10,7.01.11,7.01.12,7.01.13,7.02.02,7.02.04,7.02.05

Water quality. 1.07.07,1.07.16,1.09.02,1.09.12,1.09.17,1.09.20,2.02.19,2.04.15,2.05.29,2.06.10,2.08.03,2.08.08,2.09.06,2.09.11,2.13.07,3.03.06,3.03.07,3.03.14,3.03.20,3.03.21,3.05.02,3.05.08,3.05.09,3.05.10,3.05.14,3.05.16,3.06.07,3.07.02,3.07.10,3.08.04,3.08.06,3.08.07,3.08.08,3.08.11,3.08.17,3.08.18,3.08.22,3.09.10,3.09.12,3.09.13,3.10.11,3.10.12,3.10.15,3.10.26,3.10.27,3.11.03,3.11.18,3.11.23,3.11.26,3.11.27,3.13.04,3.13.06,3.13.07,3.13.09,3.13.11,3.13.14,3.13.23,3.15.21,3.15.35,3.16.15,4.02.02,4.02.05,4.04.13,4.07.06,4.11.04,4.11.06,4.11.07,4.11.09,4.12.02,4.12.07,4.12.09,4.13.11,4.13.12,4.14.10,4.15.01,4.15.03,4.16.04,5.02.04,5.04.15,5.07.02,7.01.06,7.01.13,7.02.04

Weight of evidence. 1.01.07,1.01.08,1.04.06,1.04.12,1.05.01,1.05.10,3.01.18,3.06.02,3.08.04,3.08.22,3.09.16,3.12.24,4.02.05,4.03.15,4.03.21,4.09.01,4.09.03,6.01.02,6.03.05

Wetlands. 1.04.11,2.04.09,2.06.04,2.12.02,2.12.16,3.10.03,3.12.22,4.02.10,4.04.12,4.04.20,4.11.15,4.14.27,7.02.05

Author Index

A

Aalizadeh, Reza. 1.10.06
Aas, Wenche. 3.15.08
Abeynayaka, Amila. 3.08.07, 3.11.26, 3.14.07
Abiakar, Farah. 4.06.28, 4.06.29
Abian, Joaquin. 3.03.09
Aboites Espinosa, Luis. 2.06.12
Abou Zeid, Lana. 1.10.08
Abrantes, Nelson. 1.07.17, 2.02.05, 2.04.15, 2.05.29, 2.08.01, 2.08.02, 2.08.03, 3.09.04, 3.09.06, 3.09.19
Abreu, Siz. 6.01.10
Abril, Meritxell. 3.03.08
Abril, Nieves. 2.13.02, 2.13.03, 4.17.02
Abu, Abdul. 6.03.08
Accolla, Chiara. 8.02.13
Acevedo, Pelayo. 2.05.07, 2.05.14
Achenbach, John. 1.07.13
Achtenhagen, Jan. 3.03.14
Adam, Daniel. 3.01.03
Adamovsky, Ondrej. 4.11.16
Ademollo, Nicoletta. 4.14.25
Adomat, Yasmin. 3.08.09
Adouani, Nouceiba. 4.14.27
Adriaanse, Pauline. 3.10.01
Adrian, Philippe. 3.10.24, 4.03.16
Adyel, Tanveer. 3.12.19
Afantitis, Antreas. 4.01.10, 4.01.11, 4.01.12
Afonso, Mariana. 2.03.03, 2.03.09
Agatz, Annika. 4.08.04, 4.16.03
Agerstrand, Marlene. 2.01.01, 2.01.02
Aguiari, Filippo. 5.04.06
Aguilar, Alex. 3.09.14
Ahmed, Fahad. 3.03.23
Ahn, Chang Hyuk. 2.10.17
Ahn, Changhyuk. 5.02.07
Ahn, Daye. 1.05.04
Ahn, Kwang-Ho. 5.07.01
Ahn, Sungwoo. 3.05.17
Ahrens, Lutz. 3.13.04
Aimola, Giorgia. 4.04.02
Ait-Aissa, Selim. 1.05.17, 4.02.11
Ait-Mouheb, Nassim. 3.07.09
Ajibade, Fidelis. 4.04.20
Ajibade, Temitope. 4.04.20
Akbari, Ali. 4.04.07
Akcha, Farida. 1.04.01, 4.12.12
Akenga, Preston. 3.07.11
Akter, Nargis. 7.01.13
Akter, Sunjida. 3.11.25
Al-Azzawi, Mohammed. 3.12.18
Alajo, Tania. 3.08.15
Albasi, Claire. 3.01.13
Albignac, Magali. 3.08.14, 3.11.19, 3.11.22
Alburo, Carlo. 1.05.22
Aldrich, Annette. 2.12.15, 3.09.24, 4.16.06, 6.02.02
Aleiferi, Eleni. 1.10.06
Aleksandryan, Anahit. 3.15.27
Alfarhan, Ahmed. 3.07.01, 3.11.27
Alhakamy, Nabil. 3.16.13
Alhama, Jose. 2.03.04
Ali, Nadeem. 3.16.11, 3.16.13
Aliani, Stefano. 3.08.06
Alinezhad, Ali. 3.13.27, 3.13.31, 3.13.32
Alix, Anne. 4.16.03, 7.02.01
Alkassab, Abdulrahim. 4.08.07
Allacker, Karen. 5.02.09
Allan, Dawn. 4.07.12
Allen, Dave. 1.01.12
Allen, Joel. 2.01.02, 2.01.16, 4.11.10
Allen, Joey. 2.04.08
Almanza, Maria. 1.10.02
Almasi, Bettina. 4.03.20
Almeida, Agostinho. 4.11.08
Almeida, Ana. 3.12.01
Almeida, Helena. 3.12.14
Almeida De Godoy, Thais. 2.11.11
Almroth, Bethanie. 1.09.05
Almunia, Christine. 1.08.04, 1.08.08, 1.08.13
Alonso-Prados, José. 3.16.16
Altmann, Korinna. 3.11.03, 3.11.13
Alton, Lesley. 2.01.03
Alvarez, Lara. 6.01.04
Alvarez Ruiz, Rodrigo. 3.06.08, 3.06.10
Alvarez-Hernandez, Sergio. 1.07.02, 2.02.18
Álvarez-Rogel, José. 2.10.09
Alvarez-Ruiz, Rodrigo. 1.07.15, 3.05.10
Alves, Artur. 2.10.15
Alves, Rodrigo Franco. 1.05.14
Alvincz, Julia. 1.03.02, 1.08.05
Alygizakis, Nikiforos. 2.05.24, 2.05.26
Alzaga, Vanesa. 2.05.14
Alzualde, Ainhoa. 1.03.05
Amadei, Andrea. 5.02.01
Amar, Saroj. 1.03.09, 1.06.08, 3.10.02
Amin, Nuhu. 7.01.13
Aminot, Yann. 1.04.01
Amorim, Monica. 2.09.04, 2.10.03, 4.03.04, 4.14.34
Amouroux, Baptiste. 3.11.19
Amoussou, Nellya. 2.04.08
Amutova, Farida. 2.09.16
An, Youn-Joo. 1.07.01, 2.04.03, 2.10.11, 3.12.02, 3.12.07, 3.12.12, 7.01.03
Ancona, Valeria. 2.09.11, 4.04.02, 4.04.10
Andersen, Sarah. 5.03.13
Andersen, Tom. 2.08.17
Andersson, Agneta. 3.15.22
Andersson, Patrik. 3.02.11
Andevski, Jovan. 2.05.25
Andrade, Edilene. 5.06.03
Andres, Sandrine. 3.09.23
Andreu, Vicente. 7.02.04
Andrews, Steven. 3.10.08
Androulakakis, Andreas. 2.05.24
Andvik, Clare. 2.05.09
Anelli, Simone. 3.15.24
Angel, Aaron. 2.12.16
Ângelo, Lucicleide. 2.03.10, 4.14.31
Antczak, Philipp. 1.06.05
Anthamatten, Eva. 4.12.06
Antonelli, Manuela. 3.03.19
Antoniotti, Sylvain. 4.09.08
Anumol, Tarun. 3.05.16, 3.13.07, 3.13.09, 3.13.10
Apetogbor, Komlan. 4.02.03
Apitz, Sabine. 5.06.02, 8.04.05
Arangio, Andrea. 3.02.07
Araujo, Cristiano. 2.01.19, 2.01.20
Arcanjo, Caroline. 2.02.12
Ardente, Fulvio. 5.02.01
Arena, Maria. 4.16.06
Arendt, Rosalie. 5.04.04
Arey, J. Samuel. 3.01.09
Arias, Carlos. 4.11.15
Arias Borrego, Ana. 2.13.03
Arjol, Miguel Ángel. 3.15.09
Arlos, Maricor. 4.12.06
Armbrust, Kevin. 2.08.12
Armbruster, Dominik. 3.03.20
Armengaud, Jean. 1.08.04, 1.08.08
Armiraglio, Stefano. 3.15.24
Armitage, James. 3.01.20, 3.01.21, 4.03.10, 4.03.17
Arndt, Devrah. 4.07.02, 4.07.03, 4.07.07
Arnie, Joshua. 2.04.11
Arnot, Jon. 3.01.20, 3.01.21, 3.04.11, 3.15.38, 4.03.10, 4.03.17
Aronsson, Henrik. 2.02.16
Arp, Hans Peter. 3.13.18
Arroyo Schnell, Alberto. 8.04.03
Arts, Gertie. 2.04.13, 2.13.09
Arulnathan, Vivek. 5.06.07
Ashauer, Roman. 2.04.06, 2.04.14, 2.09.18, 4.06.11, 4.06.12, 4.06.13, 4.06.16, 4.06.17, 4.06.18, 4.06.20, 4.06.28, 4.06.29, 4.12.05
Asimakopoulos, Alexandros. 2.05.02
Asjes, Afra. 6.01.02
Askanian, Haroutioun. 3.12.22
Askham, Cecilia. 3.08.05
Asmonaite, Giedre. 1.09.05
Asselman, Jana. 2.08.05, 7.03.04
Assem, Louise. 4.02.06
Assoumani, Azziz. 3.13.15, 3.15.17
Assumpcio, Anton. 5.05.12, 5.06.03
Audenaert, Amaryllis. 5.01.08
Audouze, Karine. 1.09.01
Auger, Lucile. 4.12.08
Auguste, Manon. 1.02.02
Aurisano, Nicolo. 5.05.08
Aus Der Beek, Tim. 3.16.15
Avelar-González, Francisco Javier. 3.09.02
Aviles, Amandine. 1.05.11
Axelman, Johan. 4.06.06
Ayala, Maddalen. 5.01.03
Ayobahan, Steve. 1.03.02, 1.08.05
Azevedo, Joana. 4.11.07, 4.11.08

B

Baas, Jan. 4.06.10
Bacchetta, Renato. 1.09.04
Bach, Vanessa. 5.04.04
Bachmann, Jean. 4.14.01
Bachmann, Till. 5.04.04
Backhaus, Thomas. 1.04.12, 2.02.16, 7.03.03
Backus, Sean. 3.15.02
Badder, Claire. 4.12.05
Baden, Christian. 2.07.01, 2.07.03
Bado-Nilles, Anne. 1.04.03, 2.05.19, 4.01.03, 4.01.04
Badry, Alexander. 2.05.24, 2.05.26
Baetscher, Roger. 2.04.06
Bagnati, Renzo. 3.15.25, 3.15.26
Baier, Roland. 3.09.21
Baig, Aijaz. 4.13.14

Bak, Emilie. 4.17.04
Baken, Stijn. 3.09.16, 4.02.06
Bakker, Joost. 6.02.06
Bakker, Ruben. 1.08.06
Baktoft, Henrik. 2.01.06
Balakrishnan, Vimal. 4.13.14
Balasch Garcia, Aleix. 3.02.10
Balbi, Teresa. 1.02.02, 2.02.11
Baldereschi, Elena. 5.02.05, 5.02.09
Balieva, Gergana. 2.12.09, 2.12.11
Ball, Jonathan. 4.14.19
Balseiro-Romero, María. 3.16.07, 3.16.08
Balzarotti, Melissa. 7.02.02
Bamber, Shaw. 2.01.15
Banas, Damien. 4.12.08, 4.12.09
Bancel, Sarah. 1.05.16
Bandow, Nicole. 3.13.02
Bangalore Narayana, Vinay Kumar. 3.11.30
Banni, Mohamed. 2.01.19
Bannick, Claus-Gerhard. 3.11.03
Barata, Carlos. 2.01.12, 2.01.13
Baratange, Clement. 4.14.30
Barbaro, Jeffrey. 3.03.02
Barbosa, Ana Cecília Rizzatti Albergaria. 3.15.16
Barbosa, Andrés. 6.01.05
Barcelo, Damia. 3.03.09, 3.07.01, 3.11.27
Barceló, Damià. 3.12.04
Barden, Ruth. 7.01.11
Bardow, André. 5.03.07
Barduk, Olga. 6.02.05
Bargelloni, Luca. 3.13.28
Barnes, David. 2.11.06
Baroni, Davide. 2.09.07
Baroth, Anju. 3.14.08
Baroudi, Moumen. 2.09.19
Barouki, Robert. 1.09.01
Barra Caracciolo, Anna. 4.04.02, 4.04.09, 4.14.13, 4.14.25
Barresi, Enzo. 3.15.08
Barreto, Ângela Maria. 2.10.03, 4.14.34
Barron, Emmanuelle. 3.05.03
Barron, Leon. 3.07.02, 3.15.23, 3.15.32
Barron, Mace. 4.01.06
Barros, Francisco. 3.15.16
Barros, Marta. 1.02.03
Barsi, Alpar. 4.06.06
Bart, Sylvain. 4.12.05
Bartell, Steven. 4.06.28, 4.06.29
Bartholomeus, Ruud. 3.03.13
Bartolomé, Mónica. 3.13.22
Bartosova, Zdenka. 2.05.16
Bartram, Abby. 6.01.04
Bartroli, Rafael. 7.01.02
Bartrons, Mireia. 2.06.01
Baschien, Christiane. 2.03.06
Basopo, Norah. 4.14.20
Basseres, Anne. 4.02.09
Battagliarin, Glauco. 3.14.01
Battocchio, Chiara. 1.02.12
Bau, Michael. 6.01.09
Baudouin, Jean-Marc. 4.12.08
Baudrimont, Isabelle. 3.02.05
Baudrimont, Magalie. 3.02.05
Bauer, Franklin. 1.05.01, 4.06.01, 4.06.02
Bauer, Reinhard. 3.08.09
Baumann, Lisa. 1.05.05, 1.05.07, 1.05.08, 1.05.20
Baussant, Thierry. 2.01.15
Bavay, Dominique. 2.11.12
Baveco, Hans. 4.06.31, 4.06.32, 4.16.03
Baveye, Philippe. 3.16.07
Bawa-Allah, Kafilat. 1.01.05
Bayat, Helena. 4.17.04
Beaudouin, Rémy. 4.01.03, 4.01.04
Beauvais, Rébecca. 2.02.06, 2.08.08
Bechmann, Renée. 2.01.15, 6.01.07
Becker, Benjamin. 3.03.17
Becker, Jeremias. 4.06.06, 4.06.07
Bednarska, Agnieszka. 2.07.04
Bedrossiantz, Juliette. 2.01.11, 2.01.12, 2.01.13, 6.05.02
Been, Frederic. 3.05.02
Beggio, Giovanni. 3.16.05
Behl, Mamta. 1.03.05
Behnisch, Peter. 2.09.07
Behnstedt, Laura. 1.05.05
Beijer, Steven. 4.10.11
Beitel, Shawn. 1.04.12
Bekker, Sebastian. 8.04.04
Belanger, Scott. 1.01.07, 4.01.08, 4.07.02, 4.07.03, 4.07.04, 4.07.09
Belaval, Marcel. 3.03.02
Belchier, Mark. 2.11.02
Belfeg, Julia. 4.04.15
Belkin, Shimshon. 3.03.17
Bell, Anna. 3.03.17
Bellanger, Cécile. 2.02.14
Bellés, Montserrat. 4.17.06, 4.17.07
Bellingeri, Arianna. 1.02.12
Bello López, Miguel Ángel. 4.17.02
Bellotti, Giovanni. 3.03.19
Beltman, Wim. 3.10.12, 3.10.17, 4.06.31, 4.06.32
Beltran, Estelle. 3.10.24, 4.03.16
Beltran De Heredia, Irene. 4.14.22
Ben Said, Olfa. 2.03.07
Benejam Vidal, Luis. 3.07.05
Benetto, Enrico. 5.04.01, 5.06.05
Benhajkassen, Nabil. 1.09.01
Bennett, Evan. 1.08.07
Benoit, Pierre. 3.04.10
Benskin, Jonathan. 3.15.28
Bereketoglu, Ceyhun. 2.01.23
Beretsou, Vasiliki G.. 3.03.11
Berg, Sanne. 2.01.02
Bergami, Elisa. 1.02.05, 1.02.15, 2.11.04, 2.11.07, 2.11.08, 2.11.11
Berger, Markus. 5.06.04
Berglund, Åsa. 2.06.06, 2.06.11
Berglund, Olof. 1.08.01
Bergtold, Matthias. 2.07.03
Berkemeier, Thomas. 3.02.03
Bermejo-Nogales, Azucena. 2.12.03
Bernal, Elena. 1.02.16
Bernal, Kevin. 1.09.01
Bernander, Maria. 3.02.11, 5.04.08
Bernardini, Ilaria. 3.13.28
Bernhardt, Emily. 2.03.08, 3.16.14
Berny, Philippe. 3.09.24
Berr, Marcus. 5.04.02
Berrojalbiz, Naiara. 3.04.04
Bertato, Linda. 1.01.10, 4.03.17
Bertold, Carolin. 4.07.11, 5.03.17
Bertolero, Albert. 2.05.22
Bertotto, Luisa. 1.03.07
Bertram, Michael. 2.01.02, 2.01.03, 4.14.15, 4.14.28
Bertrans Tubau, Luís. 3.03.08
Bervoets, Lieven. 2.05.03, 3.13.19, 3.13.23
Besenyő, Gabriella. 3.08.11
Besnardeau, Lydia. 2.02.11
Bessa, Filipa. 6.01.05
Besseau, Romain. 5.01.11
Betz, Alexander. 1.03.06
Beylot, Antoine. 5.05.10
Bezerra, Vanessa. 4.04.04
Bhattacharya, Prosun. 7.01.13
Bhuiyan, Muhammed. 4.15.03
Biales, Adam. 1.06.02
Bianchi, Laura. 1.01.17
Bicherel, Pascal. 4.05.04, 4.06.01, 4.06.02, 4.09.08
Bidleman, Terry. 3.15.02, 3.15.22, 3.15.33
Biedermann, Eynat. 3.14.01
Bigaj, Janusz. 2.10.06
Biganzoli, Fabrizio. 5.05.01
Bigott, Yvonne. 3.07.08
Bilbao, Eider. 1.02.08, 6.05.03
Bini, Luca. 1.01.17
Birarda, Giovanni. 2.11.08
Birch, Heidi. 3.01.01, 3.01.02, 3.04.05
Birkemoe, Tone. 2.09.22
Birketveit, Oystein. 6.02.05
Birkitt, Lucy. 3.15.23
Birkved, Morten. 5.03.13
Biscocho, John Paul. 1.05.22
Bissagou Koumba, Gaëlle. 3.12.22
Bjergbakke Fogh, Sigrid. 1.09.13
Bjerregaard, Poul. 1.05.11
Black, Kirsty. 5.06.10
Black, Mairi. 5.01.15, 5.05.11
Blackstone, Nicole Tichenor. 5.04.15
Blackwell, Brett. 3.03.16
Blahova, Lucie. 4.11.01
Blair, David. 2.05.10
Blakey, Alex. 3.10.23
Blanc, Etienne. 1.09.01
Blanc, Isabelle. 5.06.04
Blanchfield, Paul. 4.14.15
Blancho, Florent. 3.11.29
Blanco, Carlos. 5.01.09
Blanco, Guillermo. 2.05.06
Blank, Lars. 5.03.17
Blasco, Julian. 1.02.01, 2.01.19, 2.03.04
Blasco, Nagore. 1.02.08
Bleeker, Eric. 6.04.03
Block, Kelly. 4.02.04
Blom, Judith. 2.02.20
Bloom, Peter. 1.04.10
Bloor, Michelle. 8.05.01
Blumenroeder, Julian. 2.11.06
Blust, Ronny. 3.09.05
Boaventura, Debora. 7.04.01
Boccard, Julien. 1.08.13
Bodet, Pierre-Edouard. 2.02.09
Boerschig, Carmen. 4.06.13
Boesten, Jos. 3.10.01
Boffo, Luciano. 3.13.28
Böhle, Gisela. 1.08.05
Bohlin-Nizzetto, Pernilla. 3.02.04, 3.15.08
Bohmer, Nils. 6.05.06
Bolekhan, Anastasiia. 3.10.23
Bollinger, Eric. 4.02.12, 4.14.24
Bollino, Barbara. 3.08.08
Bolm, Anna. 3.12.27
Bolonio, David. 5.02.06
Bonato, Marco. 3.13.16, 3.13.28
Bonato, Tiziano. 3.16.05
Bondarenko, Liubov. 1.02.10, 6.04.15
Bonisoli-Alquati, Andrea. 1.04.10, 2.12.16, 3.13.24
Bonmatí, August. 5.05.12, 5.06.03

Bonnafe, Elsa. 4.14.32
Bonnard, Isabelle. 4.14.30
Bonnard, Marc. 1.04.03
Bonnell, Mark. 3.01.18, 4.13.14
Bonnot, Kévin. 3.04.10
Boogaerts, Tim. 7.01.07, 7.01.10
Boon, Nico. 3.01.12, 3.04.03, 4.04.23
Boonstra, Harry. 4.12.11
Booth, Andy. 1.07.12, 3.08.05, 3.12.01, 3.12.13, 3.12.33, 6.01.09, 6.04.12
Bopape, Matlola Abel. 1.09.11
Bordalo, Maria. 2.02.04, 2.08.15, 6.01.10
Bordós, Gábor. 3.08.11
Boreham, Rebekah. 4.14.19
Borgå, Katrine. 2.05.01, 2.05.05, 2.05.09, 2.08.17, 2.09.22, 4.06.19, 4.14.23
Borin, Sara. 3.15.26, 7.02.05
Borja, Ángel. 2.03.07
Borrell, Asunción. 3.09.14
Borrion, Aiduan. 5.01.15, 5.05.11
Bosch, Lea. 2.06.08
Bose, Aneesh. 2.01.09
Bossi, Rossana. 3.15.08
Botha, Andre. 2.12.06
Bottoms, Melanie. 2.09.17
Bouchard, Damien. 4.14.12
Boucher, Justin. 7.03.06
Bouguerra, Sirine. 2.09.10, 2.09.21
Bouillass, Ghada. 5.06.04
Boulange-Lecomte, Céline. 2.02.12
Boulant-Cirederf, Delphine. 2.09.08, 3.09.23
Boulay, Anne-Marie. 3.08.05
Boullemant, Amiel. 3.09.16, 4.02.06
Bouly, Lucie. 4.14.32
Bourhane, Zeina. 2.03.07
Bourmaud, Jean-Yves. 5.01.11
Boxall, Alistair. 2.03.11, 3.15.04, 4.07.05, 4.10.01, 4.10.02, 4.10.03
Boyd, Patrick. 2.09.20
Boyko, Volodymyr. 6.04.04
Boyle, David. 3.09.16
Braakhekke, Maarten. 4.06.32
Braaten, Hans Fredrik Veiteberg. 2.11.10
Brack, Werner. 1.04.12, 1.09.03
Bracquene, Ellen. 5.03.02
Bradascio, Margherita. 7.02.01
Bradley, Paul. 3.03.16
Braga, Tárcio. 2.03.10
Braga Alonso, Luma. 1.09.06
Brain, Richard. 2.05.17
Bramaz, Nadine. 1.01.06
Branco, Vasco. 4.12.01
Brand, Anne-Fleur. 1.04.11
Brand, Jack. 2.01.03, 2.01.10, 4.14.15
Brand, Walter. 6.04.03
Brander, Susanne. 1.06.04, 2.08.11, 2.08.12, 2.08.13, 3.12.27, 4.15.07, 4.15.08
Branford, Philip. 3.10.23
Braud, Lea. 5.01.13
Braun, Karsten. 4.10.10
Braun, Ulrike. 3.11.03, 3.11.07, 3.11.13
Braunbeck, Thomas. 1.01.07, 1.05.05, 1.05.07, 1.05.08, 1.05.20, 1.07.09, 4.01.08
Brauns, Mario. 1.04.12
Brede, Dag. 1.08.10, 2.08.09
Breedveld, Gijs. 3.13.18
Breider, Florian. 4.14.30, 4.15.10
Breitenmoser, Steve. 6.02.02
Breivik, Knut. 3.15.08
Brenchley, Jeff. 4.02.04
Brenon, Isabelle. 2.02.09
Bresson, Carole. 1.10.08
Breuer, Jörn. 3.13.25, 3.16.03
Breuer, Lutz. 3.10.17
Breuil, Marie Christine. 3.07.08
Brienza, Monica. 3.07.09
Brigden, Kevin. 3.12.26
Brill, Jessica. 4.07.02, 4.07.07, 4.07.08
Brinke, Marvin. 3.03.04
Brinkmann, Markus. 1.04.12
Brinovcar, Cassandra. 2.05.15
Brion, Francois. 1.05.17
Britton, Katy. 1.03.04
Brock, Andreas Libonati. 3.01.06
Brodie, Christopher. 3.02.12, 3.06.09
Brodin, Tomas. 2.01.02, 2.01.03, 2.01.06, 2.01.09, 2.01.10, 2.01.14, 2.01.18, 4.14.15, 4.14.28
Brönmark, Christer. 2.01.06
Bronzo, Laura. 3.11.11
Brookman-Amissah, Marianne. 1.01.04
Brooks, Amy. 2.04.07, 4.08.02, 6.03.08
Brooks, Bryan. 2.01.01, 2.01.02, 2.01.03, 4.14.15
Brorström Lundén, Eva. 3.15.08
Brotas, David. 1.01.15
Brown, Andrew Ross. 4.16.01, 4.16.02, 4.16.03
Brown, Colin. 2.09.18
Brown, David M. 3.01.08
Brown, Kevin. 4.08.02
Brown, Marie. 2.02.01, 2.02.03, 6.03.07, 6.03.08
Brown, Rebecca. 6.03.04
Brown, Trevor. 3.01.20, 3.01.21, 3.04.11, 3.15.37, 3.15.38
Brown, Wendy. 4.02.07
Brozman, Ondrej. 1.05.02
Brucet, Sandra. 2.06.01
Bruehl, Carsten. 2.06.08
Brugel, Sonia. 3.15.22
Brühl, Carsten. 2.06.07
Brumhard, Bjoern. 3.10.22
Brun, Anna Magdalene. 4.07.02, 4.07.03, 4.07.04, 4.07.07, 4.07.08
Brunetti, Matteo. 2.10.01
Brunner, Andrea. 3.05.02
Brunning, Hattie. 4.07.05
Bruns, Eric. 2.02.02, 2.04.06, 4.05.01, 4.05.02
Bub, Sascha. 3.10.17, 4.06.30, 8.02.09
Bubnyte, Rasa. 4.02.02
Bucheli, Thomas. 3.08.10
Buchinger, Sebastian. 3.03.17
Bücking, Mark. 3.13.25, 3.16.03
Buddendorf, Bas. 4.06.32
Budzinski, Hélène. 1.04.01, 3.01.13, 3.05.03, 3.13.15, 3.13.26, 3.15.17, 4.02.11, 4.12.12
Buenaventura, Nina. 3.08.12
Buer, Benjamin. 1.10.02
Bühler, Roman. 4.03.20
Buij, Ralph. 2.05.26
Bult, Tammo. 6.01.02
Bundschuh, Mirco. 2.06.05, 2.06.07, 2.06.08, 2.06.09, 3.12.23, 4.02.12, 4.03.02, 4.14.24, 6.04.17
Bunyatyan, Yurik. 3.15.27
Burk, Jaanus. 4.01.11
Burrows, Steven. 3.08.15, 3.11.09
Bury, Nic. 1.01.02
Bustamante, Paco. 2.11.02
Büter, Malte. 3.16.15
Butkovskyi, Andrii. 4.04.12
Butts-Wilmsmeyer, Carolyn. 2.07.02
Buyle, Matthias. 5.01.08
Byčenkienė, Steigvilė. 3.02.08, 3.02.09
Byns, Cara. 3.13.23
Bystricky, Maria. 5.02.04
Bæk, Kine. 2.05.01

C

C. Muñoz, Cynthia. 3.11.10
C. Silva De Assis, Helena. 1.07.20
Caballero Carretero, Patricia. 1.08.03, 1.09.09
Caban, Magda. 3.15.06
Cabernard, Livia. 5.02.02
Cachada, Anabela. 2.04.05, 4.04.11
Cagnon, Christine. 2.03.07
Cahon, Karen. 2.08.10
Cahuc, Chloé. 1.04.01
Cailleaud, Kevin. 4.02.05
Cajaraville, Miren. 1.02.03, 1.02.08
Calado, Sabrina. 1.07.20
Caldeira, Carla. 5.01.05, 5.05.01
Caldwell, Gary. 2.04.01, 2.08.16
Calero Herrera , Vanessa Katherine. 4.04.15
Calgaro, Loris. 3.07.04
Caliani, Ilaria. 1.01.17, 2.07.06
Callejón-Leblic, Belén. 2.13.02
Calore, Francesco. 3.07.04
Calviño-Vázquez, Beatriz. 3.16.07, 3.16.08
Calvo, José F.. 1.04.09
Calza, Paola. 1.09.14
Camana, Daniela. 5.04.09
Camarero, Pablo R.. 1.04.07, 1.04.09, 2.05.07, 2.05.14, 2.12.07, 2.12.13
Cameán, Ana. 1.03.12, 4.11.07, 4.11.12, 4.11.14
Camenzuli, Louise. 3.01.04, 3.01.09, 4.09.09
Caminal, Gloria. 3.04.09
Campan, Pauline. 3.10.26
Campanale, Claudia. 3.08.08, 3.08.13
Campani, Tommaso. 1.01.17, 2.07.06
Campioni, Letizia. 2.12.17
Campo, Julian. 3.06.08, 3.06.10, 3.07.01, 3.11.27, 4.08.08
Campos, Alexandre. 4.11.07, 4.11.08, 4.11.09
Campos, Bruno. 4.01.02
Campos, Diana. 3.15.18
Campos, Fábio. 6.04.14
Campos, Isabel. 1.07.17, 2.02.05, 2.03.03, 2.03.09, 2.08.01, 2.08.02, 2.08.03, 3.09.04, 3.09.06, 3.09.19
Campos-Vivanco, Aida Z.. 6.05.01
Cañas, Ana Isabel. 3.13.22
Cañaveras, José Manuel. 2.05.14
Cancio, Ibon. 6.05.03
Canesi, Laura. 1.02.02, 2.02.11
Canisius, Marie-France. 3.15.40
Canoira, Laureano. 5.02.06
Cant, Amélie. 1.04.03
Cantos, Manuel. 4.04.06
Cantu, Mark. 4.03.14
Cao, Lijia. 1.09.19
Cao, Yang. 1.08.11, 1.08.12
Cao, Yi. 8.02.14

Capistrano, Marc Aldrick. 1.05.22
Capolupo, Marco. 3.12.33
Capon, France. 4.02.06
Capozzi, Staci. 3.06.06
Cappelli, Francesca. 3.03.19, 7.01.06
Carapeto-García, Ricardo. 4.14.04, 4.14.12
Cardenas, Jorge Antonio. 2.10.16
Cardon, Mary. 3.03.16
Cardona, Dwaine. 3.05.08
Cardoso, Diogo. 2.10.05
Cardoso, Olivier. 4.12.08
Cardoso, Paulo. 3.09.20
Carere, Mario. 1.07.16, 2.05.28, 6.02.09
Carey, Sandra. 4.02.06
Caricato, Roberto. 3.02.01
Carl, Steffen. 2.03.06
Carneiro, Diana. 6.04.13
Carpentier, Patrice. 4.03.06
Carrascal, Montserrat. 3.03.09
Carrasco Del Amor, Ana Maria. 1.07.08
Carrasco Navarro, Victor. 3.12.03, 4.15.06
Carrero, Jose Antonio. 2.02.07, 4.03.19
Carriger, John. 4.01.06
Carro, Tiffany. 1.01.13, 1.01.14
Caruso, Tancredi. 2.11.08
Carvalho, Aline. 3.11.22
Carvalho, Patricia. 6.04.12
Carvalho, Pedro. 4.11.15
Casagrande, Naiara. 3.08.05
Casal, Paulo. 2.11.03
Casallanovo, Fabio. 2.09.03
Casartelli, Ilaria. 4.03.17
Casas, Gemma. 2.11.03, 3.04.04
Casazza, Marco. 4.11.05
Casillas, Alba. 3.10.04
Casimiro Elena, Ramiro. 4.14.04
Casini, Silvia. 1.01.17, 2.07.06
Cassano, Domenico. 3.12.29
Castán, Samuel. 2.12.05
Castaño, Argelia. 3.13.22
Castano-Ortiz, Jose Maria. 3.12.04
Castella, Emmanuel. 2.06.04
Castilla Alcantara, Jose Carlos. 4.04.06, 4.04.07
Castillo, Duvan. 7.02.08
Castillo, Nicholas. 4.14.28
Castle, Denise. 4.08.07
Castro, Filipe. 2.02.15
Castro, Mafalda. 3.05.04, 4.17.04
Castronovo, Sandro. 3.09.21
Catarino, Ana. 3.11.20, 3.12.05, 7.03.03
Catarino, Ana I. 7.03.01
Catteau, Audrey. 1.04.03
Cavagnino, Daniela. 3.05.09
Cayrou, Jérome. 4.02.09
Cecchinato, Caterina. 3.13.16
Cedergreen, Nina. 2.13.07, 3.05.04, 4.17.04, 8.02.14
Ceger, Patricia. 1.01.12
Cejas, Mark. 3.06.02
Cela, Rafael. 3.01.15, 3.05.06
Celis, Rafael. 7.02.07
Celo, Valbona. 3.02.07
Celsie, Alena. 4.09.05
Cermak, Janet. 2.09.06
Cerro-Gálvez, Elena. 3.11.24
Cerveny, Daniel. 2.01.03, 2.01.06, 2.01.09, 2.01.18, 4.14.15, 4.14.28
Cesarini, Giulia. 1.09.12
Cetinic, Katarina. 2.13.01
Chabanon, Marthe. 1.05.16
Chabchoubi, Imen. 1.05.05
Chabenat, Apolline. 2.02.14
Chacon, Esther. 6.01.01
Chadha, Marcus. 3.13.09
Chaideftou, Evgenia. 4.06.06
Challa Sasi, Pavankumar. 3.13.27, 3.13.31, 3.13.32
Chalon, Carole. 4.13.04
Chaos-Carballo, Zoe. 3.16.08
Chappell, Mark. 1.03.09, 3.10.02
Chardon, Clémence. 4.02.11
Charles, Sandrine. 4.01.01, 4.03.06, 4.06.04, 4.06.06, 4.06.08, 4.06.14, 4.06.15
Chatel, Amelie. 1.02.11
Chaumet, Betty. 2.03.05
Chaumot, Arnaud. 1.08.04, 1.08.08, 2.02.15
Chee, Beatrice. 4.03.14
Chefetz, Benny. 4.10.01, 7.02.02
Chen, Helen. 6.04.09
Chen, Ruiwen. 3.15.13
Cherek, Paulina. 3.12.26
Cherubini, Alessandro. 1.09.04
Chikere, Chioma. 2.10.14
Chinchilla, Jose Manuel. 2.05.07
Chingate, Edwin. 3.04.08
Chinnadhurai, Sita. 3.13.17
Chirico, Nicola. 4.03.17
Chiron, Serge. 3.07.09
Chng, Shannae. 3.16.09
Cho, Sung-Hee. 3.12.11
Cho, You Na. 3.12.25
Cho, Youna. 3.11.04, 3.11.05, 3.12.11, 3.12.30
Choi, Kyungho. 1.05.04
Choi, Youmi. 1.07.18, 3.12.11
Chojnacka, Justyna. 3.12.06
Choksi, Neepa. 1.01.12
Choubert, Jean-Marc. 3.01.13
Choukr-Allah, Redouane. 7.02.05
Chouvelon, Tiphaine. 1.04.01
Chowanski, Szymon. 2.13.08
Chowdhury, Mohammed. 3.09.16
Christ, Bastien. 6.02.02
Christensen, Guttorm. 2.11.10
Christensen, Jan. 3.05.04, 3.15.15
Christensen, Morten. 3.08.21
Christiansen, Monica. 1.05.20
Christl, Heino. 6.03.06
Christophe, Armelle. 1.05.17
Christophoridis, Christophoros. 4.11.03
Christou, Anastasis. 3.03.11
Chu, Seoyoon. 1.05.04
Ciacci, Caterina. 1.02.02
Ciccia, Théo. 4.03.11
Cicero, Giuseppe. 2.05.24
Ciesielski, Tomasz. 2.05.05, 6.01.06, 6.01.09
Cifuentes, Victor. 8.06.01
Cincinelli, Alessandra. 2.05.24, 2.05.26
Cindrić, Mario. 2.02.17
Cinelli, Marco. 6.02.03
Cione Buchviser, Ana Paola Prata. 2.09.03
Cirpus, Petra. 3.03.06
Cisar, Petr. 2.01.18
Ciscato, Maria. 3.13.28
Ciudad, Carlos. 2.05.23
Civancik Uslu, Didem. 5.03.08
Claes, Steven. 5.01.10
Clark, Bryan. 1.07.11
Clarke, Bradley. 3.13.07
Claßen, Silke. 2.02.02
Classen, Daniela. 3.01.06, 3.05.15
Claytor, Carrie. 3.09.16, 4.02.06
Clock, Jeffrey. 3.05.17
Coelho, Celeste. 3.09.04
Coelho, Laura. 2.03.03, 2.03.09
Coetzee, Helené. 2.12.18
Cofino, Wim. 3.08.24, 6.01.02
Cohan, Frederick. 3.16.01
Coimbra, Manuel. 2.10.15
Cojoc, Lorena. 3.12.04
Cojocariu, Cristian. 1.09.17, 3.15.34
Colas, Anne Sophie. 5.03.03
Cole, Talia. 4.03.14
Coll, Marta. 2.05.12
Collado, Maria Carmen. 2.13.02, 2.13.03
Collas, Frank. 4.16.04
Collin, Fabrice. 3.11.19
Collins, Jennifer. 4.06.28
Colpo, Pascal. 6.04.05
Colwell, John. 3.08.15
Comazzi, Federico. 3.13.16
Comber, Sean. 3.07.11, 4.02.06
Combi, Tatiane. 3.15.16
Conley, Justin. 3.03.16
Connolly, Mona. 1.02.16
Connon, Richard. 1.06.04, 2.08.11, 2.08.12, 4.12.03
Connors, Kristin. 1.01.07, 1.01.08, 4.01.08, 4.07.02, 4.07.03, 4.07.04, 4.07.07, 4.07.08, 4.07.09
Conrad, Arnaud. 3.09.24
Conte, Marianna. 3.02.01
Contini, Daniele. 3.02.01
Cooper, Christopher. 3.09.16, 4.02.06, 4.16.02
Cooper, Keith. 3.06.06
Coors, Anja. 4.06.16
Coosemans, Thierry. 5.02.12, 5.03.11
Coquery, Marina. 4.03.11
Cordero-De-Castro, Andrea. 2.01.19, 2.01.20
Cornelis, Geert. 3.14.04
Corrà, Francesca. 3.13.16
Corredor, Juan. 5.01.17
Correia Fernandes, Rita. 4.04.22
Corrente, Salvatore. 6.02.03
Corsi, Ilaria. 1.02.05, 1.02.06, 1.02.12, 1.02.15, 2.09.07, 2.09.09, 2.11.04, 2.11.07, 2.11.08, 2.11.09, 2.11.11
Cosio, Claudia. 1.08.04, 4.14.30
Cossu-Leguille, Carole. 3.09.18
Costa, Daniele. 5.01.12, 5.02.12
Costa, Helder. 3.06.02
Costa, Tamíris Pacheco. 5.02.08
Costa Da Silva, Victória. 1.09.06
Costantino, Valeria. 4.11.05
Coté, Karine. 4.09.07
Cotugno, Pietro. 2.09.11, 4.04.10
Coudret, Christophe. 3.11.19
Coulaud, Romain. 2.02.19
Coulon, Frederic. 2.03.07
Coumoul, Xavier. 1.09.01
Courant, Frederique. 1.08.13, 4.14.32
Courcoul, Camille. 2.04.08
Coutris, Claire. 3.08.05, 6.04.12
Couture, Patrice. 4.09.07
Covaci, Adrian. 7.01.10
Cowan, David. 3.15.32
Cowger, Win. 3.08.04, 3.08.22
Craeye, Bart. 5.01.08
Cravo-Laureau, Cristiana. 2.03.07
Crespo, María Jesús. 2.05.07
Creusot, Nicolas. 2.03.05
Creutzig, Felix. 5.01.19

Crevoisier, David. 3.10.26
Cristiano, Walter. 1.07.16, 2.05.28
Cristobal, Susana. 1.07.08
Crookes, Michael. 3.13.33
Crosland, Helena. 2.04.07, 4.08.02
Cross, Richard. 1.02.19
Crotti, Elena. 7.02.05
Crum, Steven. 3.08.24, 6.01.02
Crupi, Vincenza. 2.11.08
Cruz-Santiago, Omar. 1.04.13
Cruzeiro, Catarina. 3.07.08, 3.07.10
Cucherousset, Julien. 3.11.22
Cucurachi, Stefano. 5.01.05, 5.01.09
Cui, Rongxue. 2.04.03, 3.12.12
Cuine, Stephan. 2.09.24
Culatina, Stefano. 3.03.19
Cunningham, Brittany. 2.08.13, 4.15.07, 4.15.08
Curnick, David. 2.05.13
Curry, Jonathan. 6.01.04
Curtet, Laurence. 4.12.08
Curtis, Becky. 1.08.07
Cuypers, Ann. 1.06.06
Czub, Gertje. 6.02.01

D

D' Agostino, Antonella. 2.07.06
D'angelo, Elisa. 4.04.21, 4.11.04
D'errico, Giuseppe. 2.13.06
Da Costa, João. 3.08.25
Dabek, Ewa. 3.02.07
Dachs, Jordi. 2.11.03, 3.04.04
Dahllöf, Ingela. 2.06.03
Dai, Wencai. 4.03.05
Dalens, Jean-Marc. 4.02.09
Dalkmann, Philipp. 4.05.01
Dalla Rovere, Giulia. 3.13.28
Damalas, Dimitrios. 1.10.06
Damasceno, Evila. 1.07.07
Dang, Zhichao. 2.01.02
Daniel, Amber. 1.01.12
Daniel, Otto. 2.02.20, 2.12.15, 6.02.02
Daniele, Gaelle. 2.05.19
Danopoulos, Evangelos. 3.08.03
Dauchy, Xavier. 4.12.08, 4.12.09
Davey, Charlie. 4.14.07
Davidson, Thomas. 2.06.01
Davoli, Enrico. 3.15.25, 3.15.26
Davranche, Mélanie. 3.11.29, 3.14.03
Davy, Christina. 2.04.09
Dawson, Amanda. 3.12.20, 3.12.24
De Baat, Milo. 4.12.02, 4.12.11
De Bartolomeo, Anna Rita. 3.02.01
De Beni, Eleonora. 2.05.26
De Bin, Riccardo. 1.08.11, 1.08.12
De Blaeij, Arianne. 6.02.06
De Boer, Jacob. 3.08.06, 3.08.24
De Brito, Júlio. 4.14.31
De Castro, Nuria. 2.06.02
De Diego, Alberto. 2.02.07, 4.03.19
De Dobbeleer, Inge. 3.05.09
De Knecht, Joop. 6.02.06
De La Casa-Resino, Irene. 4.14.04
De La Torre, Adrián. 3.10.04, 3.13.12, 3.13.13, 3.15.09, 3.15.11
De Loof, Hans. 7.01.10
De Meester, Luc. 2.01.12, 2.01.13
De Meester, Steven. 5.03.08
De Nijs, Ton. 4.16.04
De Oliveira, Rhaul. 3.13.14, 4.14.06
De Palma, Cesare Pierpaolo. 2.09.11, 3.08.08, 4.04.10
De Paola, Domenico. 4.04.10
De Prins, Maarten. 7.01.10
De Rijcke, Maarten. 4.04.23, 7.03.03
De Roeck, Naomi. 7.01.07
De Santiago Martín, Ana. 7.02.06
De Schamphelaere, Karel. 3.09.17, 4.05.06, 4.06.25, 4.06.26, 4.06.27
De Senerpont Domis, Lisette. 4.17.05
De Silva, Amila. 2.05.15
De Wit, Cynthia. 3.15.28
De Witte, Bavo. 3.08.06, 3.11.20
De Zwart, Dick. 4.05.11
Deacon, Samantha. 6.01.04
Dean, Richard. 3.12.32
Debarre, Laura. 5.07.02
Debecker, Dominique. 8.05.04
Debret, Maxime. 4.02.11
Debry, Ronald. 1.06.02
Dechesne, Arnaud. 3.04.05
Decourten, Bethany. 1.06.04
Dedourge-Geffard, Odile. 2.05.19
Degli Esposti, Davide. 1.08.04, 1.08.08, 2.02.15
Dekker, Rene. 2.05.24, 2.05.26
Dekker, Stefan. 3.03.13, 4.10.01, 4.10.02
Del Cerro, Corinne. 3.08.06
Del Puerto, Oihane. 1.09.14
Dela Cruz, Joseph. 1.05.22
Delahaut, Laurence. 1.04.03, 4.14.30
Delangle, Pascale. 1.10.08
Delannoy, Matthieu. 2.09.16, 2.09.19
Della Sala, Gerardo. 4.11.05
Delnat, Vienna. 2.08.04, 2.08.05, 4.10.06
Delor Jestin, Florence. 3.12.22
Delorme, Astrid. 3.12.22
Delorme, Nicolas. 4.03.11
Delouvrier, Eleonore. 3.01.08
Delpit, Nicolas. 4.09.08
Delputte, Peter. 7.01.07
Dempster, Emma. 1.06.09
Den Broeder, Marjo. 1.05.18
Den Haan, Klaas. 4.05.11
Deng, Xin. 4.12.03
Dereudre, Eric. 7.02.01
Desforges, Jean-Pierre. 2.05.10
Desing, Harald. 5.03.05
Desmarteau, Dean. 3.16.04
Desmet, Cloe. 3.12.29, 6.04.05
Desrousseaux, Alizée. 3.15.04, 4.10.01, 4.10.02
Deutschmann, Björn. 1.04.12
Devers, Jason. 3.15.15
Devier, Marie Helene. 3.05.03
Dévier, Marie-Hélène. 3.01.13, 3.15.17, 4.12.12
Deviller, Genevieve. 4.02.06
Devriese, Lisa. 7.03.04
Deweirdt, Juliette. 3.02.05
Dewulf, Jo. 5.03.08
Dey, Cody. 2.01.09
Deydier Stephan, Laurence. 6.04.01
Dhatri, Sumukha. 5.01.19
Dherret, Lysiane. 4.03.11
Di Carlo, Marta. 2.13.06
Di Criscio, Michela. 1.06.05
Di Domenico, Kevin. 1.07.16
Di Fulvio, Fulvio. 5.02.03
Di Guardo, Antonio. 3.15.05, 3.15.24, 3.15.25, 3.15.26, 4.04.03
Di Noi, Agata. 1.01.17, 2.07.06
Diamond, Miriam. 3.11.08, 3.15.33
Dias, Ana Cláudia. 5.02.08
Diaz, Cecilia. 2.03.02
Diaz De Cerio, Oihane. 6.05.03
Diaz Navarrete, Cecilia Andrea. 2.03.01
Dickens, John. 2.08.13, 4.15.08
Diderich, Philippe. 4.13.05
Diehle, M.. 3.03.05
Dietrich, Christian. 4.13.10, 6.01.08
Dietz, Rune. 2.05.10
Diez-Ortiz, Maria. 1.02.19, 3.11.24
Diez-Quijada, Leticia. 4.11.07, 4.11.12, 4.11.14
Dik, Pim. 3.10.12
Dikšaitytė, Austra. 4.04.05
Dimitrova, Stefka. 2.12.09
Diniz, Mário. 4.12.01
Dinoi, Adelaide. 3.02.01
Dirks, Karlijn. 3.09.15
Dittmar, Stefan. 3.11.18
Dodd, Josie. 2.09.18
Doering, Jonathon. 1.04.12
Doganis, Philip. 4.01.11
Dokter, Lina. 4.04.24
Dolar, Andraz. 3.12.08, 4.15.09
Dolenec, Matej. 4.02.08
Domercq, Maria Del Prado. 3.12.17
Domingues, Ines. 2.01.17, 2.08.01, 2.08.02
Dominguez-Ramos, Antonio. 5.01.18
Domisch, Sami. 4.10.05
Dopp, Elke. 3.16.15
Doria, Halina. 6.03.09
Dorn, Alexander. 4.05.01
Dorn, Sabine. 3.03.14
Dornan, Laura. 4.13.14
Dorne, Jean. 8.01.01
Dorward, Mairi. 4.02.07
Douglas, Heather. 8.03.04
Douziech, Mélanie. 5.01.11
Dove, Alice. 3.15.02
Doya, Rio. 2.05.18
Doyon, Maurice. 5.06.07
Drabkin, Dmitri. 7.02.02
Drago, Massimiliano. 3.09.14
Drever, Mark. 3.15.30
Drewes, Jorg. 3.04.08, 3.12.18
Dreyer, Annekatrin. 4.17.08
Drobne, Damjana. 3.08.02, 3.12.08, 4.15.09
Drobniewska, Agata. 3.12.06, 4.03.12
Dröge, Rianne. 4.15.01
Drost, Wiebke. 2.05.24, 2.05.26
Drouillard, Ken. 3.15.30
Druez, Flora. 2.09.08, 3.09.23
Drummond, Jennifer. 3.11.23
Dryfhout-Clark, Helena. 3.15.08
Du, Miaomiao. 4.10.10
Du Hoffmann, Gunther. 2.04.11
Du Pasquier, David. 1.05.15
Dubey, Asmita. 4.17.05
Dubois, Grégoire. 8.04.02
Duboisset, Arnaud. 3.10.26
Dubuis, Pierre-Henri. 6.02.02
Ducrot, Virginie. 2.04.10, 2.04.12, 4.06.04
Dudefoi, William. 4.15.10
Dudoitis, Vadimas. 3.02.08
Duflot, Aurélie. 2.02.12, 2.02.19

Duflou, Joost. 5.03.02
Dufour, Vincent. 4.12.08, 4.12.09
Dufourcq Sekatcheff, Elizabeth. 2.09.24
Dugan, Nicholas. 4.11.10
Duke, Guy. 2.05.24, 2.05.25, 2.05.26
Dulsat-Masvidal, Maria. 2.05.22, 2.05.23
Dumas, Thibaut. 1.08.13
Dumollard, Remì. 2.02.11
Duplaix, Nicole. 3.12.27
Dupont, Anne. 4.14.14
Duporte, Geoffroy. 1.08.13
Duporté, Geoffroy. 4.04.13
Dupraz, Valentin. 3.01.13, 4.12.12
Duquesne, Sabine. 2.01.02, 4.06.07
Duran, Robert. 2.03.07
Dutour Sikiric, Maja. 4.02.08
Duus, Annette. 1.05.11
Dyballa, Sylvia. 1.07.04

E

E Silva, Claudia. 2.13.09
E Silva, Francisca. 4.14.17
Eastabrook, Chloe. 2.04.01, 2.08.16
Eatherall, Andrew. 3.10.23
Ebeling, Markus. 4.13.10
Eberhardt, Julian. 2.06.08
Ebert, Ina. 4.14.03
Ebinghaus, Ralf. 3.13.04
Ebke, Peter. 4.06.28, 4.06.29
Ečimović, Sandra. 4.17.03
Eckel, William. 1.01.12
Economos, Christina. 5.04.15
Ede, Sarah. 3.08.15
Eens, Marcel. 2.05.03, 3.13.19
Eeva, Tapio. 1.04.07, 1.04.09
Egan, Nicole. 4.12.03
Egardt, Jenny. 2.06.03
Egerer, Sina. 4.16.05
Eggen, Trine. 4.04.12
Egger, Barbara. 6.02.02
Eghbalinejad, Mahleh. 3.16.12
Egli, Melanie. 3.07.02
Egsmose, Mark. 3.09.24
Ehlig, Björn. 3.15.14
Eilebrecht, Elke. 1.05.13, 1.05.16, 1.08.05
Eilebrecht, Sebastian. 1.03.02, 1.08.05
Eilmus, Sascha. 2.07.01
Eisentraut, Paul. 3.11.13
Eitzer, Brian. 7.01.08
Eitzinger, Josef. 4.10.01, 4.10.02
Ekelund Ugge, Gustaf. 1.08.01
Ekner-Grzyb, Anna. 2.13.08
Ekvall, Tomas. 5.03.01
El Hadri, Hind. 3.11.14
El Wanny, Nadine. 2.09.16, 2.09.19
El-Fityani, Tamzin. 2.09.06
El-Sheikh, Mohamed. 3.07.01, 3.11.27
Elena, Mazlova. 4.04.19
Elepaño, Amalia. 1.05.22
Elfeky, Imane. 4.02.09
Elischer, Anne. 2.04.10
Eliso, Maria Concetta. 1.02.06
Eljarrat, Ethel. 2.05.12, 3.02.10, 7.01.02
Ellingsen, Ingrid. 3.15.06
Elliott, John. 3.15.30
Ellis, Laura-Jayne. 4.01.12
Ellis, Lee. 1.03.05, 1.07.13
Embry, Michelle. 1.01.07, 1.05.10, 4.01.08, 4.03.10, 4.03.17
Emmanouil, Nikolaos. 5.03.13
Emnet, Philipp. 4.06.13
Empl, Michael. 4.14.12
End, Nicole. 6.04.04
Endo, Satoshi. 3.15.36, 4.09.06
Eng, Anita. 3.13.17
Enge, Ellen Katrin. 2.05.01
English, Chris. 3.15.34
Entling, Martin. 2.06.10
Eo, Soeun. 3.08.19, 3.11.12
Eon, Mélissa. 2.03.05
Ericson, Lars. 3.15.22
Erk, Marijana. 2.02.17
Ernst, Gregor. 2.09.05
Ernstoff, Alexi. 5.05.02
Erzgraeber, Beate. 4.09.03, 4.09.04
Escher, Beate. 1.03.11, 4.12.10, 4.13.12
Esimbekova, Elena. 1.01.16, 1.09.10
España, Pilar. 3.16.16
Espin, Silvia. 1.04.07, 1.04.08, 1.04.09, 2.05.04, 2.05.25, 2.12.04, 2.12.10
Espín, Silvia. 2.05.24, 2.12.05, 2.12.08
Espindola, Evaldo. 2.01.19
Espinosa-Reyes, Guillermo. 1.04.13
Esponda, Maria. 1.02.19
Esponda, Maria Fernanda. 1.02.19, 3.11.24
Esposito, Germana. 4.11.05
Esteban, Marta. 3.13.22
Esther, Alexandra. 2.12.14
Etxebarria, Nestor. 1.09.20, 3.13.14, 4.14.22
Eulaers, Igor. 2.05.25
Eusébio, Rodrigo. 4.14.34
Evangelista, Sara. 1.05.21
Evans, Nicola. 3.03.16
Everaert, Gert. 3.11.20, 3.12.05, 7.03.01, 7.03.03, 7.03.04
Exeler, Nina. 1.10.02

F

Fabbri, Elena. 3.03.21, 3.12.33
Fabbri, Serena. 5.05.07
Faber, Daniel. 4.05.01
Faber, Jack. 4.16.01, 4.16.02, 4.16.03
Fabrello, Jacopo. 3.13.28
Fabres, Joan. 3.08.06
Fackelmann, Gloria. 3.12.34
Fadel, Ali. 4.11.03
Fagundes, Maria Teresa. 1.05.05, 1.05.07, 1.05.08
Faist, Sven. 3.08.09
Falakdin, Parisa. 3.15.05
Fallet, Manon. 1.06.05
Fantke, Peter. 3.08.05, 5.04.08, 5.05.08, 6.02.08
Farama, Emilie. 3.10.26
Farbos, Audrey. 1.06.09
Faria, João Marcos. 1.09.06
Faria, Melissa. 2.01.11
Farkas, Julia. 1.07.12, 3.12.13, 6.01.06, 6.01.09, 6.04.12
Farré, Maria-José. 3.04.08
Farre, Marinella. 3.08.06
Fasel, Melanie. 2.08.08
Fassbender, Christopher. 1.01.08
Fatta-Kassinos, Despo. 3.03.11
Fattore, Elena. 3.15.25, 3.15.26
Fattorini, Daniele. 2.13.06
Fattorini, Niccolò. 2.11.09
Faure, Olivier. 2.09.08, 3.09.23
Faure, Paul. 2.01.22
Feckler, Alexander. 4.02.12
Fedele, Andrea. 5.04.06
Federico, Antonio. 4.01.11
Feidt, Cyril. 2.09.16, 2.09.19, 4.03.23, 4.12.08, 4.12.09
Feiler, Ute. 3.03.17
Feld, Christian. 4.13.11
Feldbrügge, Michael. 5.03.17
Feliu, Jordi. 2.12.02
Fellin, Phil. 3.15.08
Fellmann, Sebastian. 2.04.10
Felten, Vincent. 4.02.10
Felton, Adam. 5.02.03
Fenet, Helene. 1.08.13, 4.14.32
Feng, Siyuan. 3.11.14
Fenner, Kathrin. 3.04.07
Feraille, Adélaïde. 5.03.03
Ferjan, Špela. 5.03.09
Fernandes, Eva. 2.06.10
Fernandes, Helena. 3.08.25
Fernández, Jesús. 3.15.09
Fernández, Maria Dolores. 2.09.14, 6.04.07
Fernández Arribas, Julio. 2.05.12
Fernández-Alba, Amadeo. 7.02.03
Fernández-Arribas, Julio. 7.01.02
Fernandez-Cruz, Maria Luisa. 1.02.16
Fernandez-Lopez, Carmen. 4.04.06
Fernandez-Torres, Ruth. 4.17.02
Fernie, Kim. 1.04.05
Ferrari, Benoit. 4.15.10
Ferrari, Benoit J.d.. 2.02.06, 2.08.08
Ferrario, Claudia. 3.13.16, 3.13.20
Ferraro, Fabrizio. 2.09.07
Ferreira, I.m.p.l.v.o.. 4.11.07
Ferreira, Inês. 2.08.01
Ferreira, Paula. 2.10.15
Ferreiro Amil, Berta. 4.04.11
Ferret, Céline. 3.15.17
Ferri, Alessandro. 2.10.01
Fetke, Janine. 1.06.02
Fett, Bastian. 5.01.14
Fettweis, Andreas. 3.09.17, 4.05.06
Fialova, Pavla. 4.12.10
Fick, Jerker. 2.01.03, 2.01.18, 4.14.28
Fidalgo, Fernanda. 2.04.05, 4.04.11
Figueira, Etelvina. 3.09.20, 3.12.14, 6.04.08
Fiket, Željka. 3.03.03, 4.02.08
Fildier, Aurélie. 2.05.19
Filipic, Metka. 4.11.13
Filker, Sabine. 4.14.24
Filser, Juliane. 2.09.13, 4.11.11
Fink, Patrick. 1.04.12
Finkbeiner, Matthias. 5.04.04, 5.06.04
Fischer, Björn. 3.14.02
Fischer, Jonas. 2.09.13
Fischer, Marten. 3.08.20
Fiskal, Annika. 3.09.21
Fitzsimons, Mark. 3.07.11
Flade, Jens. 3.10.21
Flamant, Frederic. 1.05.20
Flamenco, Raul. 3.13.24
Flerlage, Hannah. 4.10.11
Flick, Robert. 1.06.02
Flipkens, Gunter. 3.09.05
Floers, Markus. 3.03.06
Flörke, Martina. 4.10.05
Fochi, Igor. 7.01.06
Focks, Andreas. 4.06.06, 4.06.22, 4.08.03, 4.10.07

Fohrer, Nicola. 4.06.22
Fones, Gary. 3.15.32
Font, Alejandro. 2.11.11
Forbes, Valery. 7.01.04
Ford, Alex. 2.01.01, 2.01.02, 2.01.03, 2.01.21, 2.02.13
Ford, Robert. 6.02.03
Forget-Leray, Joëlle. 2.02.12
Forsell, Nicklas. 5.02.03
Fossum, Frida. 1.07.12
Foster, Karen. 4.03.10
Fostier, Julien. 3.10.26
Foudoulakis, Manousos. 1.01.13, 1.01.14
Fourcot, Aurore. 4.03.23
Fournier, Agnes. 2.09.19, 4.03.23
Fragão, Joana. 6.01.05
Franceschini, Alessandra. 3.09.26
Francisco, Rafael. 4.14.17, 4.14.18
Franco, Daniel. 6.04.07
Francoeur, Madison. 1.07.11
Frédéric, Hervant. 3.12.10
Fredricks, Timothy. 1.01.13, 1.01.14
Freeland, Joanna. 2.04.09
Freire, Mara. 4.14.17
Freitas, Marisa. 4.11.07, 4.11.08, 4.11.09
Frelon, Sandrine. 2.09.24
Friesen, Anton. 3.03.04
Frolova, Natalia. 2.11.10
Fu, Qiuguo. 4.14.21
Fuchs, Benjamin. 5.01.02
Fuchs, Mike. 3.10.14
Fuchs, Monika. 3.11.03
Fuelling, Olaf. 2.01.08
Fuertes, Inmaculada. 2.01.12
Fuisz, Tibor. 2.05.24
Fujito, Yuka. 3.13.08
Fukai, Takayuki. 3.11.26
Fukunaga, Yu. 3.08.07, 3.14.07
Fuller, Neil. 2.02.13
Funck, Matin. 3.12.18, 3.14.02
Furdek Turk, Martina. 3.04.02
Furlong, Edward. 3.03.02

G

Gaballah, Shaza. 1.03.07
Gabbert, Silke. 6.02.04, 6.02.06, 6.02.07
Gabrielli, Paolo. 3.09.26
Gabrielsen, Geir. 3.03.01
Gabsi, Faten. 4.05.02
Gachanja, Anthony. 3.07.11, 4.14.11
Gade, Annika. 5.05.04
Gaertner, Christian. 3.01.06, 3.05.15
Gaillard, Gerard. 5.02.04
Gaillard, Juliette. 4.12.12
Gaillet, Veronique. 1.08.04
Gajraj, Rudrani. 4.10.01, 4.10.02
Galán, Jose. 7.02.07
Galas, Simon. 2.09.24
Galert, Wiebke. 6.04.02
Galgani, Francois. 3.08.06
Galic, Nika. 2.05.17, 4.06.28, 4.06.29, 4.16.01
Gallampois, Christine. 3.02.11
Gallego, Julian. 1.09.05
Gallego, Sara. 3.07.08
Gallen, Michael. 3.08.15
Gallorini, Andrea. 4.14.30
Galloway, Tamara. 3.12.26
Gamba, Marco. 2.05.28
Gámiz, Beatriz. 7.02.07
Gan, Chee-Sian. 3.13.10
Gan, Lin. 1.07.14
Ganci, Aristide. 3.13.11
Gandhi, Jemi. 3.03.07
Gao, Zhenglei. 1.05.10
Garaguso, Ignazio. 3.15.35
Garbarienė, Inga. 3.02.08, 3.02.09
Garbicz, Damian. 4.14.33
Garbini, Gian. 4.04.02, 4.04.09
Garbini, Gian Luigi. 4.14.25
Garbisu, Carlos. 4.14.22
Garcia, Angeles. 1.09.08
Garcia, Jose Luis. 4.04.06
García Barrera, Tamara. 4.17.02
Garcia Mendoza, Diego. 1.04.06
García-Barrera, Tamara. 2.13.02, 2.13.03
García-Calvo, Eloy. 5.02.13, 5.03.12
Garcia-Fernandez, Antonio Juan. 1.04.07, 1.04.09, 2.05.04, 2.05.06, 2.05.24, 2.05.25, 2.12.04, 2.12.05, 2.12.08, 2.12.10
Garcia-Garin, Odei. 3.09.14
García-Martínez, María-Jesús. 5.02.06
García-Pacheco, Raquel. 5.03.12
García-Valverde, M.. 7.02.03
Garcia-Velasco, Nerea. 2.02.07, 4.03.19
Garciagomez, Concepcion. 2.09.14, 6.04.07
Gardia Parege, Caroline. 3.01.13
Gardia-Parège, Caroline. 4.02.11, 4.12.12
Gargiulo, Antonio. 2.05.04
Garman, Emily. 3.09.08, 3.09.09, 3.09.10, 3.09.11, 3.09.12, 3.09.13, 3.09.16, 4.02.06
Garrido, Itziar. 1.09.20
Gartner, Coral. 3.03.22
Gaßmann, Matthias. 3.13.25, 3.16.03
Gathmann, Achim. 6.02.01
Gauch, Roger. 2.12.15
Gaugler, Tobias. 5.06.06
Gautam, Prakash. 1.07.01
Gautier, Maud. 2.11.12
Gavalás-Olea, Antonio. 4.11.11
Gaze, William. 2.03.11
Gebbink, Wouter. 3.13.33
Gebler, Sebastian. 3.03.15, 3.10.14, 3.10.23, 4.09.03
Geels, Camilla. 3.02.06
Geffard, Alain. 1.04.03, 1.08.04, 2.05.19
Geffard, Oliver. 1.08.04, 2.02.15
Geffard, Olivier. 1.08.08, 4.03.03, 4.03.11
Gelber, Clementine. 4.02.05
Geneste, Emmanuel. 4.12.12
Georgantzopoulou, Anastasia. 6.04.12
Georgiou, Stavros. 3.12.32
Gerbinet, Saicha. 5.03.10
Gerdts, Gunnar. 3.08.17, 3.08.20, 3.08.23
Geret, Florence. 4.14.32
Gergely, Szilveszter. 3.08.11
Gergs, Andre. 2.09.05, 4.06.09, 4.06.14, 4.06.15
Gergs, Katrin. 2.02.02
Gerhard, Jana. 4.06.30
Gerhardt, Renata. 4.07.01
Gerken, Maximilian. 2.06.07
Germande, Ophélie. 3.02.05
Gerson, Jacqueline. 2.03.08, 3.16.14
Gerstle, Verena. 2.06.07, 2.06.08
Gestin, Ophelia. 4.03.03, 4.03.11
Geueke, Birgit. 3.15.31, 7.03.06
Geyer, Carina. 5.05.13
Gezahegne, Wondem. 3.10.05
Ghafoor, Abdul. 3.10.18
Ghiglione, Jean François. 3.08.14
Ghorab, Mohamed. 4.11.10
Ghoshal, Subhasis. 4.04.07
Giacoma, Cristina. 2.05.28
Giamberini, Laure. 3.09.18
Giardina, Matthew. 3.13.10
Gibon, Thomas. 5.06.05
Gießing, Benedikt. 2.01.07
Gigault, Julien. 2.11.12, 3.11.14, 3.11.29, 3.14.03
Gildemeister, Daniela. 4.14.01
Gill, Touqeer. 3.02.08, 3.02.09
Gilliland, Douglas. 3.12.29
Giménez, Valéria. 3.09.20
Giménez Verdugo, Joan. 2.05.12
Gin, Karina. 3.16.06, 3.16.09
Ginebreda, Antoni. 3.03.09
Giordano, Maria Elena. 3.02.01
Giorgetti, Alessandra. 3.08.06
Girdhari, Kyle. 3.15.33
Gismondi, Eric. 2.05.21, 3.09.25, 4.09.10
Giubilato, Elisa. 3.07.04
Giuliani, Maria Elisa. 2.13.06
Giusti-Petrucciani, Nathalie. 2.02.12
Gkotsis, Georgios. 2.05.24, 2.05.26, 3.03.11
Glaberman, Scott. 1.05.10
Gladbach, Anja. 4.13.10
Glassmeyer, Susan. 3.03.02
Glowacka, Natalia. 2.05.24, 2.05.26
Gobas, Frank. 4.03.14
Göckener, Bernd. 1.08.05, 3.13.25, 3.16.03
Godejohann, Matthias. 3.08.17
Godinez Bravo, Diego. 1.04.13
Godlewska, Paulina. 3.03.12
Golz, Lisa. 1.05.05, 1.05.07, 1.05.08
Gölz, Lisa. 1.05.20
Gomes, Susana. 2.09.04
Gomes, Tania. 1.02.04, 3.12.01, 3.12.13, 6.04.12
Gomes, Vicente. 2.02.13
Gomez, Elena. 1.08.13, 4.04.13
Gomez, Tânia. 4.14.23
Gómez Ariza, José Luis. 4.17.02
Gómez Navarro, Olga. 3.03.08, 3.15.21
Gomez Pizzaro, Benjamin. 2.03.01, 2.03.02
Gómez-Canela, Cristian. 2.01.11
Gómez-Oliván, Leobardo Manuel. 2.01.11
Gómez-Ramírez, Pilar. 2.05.04, 2.05.24, 2.05.25
Gomez-Ramirez, Pilar. 2.05.06, 2.12.04, 2.12.05, 2.12.08
Gómez-Ramos, M.m. 7.02.03
Goncalves, Ana Marta. 1.09.07, 2.04.15, 2.05.29, 2.08.14, 3.12.28
Gonçalves, Fernando. 1.07.17, 2.02.05, 2.03.03, 2.03.09, 2.04.15, 2.05.29, 2.08.14, 3.09.19
Gonçalves, Idalina. 2.10.15
Goncalves, Liliana. 2.09.10
Gonçalves, Micael F.m.. 2.10.15
Gonçalves, Nuno. 1.09.14
Goncalves, Sandra. 4.01.10
Gönczi, Mikaela. 3.10.27
Gonsior, Gundula. 4.08.10
Gony, Sylviane. 3.10.24, 4.03.16
Gonzalez, Michael. 6.02.03
Gonzalez, Patrice. 1.06.07
González, Sara. 3.13.22

Gonzalez, Susana. 2.05.02
Gonzalez Soto, Nagore. 1.02.08
Gonzalez-Alcaraz, M. Nazaret. 2.10.09, 2.10.10
Gonzalez-Andres, Veronica. 1.02.19, 3.11.24
González-Aravena, Marcelo. 2.11.08, 2.11.11
González-Baizán, Angela. 3.05.06
Gonzalez-Gaya, Belen. 1.09.20, 3.13.14, 4.14.22, 6.05.01
Gonzalez-Mille, Donaji. 1.04.13
Gorbi, Stefania. 2.13.06
Gori, Riccardo. 2.09.07
Gosselin, Anne. 4.13.14
Gota, Fabrizio. 5.01.10
Gottardo, Stefania. 6.04.05
Gottfridsson, Marie. 5.03.01, 5.04.08
Gottstein, Sanja. 2.02.17
Gouin, Todd. 3.01.20
Gould, Sophie. 1.03.03
Goulson, Dave. 2.05.20
Goussen, Benoit. 2.05.17, 4.06.09, 4.06.10, 4.06.13, 4.06.14, 4.06.15, 4.06.16, 4.08.04
Goussen, Benoît. 4.06.06, 4.06.17, 4.06.18
Graber, Marianne. 2.02.09
Grabic, Roman. 4.12.10
Granadeiro, José Pedro. 2.12.17
Grande, Cristina. 3.13.22
Grandjean, Dominique. 4.14.30
Grassl, Bruno. 3.11.14
Graumans, Martien. 3.04.06
Graumnitz, Stephanie. 4.14.10
Gray, Andrew. 3.08.04, 3.08.22
Gray, Leon. 3.03.16
Greco, Dario. 4.01.11
Greco, Luke. 3.02.07
Gredilla, Ainara. 2.02.07, 4.03.19
Green, Ashley. 5.04.14
Green, Bidemi. 2.01.21
Green, Curtis. 2.04.02
Green, John. 1.01.08, 1.01.13, 1.01.14, 2.04.11
Green, Norman. 3.08.12
Gregory, Susan. 2.11.02
Greiner, Andreas. 3.11.30
Grenni, Paola. 4.04.02, 4.04.09, 4.14.13, 4.14.25
Gresham, Alexandra. 1.04.10
Gresset-Bourgeois, Mathilde. 5.01.11
Grevé, Michael. 2.07.01
Grgić, Ivana. 1.10.01
Griethuysen, Corine. 3.10.01
Griffin, Timothy. 5.04.15
Griffiths, Megan. 2.13.07, 3.01.04, 3.01.08
Grimalt, Joan. 2.06.01
Grimm, Volker. 7.01.04
Grinsted, Lena. 2.01.21
Grischek, Thomas. 3.08.09
Grisolia, Cesar. 2.01.17
Groenendijk, Piet. 3.10.12
Groezinger, Franziska. 4.09.03, 4.09.04
Groffen, Thimo. 3.13.19, 3.13.23
Groh, Ksenia. 1.01.09, 3.15.31, 4.07.13, 7.03.06
Groleau, Paule Émilie. 4.09.07
Groslambert, Sylvie. 5.03.10
Großmann, Dietlinde. 3.10.21
Gross, Elisabeth. 2.04.08
Gross, Wolfgang. 3.11.30
Grossar, Daniela. 6.02.02
Grossman, Jarod. 3.05.16
Gruenenwald, Thomas. 5.06.01
Grung, Merete. 3.06.03, 4.01.07, 4.01.09, 4.10.01, 4.10.09, 4.14.02
Grzesiuk, Elzbieta. 4.14.33
Grzesiuk-Bieniek, Malgorzata. 4.14.33
Grzyb, Tomasz. 2.13.08
Guadie, Awoke. 4.04.20
Gual-Gimeno, Marta. 1.06.03
Guascito, Maria Rachele. 3.02.01
Guchok, Maria. 4.04.16
Gueganno, Tristan. 4.01.03
Guerin, Sarina. 3.13.26
Guerrero-Barrera, Alma Lilian. 3.09.02
Guhl, Barbara. 4.13.11
Guibert, Christelle. 3.02.05
Guilhermino, Lucia. 1.02.08
Guímaro, Hugo. 6.01.05
Guirandy, Noemie. 1.06.07
Guiraud, Michel. 2.05.24
Gunaalan, Kuddithamby. 3.12.33
Gundersen, Cathrine. 2.11.10
Gunnarsson, Josefin. 5.04.08
Günther, Nadine. 3.05.14
Guo, Zhiling. 6.04.10
Gust, Kurt. 1.03.09, 1.06.08, 3.10.02
Gutiérrez-Expósito, Carlos. 2.01.07
Gutierrez-Praena, Daniel. 4.11.12
Guven, Olgac. 3.12.21
Guy, Martha. 4.13.14
Gwinnett, Claire. 3.06.07, 3.14.05
Gwosdz, Frank. 7.03.06

H

Ha, My Kieu. 4.01.11
Ha, Sung Yong. 3.11.05
Haas, Julian. 1.10.02, 7.04.01
Habekost, Maike. 4.09.03
Habraeken, Nikki. 1.05.18
Hackenberger, Branimir. 8.02.12
Hacker, Christian. 3.12.26
Hackett, Michael. 2.04.07
Hader, John. 4.10.01
Hagan, Ama. 3.16.01
Hageman, Kimberly. 3.10.07, 3.15.13
Hager, Jutta. 2.02.02
Haglund, Peter. 3.02.11
Háhn, Judit. 3.08.11
Hahn, Stefan. 3.01.08, 3.01.19, 3.03.05, 4.09.01, 6.02.07, 6.03.01
Hahne, Joerg. 2.01.07
Haigis, Ann-Cathrin. 1.07.14
Hajjar, Carla. 3.08.05
Hakola, Hannele. 3.15.08
Hakulinen, Hanna. 1.10.07
Halder, Marlies. 1.01.08
Hale, Sarah. 3.13.18
Halfpenny, Richard. 3.14.05
Hallgrimsson, Gunnar Thor. 2.05.25
Hallmann, Anna. 3.15.06
Halsband, Claudia. 3.08.12
Halse, Anne. 3.02.04
Hama, Jawameer. 2.13.07
Hamani, Vincent. 2.02.09
Hamers, Robert. 1.08.07, 2.04.02
Hamers, Timo. 1.09.16
Hamm, Jonathan. 1.01.12
Hammel, Klaus. 3.10.09, 3.10.11, 4.05.01
Hammer, Jort. 4.09.06
Hammers-Wirtz, Monika. 6.03.02
Hammershoj, Rikke. 3.04.05
Hammoud, Noura. 4.11.03
Han, Gi Myung. 3.11.04, 3.11.05, 3.11.12, 3.12.11, 3.12.25, 3.12.30, 3.13.21
Handley, John. 1.01.08
Häner, Andreas. 4.14.09
Hanesch, Susanne. 5.01.06
Hanke, Irene. 6.02.02
Hansen, Bjørn Henrik. 1.07.12, 4.03.22, 6.01.06, 6.01.09
Hansen, John. 4.11.16
Hansen, Martin. 1.05.20, 4.07.07
Hansson, Lars-Anders. 2.01.06
Hansul, Simon. 3.09.17, 4.05.06
Hao, Chunyan. 3.15.07
Happel, Oliver. 3.03.20
Haque, Rehnuma. 7.01.13
Hardege, Joerg. 2.11.05
Hardy, Ingrid. 3.15.40
Haring, Herman. 4.11.10
Harkai, Péter. 3.08.11
Harner, Tom. 3.02.07, 3.13.17, 3.15.07, 3.15.12, 3.15.13, 4.13.14
Haro Castuera, Amparo. 4.14.04
Harper, Bryan. 2.08.13, 4.15.07, 4.15.08
Harper, Stacey. 2.08.13, 4.15.07, 4.15.08
Harpprecht, Carina. 5.01.02
Harre, Kathrin. 3.08.09
Harrison, Eleanor. 3.14.05
Hart, Andy. 8.03.02
Hartig, Florian. 6.06.02
Hartin, Kathleen. 4.13.14
Hartmann, Nanna. 3.08.01, 3.08.02
Hartmann, Vanessa. 3.03.20
Hasenbein, Simone. 4.12.03
Hassan, Sameer. 2.02.16
Hassellöv, Ida-Maja. 2.06.03
Hassold, Enken. 6.03.02
Hauck, Mara. 5.03.09
Hauschild, Michael. 5.04.05, 5.05.04, 5.05.05, 5.05.07, 5.05.09
Hauser, Thomas. 4.11.11
Hayes, Sean. 4.02.07
He, Jia. 3.09.13
Heckel, Gerald. 4.03.20
Hecker, Markus. 1.04.12
Hedgepeth, William. 3.13.08
Hedgpeth, Bryan. 3.01.09
Heger, Sebastian. 4.07.11, 4.10.10, 5.03.17
Heidari, Mohammad. 5.06.07
Heiger-Bernays, Wendy. 4.13.07
Heikkinen, Harri. 1.10.07
Hein, Arne. 4.14.01, 4.14.10
Heine, Simon. 4.05.02, 4.06.21
Heinemeyer, Ute. 3.14.01
Heinen, Marius. 3.10.12
Heinlaan, Margit. 3.12.08
Heinze, Wiebke Mareile. 3.14.04
Helander, Björn. 2.05.25
Helberg, Morten. 2.05.01
Helbig, Christoph. 5.06.06
Heller, Claudia. 3.11.03
Hellpointner, Eduard. 4.05.01
Hellström, Anna-Karin. 5.04.08
Hellström, Gustav. 2.01.03, 2.01.06
Hellweg, Stefanie. 5.02.02, 5.02.03, 7.03.05
Helm, Paul. 3.11.08
Helmholz, Lissa. 3.09.21
Helmus, Rick. 3.03.13
Hendriks, Jan. 2.05.27, 3.01.17, 3.04.06, 4.03.09, 4.06.24, 4.16.04

Hennebert, Pierre. 3.16.05
Hennecke, Dieter. 3.01.04, 3.01.06, 3.01.07, 3.01.08, 3.01.19, 3.05.15, 3.14.02, 4.09.01, 7.02.02
Hennekens, Stephan. 2.04.13
Hennequin, Thomas. 5.01.14, 5.04.11
Henriksen, Cecillie. 4.17.04
Henriques, Isabel. 1.07.07, 2.10.05, 2.10.12, 2.10.13
Henry, Betsy. 6.06.01
Henry, Jason. 2.01.10
Henry, Theodore. 7.03.01
Hentati, Olfa. 2.09.10
Herbera, Xavier. 2.06.02
Hercog, Klara. 4.11.13
Hermann, Markus. 2.08.07, 4.10.01, 4.10.04
Hermens, Joop L.m.. 4.05.11
Herrala, Mikko. 3.07.06, 3.12.16
Herrchen, Monika. 3.03.05
Herrera, Lucía. 3.05.13, 7.02.04
Herrero Villar, Marta. 2.12.07, 2.12.12, 2.12.13
Herrig, Ilona. 3.09.21
Herrmann, Marco. 3.10.06
Herruzo, Ana María. 2.03.04
Herweyers, Robin. 3.04.03
Herwijnen, Rene. 4.14.12
Herzke, Dorte. 2.05.25, 3.08.06
Hessel, Ellen. 1.03.05
Hesser, Franziska. 5.05.06
Hetheridge, Malcolm. 4.14.19
Heuschele, Jan. 2.08.17, 4.06.19
Hewitt, Mark. 4.13.14
Hickmann, Silke. 4.14.12
Hidding, Bjoern. 6.04.04
Hilário, Sandra. 2.10.15
Hildenbrand, Jutta. 5.04.08
Hill, Bridgett. 1.03.04, 1.03.05
Hilscherova, Klara. 1.05.02, 1.05.03, 3.02.03, 4.11.01, 4.12.10
Hilt, Sabine. 2.04.08
Hindle, Ralph. 3.13.07
Hinojosa, Maria Gracia. 1.03.12
Hintelmann, Holger. 2.11.10
Hipfner, Mark. 3.15.30
Hirata, Akiko. 5.05.03
Hischier, Roland. 3.02.02, 5.03.05, 5.03.06, 5.04.02, 5.04.10, 7.01.01
Hiskia, Anastasia. 4.11.03
Hjermann, Dag. 6.01.07
Hjort, Markus. 4.02.05
Hjort-Gregersen, Helle. 1.05.11
Hladik, Michelle. 2.08.12
Hochstrasser, Markus. 6.02.02
Hodapp, Bettina. 2.09.15
Hodges, Juliet. 6.01.03
Hodson, Mark. 3.14.06, 4.06.20
Hofer, Rachel. 3.03.16
Hoffmann, Frauke. 2.01.02
Hofman, Jakub. 3.16.12
Hofman, Sebastian. 2.10.06
Hofmann, Thilo. 3.11.16
Hoger, Stefan. 1.05.12, 2.09.15, 3.01.03, 4.14.14
Hogstrand, Christer. 1.01.02, 1.01.05
Holbech, Bente. 1.05.11
Holbech, Henrik. 1.05.06, 1.05.07, 1.05.08, 1.05.11, 1.05.20
Holgado, Cristina. 3.01.14
Holgersson, Vanessa. 1.01.04
Hölker, Franz. 2.04.08
Holland, Erika. 4.12.03
Hollender, Juliane. 4.03.02, 4.05.05, 4.12.06, 4.14.21
Hollert, Henner. 1.03.02, 1.04.12, 1.07.03, 1.07.14, 1.08.05, 2.01.02, 3.03.20, 4.05.01, 4.07.11, 4.10.10, 4.17.03, 5.03.17
Holmes, Breanne. 6.03.04
Holmes, Christopher. 2.07.02, 3.10.16, 3.10.19, 4.05.08, 4.05.09, 4.05.10, 6.01.03
Holmes, Claire. 3.10.16
Holmlund, Per. 3.06.03
Holmquist, Hanna. 5.04.08
Holmstrup, Martin. 4.03.05
Holten, Roger. 4.10.09
Holton, Elizabeth. 7.01.11
Holtzapple, David. 1.03.09
Hommen, Udo. 4.06.06, 4.06.23, 8.02.06
Honda, Tomonori. 5.04.12
Hong, Chang Pyo. 3.12.11
Hong, Sang Hee. 2.11.08, 3.08.19, 3.11.04, 3.11.05, 3.11.12, 3.12.11, 3.12.25, 3.12.30, 3.13.21
Honti, Mark. 3.04.07
Hoogeweg, Cornelis. 3.10.18, 3.16.04
Hopkins, Kailee. 1.04.05
Horemans, Nele. 1.06.06
Hosen, Md. 5.02.12, 5.03.11
Hosner, Peter. 2.05.24
Hossain, Alamgir. 7.01.13
Hossain, Murshid. 3.05.05
Hostettler, Lu. 1.01.01, 1.01.03, 4.03.08, 4.03.15
Houtman, Corine. 1.09.16
Hovland Steindal, Eirik. 2.11.10
Howey, Xia Meng. 1.03.07
Hsieh, Jui. 1.03.05
Hsu-Kim, Heileen. 3.16.14
Hu, Kaidi. 3.04.09
Huang, Anna. 4.10.07
Huang, Lei. 5.05.08, 6.02.08
Huang, Peng. 4.06.05, 4.17.01
Huang, Weichun. 1.06.02
Huarte, Ainhoa. 3.13.14
Hubbard, Laura. 3.03.16
Huben, Michael. 3.03.05
Huber, Carolin. 1.09.03
Hübner, Uwe. 3.04.08
Hucher, Nicolas. 2.02.19
Hufenus, Rudolf. 3.11.01
Hufnagl, Benedikt. 3.08.18
Hughes, Christopher. 3.01.04, 3.01.07, 3.01.08, 3.01.19, 4.09.01
Hughes, Gregory. 3.10.23
Huijbregts, Mark. 3.01.17, 5.03.09, 5.04.11
Hulgard, Katrine. 1.05.11
Hulthén, Kai. 2.01.06
Hultman, Maria. 1.01.03, 3.08.12, 6.04.12
Hund-Rinke, Kerstin. 2.03.01, 2.03.02
Hung, Hayley. 3.15.07, 3.15.08
Hunt, Alistair. 3.12.32
Hunt, Kathy. 3.13.07
Hunter, Deborah. 1.03.04
Huovinen, Marjo. 3.12.16
Hurley, Jessica. 2.11.05
Hurley, Rachel. 3.08.12
Huszarik, Maike. 2.06.10
Huthwelker, Thomas. 3.13.01
Hutton, Sara J.. 2.08.12, 2.08.13, 4.15.08
Huysveld, Sophie. 5.03.08
Hvorslev, Peter. 1.05.12

I

Iakovides, Iakovos. 3.03.11
Ibarra, Jone. 1.07.04
Ibrahim, Lara. 4.06.11
Idris, Oladayo. 2.09.12
Igartua, Amaia. 3.12.01
Igos, Elorri. 5.06.05
Ikenaka, Yoshinori. 2.05.18
Ilizaliturri-Hernandez, Cesar. 1.04.13
Indiketi, Nishodi. 4.09.07
Infante, Octavio. 2.05.23
Inostroza, Pedro. 2.02.16
Insinga, Logan. 3.10.19
Iordachescu, Lucian. 3.11.06, 3.11.17
Iori, Silvia. 3.13.28
Ippolito, Alessio. 4.08.03
Irabien, Angel. 5.01.18
Irazola-Duñabeitia, Mireia. 1.02.08
Irlam, Rachel. 3.15.32
Isemer, Rena. 2.04.10, 2.04.12
Ishizuka, Mayumi. 2.05.18
Islam, Md Tahmidul. 7.01.13
Islam, Md. Mazharul. 3.05.05, 3.11.25
Islam, Mohammed. 2.01.19
Islam, Rafiquel. 2.02.10
Islam, Rezaul. 6.04.17
Islam, Sharif. 2.05.24
Ismail, Iqbal. 3.16.11, 3.16.13
Isnard, Hélène. 1.10.08
Ito, Koji. 5.04.13
Itsubo, Norihiro. 3.08.07, 3.11.26, 3.14.07, 5.01.20, 5.03.15, 5.04.12, 5.04.13, 5.05.03, 7.01.05
Iturria, Iñaki. 1.01.11, 1.05.09
Itzel, Fabian. 3.12.18, 3.14.02, 4.17.08
Iuretig, Alain. 4.12.09
Ivanovic, Tijana. 5.04.10
Izagirre, Urtzi. 2.02.07, 4.03.19, 6.05.03

J

Jackson, Michelle. 4.10.04
Jacob, Stefanie. 2.01.02
Jacobs, Cor. 3.10.01
Jacobs, Lotte. 7.01.07
Jaegers, Jeremy. 4.09.10
Jagadeesan, Kishore Kumar. 7.01.09, 7.01.11
Jager, Tjalling. 2.08.17, 4.06.07, 4.06.09, 4.06.12, 4.06.13, 4.06.16, 4.06.17, 4.06.18, 4.06.19, 4.12.05, 6.01.06, 6.01.07
Jahnke, Annika. 4.03.01, 6.05.07
Jaka, Oihane. 1.05.09
Jakob, Felix. 4.07.11
Jakobs, Arthur. 5.05.14
Jakoby, Oliver. 4.06.06, 4.06.11
James, Richard. 1.03.09
Jänes, Jaak. 4.01.11
Jang, Mi. 3.11.04, 3.11.05, 3.12.11, 3.12.25, 3.12.30, 3.13.21
Jans, Daniela. 2.04.10, 2.04.12
Jansman, Hugh. 2.05.26
Janssen, Colin. 3.11.20, 7.03.03, 7.03.04
Janssen, Elisabeth. 4.11.06
Janssen, Sharon. 4.06.26
Jantunen, Liisa. 3.15.02, 3.15.33
Jarosz, Emil. 2.04.01, 2.08.16
Jarque, Sergio. 1.07.04
Jartun, Morten. 3.13.18
Järvelä, Essi. 3.12.16
Jarvis, Nicholas. 3.10.27
Jaskulak, Marta. 1.08.02, 2.10.06
Jaspers, Veerle. 1.04.04, 1.04.11, 2.05.02, 2.05.05, 2.05.16, 2.05.25

Jayaraman, Saro. 1.07.11
Jeantet, Alexis. 3.10.20
Jeffers, Terry. 3.05.08
Jeffries, Aaron. 1.06.09
Jekel, Martin. 3.11.18
Jellyman, Juanita. 2.12.16
Jemec, Anita. 3.08.02, 3.12.08, 4.15.09
Jene, Bernhard. 3.10.18
Jenner, Karen. 1.01.01, 4.03.08, 4.03.15
Jenssen, Bjorn Munro. 3.13.18, 6.01.09
Jeong, Jongwook. 3.11.05, 3.12.30
Jeong, Seung-Woo. 1.07.01
Jeong, Yoonah. 4.15.02, 5.07.01
Jepson, Paul. 2.05.13
Jessl, Sarah. 3.11.16, 3.14.01
Jesus, Fatima. 2.03.03, 2.03.09, 3.09.19
Jetten, Mike. 3.04.06
Jewell, Kevin. 3.15.14
Jhutty, Manpreet. 4.03.14
Ji, Kyunghee. 1.07.06
Jieh, Phoenix. 7.03.06
Jimenez, Begona. 2.11.03
Jiménez, Laura. 2.02.07
Jiménez, Laureano. 5.06.03
Jiménez, Pedro. 1.04.07, 2.05.04, 2.12.04, 2.12.05, 2.12.08
Jiménez-Oyola, Samantha. 5.02.06
Jin, Bosen. 3.13.27
Jing, Yuying. 3.10.03, 4.04.12
Joaquim-Justo, Celia. 4.09.10
Jobling, Susan. 2.05.13
Joerss, Hanna. 3.13.04
Johann, Sarah. 1.07.03, 3.03.20, 4.07.11, 5.03.17
Johansen, Jon Eigill. 3.08.06, 3.08.24
Johansson, Magnus. 5.04.08
Johansson, Ulf. 2.05.24
Jöhncke, Ulrich. 3.01.06
Johnson, Dave. 3.10.19
Johnson, Magnus. 2.11.06
Johnson, Tamara. 1.01.12
Johnston, Alice. 7.01.04
Johnston, Dara. 7.01.13
Johnston, Robert. 6.06.01
Jolliet, Olivier. 3.08.05, 5.05.08, 6.02.08
Jonander, Christina. 2.06.03
Jonas, Kristina. 4.12.04
Jones, Jennifer. 4.11.10
Jones, Zoe. 2.02.01, 2.02.03, 6.03.07, 6.03.08
Jong, Mui-Choo. 3.16.09
Jonkers, Tim. 1.09.16
Jonsson, Annie. 1.08.01
Jonsson, Micael. 2.06.11
Jordan, Randy. 3.06.02
Jornod, Florence. 1.09.01
Jos, Angeles. 1.03.12, 4.11.07, 4.11.12, 4.11.14
Jouannais, Pierre. 5.01.03, 5.01.04
Jourdain, Eve. 2.05.09
Journel, Blandine. 3.10.24, 4.03.16
Joyce, Shelby. 1.01.04
Jubault, Severine. 3.10.24, 4.03.16
Juknys, Romualdas. 4.04.05
Jung, Jee Hyun. 1.07.18, 3.09.22, 3.12.11
Jung, Jinhong. 5.07.01
Jungmann, Dirk. 4.14.10
Junior, Carlos. 3.09.04
Junttila, Ville. 3.15.20
Jurga, Natalia. 2.13.08
Jurjanz, Stefan. 2.09.16
Just, Hildegard. 3.13.25, 3.16.03
Juvonen, Risto. 3.07.06

K

Kadi, Mohamed. 3.16.11, 3.16.13
Kadziński, Miłosz. 6.02.03
Kaegi, Ralf. 3.08.06, 3.08.10, 6.04.12
Kairigo, Pius. 4.14.11
Kaisarevic, Sonja. 1.03.10
Kaiser, Christina. 4.05.01
Kaiser, Florian. 4.08.10
Kaka, Hussain. 2.10.08
Kalčíková, Gabriela. 4.07.10, 4.15.05, 6.04.17
Kallenborn, Roland. 3.03.01, 3.15.06
Kalman, Judit. 1.09.08
Kalogerakis, Nicolas. 7.02.05
Kaloudis, Triantafyllos. 4.11.03
Kalyabina, Valeriya. 1.01.16
Kamel, David. 3.07.10
Kamminga, Leonie. 1.05.18
Kämpfer, Christoph. 4.07.11, 5.03.17
Kamstra, Jorke. 1.05.18, 1.06.03
Kandrapu, Vinod. 6.04.17
Kane Driscoll, Susan. 3.05.17
Kannan, Andrew. 7.01.09, 7.01.11
Karadimou, Ioanna. 4.17.04
Karatzas, Pantelis. 4.01.11
Kariuki, Jackline. 1.06.06
Karna, Ranju. 1.03.09
Kascak, Alex. 6.06.01
Kaserzon, Sarit. 3.08.15, 3.12.26
Kasprzyk-Hordern, Barbara. 7.01.09, 7.01.11
Kassotis, Christopher. 7.03.06
Kästner, Matthias. 3.01.06, 3.10.03, 4.04.12
Kataba, Andrew. 2.05.18
Kätelhön, Arne. 5.03.07, 5.04.07
Katsiadaki, Ioanna. 1.05.15, 1.05.16
Katsumiti, Alberto. 1.02.03
Kattwinkel, Mira. 4.06.06
Kavelaars, Marwa. 2.05.03
Kayode, Jegede. 2.13.09
Kazakova, Julia. 4.17.02
Kecorius, Simonas. 3.02.08
Keeble, Nick. 6.03.03
Kehrein, Nils. 3.10.11
Keijzers, Rineke. 4.13.02
Keitel-Gröner, Frederike. 2.01.15
Keiter, Steffen. 1.06.05
Keizer, Jan Jacob. 1.07.17, 3.09.04, 3.09.06
Kelényi, Janka. 3.11.06
Kelley, Barbara. 3.01.09
Keltsch, Nils. 6.01.08
Kênida Lessa Martins, Lunara. 1.09.06
Kennedy, Alan. 1.03.09, 1.06.08
Kenny, Kate. 4.02.04
Kermarrec, Lenaïg. 4.02.09
Kermen, Florence. 1.07.12
Kerndorff, Alexander. 3.11.03
Keyte, Ian. 3.13.33
Khachatryan, Artak. 3.15.27
Khachatryan, Lusine. 3.13.03
Khan, Pathan Moshin. 4.07.04
Khan, Tazeen. 3.14.06
Khodaparast, Zahra. 2.10.05, 2.10.13, 4.01.10
Kholodkevich, Sergey. 2.02.08
Kicheeva, Arina. 1.02.09, 1.02.10
Kiemle, Annika. 4.08.05, 4.08.06
Kienle, Cornelia. 3.03.18
Kierski, Michael. 3.05.17
Kilgallon, John. 6.01.03
Kiljunen, Harri. 1.10.07
Kille, Peter. 1.01.02
Kim, Dokyung. 2.10.11, 3.12.02, 3.12.07
Kim, Haemi. 3.12.02, 3.12.07
Kim, I-Tae. 5.07.01
Kim, Il Hoon. 3.12.25
Kim, Ji Hyun. 1.05.04
Kim, Lia. 1.07.01, 3.12.12
Kim, Min Ji. 1.09.01
Kim, Min Joo. 1.05.04
Kim, Min Seop. 3.12.25
Kim, Moonkoo. 1.07.18, 3.09.22
Kim, Sujin. 1.05.04
King, Peter. 3.12.32
King, Taj. 1.03.09
Kinross, Ashlie. 3.10.07
Kipferler, Nora. 2.04.08
Kirchner, Julia. 3.05.14
Kirin, Maxim. 2.02.08
Kirk, Jane. 2.11.10
Kiruyshina, Anastasiya. 4.04.15
Kisand, Vambola. 3.12.08
Kitami, Tomoya. 5.04.13
Kitanovski, Zoran. 3.02.03
Kittner, Maria. 3.11.13
Kizgin, Ali. 3.03.18
Kjellberg, Matti. 1.10.07
Klaassen, Marcel. 1.04.11
Klaminder, Jonatan. 4.14.28
Klaper, Rebecca. 1.08.07, 2.04.02
Klaschka, Ursula. 6.02.10
Klein, Judith. 3.01.19, 4.06.06, 4.06.23
Klein, Michael. 3.01.19, 3.03.05, 3.10.18, 3.10.21, 6.02.07
Kleinstreuer, Nicole. 1.01.12
Kloas, Werner. 2.01.02
Klöcker, Clara. 5.05.01
Kluver, Nils. 1.03.01, 1.03.05, 1.03.07, 2.01.02
Knaebe, Silvio. 4.08.05, 4.08.06, 4.08.10
Knaeps, Els. 7.03.04
Knapen, Dries. 1.05.07, 1.05.08, 1.05.20
Knigge, Thomas. 2.02.14
Knight, Tiffany. 2.07.05
Kniuipyte, Inesa. 4.04.05
Knoop, Oliver. 3.12.18
Knudsmark Sjoholm, Karina. 3.01.01, 3.04.05
Knudtzon, Nina. 2.05.01
Koch, Bianca. 5.02.11
Koch, Josef. 4.06.06, 4.06.27
Koelmans, Albert. 7.03.03
Koelmel, Jeremy. 7.01.08
Koenig, Maria. 4.12.10
Koenig, Natacha. 1.08.08
Koestel, Johannes. 3.14.04
Köhler, Heinz. 1.10.06
Köhler, Petra. 2.04.10, 2.04.12
Kojima, Fujio. 3.08.07, 3.11.26, 3.14.07
Kok, Kasper. 4.10.02
Kolbenschlag, Sara. 2.06.05, 2.06.07, 2.06.08, 2.06.09
Kolesnik, Olga. 4.04.14
Kolpin, Dana. 3.03.02, 3.03.16
Kondic, Todor. 4.13.05
Könemann, Sarah. 1.03.06
Konestabo, Heidi Sjursen. 2.08.17, 2.09.22
König, Azora. 1.05.16, 1.08.05
Konig, Fabian. 3.11.07

König, Yannis. 3.03.20
Konuspayeva, Gaukhar. 2.09.16
Kool, Jeroen. 1.09.16
Koopman, Remon. 4.16.04
Kooter, Ingeborg. 4.15.01
Köppe, Toni. 3.15.14
Kopylova, Kseniya. 1.01.16
Koraltan, Idris. 3.12.21
Korkaric, Muris. 6.02.02
Koroma, Michael. 5.02.12
Kosak, Lena. 1.05.16
Koschorreck, Jan. 2.05.24, 2.05.26, 3.13.02, 3.15.14, 4.03.21
Köser, Jan. 4.11.11
Kosfeld, Verena. 4.03.13, 4.03.21
Kotschik, Pia. 2.09.01, 2.09.02
Koureas, Dimitris. 2.05.24
Kovaleva, Ekaterina. 4.04.16
Kovalevskaya, Liubov. 6.04.15
Kovel, Ekaterina. 1.02.09, 1.02.10
Kowalczyk, Janine. 3.13.25, 3.16.03
Koyamparambath, Anish. 5.03.04
Kozlova, Irina. 4.04.15
Kraak, Michiel. 3.09.15, 4.04.24, 4.12.02, 4.12.11, 4.14.07
Kracht, Oliver. 3.06.09
Kraetzig, Gwendolin. 6.03.07
Kraft, Philipp. 3.10.17
Kramer-Schadt, Stephanie. 4.06.07
Kramm, Johanna. 7.03.01, 7.03.02
Kratasyuk, Valentina. 1.01.16, 1.09.10
Krause, Stefan. 3.11.23, 3.12.10
Krauss, Martin. 1.09.03, 4.04.12
Krebs, Florian. 3.10.13, 3.10.17
Kress, Holger. 3.11.30
Kreuger, Jenny. 3.10.27
Kreutzer, Georg. 1.01.01, 4.03.15
Krier, Jessy. 4.13.05
Kringstad, Alfhild. 3.06.03
Kristensen, Jan Bolding. 2.05.24
Kristensen, Torstein. 6.01.06
Kriszt, Balázs. 3.08.11
Kroglund, Ingvild. 2.05.05
Krone, Oliver. 2.05.24, 2.05.25
Kroon, Frederieke. 3.12.20, 3.12.24
Krueger, Henry. 2.04.11
Krug, Harald. 6.05.06
Krupinski Emerenciano, Andrews. 1.02.15, 2.11.08, 2.11.11
Ksibi, Mohamed. 2.09.10
Kube, Christine. 4.17.08
Kuddithamby, Gunaalan. 3.11.17
Kudryasheva, Nadezda. 1.02.09
Kudryasheva, Nadezhda. 1.02.10, 4.04.14
Kuehnel, Dana. 3.08.02, 6.05.07
Kuehr, Sebastian. 6.04.12
Kühnel, Dana. 6.05.06
Kuhr, Sebastian. 1.02.14
Kukkola, Anna. 3.11.23, 3.12.10
Kukkonen, Jussi. 3.12.03, 4.15.06
Kukucka, Petr. 3.02.03
Kulachkova, Svetlana. 4.04.15
Kulikova, Olga. 4.04.19
Kumar, Darmit. 6.05.04
Kumari, Santosh. 3.14.08
Kundu, Kankana. 3.01.12, 3.04.03, 4.04.23
Kurianska-Piatek, Ligia. 2.10.06
Kurmayer, Rainer. 4.11.02
Kurzweg, Lucas. 3.08.09
Küster, Eberhard. 1.07.19
Kuster, Thomas. 6.02.02
Kutscher, Daniel. 1.09.17
Kuzikova, Irina. 2.09.23
Kuzmenko, Kateryna. 5.03.03
Kuznetsova, Sofia. 4.04.15
Kuznetsova, Tatiana. 2.02.08
Kvæstad, Bjarne. 1.07.12, 6.01.06
Kwak, Jin Il. 1.07.01, 3.12.12, 7.01.03
Kwaśniewski, Sławomir. 3.15.06
Kwon, Ba Reum. 1.05.04
Kydralieva, Kamila. 1.02.10, 6.04.15

L

La Spina, Rita. 3.12.29
Laanen, Paulus. 1.06.06
Labadie, Pierre. 3.13.15, 3.13.26, 3.15.17
Labille, Jerome. 1.02.17
Labrune, Celine. 3.08.14
Lacchetti, Ines. 1.07.16, 6.02.09
Lachat, Ambroise. 5.03.03
Lachaux, Nicolas. 3.09.18
Lackmann, Carina. 4.17.03
Lacorte, Silvia. 2.05.02, 2.05.22, 2.05.23, 2.12.17
Lacoue-Labarthe, Thomas. 4.03.03, 4.03.11
Laczko, Endre. 4.14.21
Ladak, Adam. 3.05.08, 3.15.34
Ladner, Samia. 1.10.09
Laforsch, Christian. 3.08.18, 3.11.30
Lagadic, Laurent. 1.05.10, 2.02.02, 6.03.04
Lagisz, Malgorzata. 4.14.15
Lahive, Elma. 3.14.04, 4.12.05
Lahtela-Kakkonen, Maija. 3.12.16
Lai, Frederic. 5.05.10
Laissaoui, Abdelmourhit. 2.01.19
Lam, Monica. 4.07.02, 4.07.03, 4.07.04, 4.07.09
Lama, Virginia. 5.03.05
Lamb, Aaron. 3.15.34
Lambright, Christy. 3.03.16
Lammel, Gerhard. 3.02.03
Lammel, Tobias. 1.01.04, 1.02.17, 1.09.05, 6.04.06
Lämmer, René. 3.13.25, 3.16.03
Lamon, Lara. 3.07.04
Lamoree, Marja. 1.05.21, 1.09.16
Landaburu-Aguirre, Junkal. 5.02.13, 5.03.12
Landis, Wayne. 2.11.10, 3.12.31, 4.01.05, 4.01.08
Lane, Taylor. 4.10.01
Lang, Christiane. 4.07.01
Lang, Imke. 4.11.11
Langberg, Hakon. 3.13.18
Langer, Miriam. 2.01.05, 3.03.18
Lankin, Anton. 4.04.01
Lanno, Roman. 4.09.02
Lansdale, Kelsey. 3.16.14
Lanzén, Anders. 2.03.07
Lapczynski, Aurelia. 4.09.08
Lapushkin, Maxim. 4.04.01
Larrea, Gustavo. 5.04.01
Larroze, Severine. 6.04.16
Larson, Shawn. 3.12.27
Larson, Venus. 3.13.05
Lasdin, Katherine. 3.12.27
Laskowski, Ryszard. 4.16.06
Lasters, Robin. 3.13.19
Lastumäki, Anu. 1.10.07
Lau, Danny. 2.06.11
Laudadio, Elizabeth. 1.08.07, 2.04.02
Laue, Heike. 1.01.01, 1.01.03, 4.03.08, 4.03.15
Laughrey, Zachary. 4.11.16
Lauper, Benedikt. 4.12.06
Laurent, Alexis. 5.04.05, 5.05.04
Laurent-Martin, Fabrice. 3.07.08
Lauri, Pekka. 5.02.03
Lautz, Leonie. 4.16.04
Lava, Roberto. 3.13.16
Laviale, Martin. 2.04.08, 4.02.10
Lavoie, Jérôme. 3.08.05
Lawler, Sharon. 4.12.03
Lawrence, Alan. 4.08.02
Lazarova, Asenova. 2.12.09
Lazorchak, James. 2.01.02, 2.01.16, 4.02.01, 4.11.10
Lazzari, Lorenza. 1.09.04
Le Carlier, Christian. 2.11.12
Le Cor, Francois. 4.12.08, 4.12.09
Le Foll, Frank. 2.02.19
Le Menach, Karyn. 3.05.03
Le Menach, Karyn. 3.15.17
Lebeau, Emmanuelle. 2.05.19
Leblanc, Denis. 3.03.02
Lèche, Alvina. 2.05.21
Lechuga, Juan Luis. 1.09.20
Lecomte-Pradines, Catherine. 2.09.24
Ledesma, Mariana. 2.01.02
Lee, David. 3.05.08
Lee, Jungeun. 1.03.11, 1.05.04
Lee, Patrick. 3.15.07
Lee, Saeromi. 2.10.17, 5.02.07
Lee, Soyoung. 4.15.04
Lee, Sujin. 1.07.06
Lee, Tae-Yang. 2.10.11, 3.12.02
Lee, Ye-Eun. 5.07.01
Lee, Yung-Shan. 4.03.14
Lee Behrens, Hanna. 6.01.01
Leflaive, Joséphine. 2.04.08
Lega, Massimiliamo. 4.11.05
Legler, Juliette. 1.05.18, 1.06.03
Legradi, Jessica. 1.03.05, 1.07.14
Lehto, Mikko. 2.02.20
Lehtonen, Kari. 1.10.07
Lehtonen, Marko. 3.07.06
Leicher, Thorsten. 7.04.01
Leicht, Kathrin. 3.14.04
Leinaas, Hans Petter. 2.09.22
Leite, Gonçalo. 2.10.02
Leivits, Madis. 2.05.24
Lemieux, Heather. 2.09.20
Lemkine, Gregory. 1.05.15, 1.05.16
Lencioni, Valeria. 1.04.14, 3.09.26
Lenton, Timothy. 5.05.07
Lenz, Markus. 3.09.01
León-Ortega, Mario. 1.04.07, 1.04.09
Léonard, Angélique. 5.03.10
Leonards, Pim. 1.05.21, 1.07.14, 4.02.05
Leopold, Annegaaike. 8.05.01
Lepot, Bénédicte. 3.15.17
Leroy, Delphine. 3.15.40, 4.13.04
Leroy, Eric. 3.08.14
Leslie, Heather. 3.08.24
Lestremau, François. 3.13.15, 3.15.17
Letcher, Robert. 1.04.05, 2.05.10, 4.13.14
Letourneau, Eliza. 3.16.14
Lettieri, Teresa. 8.06.03
Leusch, Frederic. 2.02.10
Leuthold, David. 1.03.01, 1.03.07

Leuven, Rob. 4.16.04
Leverett, Dean. 3.09.16, 6.03.04
Levert, Luc. 4.09.07
Levi, Taal. 3.12.27
Lexén, Jenny. 3.02.11
Lharidon, Jacques. 8.04.06
Li, Eric. 5.06.07
Li, Henrik. 3.15.08
Li, Kun. 3.15.07
Li, Li. 3.01.20, 3.01.21, 3.15.07
Li, Mengting. 3.09.07
Li, Shanghua. 3.03.15
Li, Shao-Meng. 3.15.07
Li, Wenlong. 3.15.07
Li-Beisson, Yonghua. 2.09.24
Liang, Ruoyu. 4.05.07
Liberatori, Giulia. 1.02.05, 2.09.07, 2.09.09
Libert, Pierre-Nicolas. 3.15.40
Lidman, Johan. 2.06.06, 2.06.11
Liess, Matthias. 4.06.07
Liggio, John. 3.15.07
Ligocki, Isaac. 4.14.15
Ligthart, Jerker. 4.07.13
Liguori, Renato. 1.09.19
Lillicrap, Adam. 1.01.03, 1.01.07, 4.01.08, 4.14.23, 6.01.01, 6.04.12
Lima Da Silveira, Augusto. 1.07.20
Lin, Elizabeth. 7.01.08
Lin, Haodong. 5.01.15, 5.05.11
Lin, Yan. 3.06.06
Linares, Victòria. 4.17.06, 4.17.07
Lind, Ole. 2.08.09
Liñero, Olaia. 2.02.07
Link, Moritz. 2.06.10
Linnemann, V.. 3.03.05
Lionetto, Maria Giulia. 3.02.01
Littler, Hannah. 1.06.09
Liu, Guanghai. 5.01.21
Liu, Qifan. 3.15.07
Liu, Runya. 5.05.03
Liu, Runze. 1.05.03
Liu, Shushen. 1.09.15, 4.06.05
Liu, Wei. 2.06.04, 3.09.07
Lizano-Fallas, Veronica. 1.07.08
Lizin, Sebastien. 5.01.10
Llewellyn, Neville. 3.13.06
Lloret Lloret, Elena. 2.05.12
Lobinski, Ryszard. 4.11.03
Lode, Torben. 2.08.17, 4.06.19
Löder, Martin. 3.08.18
Loeffler, Thomas. 4.02.12
Loetscher, Matthias. 4.14.14
Loftin, Keith. 4.11.16
Lohmann, Rainer. 3.15.02
Lohninger, Hans. 3.08.18
Loibner, Andreas. 3.04.05
Loiseau, Ludovic. 3.10.19
Loizeau, Jean-Luc. 4.14.30
Lola, Ma. Suzanneth Epifania. 1.05.22
Longoni, Orietta. 7.01.06
Lonneville, Britt. 7.03.03
Looky, Alexandra. 4.03.10
Lopes, Célia. 1.05.19
Lopes, Christelle. 4.03.03, 4.03.06, 4.03.11
Lopes, Isabel. 1.01.15, 2.08.01, 2.08.02, 2.08.03, 2.08.10, 4.07.06, 4.14.17, 4.14.18
Lopes, Joana. 2.10.15
Lopes, Sónia. 2.10.05
Lopes Peixoto, Sara Cristiana. 1.07.07, 2.10.12, 2.10.13
López, Diana. 3.07.05, 3.15.21
López, Isabel. 4.14.06
López, María. 3.02.10
Lopez Antia, Ana. 2.05.03, 2.12.02
López De Alda, Miren. 3.04.09
Lopez Mangas, Alba. 2.01.05
López Moreira Mazacotte, Grégorio. 2.04.08
Lopez-Antia, Ana. 2.05.24
Lopez-Cabeza, Maria Del Rocio. 3.16.12
López-Perea, Jhon. 2.12.02
Lord, Heather. 3.13.03
Lorenz, Claudia. 3.08.20, 3.11.17
Lorke, Andreas. 3.10.14, 4.14.24
Loro, Francesco. 3.16.05
Losacco, Daniela. 2.09.11, 4.04.02
Losada Carrillo, Maria Paula. 1.02.03
Lot, Marie-Claire. 4.02.09
Lotufo, Leticia. 1.07.07
Loubet, Philippe. 5.06.08
Loureiro, Susana. 1.07.07, 2.10.02, 2.10.04, 2.10.05, 2.10.07, 2.10.10, 2.10.12, 2.10.13, 2.10.15, 4.01.10, 4.04.22, 6.04.13, 6.04.14
Lourenco, Joana. 2.09.10, 3.08.25
Lourenco, Rui. 2.05.24, 2.05.25
Louw, Marissa. 3.05.11
Löw, Fabian. 3.10.16
Lowery, Morgan. 1.03.04
Lowit, Michael. 1.01.12, 8.01.03
Lozano, Anthony. 3.10.23
Lucia, Giulia. 2.13.06
Luederwald, Simon. 6.04.04
Luhua, You. 3.16.06
Luit, Richard. 6.02.06
Lundqvist, Johan. 3.05.04
Luquot, Linda. 4.04.13
Lurling, Miquel. 4.17.05
Lusher, Amy. 3.08.05, 3.08.06, 3.08.12, 3.08.24, 3.11.11
Lutton, Antony. 3.09.26
Luzardo, Octavio. 1.04.09
Luzi-Thafeni, Luleka. 3.05.12
Lyche, Jan. 2.05.09
Lykkemark, Jeanette. 3.08.21
Lynch, Iseult. 3.11.23, 3.12.09, 4.01.10, 4.01.11, 4.01.12, 6.04.10
Lyngstad, Inger. 3.12.01, 3.12.13
Lyon, Delina. 3.01.04, 4.09.09
Lyons, Sean. 3.10.07
Lythgo, Christopher. 3.09.24

M

M. González, Enrique. 3.09.14
Maack, Gerd. 1.05.13, 2.01.01, 2.01.02, 4.14.10
Maboeta, Mark. 2.09.12, 2.10.08
Macfarlane, Geoff. 2.02.10
Machado, Ana. 2.02.04
Machera, Kyriaki. 4.16.06
Maciejczak, Angelika. 4.03.12
Mackay, Donald. 4.09.05
Macken, Ailbhe. 3.06.03, 4.14.23, 6.04.12
Macleod, Matthew. 3.12.17, 4.10.01
Macorps, Nicolas. 3.13.15, 3.13.26
Madaschi, Laura. 1.09.04
Maddison, Linda. 4.13.14
Madeiros, Jeremy. 2.12.17
Madhu, Kavya. 5.01.19
Madia, Federica. 8.01.04
Madsen, Anders. 1.01.07, 4.01.08
Madureira, Tânia Vieira. 1.05.19
Maes, Ben. 5.01.08
Maffini, Maricel. 7.03.06
Magalhães, Rui. 3.08.25
Maggi, Lorenzo. 3.16.05
Magoga, Giulia. 2.10.01
Mahat, Sabnam. 4.03.20
Mahe, Kelig. 1.04.01
Mahfouz, Celine. 3.15.19
Mahmud, Md. 4.04.21
Mahmud, Zahid Hayat. 7.01.13
Mahú, Francisco. 3.02.13
Maia, Frederico. 4.04.22, 6.04.13
Maillet-Mezeray, Julie. 3.10.20
Maillot-Maréchal, Emmanuelle. 4.02.11
Mainelis, Gediminas. 3.02.08
Mair, Magdalena. 6.06.02
Major, Kaley. 1.06.04
Makombe, Martin. 3.05.07, 3.09.03
Malarvannan, Govindan. 3.16.11, 3.16.13
Malatji, Katlego Mafura. 3.05.11
Malcomber, Ian. 8.05.06
Malgouyres, Jean-Michel. 4.14.32
Malheiro, Catarina. 2.10.02, 2.10.05, 2.10.07, 2.10.10, 2.10.13, 2.10.15
Mallory, Mark. 3.12.34
Mallula, Swetha. 3.13.05, 3.13.27
Malm, Lisa. 2.06.11
Malpei, Francesca. 3.03.19
Maltby, Lorraine. 4.05.07, 4.05.08, 4.05.09, 4.05.10, 4.10.03, 4.16.01, 4.16.02, 4.16.03
Malzahn, Arne. 6.01.09
Mamun, M Iqbal Rouf. 3.11.25
Mamy, Laure. 3.04.10
Man Kit Yu, Richard. 2.02.10
Manasfi, Rayana. 3.07.09
Mancini, Laura. 1.07.16, 2.05.28, 6.02.09
Mańczak, Dominika. 2.13.08
Manfrin, Alessandro. 2.06.07
Mangold-Doring, Annika. 4.10.01, 4.10.04, 4.10.07, 8.02.11
Manier, Nicolas. 1.02.11, 2.09.08, 3.09.23
Mannio, Jaakko. 3.15.20
Manno, Clara. 2.11.01, 2.11.07, 6.01.05
Manzardo, Alessandro. 5.01.21, 5.04.06, 5.04.09
Maor, Yehoshua. 7.02.02
Mapelli, Francesca. 7.02.05
Marcelino, Rocky. 1.05.22
Marchese, Enrico. 3.07.04
Marchese, Melissa. 3.16.14
Marchesini, Paolo. 7.02.01
Marcomini, Antonio. 3.07.04
Marcotullio, Sandro. 4.11.06
Marfil-Vega, Ruth. 3.13.08
Maria, Vera. 2.10.03, 4.14.34
María-Mojica, Pedro. 2.05.04, 2.05.25, 2.12.04, 2.12.05, 2.12.08, 2.12.10
Marin, Maria Gabriella. 3.13.28
Mariussen, Espen. 3.02.04
Markert, Nele. 1.04.12, 4.13.11
Markiewicz, April. 4.01.05
Marle, Pierre. 2.06.04
Marneffe, Yves. 3.15.40, 4.13.04
Marochko, Tatiana. 6.04.15
Marquardt, Clarissa. 6.05.06
Marques, João. 1.09.07, 2.04.15, 2.08.14, 3.12.28
Marques, Paula. 6.04.08
Marques, Sérgio. 1.07.17, 2.03.03, 2.03.09
Marques Dos Santos, Luis

Fernando. 1.02.15
Marsh, Paul. 3.09.16
Marshall, Stuart. 4.05.08, 4.05.09, 4.05.10, 4.16.01, 4.16.02, 4.16.03
Marson, Alessandro. 5.04.06
Martella, Monica Beatriz. 2.05.21
Martellini, Tania. 2.05.24, 2.05.26
Martí, Celia. 1.05.09
Martin, Bram. 3.04.03
Martin, Edith. 5.05.02
Martin, Helen. 3.11.21
Martin, Jake. 2.01.03, 2.01.10, 4.14.15
Martin, Nicolas. 1.02.17, 6.04.06
Martin, Olwenn. 7.03.06
Martin, Stephan. 3.05.03
Martin, Thomas. 4.06.20
Martin-Aparicio, Alberto. 3.01.04, 3.01.07, 3.01.10, 4.09.09
Martináková, Anetta. 1.05.03
Martínez, Francisco. 7.01.12
Martínez, María De Los Ángeles. 3.10.04, 3.13.12, 3.13.13, 3.15.09, 3.15.11
Martinez, Mathieu. 3.09.01
Martinez, Ruben. 1.06.01, 1.06.03
Martinez Bueno, M.j.. 7.02.03
Martínez Hernández, Virtudes. 7.02.06
Martinez-Frances, Elena. 3.08.24
Martínez-García, Lorena. 7.01.12, 7.02.06
Martinez-Guitarte, Jose-Luis. 1.04.14, 1.08.03, 1.09.08, 1.09.09, 2.02.05
Martinez-Haro, Monica. 2.05.07, 2.05.14, 2.12.07
Martínez-López, Emma. 2.05.25
Martínez-Varela, Alicia. 2.11.03
Martins, Adrielle. 3.15.16
Martins, Duarte. 6.05.04
Martins, Marta. 4.12.01
Martins, Nicole. 3.08.25
Martins, Roberto. 4.04.22, 6.04.13, 6.04.14
Martinson, John. 1.06.02
Martulli, Alessandro. 5.01.10
Marvuglia, Antonino. 5.04.01
Marx, Michael. 2.07.01, 2.07.03
Masiero, Luciano. 3.13.28
Maspero, Angelo. 3.15.25, 3.15.26
Massarelli, Carmine. 2.09.11, 3.08.08, 3.08.13
Massei, Riccardo. 1.07.03, 1.07.19
Masset, Thibault. 4.15.10
Massey, Paul. 3.10.23
Massini, Giulia. 4.14.13
Massop, Harry. 3.10.12
Mateo, Rafael. 1.04.07, 1.04.09, 2.05.24, 2.05.25, 2.12.12
Mateo Soria, Rafael. 2.05.07, 2.05.14, 2.05.22, 2.05.23, 2.12.02, 2.12.07, 2.12.13
Mateos-Miranda, Miguelina. 3.16.16
Matezki, Steffen. 4.06.07, 4.16.05
Mathys, Alexander. 5.04.14
Matias, Ricardo. 2.11.02
Matos, Beatriz. 4.12.01
Matozzo, Valerio. 3.13.28
Matsui, Tetsuya. 5.05.03
Matsukami, Hidenori. 4.09.06
Mattelin, Valerie. 3.01.12, 3.04.03
Mattern, Andreas. 6.05.06
Mattern, Derek. 3.15.35
Mattie, David. 1.03.09
Matzke, Marianne. 1.02.19
Mauduit, Florian. 2.08.11
Mauffret, Aourell. 1.04.01
Maul, Jonathan. 1.01.13, 1.01.14, 2.05.17
Maurice, Nicolas. 4.14.27
Maus, Christian. 2.07.01, 2.07.03
Mavandadi, Farah. 3.13.10
Mavruk, Sinan. 3.12.21
Mayer, Philipp. 3.01.01, 3.01.02, 3.04.05
Mayol, Marta. 2.01.11
Mayser, Jan Peter. 3.11.21
Mazerolles, Vanessa. 4.03.06
Mazón, Maria José. 1.01.11, 1.05.09
Mazurier, Arnaud. 3.16.07
Mazzella, Nicolas. 2.03.05
Mazzoli, Carola. 2.09.07
Mccallum, Erin. 2.01.03, 2.01.06, 2.01.09, 4.14.15, 4.14.28
Mccann Smith, Eliana. 1.04.04, 2.05.16
Mccobb, Timothy. 3.03.02
Mcdonnell, Kevin. 5.01.13
Mcfarlin, Kelly. 3.01.09
Mcfee, Ashley. 2.05.15
Mcgoldrick, Daryl. 4.13.14
Mckernan, John. 6.02.03
Mckie, Brendan. 3.12.23
Mckinney, Melissa. 1.04.05, 2.05.10
Mcnabb, Nicole. 1.07.11
Mcvey, Emily. 8.02.01
Mcwilliams, Laura. 3.06.02
Medlock Kakaley, Elizabeth. 3.03.16
Medunić, Gordana. 4.02.08
Meehan, Kelley. 3.12.20
Meekel, Nienke. 3.05.02
Meer, Froukje. 4.12.11
Meer, Tom. 3.09.15, 4.04.24
Meerwijk, Thomas. 3.09.15
Mees, Jan. 7.03.03
Meesters, Johannes. 6.04.03
Meffe, Raffaella. 7.02.06
Mehinto, Alvine. 1.06.04
Meide, Marc. 5.01.07
Meier, Jutta. 3.09.21
Meinecke, Stefan. 3.11.07
Meisterjahn, Boris. 3.01.04, 3.01.07
Melagraki, Georgia. 4.01.10, 4.01.11, 4.01.12
Meland, Sondre. 3.06.03
Melo, Madson Silveira. 3.09.25
Melvin, Steven D.. 2.01.02, 2.02.10
Mena, Freylan. 2.01.19
Ménard, Dominique. 4.12.12
Menéndez, Margarita. 2.06.02
Meng, Shandong. 2.08.04
Meng, Yuan. 1.10.03
Menger, Frank. 3.13.04
Mentzel, Sophie. 4.01.09, 4.10.01, 4.10.09
Merchan, Angel. 5.01.16
Mermillod-Blondin, Florian. 3.12.10
Merrington, Graham. 3.09.09, 3.09.11, 3.09.12, 3.09.13, 4.02.06
Mertens, Jelle. 4.02.06
Mesquita, Andreia Filipa. 2.04.15, 2.08.14
Messagie, Maarten. 5.01.12, 5.02.12, 5.03.11
Messo, James. 4.14.11
Mestre, Diana. 6.04.01
Mestrot, Adrien. 4.03.20
Meyer, Horatio. 3.10.11
Meyer, Lars. 3.14.01
Meyer, Stéphanie. 1.03.06
Meyer, Toby. 5.01.10
Meyers, Nelle. 3.11.20
Meys, Raoul. 5.03.07, 5.04.07
Miaz, Luc. 3.15.01
Michán, Carmen. 2.03.04
Michel, Amandine. 3.03.06
Michelangeli, Marcus. 2.01.03, 2.01.10, 4.14.15
Michelangeli, Maria Elisabetta. 1.02.04
Michenkova, Olga. 1.10.07
Middleton, Elizabeth. 3.09.08, 3.09.09, 3.09.10, 3.09.11, 3.09.12, 3.09.13, 3.09.16, 4.02.06
Mielecki, Damian. 4.14.33
Miglioli, Angelica. 2.02.11
Miguez, Bettina. 3.10.15
Mihan, Christine. 2.04.10
Mikac, Nevenka. 3.04.02
Mikołajczyk, Łukasz. 2.07.04
Mikusova, Petra. 4.12.10
Milan, Massimo. 3.13.28
Miles, Laura. 3.11.21
Miles, Mark. 4.06.10, 4.08.06
Mill, Jonathan. 1.06.09
Miller, Jessica. 3.12.27
Miller, Michaela. 3.12.24
Miller, Rachael. 3.06.07
Miller, Thomas. 3.15.23
Mills, Graham. 3.15.32
Mills, Lesley. 1.07.11
Mills, Marc. 3.03.02
Miltner, Anja. 3.10.03, 4.04.12
Miñana-Prieto, Rafael. 1.07.04
Minderyte, Agne. 3.02.08
Misiewicz, Anna. 2.07.04
Miškelytė, Diana. 4.10.08
Mit, Corentin. 4.01.03, 4.01.04
Mitchell, Connie. 1.05.10
Mitchell, Kerry. 3.09.02
Mitrano, Denise. 1.09.05, 3.12.26, 3.14.04, 7.03.07
Moe, Jannicke. 1.01.07, 1.08.11, 1.08.12, 2.11.10, 4.01.07, 4.01.08, 4.01.09, 4.10.01, 4.10.09, 4.14.02, 6.01.07
Mohaimani, Aurash. 1.08.07
Mohanraj, John. 3.11.30
Mohapatra, Sanjeeb. 3.16.06
Mohr, Silvia. 2.01.02, 2.03.06
Molina, Serena. 5.02.13
Molina-Hernández, Verónica. 4.11.14
Möller, Nadja. 6.05.06
Monclus, Laura. 1.04.04, 2.05.16
Monira, Sirajum. 4.15.03
Monperrus, Mathilde. 3.04.02
Monsinjon, Tiphaine. 2.02.14
Montagna, Matteo. 2.10.01
Monteiro, Bruna. 4.14.17
Monteiro, Bruna Ariana. 4.14.18
Monteiro, Pedro. 2.10.05, 2.10.13
Monteiro, Silvia. 3.08.25
Montemayor, Erica. 5.05.12, 5.06.03
Montemurro, Nicola. 3.03.08, 3.07.05, 3.07.07, 3.07.08, 3.07.09, 3.07.12, 3.15.21
Monterroso, Carmela. 3.16.07, 3.16.08
Montforts, Mark. 4.14.12
Monti, Carlo. 3.06.04, 3.06.05
Mooij, Dewi. 3.01.17
Moon, Ye Lim. 3.12.25

Moon, Ye Lim. 3.13.21
Moore, David. 6.06.01
Moore, Eric. 2.03.08
Moore, Karen. 1.06.09
Moore, Lee. 1.06.08
Morales Morales, Lorenzo. 2.10.16
Morash, Michael. 1.07.13
Moreau, Stéphane. 1.09.02, 3.13.08
Moreno, Ignacio. 2.03.04
Moreno, Teresa. 3.02.10, 7.01.02
Moreno Gutiérrez, Rosa Martha. 4.14.29
Morgado, Fernando. 6.01.10
Morgado, Rui. 2.10.02, 2.10.04, 2.10.05, 2.10.07, 2.10.12, 2.10.13
Morganti, Michelangelo. 3.13.20
Morigi, Caterina. 3.11.11
Morin, Soizic. 2.03.05
Moriniere, Jerome. 2.07.07
Moriniere, Kirsten. 2.07.07
Morley, Simon. 2.11.05
Moro, Gema. 4.17.02
Moron Asensio, Ruben. 4.11.02
Morosini, Cristiana. 3.15.24, 3.15.25
Morr, Charlotte. 6.05.05
Morthorst, Jane. 1.05.07, 1.05.11
Moscovici, Liat. 3.03.17
Mostoni, Silvia. 5.01.14
Motoshita, Masaharu. 5.04.03, 5.04.04
Mounède, François. 4.02.09
Mouneyrac, Catherine. 1.02.11, 1.02.13
Movalli, Paola. 2.05.24, 2.05.25, 2.05.26
Moyano, Rosario. 4.11.14
Mueller, Axel. 3.11.13
Mueller, Jochen. 3.03.22, 3.03.23
Mueller, Olaf. 1.08.07
Muir, Derek. 3.15.01, 3.15.02
Mulder, Martin. 3.10.12
Mullen, Alexander. 5.04.08
Müller, Wendt. 2.05.03
Multari, Gauthier. 4.03.06, 4.06.04
Multsch, Sebastian. 3.10.10, 3.10.13, 3.10.17, 3.10.21, 3.10.27, 4.06.32
Muncke, Jane. 3.15.31, 4.07.13, 7.03.06
Muñiz-González, Ana-Belen. 1.04.14, 2.02.05
Munne, Antoni. 8.06.02
Munoz, Cynthia. 2.05.27
Muñoz, Isabel. 2.06.02
Muñoz-Carpena, Rafael. 3.10.09, 3.10.10, 3.10.11
Muñoz-Castro, Clara. 1.03.12
Munschy, Catherine. 1.04.01
Murano, Carola. 1.02.05
Muriana, Arantza. 1.01.11, 1.03.05, 1.05.09
Murillo, Laurence. 2.02.09
Muriuki, Cecilia. 4.14.11
Murn, Campbell. 2.12.06
Murphy, Fionnuala. 5.01.13
Murray, Aimee. 2.03.11
Murray, Laura. 2.03.11
Muz, Melis. 4.03.01
Muzandu, Kaampwe. 2.05.18
Mysara, Mohamed. 1.06.06

N

Nacci, Diane. 1.07.11
Nadin, Caroline. 3.15.40
Nagar, Nupur. 2.06.01
Nagesh, Poornima. 4.10.01, 4.10.02
Nagler, Marisa. 3.03.20
Nahar, Nilufar. 3.05.05
Nakagawa, Shinichi. 2.01.03, 4.14.15
Nakajima, Fumiyuki. 4.15.04
Nakajima, Kenichi. 5.04.03
Nakamura, Hiroyuki. 5.01.20
Nakamura, Keiji. 5.03.15
Nakata, Hokuto. 2.05.18
Nakayama, Shota. 2.05.18
Nansai, Keisuke. 5.04.03
Narain-Ford, Dominique. 3.03.13
Nason, Sara. 7.01.08
Natsch, Andreas. 1.01.01, 4.03.08, 4.03.15
Natumi, Regiane. 4.11.06
Nau, Jean-François. 2.09.08, 3.09.23
Nau, Katja. 6.05.06
Nauen, Ralf. 1.10.02, 7.04.01
Nauts, Robin. 1.06.06
Navarre, Nicolas. 5.01.05
Navarrete-Gutierrez, Tomas. 5.04.01
Navarro, Irene. 3.10.04, 3.13.12, 3.13.13, 3.15.09, 3.15.11
Navarro, Joaquín. 2.05.21
Navarro-Martin, Laia. 1.06.01, 1.06.03
Navas, Isabel. 2.05.04, 2.12.04, 2.12.05, 2.12.08
Navas, José María. 2.12.03
Navon, Gal. 1.10.05
Nałęcz-Jawecki, Grzegorz. 4.03.12
Nałęcz– Jawecki, Grzegorz. 3.12.06
Ndebele, Donald. 4.14.20
Ndungu, Kuria. 6.04.12
Neale, Peta. 4.13.12
Neep, David. 3.15.32
Nefedova, Natalia. 4.04.15
Negley, Tim. 3.06.04, 3.06.05
Nel, Holly. 3.11.23, 3.12.10
Nellström, Maja. 5.03.01
Nelms, Simon. 1.09.17
Nelson, Caroline. 6.04.16
Nemecek, Thomas. 5.02.04, 5.04.14, 5.06.09
Nendza, Monika. 6.02.07
Nenes, Athanasios. 3.02.07
Nentwig, Nina. 4.11.11
Nepstad, Raymond. 4.03.22
Nerentorp Mastromonaco, Michelle. 3.15.08
Nerland Bråte, Inger Lise. 3.08.12
Neuparth, Teresa. 2.02.15
Neuweiler, Reto. 6.02.02
Neves, Joana. 2.10.05, 2.10.13
Neves, Márcia. 4.14.17
New, Lee-Sun. 3.13.10
Neyts, Marijke. 3.04.03
Ng, Keng Tiong. 3.07.02
Ngumba, Elijah. 4.14.11
Nguyen, Brian. 3.11.14
Ngwenya, Sasha. 4.14.20
Nhu Thuy, Trang. 5.03.08
Nibamureke, Marie Clémentine Uwineza. 1.09.11
Nickel, Stefan. 4.17.08, 4.17.09
Nickisch, Dirk. 4.06.11, 4.06.12
Nicol, Liz. 3.13.33
Nielsen, Asbjørn. 3.08.21, 3.11.17
Nielsen, Torkel. 3.11.17
Niemikoski, Hanna. 1.10.07
Niemuth, Nicholas. 1.08.07
Nietch, Christopher. 4.11.10
Nihouarn, Thomas. 5.01.11
Nika, Maria-Christina. 2.05.24, 2.05.26, 3.03.11
Nikolopoulou, Varvara. 2.05.24, 2.05.26
Nilsson, Anders. 2.01.06
Nilsson, Johan. 5.03.01
Niu, Shan. 3.15.13
Niu, Zhiyue. 3.12.05
Nogueira, Antonio. 3.09.19
Nogueira, Keite. 2.03.10
Nogueira, Verónica. 2.09.21, 3.08.25
Nolte, Tom. 3.01.17, 3.04.06, 4.16.04
Norberg-King, Teresa. 4.02.01
Nordheim, Eirik. 4.02.06
Nordtug, Trond. 3.15.06, 6.01.06
Norling, Magnus. 6.01.07
Nouairi, Jihen. 2.09.21
Nouri Sharikabad, Mohammad. 4.01.07, 4.14.02
Novak, Jiri. 1.05.02, 1.05.03, 3.02.03, 4.12.10
Novak, Lion. 1.10.05
Novakova, Zuzana. 3.02.03
Nowack, Bernd. 3.02.02, 3.11.01, 3.11.02, 5.03.06, 6.04.11
Nowak, Karolina. 3.10.03, 4.04.12
Nozal, Leonor. 7.01.12
Nunes Cardoso, Diogo Filipe. 2.10.04, 2.10.07, 2.10.13
Nunes Guimarães, Lucas. 1.09.06
Nutter, Amy. 3.05.08
Nuutinen, Aino. 4.15.06
Nyarko, Kwasi. 4.13.14
Nygård, Torgeir. 2.05.02
Nys, Charlotte. 3.09.08, 3.09.12, 3.09.16
Nzube, Silumko. 1.09.18
Nøst, Ole Anders. 6.01.07

O

O'brien, Jake. 3.03.22, 3.03.23, 3.11.09
O'conner, Wayne A.. 2.02.10
O'connor, Isabel. 3.10.21, 4.06.28, 4.06.29
O'connor, Niamh. 4.06.22
Obal, Terry. 3.13.03
Oberbeck, Lars. 5.01.09
Obrador, Ana. 2.09.14
Obrien, Stacey. 3.08.15, 3.11.09
Odenkirchen, Edward. 1.01.12
Oellers, Johanna. 2.07.03
Ogada, Darcy. 2.12.06
Ogawa, Takahiro. 2.05.18
Ogungbemi, Afolarin. 1.07.19
Oh, Byung-Chul. 1.05.04
Ohashi, Haruka. 5.05.03
Ohgaki, Masataka. 3.11.26
Ohms, Pernille. 5.04.05
Okamura, Tetsuro. 1.05.16
Okeme, Joseph. 3.15.33
Okoffo, Elvis. 3.08.15, 3.11.09
Okoniewski, Nicole. 1.07.05
Okoye, Amara. 2.10.14
Okpokwasili, Gideon. 2.10.14
Olabemiwo, Fatai. 3.16.01
Olafsdottir, Kristín. 3.15.08
Olesik, John. 3.09.26
Oleszczuk, Patryk. 3.03.12
Olin, Jeanene. 1.03.04
Olivares, Maitane. 1.09.20
Oliveira, Flavio. 4.11.07, 4.11.08
Oliveira, Gonçalo. 2.10.15
Oliveira, Jacinta. 1.07.07, 2.08.15, 2.10.12
Oliveira, Miguel. 1.01.15, 2.01.17, 3.12.14, 4.07.06

Oliveira, Sofia. 6.05.04
Oliveira, Tânia. 6.04.13
Ollikainen, Elisa. 4.03.07
Olmastroni, Silvia. 2.11.09
Olmo, Lluc. 3.04.09
Olsen, Kristine. 4.01.07, 4.14.02
Olsson, Jelena. 5.04.08
Olsvik, Pål. 6.01.06, 6.01.09
Opeolu, Beatrice. 4.02.03
Opute, Prosper. 2.09.12, 2.10.08
Ordoñez, Víctor. 1.07.04
Orford, Jack. 4.14.15
Orimoloye, Israel. 2.09.12
Oró-Nolla, Bernat. 2.05.02, 2.12.17
Ortega, Marcelo. 5.02.06
Ortega-Calvo, Jose Julio. 4.04.03, 4.04.06, 4.04.07, 4.04.08, 7.02.02
Ortiz, Inmaculada. 5.01.17
Ory, Pascaline. 2.02.09
Ostovich, Eric. 2.04.02
Otero, Vanessa. 6.01.05
Otitoloju, Adebayo. 1.01.05
Otte, Jens. 4.05.08, 4.05.09, 4.05.10
Ottenbros, Anne. 5.03.09
Oudra, Brahim. 4.11.09
Ovadnevaitė, Jurgita. 3.02.08
Ovcharova, Viktoriia. 1.07.09, 2.04.12
Overjordet, Ida Beathe. 3.03.01, 3.15.06
Owen, Stewart. 3.15.23, 4.14.19
Owsianiak, Mikolaj. 5.05.07, 5.05.09
O'brien, Kate. 5.05.02

P

Padilla, Stephanie. 1.03.04, 1.03.05, 2.01.02
Paetzold, Ulrich. 5.01.10
Paez, Martha. 7.02.08
Pagkaliwagan, Aline Karen. 1.05.22
Pajalic, Oleg. 5.04.08
Palluel, Olivier. 1.04.03
Palmeira Pinto, Letícia. 1.02.15, 2.11.08, 2.11.11
Palmisano, Giovanni. 3.15.25, 3.15.26
Palotai, Zoltán. 3.08.11
Palt, Martin. 4.13.11
Palumbo, Anna. 1.02.05
Palumbo, Maria Teresa. 3.03.19, 7.01.06
Pan, William. 3.16.14
Panagopoulou, Eleni. 1.10.06
Pandard, Pascal. 1.05.17, 4.03.13
Panella, Marta. 2.10.01
Pannetier, Pauline. 1.05.05, 1.05.07, 1.05.08
Pantelyushin, Stanislav. 1.07.05
Panter, Grace. 6.03.04
Paoli, Francesca. 3.09.26
Papa, Ester. 1.01.10, 4.03.10, 4.03.17
Papacharalampos, Konstantinos. 3.11.06, 3.11.17
Papadiamantis, Anastasios. 4.01.10, 4.01.11
Pape, Lydia. 3.10.13
Paquin, Paul. 4.02.02
Parakal, Katherine. 4.09.02
Paraskevopoulou, Aikaterina. 4.11.03
Paraso, Michelle Grace. 1.05.22
Pardon, Patrick. 3.05.03, 3.15.17
Paris, Severine. 4.14.30
Park, Jae-Roh. 2.10.17, 5.02.07
Park, Young Joo. 1.05.04
Parker, Matthew. 2.01.21
Parmentier, Koen. 3.04.03, 4.04.23
Parnis, J. Mark. 3.15.13, 3.15.33, 4.09.05
Parolini, Marco. 3.13.20
Parra, Gema. 2.01.20
Parsons, John. 4.05.11
Pascariello, Simona. 3.03.19, 3.13.20
Pasquini, Laure. 4.12.08
Passer, Alexander. 5.02.09
Passoni, Alice. 3.15.25, 3.15.26
Pastorok, Robert. 4.06.28, 4.06.29
Patankar, Shreyas. 3.08.16
Patarnello, Tomaso. 3.13.28
Patinha, Carla. 4.04.11
Patrolecco, Luisa. 4.04.09, 4.14.13, 4.14.25
Pattison, David. 3.15.15
Patureau, Dominique. 3.04.10
Pauliuk, Stefan. 5.01.19, 5.05.14
Pauly, Danièle. 3.09.18
Pauna, Valentina. 3.08.05
Pauraite, Julija. 3.02.08, 3.02.09
Pauwels, Olivier. 2.05.24
Pavia, Marco. 2.05.24
Pavlaki, Maria. 1.07.07, 2.10.05, 2.10.13
Payne, Katie. 6.06.01
Pearson, Randall. 2.07.02
Peccia, Jordan. 7.01.08
Peck, Vicky. 2.11.07
Peck, Victoria. 4.03.24
Pecquerie, Laure. 8.02.02
Pederson, Emily. 2.08.12, 2.08.13, 3.12.27, 4.15.08
Pedolin, Dario. 5.06.09
Peeters, Edwin. 2.08.07
Peeters, Jef. 5.03.02
Peijnenburg, Willie. 5.01.09, 6.04.03
Peinado, Beatriz. 7.01.12, 7.02.06
Peiry, Jean-Luc. 3.12.22
Peither, Armin. 1.05.12
Peixoto, Sara. 2.10.05
Pell, Albert. 1.10.08
Pelletier, Nathan. 5.06.07
Peña Herrera, Juan. 3.07.05
Penoni, Andrea. 3.15.25
Perdomo E., E. Alejandro. 5.05.06
Pereao, Omoniyi. 4.02.03
Pereira, Andreia. 2.10.07
Pereira, Gloria. 2.05.24
Pereira, Inês. 1.05.19
Pereira, Joana Luisa. 1.07.17, 3.09.19, 6.05.04
Pereira, Maria. 2.11.02
Pereira, Patrícia. 3.09.06
Pereira, Ruth. 2.04.05, 2.09.10, 2.09.21, 3.08.25, 4.04.11, 6.05.04
Pereiro, Patricia. 1.02.07
Perez, Michelle. 3.12.20
Pérez, Sandra. 3.03.08, 3.07.05, 3.07.07
Perez, Sandra. 3.07.08, 3.07.09, 3.07.12, 3.15.21
Pérez López, Carlos. 3.03.09
Perez-Albaladejo, Elisabet. 3.12.15
Pérez-López, Paula. 5.01.11
Pérez-Lopez, Paula. 5.06.04
Pérez-Mayán, Leticia. 3.01.15, 3.05.06
Perez-Rojas, Alberto. 1.07.02, 2.02.18
Perillo, Vitoria. 2.01.17
Perkola, Noora. 3.15.20
Perren, Sarah. 6.02.02
Peruzza, Luca. 3.13.28
Pescatore, Tanita. 4.04.09, 4.14.25
Pestana, João. 2.02.04
Peters, Adam. 3.09.08, 3.09.09, 3.09.10, 3.09.11, 3.09.12, 3.09.13, 3.09.16, 4.02.06
Petersen, Karina. 1.01.03, 2.08.09, 2.08.16, 4.01.09, 4.14.23, 6.04.12
Petrović, Marija. 4.02.08
Petrovic, Mira. 1.10.01, 2.13.05
Pfefferle, Jakob. 1.07.03
Pfenninger, Markus. 6.03.09
Pfister, Stephan. 5.02.02, 5.02.03, 5.05.01
Pfohl, Patrizia. 3.11.16, 3.14.01
Pham, Christopher. 3.12.34
Philipp, Matthias. 3.08.10
Philippe, Lemaire. 3.10.24, 4.03.16
Philippot, Maeva. 5.02.12, 5.03.11
Piarulli, Stefania. 1.07.12, 3.12.13, 6.01.09
Picard, Céline. 2.02.19
Piccini, Benjamin. 1.05.17
Pich, Andrij. 4.07.11
Pico, Yolanda. 1.07.15, 3.05.10, 3.05.13, 3.06.08, 3.06.10, 3.07.01, 3.11.27, 4.08.08, 7.02.04
Picolo, Victor. 2.01.17
Pieper, Silvia. 2.09.01, 2.09.02, 3.09.24, 4.06.07, 4.16.05, 4.16.06
Pierron, Fabien. 1.06.07
Pietz, Sebastian. 2.06.05, 2.06.09
Pihlaja, Tea. 4.03.07
Pilgrim, Erik. 1.06.02
Pimparel, Ines. 6.04.16
Piña, Benjamin. 1.06.01, 1.06.03
Pinheiro, Carlos. 4.01.10
Pinlova, Barbora. 3.11.01
Pinto, Edgar. 4.11.07, 4.11.08
Pinto, Gloria. 2.10.05, 2.10.13
Pintonello, Gilberto. 3.05.09
Piombino, Matteo. 2.10.01
Pipal, Marek. 4.11.01
Pires, Adília. 3.09.20, 3.12.14, 6.04.08
Pires, Sílvia. 2.08.15
Piringer, Gerhard. 5.02.10
Pivato, Alberto. 3.16.05
Pizzol, Massimo. 5.01.03, 5.01.04
Plassmann, Merle. 3.15.28
Plauškaitė, Kristina. 3.02.08, 3.02.09
Plautz, Stephanie. 1.01.13, 1.01.14
Plytycz, Barbara. 2.10.06
Pochee, Sureiya. 4.02.07
Pochet, Cécile. 4.14.27
Poiger, Thomas. 6.02.02
Polazzo, Francesco. 4.10.01, 4.10.04, 4.10.05
Polcher, Alexandra. 6.03.01
Polesello, Stefano. 3.03.19, 3.13.16, 3.13.20, 3.13.28, 7.01.06
Polst, Bastian. 2.04.08
Polverino, Giovanni. 2.01.03
Ponce Ortíz, Rafael. 2.10.16
Ponogaybo, Ksenia. 4.04.18
Pons, Marie. 4.02.10
Pons, Marie-Noelle. 4.14.27
Ponti, Jessica. 3.12.29
Poot, Anton. 3.10.18
Porcher, Jean Marc. 1.04.03
Porcher, Jean-Marc. 2.05.19

Poret, Agnes. 2.02.19
Porte, Cinta. 3.12.15
Posacka, Anna. 3.08.16
Posada, Rosa. 4.04.08
Posada-Baquero, Rosa. 4.04.03, 4.04.06
Pöschl, Ulrich. 3.02.03
Posthuma, Leo. 4.13.02, 4.16.04, 8.05.03
Postiglione, Augustus. 3.13.05
Postigo, Cristina. 3.04.09
Postma, Jaap. 4.02.05, 4.13.02
Potokar, Anja. 4.11.15
Potter, Elaine. 2.05.26
Potthoff, Annegret. 3.08.02
Potyka, Ute. 3.11.28
Poulsen, Rikke. 1.05.20
Pradel, Alice. 2.11.12
Pradhan, Ajay. 2.01.23
Praetorius, Antonia. 3.12.17, 4.14.07
Prakash, Halan. 3.03.07
Pramanik, Biplob. 4.15.03
Prats, Eva. 2.01.11
Preisinger, Christian. 1.07.14
Prenzato, Massimiliano. 3.13.16
Preud'homme, Hugues. 3.15.19
Preuss, Thomas. 2.02.02, 2.04.06, 2.04.07, 4.05.02, 4.06.06, 4.06.10, 4.06.21, 4.06.23, 4.08.04
Previsic, Ana. 2.13.01, 2.13.05
Prevot, Alessandra. 1.09.14
Preziosi, Damian. 4.06.28, 4.06.29
Pribylova, Petra. 3.02.03
Price, Anna. 1.03.05
Pries, Julia. 3.11.18
Prieto, Ailette. 1.09.20, 3.13.14
Prieto, Ana Isabel. 1.03.12, 4.11.07
Prieto Amador, Marina. 1.08.03, 1.09.09
Primpke, Sebastian. 3.08.06, 3.08.17, 3.08.20, 3.08.23
Princz, Juliska. 2.09.20, 6.04.09
Pritsolas, Joshua. 2.07.02
Priya, Vernita. 4.11.08
Prodana, Marija. 2.10.02, 2.10.04, 2.10.05, 2.10.07, 2.10.10, 2.10.13, 2.10.15
Profita, Marilin. 3.03.21
Proia, Lorenzo. 3.03.08
Prokes, Roman. 4.12.10
Prosser, Chris. 4.09.09
Protano, Giuseppe. 1.02.12
Provencher, Jennifer. 3.12.34
Prudnikova, Eva. 4.04.15
Prygiel, Jean. 1.04.03
Puccini, Monica. 2.09.07
Pucheux, Nicolas. 2.09.08, 3.09.23
Puertas, Ana. 1.01.11, 1.05.09
Puerto, Maria. 4.11.12
Puglisi, Raechel. 1.05.10
Puig-Samper Naranjo, Gonzalo. 5.02.06
Pyke, James. 3.13.07
Pyle, Greg. 2.01.02

Q

Qin, Feng. 3.13.11
Qiu, Yanling. 1.10.03
Quambusch, Anja. 6.05.05
Quevarec, Loïc. 2.09.24
Quik, Joris. 6.02.06, 6.04.02, 6.04.03
Quinteiro, Paula. 5.02.08
Quireyns, Maarten. 7.01.10

R

Radix, Pascal. 4.08.06
Ragaert, Kim. 5.03.08
Ragas, Ad. 2.05.27, 3.01.17, 3.04.06, 4.03.09, 4.06.24
Raghunatha Reddy, Lipi. 3.09.21
Rahman, Mahbub. 7.01.13
Rahman, Mohammed Ziaur. 7.01.13
Rahman, Ziaur. 7.01.13
Raikar, Laxman. 3.03.07
Rainsford, Caroline. 3.12.32
Raisanen, Riikka. 3.07.06
Rajagopalan, Neethi. 5.01.10
Rakel, Kim. 2.09.05
Raldúa, Demetrio. 2.01.11, 2.01.12, 2.01.13
Rall, Björn. 4.06.11, 4.06.12
Rama, Fabrizio. 3.10.22
Ramello, Gloria. 2.05.24
Ramesh, Prasanth Babu. 3.12.19
Ramil, María. 3.01.15, 3.05.06
Ramírez Acosta, Sara. 2.13.02, 4.17.02
Ramirez-Vargas, Carlos. 4.11.15
Ramos-Gómez, Magdalena Samanta. 3.09.02
Ramos-Rodríguez, Eloísa. 2.01.20
Ramsey, Michael-Thomas. 4.08.09
Ramsperger, Anja. 3.11.30
Rapp-Wright, Helena. 3.07.02
Raqib, Rubhana. 7.01.13
Rascio, Ida. 4.04.02
Rashed, Ahmed. 7.02.05
Rashid, Muhammad. 3.16.13
Rasmussen, Matilda. 2.06.03
Raspa, Giuseppe. 3.15.24
Ratcliffe, Norman. 6.01.05
Rathgeber, Andreas. 5.06.06
Rathjens, Hendrik. 3.10.15
Raths, Johannes. 4.03.02, 4.05.05
Ratier, Aude. 4.03.06, 4.06.04
Rattner, Barnett. 2.12.01
Rattray, Chris. 3.15.34
Rauert, Caren. 4.03.13, 4.03.21
Rauert, Cassandra. 3.11.09, 3.15.12
Rauseo, Jasmin. 4.04.09, 4.14.13, 4.14.25
Rawlings, Jane. 4.07.03, 4.07.09
Ré, Ana. 1.07.17, 3.09.19
Re, Ana. 2.02.05
Re, Ilaria. 7.02.02
Reche, Cristina. 3.02.10
Redman, Aaron. 4.02.05, 4.09.09
Redžović, Zuzana. 2.02.17
Reed, Melissa. 4.06.06
Refseth, Gro. 6.01.07
Reger, Luisa. 1.05.05
Regnery, Julia. 3.03.04
Regoli, Francesco. 2.13.06
Rehkamper, Mark. 6.04.12
Reichenberger, Stefan. 3.10.09, 3.10.10, 3.10.11, 3.10.13, 3.10.17, 3.10.21, 3.10.27, 4.06.06
Reifferscheid, Georg. 3.03.04, 3.03.17
Reijerkerk, Tristan. 3.09.15
Reiling, Kevin. 3.14.05
Reilly, Katie. 3.12.09, 6.04.10
Reina Joviano, Willian. 1.02.15, 2.11.08, 2.11.11
Reinwald, Hannes. 1.03.02, 1.08.05
Remili, Anais. 2.05.10
Renault, David. 4.14.30
Rendal, Cecilie. 2.04.06, 2.04.14, 4.06.06
Rendón-Von Osten, Jaime. 6.01.10
Renpenning, Julian. 1.09.17, 3.02.12, 3.05.08, 3.05.09, 3.06.09, 3.15.34
Resseler, Herbert. 3.10.22
Reunamo, Anna. 1.10.07
Reyero, Natalia. 2.01.11
Reza, Md. Shahed. 3.05.05
Rhodes, Ileana. 3.06.01
Ribeiro, Ana Beatriz. 3.15.18
Ribeiro, Francisca. 3.08.15, 3.11.09, 3.12.26
Ribeiro, Sonia. 4.04.11
Ribeiro Rosa, Amanda. 1.02.15
Richard, Claire. 3.01.14
Richardson, Alexandra. 3.15.32
Richardson, Kara. 2.02.13
Richardson, Katherine. 5.05.05
Rico, Andreu. 3.13.14, 4.10.01, 4.10.04, 4.10.05, 4.14.06, 8.02.07
Rieder, Jessica. 1.05.16
Riego Sintes, Juan. 6.04.05
Riegraf, Carolin. 3.03.17
Riem-Galliano, Louna. 3.11.22
Rigato, Jacopo. 7.01.06
Rios Miguel, Ana. 3.04.06
Risalde, Maria Angeles. 2.05.14
Risso, Wagner. 4.04.04
Rist, Sinja. 3.08.01
Ritter, Amy. 3.16.04
Riva, Francesco. 7.02.05
Riva, Valentina. 7.02.05
Rivero, María José. 5.01.17
Rivero Arze, Andrea. 1.02.11
Rivetti, Claudia. 4.01.02
Rixrath, Doris. 5.02.10
Rizzi, Cristiana. 4.14.06
Robert, Alexandrine. 2.02.14
Roberts, Sarah. 2.11.10
Robey, Darren. 3.11.15
Robinson, Alex. 4.12.05
Robinson, Paige. 1.06.09
Robinson, Stacey. 2.05.15
Robuchon, Marine. 8.04.02
Rocca, Jennifer. 2.03.08
Rocha, Ana. 1.07.17
Rocha, Carolina. 1.09.07
Rocha, Eduardo. 1.05.14, 1.05.19, 3.10.25, 3.15.18
Rocha, Juliana Delatim Simonato. 4.04.04
Rocha, Maria João. 1.05.14, 3.10.25, 3.15.18
Rocha, Rui. 2.08.15
Rocha-Santos, Teresa. 3.08.25
Roche, Baptiste. 5.01.11
Rocher, Vincent. 3.13.26
Röck, Martin. 5.02.09
Rodea-Palomares, Ismael. 4.13.15
Rodenburg, Lisa. 3.06.06
Röder, Nina. 2.06.05, 2.06.08, 2.06.09, 2.06.10
Rodgers, Tim. 3.15.33
Rodrigues, Andreia. 2.08.15
Rodrigues, Mariana. 2.05.29
Rodrigues, Sandra. 2.10.05, 2.10.13
Rodrigues, Sónia. 2.10.05, 2.10.13
Rodriguez, Amaia. 3.12.13
Rodríguez, Isaac. 3.01.15, 3.05.06
Rodríguez, Juan José. 3.13.22
Rodriguez, Simon. 4.14.23
Rodriguez, Yasmina. 3.12.34
Rodriguez Ruiz, Amaia. 6.04.01
Rodriguez Seijo, Andres. 4.04.11
Rodriguez Unamuno, Virginia. 6.04.01

Rodriguez-Mozaz, Sara. 3.12.04
Rodríguez-Ruiz, Ángela. 2.01.19, 2.01.20
Roeben, Vanessa. 4.08.04
Roelofs, Dick. 1.08.06
Roesch, Philipp. 3.13.01
Roessink, Ivo. 2.02.02, 4.06.10, 4.12.11
Roig, Francesc. 3.07.07, 3.15.21
Rojo-Nieto, Elisa. 4.03.01
Rolando, Ludovica. 4.04.02, 4.04.09
Rollin, Marc. 2.02.19
Romano, Dolores. 4.07.13
Romano, Maria Pia. 3.02.01
Romeo, Daina. 3.02.02
Romero, Ferran. 3.12.04
Roncari, Francesco. 6.04.05
Rönnefahrt, Ines. 4.14.03
Roodt, Alexis. 2.06.05, 2.06.09
Roos, Andre. 8.02.03
Rorat, Agnieszka. 2.10.06
Roß-Nickoll, Martina. 4.07.11, 4.10.10, 5.03.17
Rosa, Francesca. 5.02.03, 5.05.01
Roscales, Jose Luis. 2.11.03
Roscioli, Claudio. 7.01.06
Rose, Nicholas. 3.06.04, 3.06.05
Roslev, Peter. 1.09.13, 1.09.14
Ross, Peter. 3.08.16
Rossbach, Andrea. 2.01.08
Rossi, Vincent. 5.05.02
Rosso, Lorenzo. 1.09.04
Rota, Emilia. 2.11.08
Rotchell, Jeanette. 3.08.03
Roth, Christian. 3.14.01
Roth, Sabrina. 4.10.01, 4.10.04
Roussel, Erwan. 3.12.22
Rousselle, Christophe. 1.09.01
Roux, Clément. 3.11.19
Rouxel, Julien. 1.04.01
Rowenczyk, Laura. 3.11.14
Roxburgh, Lizanne. 2.12.06
Roy, Jean-Francois. 3.05.16
Roy, Kunal. 4.07.04
Roy, Rajdeep. 6.04.17
Roychand, Rajeev. 4.15.03
Rozhko, Tatiana. 4.04.14
Rozman, Marko. 1.10.01, 2.13.01, 2.13.05
Rozman, Ula. 4.07.10, 4.15.05
Rubbini, Davide. 1.03.05
Rubio Montejano, Consuelo. 4.14.04
Rubio-Brotons, Maria. 1.07.04
Rubolini, Diego. 3.13.20
Rudnicka, Dominika. 8.02.14
Ruedel, Heinz. 4.03.21
Rüegg, Joelle. 1.06.05
Ruhl, Aki. 3.11.18
Ruivo, Raquel. 2.02.15
Ruiz Romera, Estilita. 1.09.20, 4.14.22
Rumayor, Marta. 5.01.17, 5.01.18
Rumkee, Jack. 2.09.18, 4.08.04
Rummel, Christoph. 6.05.07
Rumohr, Quintana. 2.07.03
Rundberget, Thomas. 3.13.18
Runkel, Robert. 3.11.23
Ruokojärvi, Päivi. 3.15.20
Rusconi, Francesco. 1.09.04
Rusconi, Michele. 7.01.06
Russ, Anja. 6.03.06
Russo, Simona. 3.03.19
Russu, Anzhela. 2.09.23
Ruus, Anders. 2.05.01, 4.14.23
Ryan, Adam. 3.09.16, 4.02.06
Ryan, Kristen. 1.03.05
Ryberg, Morten. 5.04.05, 5.05.05
Rychen, Guido. 4.03.23
Rydberg, Maria. 5.03.01
Rydberg, Tomas. 5.03.01, 5.04.08
Rysä, Jaana. 3.07.06, 3.12.16
Ryser, Marie. 4.03.20

S

Sá, Carina. 3.09.20, 6.04.08
Saad, Noha. 2.01.23
Saarimäki, Laura. 4.01.11
Saaristo, Minna. 2.01.02, 2.01.03
Sachana, Magdalini. 1.03.05
Sadler, Jon. 3.12.09
Sadowski, Jan. 2.12.14
Sadutto, Daniele. 3.05.13, 3.06.10, 7.02.04
Saenen, Eline. 1.06.06
Saggese, Miguel. 1.04.10
Sahm, René. 2.01.02
Sahm, Rene. 2.03.06, 8.02.04
Saini, Amandeep. 3.13.17, 3.15.07, 3.15.13
Sakka, Yvonne. 4.11.11
Sakkas, Vasilios. 3.01.14
Sakuma, Satoru. 7.01.05
Sakurai, Maki. 1.05.16
Sala, Alberto. 7.01.06
Sala, Serenella. 5.01.05, 5.02.05, 5.02.09, 5.05.01
Sala, Serenella. 5.02.01, 8.05.02
Sala Solà, Berta. 2.05.12
Salamova, Amina. 3.15.10
Salas Redondo, Caterin. 5.01.09
Salbu, Brit. 1.08.10, 2.08.09
Salerno, Franco. 7.01.06
Salgó, András. 3.08.11
Salieri, Beatrice. 3.02.02
Salinas, Gabriela. 1.03.02, 1.08.05
Saling, Peter. 5.04.08, 5.06.01
Sallach, Jonathan. 3.15.04, 4.07.05
Salomon, Morgane. 3.15.17
Salvatierra, David. 2.01.19, 2.01.20
Salve, Nupur. 3.03.07
Samanipour, Saer. 6.04.12
Samarra, Filipa. 2.05.10
Sambrook Smith, Greg. 3.11.23
Samouelian, Anatja. 3.10.26
Samsonov, Dmitry. 3.15.08
Sanan, Toby. 4.11.10
Sánchez, Blanca. 4.12.04
Sánchez, Mª Blanca. 7.01.12, 7.02.06
Sánchez Soberón, Francisco. 4.17.06, 4.17.07
Sanchez Virosta, Pablo. 1.04.08, 1.04.09
Sanchez-Arguello, Paloma. 2.09.14, 6.04.07
Sanchez-Barbudo, Ines. 2.12.07, 2.12.13
Sánchez-Mico, Maria. 1.03.12
Sánchez-Vega, Elena. 2.05.04
Sánchez-Virosta, Pablo. 1.04.07, 2.05.04, 2.05.24, 2.05.25, 2.12.04, 2.12.05, 2.12.08
Sanders, Gordon. 1.01.01, 4.03.08, 4.03.15
Sanderson, Hans. 4.07.02, 4.07.03, 4.07.04, 4.07.07, 4.07.08
Sandoval Herrera, Natalia. 1.04.02, 2.01.22
Sangines De Carcer, Paula. 5.05.02
Sangion, Alessandro. 3.01.20, 3.01.21, 3.04.11, 3.15.38, 4.03.10, 4.03.17
Santana, Marina. 3.12.20, 3.12.24
Santillán-Saldivar, Jair. 5.06.06
Santini, Saul. 2.05.26
Santo, Nadia. 1.09.04
Santore, Robert. 3.09.10
Santos, Catia. 2.10.05, 2.10.13
Santos, Diogo. 2.08.02
Santos, Eduarda. 1.06.09
Santos, Fatima. 4.03.04
Santos, Joana. 2.10.03, 4.14.34
Santos, Lucia. 3.12.04
Santos, Maria João. 4.14.17
Santos, Miguel. 2.02.15
Santos, Niedja. 2.01.17
Santos, Paulo. 6.05.04
Santos, Sara Filipa. 3.10.25
Santovito, Gianfranco. 3.13.28
Sanye-Mengual, Esther. 5.02.01, 5.02.05, 5.05.01
Sanz, Paloma. 3.10.04, 3.13.12, 3.13.13, 3.15.09, 3.15.11
Sarajlić, Nermina. 2.05.24
Sarimveis, Haralambos. 4.01.11
Sarker, Pratim. 7.01.13
Sarma, Ss.. 4.14.29
Sarno, Antonio. 3.03.01, 3.15.06
Sarrà, Montserrat. 3.04.09
Sartelet, Hervé. 1.08.04
Sarti, Chiara. 2.05.26
Sassmann, Tim. 4.07.11
Sauer, Pavel. 4.12.10
Sauer, Theodor. 3.06.02
Saunders, David. 3.01.10, 3.04.05, 4.09.09
Sawada, Haruki. 7.01.05
Saxena, Sumit. 3.12.19
Sayen, Stéphanie. 4.14.30
Sbokos, Georgios. 2.05.24
Scalici, Massimiliano. 1.09.12
Scarpato, Silvia. 4.11.05
Schad, Thorsten. 2.07.01, 2.07.02, 3.10.16, 3.10.17, 4.06.30, 4.06.31, 4.06.32
Schade, Michael. 2.07.03
Schaefer, Sabine. 3.03.04, 3.03.17
Schaefers, Christoph. 1.05.13
Schäfer, Jörg. 4.03.19
Schäfer, Ralf. 4.06.03
Schäfers, Christoph. 1.03.02, 1.08.05
Schäffer, Andreas. 3.01.03, 3.01.06, 3.05.15
Schapaugh, Adam. 1.01.13, 1.01.14
Scharmüller, Andreas. 4.06.03
Schatten, Rene. 7.02.02
Schaubroeck, Thomas. 5.06.05
Schauer, Raphael. 5.02.10
Schebek, Liselotte. 5.01.06, 5.02.11, 5.05.13
Scheepers, Paul. 3.04.06
Scheiber, Laura. 3.15.21
Schellenberger, Steffen. 5.04.08
Schenke, Detlef. 2.12.14
Scherbak, Nikolai. 1.06.05
Schertzinger, Gerhard. 3.03.20
Schestak, Isabel. 5.06.10
Schiavone, Valentina. 1.03.05, 1.07.04
Schierz, Petra Ariette. 3.05.17
Schipper, Kerstin. 5.03.17
Schirmer, Kristin. 1.01.06, 1.01.07, 1.01.09, 1.07.05, 4.01.08, 4.15.10
Schirrmeister, Sven. 3.08.09
Schiwy, Sabrina. 3.03.20
Schlechtriem, Christian. 1.02.14, 4.03.13, 4.03.21, 6.03.01
Schleicher, Zachary. 6.01.03

Schlekat, Christian. 3.09.08, 3.09.09, 3.09.10, 3.09.11, 3.09.12, 3.09.13, 3.09.16, 4.02.06
Schlezinger, Jennifer. 4.13.07
Schlich, Karsten. 2.03.01, 2.03.02
Schlichting, Rita. 1.03.11, 4.12.10
Schlösinger, Anne. 1.10.06
Schluesener, Michael. 3.05.14
Schmalz, Holger. 3.11.30
Schmid, Cora. 3.16.15
Schmid, Simon. 1.08.09
Schmidt, Danina. 3.03.18
Schmidt, Jana. 3.01.06
Schmidt, Jürgen. 4.06.28, 4.06.29
Schmidt, Thomas. 2.09.15, 4.14.14
Schmidt, Torsten. 3.12.18, 3.14.02
Schmitt, Tobias. 3.12.23
Schmitt-Jansen, Mechthild. 2.04.08
Schmitz, Markus. 1.04.12
Schmitz, Susanne. 4.14.03
Schmoldt, Angela. 1.08.07
Schmolke, Amelie. 4.06.28, 4.06.29
Schmutz, Beat. 3.03.20
Schmutz, Mélanie. 5.03.06, 7.01.01
Schneidewind, Uwe. 3.11.23
Schnell, Sabine. 1.01.02
Schnitzler, Frauke. 3.03.14
Schnurr, Jacob. 4.03.02
Schoenenberger, Rene. 1.01.09
Schoenfeld, Jens. 6.03.01
Schokker, Arjen. 3.09.15
Scholliers, Niklas. 5.05.13
Scholz, Stefan. 1.01.07, 1.03.01, 1.03.11, 1.07.19, 2.01.02, 4.01.08
Scholz-Boettcher, Barbara. 3.08.20
Scholz-Starke, Bjoern. 6.03.02
Schönenberger, René. 1.01.06
Schoonjans, Reinhilde. 6.04.03
Schopel, Miriam. 3.13.33, 6.03.01
Schoyen, Merete. 3.11.11
Schrader, Hannah. 1.07.11
Schreiner, Verena. 4.06.03
Schreitmüller, Jörn. 1.05.12
Schrijvers, Dieuwertje. 5.06.08
Schröder, Stephan. 3.11.28
Schröder, Tom. 3.03.15
Schröder, Winfried. 4.17.08
Schroeder, Peter. 3.07.08, 3.07.10
Schroeder, Winfried. 4.17.09
Schuhmacher, Marta. 4.17.06, 4.17.07
Schuijt, Lara. 2.01.04
Schulz, Isabelle. 6.01.01
Schulz, Manoj. 3.05.14
Schulz, Ralf. 2.06.05, 2.06.09, 2.06.10, 3.12.23, 4.02.12, 6.04.17
Schulz, Wolfgang. 3.03.06
Schulze, Tobias. 1.04.12, 1.09.03
Schürch, Stephanie. 6.02.02
Schuster, Hanna. 2.02.01, 2.02.03, 6.03.07, 6.03.08
Schuster, Jasmin. 3.13.17, 3.15.12
Schuwirth, Nele. 4.06.28, 4.06.29, 8.02.08
Schwarz, Anna. 5.03.09
Schwarz, Christine. 4.14.12
Schwarz, Simon. 4.14.01
Schweiger, Nicole. 1.03.01, 1.03.07
Schweizer, Mona. 1.10.06
Schwenk, Klaus. 2.06.05, 2.06.09
Schwertfeger, Dina. 6.04.09
Schwirn, Kathrin. 6.04.02
Schymanski, Emma. 4.13.05
Scipioni, Antonio. 5.01.21, 5.04.09
Scott-Fordsmand, Janeck. 2.09.04, 4.03.04
Scotti, Roberto. 5.01.14
Scroggins, Rick. 2.09.20, 6.04.09
Sebastian, Aleksandra. 3.02.11
Sebire, Marion. 1.05.15, 1.05.16
Seco, José. 2.11.02
See, Mary Jean. 1.06.02
Segarra, Amelie. 2.08.11, 2.08.12
Segelle, Steffie. 3.10.24, 4.03.16
Segner, Helmut. 1.05.16
Sehnal, Ludek. 4.11.01
Seidel, Erika. 4.05.01
Seiler, Thomas-Benjamin. 1.04.12, 4.17.03
Seitz, Wolfram. 3.03.06
Selck, Henriette. 1.02.13
Seller, Carolin. 3.04.07
Sellin Jeffries, Marlo. 1.05.15
Selma-Royo, Marta. 2.13.02, 2.13.03
Semenzin, Elena. 3.07.04
Senan-Salinas, Jorge. 5.02.13, 5.03.12
Sendra, Marta. 1.02.01, 1.02.07, 2.03.04
Sengar, Ashish. 4.14.08
Sengupta, Sagnik. 2.09.22
Sengupta, Soumi. 4.11.07
Seo, Seongbong. 3.11.12
Seppänen, Suvi. 3.12.03
Serpa, Dalila. 2.03.03, 2.03.09, 3.09.19
Serra, Angela. 4.01.11
Serra, Noemí. 4.17.06, 4.17.07
Serrão, Joana. 3.08.25
Sesin, Verena. 2.04.09
Severin, Kevin. 3.13.25
Sevilla-Morán, Beatriz. 3.16.16
Sewell, Fiona. 1.05.10
Sezutsu, Hideki. 5.01.20
Shafer, Timothy. 1.03.05
Shahpoury, Pourya. 3.02.03, 3.02.07
Shakibay, Dror. 3.03.17
Shao, Ying. 1.04.12
Sharapova, Maria. 6.04.15
Sharpe, Emma. 3.12.31
Shaw, Emily. 4.13.08
Shen, Yu. 1.08.07
Shenkar, Noa. 1.10.05
Sherborne, Neil. 2.09.18, 4.06.17, 4.06.18
Shim, Won Joon. 3.08.19, 3.11.05, 3.12.11, 3.12.30
Shim, Wonjoon. 2.11.08, 3.11.04, 3.11.12, 3.12.25, 3.13.21
Shin, Dong-Chul. 5.07.01
Shin, Dongju. 1.07.18
Shin, Dongju. 3.09.22, 3.12.11
Shoeb, Mohammad. 3.05.05, 3.11.25
Shoemaker, Jody. 3.03.02
Shomroni, Orr. 1.08.05
Shore, Richard. 2.05.24, 2.05.25, 2.05.26, 2.12.12
Shrestha, Prasit. 3.01.04
Shukla, Shobha. 3.12.19
Siddiqui, Samreen. 2.08.12, 2.08.13, 4.15.08
Siebler, Flora. 5.01.05
Sigurðsson, Árni. 3.15.08
Sih, Andy. 2.01.03
Sikanen, Tiina. 4.03.07
Silva, Ana. 2.10.07, 2.10.10
Silva, Daniel. 2.01.19
Silva, Daniela. 3.12.28
Silva, Jose Roberto. 1.02.15, 2.11.08, 2.11.11
Silva, Marta. 3.12.14, 6.04.08
Silva, Monica. 2.12.17
Silva, Patricia. 4.01.10
Silva, Rui. 3.08.25
Silva De Assis, Helena. 2.03.10, 4.14.31
Silva De Souza Nunes, Gabriel. 3.13.14, 4.14.06
Simaitis, Joris. 5.05.11
Šimić, Antonio. 4.17.03
Simon, Franz. 3.13.01
Simon, Laurent. 3.12.10
Simon, Olivier. 1.06.07
Simonetti, Silvia. 2.11.09
Simonin, Marie. 2.03.08
Sims, Ian. 2.04.06
Sims, Natalie. 7.01.11
Sinclair, Tom. 4.10.03
Singer, Alexander. 4.06.11, 4.06.12
Singer, Heinz. 2.01.05, 4.12.06
Singh, Randolph. 4.13.05
Sinisalo, Jade. 4.03.07
Sinkko, Taija. 5.02.05
Siol, Antje. 4.11.11
Sipos, Levente. 1.08.05
Sittig, Stephan. 3.10.10, 3.10.21
Six, Johan. 5.06.09
Skancke, Jørgen. 3.15.06, 4.03.22
Skov, Henrik. 3.15.08
Slaby, Sylvain. 4.12.08, 4.12.09
Slaveykova, Vera. 2.06.04, 3.09.07
Slim, Kamal. 4.11.03
Slinde, Gøril. 3.13.18
Slobodnik, Jaroslav. 2.05.24, 2.05.26, 8.03.05
Slomberg, Danielle. 1.02.17
Slootweg, Chris. 4.10.11
Slootweg, Jaap. 4.13.02
Slotsbo, Stine. 4.03.05
Smetana, Sergiy. 5.04.14
Smit, Els. 2.01.02
Smit, Mathijs. 4.02.07
Smit, Tom. 3.10.12
Smith, Andrew. 3.06.09
Smith, Balthasar. 6.02.01
Smith, Jamie. 2.02.01, 2.02.03, 6.03.07, 6.03.08
Smith, Robert. 4.16.06
Smolarz, Katarzyna. 3.15.06
Smolders, Erik. 3.09.17, 4.05.06
Smutna, Marie. 4.11.01, 4.12.10
Smyth, Shirley Anne. 4.13.14
Snape, Jason. 2.03.11, 5.04.08
Sneekes, Andrea. 6.01.02
Soares, Amadeu. 1.07.07, 2.10.02, 2.10.04, 2.10.07, 2.10.12, 2.10.15, 4.04.22, 6.01.10
Soares, Cristiano. 2.04.05, 4.04.11
Sobek, Anna. 4.10.01, 4.10.04, 4.12.04
Sobotka, Jaromir. 4.12.10
Sobral, Paula. 6.01.05
Sobrino-Figueroa, Alma. 1.07.02, 2.02.18, 2.06.12, 4.14.29
Socher, Martin. 3.08.09
Soggiu, Maria Eleonora. 6.02.09
Sokołowski, Adam. 3.15.06

Solé, Magali. 4.06.07
Solga, Andreas. 4.05.02
Solhaug, Knut Asbjørn. 2.04.04, 2.08.09
Solís, Juan Carlos. 1.01.11
Solis, Marina. 2.06.02
Som, Claudia. 5.03.06, 5.04.10, 7.01.01
Somerset, Vernon. 1.09.18, 3.05.07, 3.05.11, 3.05.12, 3.09.03
Sommer, Simone. 3.12.34
Song, You. 1.08.09, 1.08.10, 1.08.11, 1.08.12, 2.04.01, 2.04.04, 2.08.09, 2.08.16
Song, Young Kyoung. 3.11.12
Song, Youngkyoung. 3.08.19
Sonne, Christian. 2.05.10, 2.05.25, 6.01.09
Sonnemann, Guido. 5.03.04, 5.06.06, 5.06.08
Soon, Zhi Yang. 1.07.18, 3.09.22
Sopko, Xiaoyi. 1.01.13, 1.01.14
Sorensen, Lisbet. 3.12.01
Soriano, Yolanda. 3.05.10
Soto, Ana. 3.16.08
Soto, Eva Patricia. 3.13.22
Soto, Manuel. 2.02.07, 4.03.19, 6.05.03
Soursou, Vasiliki. 3.07.01, 4.08.08
Sousa, Ana Catarina. 4.14.17, 4.14.18
Sousa, José Paulo. 4.16.06
Sowa, Christopher. 1.09.02, 3.11.28
Spaan, Kyra. 3.15.28
Spagnuolo, Antonietta. 1.02.06
Spani, Federica. 1.09.12
Sparham, Chris. 3.01.01
Spataro, Francesca. 4.04.09, 4.14.13, 4.14.25
Spehlmann, Max. 2.04.02
Speichert, Gunther. 3.01.06
Spiralski, Filip. 3.15.06
Spirinckx, Carolin. 5.01.10
Sprankle, Catherine. 1.01.12
Spriet, Jan. 5.06.10
Springer, Timothy. 2.04.11
Spry, Douglas. 2.09.06
Spurgeon, David. 1.02.19, 4.12.05
Spycher, Simon. 3.10.21, 4.06.28, 4.06.29
St. Romain, Scott. 2.08.12
Stachowski, Sabine. 4.12.12
Stadnicka-Michalak, Julita. 1.01.06
Stahl, Thorsten. 3.13.25, 3.16.03
Štampar, Martina. 4.11.13
Standerwick, Richard. 7.01.11
Stanisic, Lucija. 4.03.20
Stankiewicz, Paulina. 4.03.12
Stante, Michele. 3.03.19
Stapleton, Ken. 4.14.12
Staub, Pierre-François. 3.15.17
Stauch, Claudia. 5.01.14
Steevens, Jeff. 2.01.02
Stefani, Fabrizio. 7.01.06
Stehle, Sebastian. 4.02.12
Steinbach, Christoph. 6.05.06
Steinemann, Anne. 6.02.10
Steiner, Magdalena. 2.02.20
Steiniche, Tessa. 2.05.08
Stellner, Laura. 5.04.07
Stemmer, Michael. 3.10.18
Stenrød, Marianne. 4.01.09, 4.10.09
Stephansen, Diana. 3.11.17
Sterckx, Sindy. 7.03.04
Stern, Alexandra. 5.04.15
Štern, Alja. 4.11.13
Steubing, Bernhard. 5.01.01, 5.01.02, 5.01.07
Stibany, Felix. 6.03.02
Stibor, Herwig. 2.04.08
Stinckens, Evelyn. 1.05.07
Stinson, Sarah. 4.12.03
Stipanicev, Marko. 6.02.05
Stoddart, Gilly. 1.01.08
Stoica, Elena. 3.08.06
Stoks, Robby. 2.08.04, 2.08.05, 2.08.06, 4.10.06
Stornaiuolo, Mariano. 4.11.05
Stouffer, Philip. 2.12.16
Strand, Jakob. 3.08.06
Strasser, Vida. 4.02.08
Stratmann, Heidi. 6.04.04
Strauss, Tido. 4.06.06, 4.06.28, 4.06.29, 4.06.30
Streissl, Franz. 4.16.06
Strijakova, Elena. 4.04.01
Strobel, Bjarne. 2.13.07
Strotkötter, Lisa. 2.06.09
Struijs, Jaap. 4.05.11
Stubblefield, William. 3.09.16
Studholm, Katherine. 3.15.30
Sturba, Lucrezia. 2.09.07, 2.09.09
Stürzenbaum, Stephen. 3.15.32
Styles, David. 5.06.10
Styles, Renee. 1.03.09, 3.10.02
Sudhakaran, Raja. 4.11.08
Sudheshwar, Akshat. 5.03.06
Sugiyama, Genta. 5.04.12
Suh, Sangwon. 5.03.07
Sullivan, Kristie. 8.01.02
Summan, Ahmed. 3.16.11, 3.16.13
Sundh, Henrik. 1.09.05
Sundh, Ingvar. 4.16.06
Sundin, Josefin. 2.01.03, 4.14.15
Sur, Robin. 3.10.06, 3.10.09, 3.10.10, 3.10.11, 3.10.12, 3.10.15, 3.10.18, 4.08.06
Sushkova, Alesia. 6.04.16
Suter, Marc. 1.01.09
Suzuki, Hayato. 3.14.07
Svendheim, Linn. 6.01.06
Svendsen, Claus. 1.02.19, 4.12.05
Sverko, Ed. 3.15.08
Swaegers, Janne. 2.08.05
Swarowsky, Klaus. 4.16.05
Sweeney, Paul. 3.10.18, 3.10.19, 4.05.08, 4.05.09, 4.05.10
Sweeney, Paul. 3.10.22
Syberg, Kristian. 3.11.17
Szabo, Drew. 3.13.07
Szabó, Éva. 3.08.11
Szoboszlay, Sándor. 3.08.11
Szpunar, Joanna. 4.11.03
Sørensen, Lisbet. 3.03.01, 3.12.13, 3.12.33, 3.15.06
Sørli, Jorid. 3.13.29
Słowiński, Roman. 6.02.03

T

Taboureau, Olivier. 1.01.10
Taggart, Mark. 2.12.12
Takahashi, Kiichiro. 7.01.05
Takenaka, Miyuki. 3.11.26
Tal, Tamara. 1.03.01, 1.03.07
Talal, Ghanem. 2.09.13
Talavera, Francisca. 2.05.14
Tamarit, Carmen. 5.02.13
Tamm, Kaido. 4.01.11
Tan, Hung. 2.01.03, 4.14.15
Tanchev, Dimitar. 2.12.11
Tandy, Elizabeth. 6.01.03
Tang, Jianhui. 3.13.04
Tanguay, Robyn. 1.03.05
Tapie, Nathalie. 4.12.12
Tarazona, Jose. 6.04.03
Tardif, Genevieve. 4.13.14
Tasnim, Khandoker Tahmina. 3.11.25
Tauler, Roma. 1.06.01, 3.03.09
Taylor, Nadine. 2.02.01, 2.02.03, 6.03.07, 6.03.08
Taylor, Sabrina. 2.12.16
Taylor, Seamus. 6.03.07
Tebby, Cleo. 4.01.03
Tedim, João. 4.04.22, 6.04.16
Teien, Hans-Christian. 2.04.01, 2.04.04, 2.08.16
Teigeler, Matthias. 1.05.13
Teijón, Gloria. 7.02.06
Teixeira, Camilla. 3.15.02
Teixeira Sabóia-Morais, Simone. 1.09.06
Tell, Joan. 4.02.04
Tenji, Dina. 1.03.10
Tenório Botelho, Marina. 2.01.21, 2.02.13
Teodorovic, Ivana. 1.03.10
Ter Halle, Alexandra. 3.08.14, 3.11.22
Ter Halle, Alexandra. 3.11.19
Ter Horst, Mechteld. 3.10.01
Ter Laak, Thomas. 4.14.07
Terekhova, Vera. 4.04.15, 6.04.15
Terentjev, Petr. 2.11.10
Ternes, Thomas. 3.05.14, 3.09.21, 3.15.14, 6.01.08
Terriente, Javier. 1.03.05, 1.07.04
Terytze, Konstantin. 7.02.02
Terzaghi, Elisa. 3.15.05, 3.15.24, 3.15.25, 3.15.26, 4.04.03
Tessaro, Gloria. 3.04.08
Teta, Roberta. 4.11.05
Tettenhorst, Daniel. 4.11.10
Teunkens, Bert. 7.03.04
Thai, Phong. 3.03.22
Thelakkat, Mukundan. 3.11.30
Theys, Charlotte. 2.08.06
Thiele, Karen. 6.02.04
Thit Jensen, Amalie. 1.02.13
Thoeny, William. 4.11.10
Thomaidis, Nikolaos S.. 1.10.06, 2.05.24, 2.05.26, 3.03.11
Thomas, Jeff. 4.02.02
Thomas, Kai. 3.10.21
Thomas, Kevin. 3.03.22, 3.03.23, 3.08.15, 3.11.09, 3.12.26, 6.04.12
Thomas, Paul. 1.05.01, 4.05.04, 4.06.01, 4.06.02, 4.09.08
Thompson, Amibeth. 2.07.05
Thompson, Matthew. 4.01.06
Thorbek, Pernille. 4.05.08, 4.05.09, 4.05.10, 4.06.06, 4.08.04, 4.16.03, 7.01.04
Thorenz, Andrea. 5.06.06
Thorstensen, Helene. 2.05.01
Thulke, Hans-Herrmann. 7.01.04
Tierbach, Alena. 1.01.09
Tietjen, Lars. 6.04.02
Tiktak, Aaldrik. 3.10.18
Tilbrook, Martin. 3.10.05
Timmer, Niels. 3.01.10
Timoner, Pablo. 2.06.04
Tincani, Flavio. 2.09.03
Tindall, Andrew. 1.05.15, 1.05.16
Tiselius, Peter. 2.06.03
Tisler, Selina. 3.05.04
Tiso, Till. 5.03.17
Titelman, Josefin. 2.08.17
Titova, Jelizaveta. 3.12.08
Toapanta, Tania. 3.11.09

Tobi, Hilde. 6.02.04
Tobino, Tomohiro. 4.15.04
Todorov, Zlatomir. 1.05.01
Tollefsen, Knut Erik. 1.08.09, 1.08.10, 1.08.11, 1.08.12, 2.04.01, 2.04.04, 2.08.09, 2.08.16, 4.01.07, 4.01.09, 4.10.09, 4.14.02
Tomic, Tanja. 1.03.10
Toniolo, Sara. 5.04.09
Toorman, Erik. 7.03.04
Toose, Liisa. 3.01.20, 3.01.21
Töpel, Alexander. 4.07.11
Topfer, Till. 2.05.24
Topping, Christopher. 4.16.06
Torgashina, Irina. 1.09.10
Torres, María. 2.12.04
Toschki, Andreas. 2.07.03
Tourinho, Paula. 4.03.04
Tournebize, Julien. 3.10.20
Tousova, Zuzana. 4.12.10
Town, Raewyn. 3.09.05
Toyomaki, Haruya. 2.05.18
Trapp, Stefan. 3.01.06
Trathan, Phil. 6.01.05
Tremolada, Paolo. 1.09.04
Tremolet, Gauthier. 2.02.12
Treu, Gabriele. 2.05.24, 2.05.26
Triantis, Theodoros. 4.11.03
Triebskorn, Rita. 1.10.06
Trier, Xenia. 3.13.29
Triffault-Bouchet, Gaëlle. 4.09.07
Trijau, Marie. 2.05.17, 4.06.13, 4.06.14, 4.06.15, 4.06.17, 4.06.18
Trofimov, Sergey. 4.04.16
Tromp, Peter. 4.15.01
Troublé, Romain. 8.04.07
Truax, Jim. 1.01.12
Trujillo, Abraham. 6.01.01
Truong, Lisa. 1.03.05
Trznadel, Maciej. 1.03.03
Tscharke, Benjamin. 3.03.22, 3.03.23
Tsoumanis, Andreas. 4.01.10, 4.01.11
Tsygankova, Elizaveta. 4.04.19
Tudesque, Loic. 3.11.22
Tuerk, Jochen. 3.12.18, 3.14.02, 4.17.08
Tufenkji, Nathalie. 3.11.14
Tuhkanen, Tuula. 4.14.11
Tuma, Axel. 5.06.06
Tumolo, Marina. 4.04.10
Turano, Marta. 2.09.09
Turgut, Cafer. 3.01.16
Turiès, Cyril. 1.04.03, 2.05.19
Turja, Raisa. 1.10.07
Turolla, Andrea. 3.03.19
Tursi, Angelo. 2.09.11, 4.04.10
Tuthorn, Mario. 3.02.12, 3.06.09
Tüzün, Nedim. 2.08.06
Twiddy, Maureen. 3.08.03
Tyler, Charles. 1.03.03, 2.01.03, 4.14.19
Tysklind, Mats. 3.15.22

U

Uchanov, Pavel. 4.04.15, 6.04.15
Uchida, Yoshitaka. 2.05.18
Udiković-Kolić, Nikolina. 3.03.03
Ulrich, Uta. 4.06.22
Umbuzeiro, Gisela. 2.02.13, 3.07.06
Uotila, Elina. 3.01.05
Urban, Noel. 4.13.08
Uricchio, Vito Felice. 2.09.11, 3.08.08, 3.08.13, 4.04.02, 4.04.10
Urionabarrenetxea, Erik. 2.02.07, 4.03.19
Urra, Julen. 1.09.20, 4.14.22
Usman, M.. 3.03.05

V

Vaccari, Lisa. 2.11.08
Väinölä, Risto. 2.05.24
Vaj, Claudia. 2.10.01, 7.02.01
Valbonesi, Paola. 3.03.21, 3.12.33
Valdehita, Ana. 1.02.16
Valdivia, Meica. 3.09.14
Valhondo, Cristina. 4.04.13
Vallier, Manon. 3.12.10
Vallotton, Nathalie. 4.07.12
Valsami-Jones, Eugenia. 4.01.10, 4.01.11
Valsecchi, Sara. 3.03.19, 3.13.16, 3.13.20, 3.13.28, 7.01.06
Valsesia, Andrea. 3.12.29, 6.04.05
Valverde, Irene. 2.05.04, 2.12.04, 2.12.05, 2.12.08, 2.12.10
Valverde, Pablo. 1.01.13, 1.01.14
Vamshi, Raghu. 3.16.04
Van Acker, Karel. 5.03.16
Van Aerle, Ronny. 1.06.09
Van Amerongen, Jantien. 3.09.15
Van Assche, Frank. 4.02.06
Van Bavel, Bert. 3.08.06, 3.08.24
Van Bergen, Tamara. 3.01.17, 3.04.06
Van Beusekom, Bas. 3.09.15
Van Brocklin, Jennifer. 3.12.27
Van Damme, Stefan. 7.03.04
Van De Meent, Dik. 4.05.11
Van Den Berg, Erik. 3.10.18
Van Den Berg, Hans. 1.04.06
Van Den Berg, Sanne. 2.01.16, 4.01.02, 4.10.05
Van Den Brink, Nico. 1.04.06, 2.05.25, 2.05.26
Van Den Brink, Paul. 2.01.04, 2.08.07, 4.01.02, 4.10.01, 4.10.04, 4.10.05, 4.10.07, 4.14.16, 4.16.01, 4.16.02, 4.16.03
Van Den Oever, Anne. 5.01.12
Van Der Drift, Bram. 5.03.09
Van Der Horst, Charlton. 3.05.07, 3.09.03
Van Der Hulst, Mitchell. 5.03.09
Van Der Mije, Steven. 2.05.24, 2.05.26
Van Der Oost, Ron. 4.12.07
Van Der Sluijs, Jeroen. 8.03.03
Van Dijk, Joanke. 4.10.01, 4.10.11, 8.05.01
Van Dingenen, Imke. 1.05.20
Van Donk, Ellen. 4.17.05
Van Duursen, Majorie. 1.05.21
Van Ee, Nathan. 2.04.02
Van Egmond, Roger. 3.01.01
Van Eupen, Michiel. 2.04.13
Van Gestel, C.a.m.. 1.08.06, 2.09.17, 2.09.22, 4.03.04, 4.03.05
Van Haelst, Sven. 4.04.23
Van Hall, Bart. 2.09.17
Van Harmelen, Toon. 5.03.09
Van Hattum, Bert. 2.05.25
Van Landuyt, Josefien. 3.04.03, 4.04.23
Van Mourik, Louise. 3.08.24
Van Nuijs, Alexander. 7.01.07
Van Nuijs, Alexander. 7.01.10
Van Sebille, Erik. 7.03.03
Van Sprang, Patrick. 4.06.25, 4.06.26, 4.06.27
Van Straalen, Nico M.. 4.05.11
Van Vlimmeren, Lotte. 5.01.14
Van Wezel, Annemarie. 3.03.13, 4.10.01, 4.10.11, 4.14.07, 8.03.01
Van Zelm, Rosalie. 3.01.17, 3.04.06, 5.01.14, 5.04.11
Van Zwieten, Remy. 3.09.15
Vandegehuchte, Michiel. 3.12.05, 7.03.03
Vandenbulcke, Franck. 1.08.02, 2.10.06
Vangampelaere, Geert. 3.15.29
Vangeluwe, Didier. 2.05.24
Vanhanen, Joonas. 3.02.11
Vanninen, Paula. 1.10.07
Vannuccini, Maria Luisa. 2.09.07, 2.09.09
Vannuci-Silva, Monizze. 2.02.13
Vara, Manuel. 3.04.09
Varela, Alicia. 3.04.04
Varsou, Dimitra-Danai. 4.01.12
Vartti, Vesa-Pekka. 3.15.20
Vasconcelos, Vitor. 4.11.07, 4.11.08, 4.11.09
Vasilyeva, Galina. 4.04.01
Vassilenko, Ekaterina. 3.08.16
Vasumini, Ivo. 3.03.21
Vazakidou, Paraskevi. 1.05.21
Vazquez-Sune, Enric. 3.15.21
Vea, Eldbjorg. 5.05.05
Vega, Milagros. 3.01.05
Veldhuizen, Ab. 3.10.12
Velicogna, Jessica. 2.09.20, 6.04.09
Velki, Mirna. 1.04.12, 4.17.03
Venancio, Catia. 2.08.03, 2.08.10, 4.07.06, 4.14.17, 4.14.18
Venditti, Iole. 1.02.12
Venier, Marta. 2.05.08, 3.15.10
Venuti, Valentina. 2.11.08
Verbruggen, Eric. 6.02.06
Verburg, Femke. 1.09.16
Verdonschot, Piet. 4.04.24
Verellen, Evelien. 5.02.09
Vergauwen, Lucia. 1.05.07, 1.05.08, 1.05.20
Verhaegen, Yves. 4.09.09
Verheyen, Julie. 2.08.06, 4.10.06
Vermang, Bart. 5.01.10
Vermazeren, Arend. 7.02.01
Vermeiren, Peter. 2.05.27, 3.11.10, 4.06.06, 4.06.24
Verney, Vincent. 3.12.22
Verones, Francesca. 3.08.05
Versteegen, Elien. 4.14.16
Verweij, Peter. 2.04.13
Veseli, Marina. 2.13.05
Vestel, Jessica. 4.02.04
Viaene, Karel. 3.09.16, 4.06.25, 4.06.26, 4.06.27
Viana, Mar. 3.02.10
Vianello, Alvise. 3.08.23, 3.11.06, 3.11.17
Vicario, Silvia. 1.07.12
Vieira, Hugo. 2.08.15, 6.01.10
Vighi, Marco. 4.14.06
Vighi, Morgana. 3.09.14
Vignati, Davide. 3.09.18
Vignet, Caroline. 4.14.32
Vija, Heiki. 3.12.08
Vijayanandan, Arya. 4.14.08
Vijayaraj, Vinita. 2.04.08
Vijendra, Priyesha. 2.05.20
Vijver, Martina. 3.08.05
Vike-Jonas, Kristine. 2.05.02
Vikingsson, Gisli. 2.05.10
Vila, Maria. 3.02.13
Vila-Costa, Maria. 2.11.03, 3.04.04, 6.05.01
Vilenica, Marina. 2.13.05
Villa, Sara. 3.03.19, 4.14.06
Villalobos, Sergio. 4.09.09

Villaverde, Juan Jose. 3.16.16
Villenave, Eric. 3.05.03
Villenueve, Daniel. 3.03.16
Vincent, Tomoko. 1.09.17
Vinhas, André. 2.10.07
Viñuelas, Jose Alberto. 2.05.07, 2.05.14
Visca, Andrea. 4.04.09, 4.14.13
Visnapuu, Meeri. 3.12.08
Vitale, Dyana. 1.07.15
Vitolo, Sandra. 2.09.07
Vitorica, Javier. 1.03.12
Vives-Ferrándiz Sánchez, Carmen. 2.01.07
Viza, Aida. 2.06.02
Vlachopoulos, Konstantinos. 2.05.24
Vlaeminck, Karel. 4.06.25, 4.06.27
Vlasenko, Alexander. 3.15.08
Vogel, Christian. 3.13.01
Voisin, Anne-Sophie. 2.08.08
Voldoire, Olivier. 3.12.22
Völkel, Wolfgang. 3.01.03
Völker, Carolin. 7.03.01, 7.03.02
Volker, Doris. 6.04.02
Vollertsen, Jes. 3.08.21, 3.08.23, 3.11.06, 3.11.17
Voltz, Marc. 3.10.26
Volz, Sina. 1.05.06, 1.05.20
Vom Berg, Colette. 1.03.06
Vom Berg, Johannes. 1.07.05
Von Der Ohe, Peter. 1.10.06
Von Hellfeld, Rebecca. 1.07.09
Vonk, Annigje. 2.01.04
Vorkamp, Katrin. 3.15.08
Voronina, Liudmila. 4.04.18
Vossen, Laura. 2.01.14
Voyiatzis, Evangelos. 4.01.10, 4.01.11
Vrana, Branislav. 4.12.10
Vrezec, Al. 2.05.24, 2.05.25
Vughs, Dennis. 3.05.02
Vulin, Irina. 1.03.10
Vulliet, Emmanuelle. 2.05.19, 3.15.17, 4.12.08

W

Wagenaar, Ina. 1.09.11, 2.12.18
Wäger, Patrick. 5.04.02
Wagner, Martin. 1.04.04, 2.05.16, 3.12.01, 3.12.13
Wahl, Aurelie. 3.14.03
Waichman, Andrea. 4.14.06
Waichman, Andrea Viviana. 3.13.14
Wal, Bas. 4.13.02
Waldvogel, Ann-Marie. 6.03.09
Walecka, Dominika. 3.15.06
Walker, Lee. 2.05.24, 2.05.26
Wallace, Derek. 3.10.05
Waller, Cath. 2.11.06
Walter-Rohde, Susanne. 1.05.13, 4.03.13, 6.03.01
Walther, Grit. 5.01.16
Waluda, Claire. 6.01.05
Wan, Yuchao. 3.15.33
Wang, Aijie. 4.04.20
Wang, Hong Cheng. 4.04.20
Wang, Jiaqi. 4.03.09, 4.16.04
Wang, Magnus. 4.06.06, 4.13.10, 8.02.10
Wang, Neil. 3.01.07, 4.09.09
Wang, Shaorui. 2.05.08
Wang, Tiantian. 3.12.15
Wang, Xianyu. 3.11.09
Wang, Zejun. 1.09.15
Wang, Zhanyun. 7.03.05
Wania, Frank. 3.01.20
Warwick, Oliver. 4.16.01
Wasserman, Michael. 2.05.08
Watari, Takuma. 5.04.03
Watkins, Mathew. 3.08.16
Watzke, Jörg. 4.03.01
Waugh, Courtney. 1.04.11
Wawryniuk, Milena. 4.03.12
Wazne, Mohammad. 3.12.10
Weber, Andrea. 4.03.20
Weber, Miriam. 3.08.05
Weber, Yanik. 3.01.03
Webster, Thomas. 3.13.29, 4.13.07
Weeks, Jason. 4.14.12
Wehmas, Leah. 1.03.07
Weichert, Fabian. 4.07.11, 5.03.17
Weidemann, Eva. 3.13.25, 3.16.03
Weighman, Kristi. 4.06.27
Weinfurtner, Karl-Heinz. 3.14.02, 7.02.02
Weitere, Markus. 1.04.12
Welch, Kenneth. 1.04.02, 2.01.22
Welch, Samuel. 4.01.07, 4.10.01, 4.14.02
Welden, Natalie. 3.08.01
Wellmitz, Joerg. 3.13.02
Welte, Cornelia. 3.04.06
Weltje, Lennart. 1.01.08
Wentzell, Jeremy. 3.15.07
Wenzel, Mike. 3.14.02, 4.17.08
Werner, Inge. 2.08.08
Wernham, Chris. 2.05.24, 2.05.25
Werschmöller, Simon. 7.03.02
Wess, Ralf Arno. 4.14.14
Weyers, Arnd. 4.13.10, 4.13.15
Whale, Graham. 3.01.08, 3.01.19, 4.09.01
Wheeler, James. 1.05.10
White, J. 1.06.04
White, Jason. 1.08.07
Whitehead, Andrew. 1.07.11
Whitehouse, Paul. 4.16.02
Whiting, Rob. 3.13.33
Whittaker, Andrew. 3.08.15
Wick, Arne. 1.09.19, 3.05.14, 3.09.21, 3.15.14, 6.01.08
Wick, Peter. 3.02.02
Widdowson, Caroline. 3.11.21
Wieczorek, Matthias. 3.12.23
Wiemann, Astrid. 3.01.06
Wiesinger, Helene. 7.03.05
Wiesner, Mark. 3.11.14
Wiesner, Yosri. 3.11.07
Wiest, Laure. 3.15.17, 4.12.08
Wietor, Jean-Luc. 8.05.05
Wietzoreck, Marco. 3.02.03
Wijntjes, Christiaan W.. 3.01.03
Wild, Romy. 1.04.12
Wilhelm, Manon. 6.04.16
Willems, Hans. 3.10.22
Williams, A. Prysor. 5.06.10
Williams, Richard. 4.10.03
Williams, Rosie. 2.05.13
Wilson, Iain. 3.09.09, 3.09.11, 3.09.12, 3.09.16, 4.02.06
Wilson, Simon. 3.15.08
Wimmer, Martin. 8.05.03
Winberg, Svante. 2.01.14
Winchell, Michael. 3.10.15
Winkler, Anna. 1.09.04
Winter, Matthew. 1.03.03
Wipfler, Louise. 3.10.17, 4.06.31, 4.06.32
Wirth, Judith. 6.02.02
Witt, Johannes. 2.04.06, 4.05.02, 4.06.21, 4.06.23
Wittwer, Philipp. 3.13.01
Wlodkowic, Donald. 2.01.10
Wogram, Joern. 4.16.05
Wohlleben, Wendel. 3.11.16, 3.14.01, 6.04.04, 7.03.07
Wojciechowska, Zofia. 3.12.06
Wolf, Carmen. 3.14.02, 4.17.08
Wolf, Christian. 2.01.07, 2.01.08
Wolf, Jeffrey. 1.05.10, 2.04.11, 6.03.05
Wolf, Raoul. 1.01.07, 2.08.16, 4.01.08
Wolf, Yvonne. 4.05.02, 6.03.04
Wolff, David. 1.09.19
Wollenberg Valero, Katharina. 2.11.05
Wong, Bob. 2.01.02, 2.01.03, 2.01.10, 4.14.15
Wong, Fiona. 3.15.08
Wood, Carmen. 1.03.07
Woodland, Cindy. 1.03.05, 1.07.13
Woods, Hope. 4.07.08
Worrell, Ernst. 5.03.09
Woutersen, Jasper. 3.01.10
Wu, Dan. 4.06.04
Wu, Fengchang. 3.09.13
Wu, Jingcun. 3.13.11
Wu, Junzhang. 5.01.21
Wulf, Sven-Erik. 3.09.21
Wurzbacher, Christian. 1.09.19, 3.04.08
Włodarska-Kowalczuk, Maria. 3.15.06

X

Xavier, Jose. 2.11.02, 6.01.05
Xiao, Feng. 3.13.05, 3.13.27, 3.13.31, 3.13.32
Xie, Li. 2.04.04, 2.08.09, 2.08.16
Xiong, Chan. 2.03.12
Xu, Xiaojuan. 1.10.03
Xuereb, Benoit. 2.02.19

Y

Yabe, John. 2.05.18
Yakovenko, Nadiia. 3.11.19
Yakushev, Evgeniy. 2.11.10
Yalcın, Melis. 3.01.16
Yamamoto, Hiroshi. 1.05.15
Yamamoto-Flores, Laura. 3.09.02
Yamazaki, Kunihiko. 4.13.06, 4.14.05
Yang, Gongda. 2.02.13
Yang, Tong. 3.11.02
Yang, Yongxiang. 5.01.07
Yeste, Pilar. 1.02.07
Yildirim, Aylin. 3.14.02
Yli-Öyrä, Johanna. 3.07.06
Yohannes, Yared. 2.05.18
Yokoi, Ryosuke. 5.04.03
Yoon, Tae Hyun. 4.01.11
Young, Sarah. 2.05.15
Young, Steven. 5.03.04
Yu, Jasmine. 3.11.08

Z

Zakharova, Liubov. 4.08.04
Zaldibar, Beñat. 2.02.07, 6.05.03
Zaltauskaite, Jurate. 4.04.05, 4.10.08
Zamora-López, Antonio. 1.04.07
Zamora-Marín, José Manuel. 1.04.07
Zanardini, Elisabetta. 3.15.24, 3.15.25, 3.15.26
Zany, Camille. 1.05.15
Zapevalov, Mikhail. 3.15.08
Zarafshani, Hanie. 5.03.16
Zarragoitia, Maren. 6.05.03
Zayter, Mariam. 3.15.19
Zaytseva, Tatyana. 2.09.23

Zegura, Bojana. 4.11.13
Zervou, Sevasti-Kiriaki. 4.11.03
Zgheib, Elias. 1.09.01
Zhang, Lihong. 2.03.11
Zhang, Qiyun. 4.14.26
Zhang, Xianming. 3.01.20, 3.15.07
Zhang, Zheng Wei. 3.02.07
Zheng, Keke. 1.08.10
Zheng, Qiuda. 3.03.22
Zheng, Yuanfang. 6.04.11
Ziegler, Korinna. 6.03.02
Ziegler, Michael. 2.01.02
Ziv, Lisa. 4.02.04
Zocher, Anna Lena. 6.01.09
Zoppo, Marina. 1.07.05
Zuber, Martin. 3.08.10
Zubrod, Jochen. 4.02.12, 4.14.24
Zukowski, Jolanta. 3.01.09
Zuliani, Filippo. 5.04.06, 5.04.09
Zuloaga, Olatz. 1.09.20
Zumbado, Manuel. 1.04.09
Zupanic, Anze. 1.03.06
Zwanzig, Martin. 8.02.05
Zyambo, Golden. 2.05.18

Ø

Østnes, Jan Eivind. 2.05.05
Øverjordet, Ida. 3.12.01

SETAC Africa
SETAC Europe

Avenue des Arts, 53
B-1000 Brussels
Belgium
T +32 2 772 72 81
setaceu@setac.org

SETAC Latin America
SETAC North America

PO Box 12804
Pensacola, FL 32591-2804
United States
T +1 850 469 1500
setac@setac.org

SETAC Asia-Pacific

27/2 Masthead Drive
Cleveland Qld 4163
Australia
ap@setac.org

setac.org

Environmental Quality Through Science®