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Dermal uptake and percutaneous penetration of ten flame retardants in a human skin *ex vivo* model

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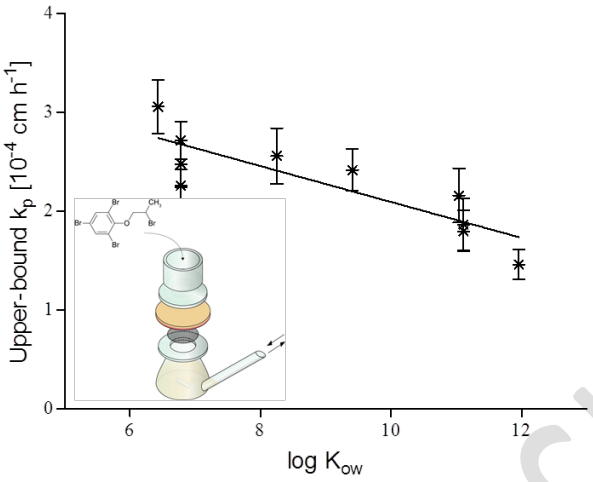
Abstract

The dermal uptake and percutaneous penetration of ten organic flame retardants was measured using an *ex vivo* human skin model. The studied compounds were DBDPE, BTBPE, TBP-DBPE, EH-TBB, BEH-TEBP, α, β and γ-HBCDD as well as syn- and anti-DDC-CO. Little or none of the applied flame retardants was recovered in either type of the receptor fluids used (physiological and worst-case). However, significant fractions were recovered in the skin depot, particularly in the upper skin layers. The primary effect of the worst-case receptor fluid was deeper penetration into the skin. The recovered mass was used to calculate lower- and upper-bound permeability coefficients k_p. Despite large structural variation between the studied compounds, a clear, significant decreasing trend of k_p was observed with increasing log K_{ow}. The results indicate that the dermis may provide a significant barrier for these highly lipophilic compounds. However, based on our results, dermal uptake should be considered in exposure assessments, though it may proceed in a time-lagged manner compared to less hydrophobic compounds.

Graphical abstract



Keywords: NBFR, brominated flame retardants, HBCDD, skin deposition, dermal exposure, Dechlorane Plus

1. Introduction

The ban and phasing out of Penta and Octa mixtures of polybrominated diphenyl ethers (PBDEs) and their inclusion in the Stockholm Convention (United Nations, 2009) have potentially led to a shift in use of organic flame retardants (FRs) from PBDEs towards alternative flame retardants (Covaci et al., 2011). While exposure and effects of PBDEs are relatively well documented (Fromme, 2016; Linares et al., 2015; Lyche et al., 2015), few studies on exposure and toxicology of the alternative flame retardants have been published. Among the alternatives are decabromodiphenyl ethane (DBDPE); 1,2-bis(4,2,4-tribromophenoxy) ethane (BTBPE); 2,3-dibromopropyl-2,4,6-tribromophenyl ether (TBP-DBPE); 2-ethylhexyl-2,3,4,5-tetrabromo-benzoate (EH-TBB); bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (BEH-TEBP); other related compounds of interest include α, β and γ- hexabromocyclododecane (HBCDD) as well as syn- and anti-Dechlorane Plus (DDC-CO), their structure and estimated basic properties are given in Table S1.

DBDPE has been used as a flame retardant since the early 1990s, with the same commercial applications as BDE-209 (Covaci et al., 2011; Kierkegaard et al., 2004) while no information is available on the current production and use of TBP-DBPE (Vetter et al., 2010). BTBPE is used to replace OctaBDE (Hoh et al.,

2005) and can be degraded to 2,4,6-tribromophenol (Hakk et al., 2004), which is an endocrine disrupting chemical (Deng et al., 2010). Mixtures containing BEH-TEBP and EH-TBB have been used as replacements of PentaBDE (e.g. Firemaster 550), but BEH-TEBP has also been used as a flame retardant on its own (Stapleton et al., 2008). It has been demonstrated that BEH-TEBP and EH-TBB have estrogenic disruption potential and that metabolites of BEH-TEBP exhibit rodent toxicity (Saunders et al., 2013; Springer et al., 2012). HBCDD has been recognized as an environmental problem for more than ten years (de Wit, 2002) and is now globally regulated through the Stockholm Convention, with exemptions of use in expanded and extruded polystyrene in buildings (UNEP, 2013). DDC-CO was introduced in the mid-1960s as a replacement flame retardant for Mirex, but unlike Mirex, it has not been used as an insecticide (Hoh et al., 2006).

Dermal uptake and percutaneous penetration of topical applied pharmaceuticals are widely studied though other chemicals particularly pesticides have also received some attention (WHO, 2006). Dermal uptake is most efficient for compounds with a moderate hydrophilicity as well as lipophilicity. This is due to the different layers of the skin i.e. the stratum corneum and epidermis generally containing a lot of lipids, while the deeper layers contain more water (WHO, 2006). The percutaneous penetration and dermal uptake of highly lipophilic compounds (particularly those with log K_{ow} > 7), which includes many of the SVOCs found in indoor environments, have only rarely been studied (Zhou et al., 2013). Generally, they have been found to accumulate in the skin with limited penetration (Chu et al., 1996; Zhou et al., 2013). The expected percutaneous penetration of the studied FRs is low, but since the exposure is continuous uptake and penetration may become relevant over time.

Associations between levels of the better studied PBDEs in indoor dust, hand wipes and biomarkers of exposure (breast milk, serum, placenta) have demonstrated that indoor exposure is an important route of exposure (Frederiksen et al., 2010; Vorkamp et al., 2011b; Watkins et al., 2011; Wu et al., 2007). Such research cannot, however, disentangle the contribution of inadvertent dust ingestion from dermal exposure. Modeling suggests that dust ingestion may be the more important of the two for most exposure groups (Lorber, 2008; Trudel et al., 2011), but these calculations are hampered by significant uncertainties, for example poorly known dust ingestion rates. More recently, the importance of dermal uptake of flame

retardants and other semi-volatile organic compounds (SVOCs) via skin contact with products and contaminated surfaces as well as direct absorption from indoor air have been discussed as potentially significant routes of exposure (Carignan et al., 2013; Weschler and Nazaroff, 2014). For replacement flame retardants, we have the additional challenge of little or no data on basic parameters needed to understand dermal absorption. The present paper helps fill this gap by providing estimates of dermal uptake and skin permeability for a number of the brominated FRs as well as dechlorane plus.

2. Materials and Methods

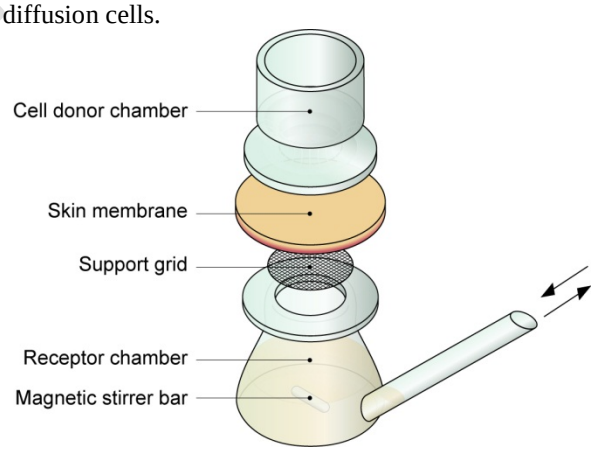
2.1. Dermal uptake model

Human skin was sampled from five female donors (age 33-43 y) that underwent plastic surgery. The donors were given complete anonymity and only registered according to age, gender, date of sampling, skin region, and size of skin patch. The regional ethics committee was informed about the study and no permission was necessary as the skin is considered waste material. The patients had given informed consent prior to skin sampling. The skin patches were primarily from the abdominal region, but also back and breast patches were used. The skin samples were stored at -20 ºC for periods not exceeding twelve months, which has proven to keep the barrier properties of the skin and not significantly change the water permeability (Bronaugh et al., 1986). The skin was thawed at room temperature and trimmed of subcutaneous fat. Accordingly, full-thickness skin with a median thickness of 0.8 mm was used.

Dermal uptake was studied in Franz diffusion cells (Figure 1) as described in OECD guideline 428 (OECD, 2004). Our system has previously described for dermal uptake of pesticides (Nielsen et al., 2009) and pharmaceuticals (Holmgaard et al., 2013). The cells were kept in a water bath ensuring a skin surface temperature of approximately 32ºC with continuous individual magnetic stirring. The mean diffusion area was 2.64 cm^2/cell and the mean receptor chamber volume was 16.6 ml. Two types of receptor fluids were used: a physiological relevant receptor fluid (PHY) consisting of an aqueous solution of 0.9% NaCl, 5% bovine serum albumin, 40 mg/l hexamycin and Na_2HPO_4 buffer (to pH 7.4); and a worst-case receptor fluid (WOC) consisting of 50% ethanol in water, which is known to increase epidermal permeability significantly (Pelling et al., 1997). The worst case receptor fluid was used as an extra measure of penetration since low

penetration with the physiological receptor fluid was expected. If nothing was absorbed using the worst case receptor, dermal uptake would be considered not to be a significant pathway. After mounting of the skin, 5 ml isotonic saline was added to the donor chamber and left overnight for hydration of the skin. Before starting experiments, the skin integrity was checked by measuring the capacitance (Lutron DM-9023, Acer AB, Sweden), which should not exceed 100 nF. After ensuring the integrity of the skin, the isotonic saline was removed and the compounds (purchased from Cambridge Isotope Laboratories (HBCDD), purity ≥ 97% and Wellington Laboratories (remaining compounds), purity >98%) were added to the donor chamber. At the same time the same volume was spiked to laboratory vials (three replicates) to define initial conditions. The compounds were loaded in 500 µl ethanol (with 20% isooctane residue) at two levels of 10-100 and 50-300 ng/cell depending on LOQ of the individual compounds, aiming for positive detection and quantification of 1% transfer to the receptor fluid at the low level. The cells were covered with parafilm and set in the water bath for 72 h; the temperature was checked daily. At the end of the experiment, the residue in the donor chamber was collected by gently drying the skin using cotton swabs, then the skin and donor chamber was gently washed twice with hexane soaked cotton swabs, and finally the skin was wiped with dry cotton swabs, all swaps were collectively analyzed as remains in the donor chamber. Afterwards isotonic saline was again added and the capacitance measured to ensure continued barrier integrity. The cells were dismounted and the upper skin layers (epidermis) were separated from of the remaining skin fraction (dermis). Finally, the entire volume of receptor fluid was sampled, and the chamber was rinsed with approximately 1 ml of fresh receptor fluid.

Figure 1. Composition of Franz diffusion cells.



The samples were extracted by sonication, cleaned up on multilayer glass columns and analyzed by either GC-MS (ECNI) or LC-MS-MS using previously described methods (Vorkamp et al., 2011a; Vorkamp et al., 2015). Details on extraction, clean-up, instrumental analysis and detection limits are given in the Supplementary material.

2.2. QA/QC

For every five Franz cells one blank cell was included and only spiked with solvents; it was fractioned into the same constituents as the spiked cells and analyzed along with the respective compartments from the test cells. The analyzed FRs were rarely detected in the unspiked samples; if detected the levels were very low, but the LOQ was raised above the highest blank for the entire batch. In addition, a laboratory blank (Hydromatrix®) was included in each batch of samples, here low levels (<LOQ) of TBP-DBPE, BTBPE and DDC-CO were occasionally detected. Due to the lack of appropriate SRM material, at least one spiked sample of either dermis, epidermis or receptor fluid was used for quality control in each batch. On average, the measured amount in the spiked control samples was 109% of the applied amount. The average recovery of the analyses (using CB-198 as recovery standard) was 98%, ranging between 73-121%.

An adhesion test was made to test if some of the applied chemicals were quickly bound to the glass surface of the receptor chamber and therefore not available for dermal uptake during the experiment. Five cells were set up and spiked with 500 µl of the spike solution as described above, omitting only the receptor fluid; in addition one blank spiked with pure solvent was included. The cells were left for 60 min after which excess fluid was removed with cotton swaps, followed by washing of the donor cell glass with hexane soaked cotton swaps. Following the regular wash, the donor chambers were dismounted and rinsed three times with hexane:dichloromethane (1:1 v/v), which was collected. Internal standards were added and the adhesion samples were reconstituted and analyzed. On average, 16% of the applied dose stuck to the glass in a way that it would not be readily available for dermal uptake, approximately half of this would be sampled along with the remaining donor chamber during the regular wash; the variation between the compounds was relatively small (Table S2). On average, 70% of the applied dose was still available for uptake (in solution)

after 1 h, confirming that actual infinite dosing was present. Detailed results of the adhesion test are given in Table S2.

2.3. Calculations

The flux, *J*, was calculated as an average flux over the entire experimental period. Furthermore, both a lower- and upper-bound flux was calculated based on different assumptions of the fate of the FRs in the skin depot. The lower bound flux, J_{min}, only considers the absorbed fraction, here defined as the mass recovered in the receptor fluid as well as the deeper layers of the skin (dermis). The upper-bound flux, J_{max}, includes the absorbable dose, which is defined by OECD (OECD, 2004) as the mass of "*that present on or in the skin following washing*" i.e. in the receptor fluid and the entire skin depot (epidermis+dermis). However, the depot in the upper layers may either become systemically available with time or be excluded by desquamation, therefore, J_{max} may be an overestimate of the actual flux through the skin.

Lower- and upper-bound fluxes, *J*, were calculated using the following equations:

$$\text{(Eq. 1)}\quad J_{lower} = \frac{absorbed\ dose}{A \cdot t} = \frac{m_S + m_R}{A \cdot t}$$

where m_S and m_R are the mass recovered in deeper skin layers (dermis) and receptor fluid, respectively, *A* is the area for diffusion, and *t* is the exposure duration.

$$\text{(Eq. 2)}\quad J_{upper} = \frac{absorbable\ dose}{A \cdot t} = \frac{m_E + m_S + m_R}{A \cdot t}$$

where m_E is the mass recovered in the upper skin layers (epidermis).

However, since the flux is dependent on the concentration in the donor chamber the permeability coefficient k_p, as described by Niedorf et al. (2008) was calculated for better comparison between compounds applied in different concentrations. In our case it is a pseudo-k_p as average and not maximum flux is used. The lower- and upper-bound average fluxes resulted in lower- and upper-bound k_p values:

$$\text{(Eq. 3)}\quad k_{p,lower} = \frac{J_{lower}}{C}$$

$$\text{(Eq. 4)}\quad k_{p,upper} = \frac{J_{upper}}{C}$$

where C is the concentration in the donor chamber (as the concentration in the donor chamber far exceeds that of the receptor chamber).

Log K_{ow} at 32°C was calculated using the SPARC online calculator v.6.1. Linear regression and an F-test of the slope's deviation from zero was performed in GraphPad Prism 5.

3. Results

3.1. Mass distribution and recovery

The average mass recoveries for the analyzed FRs were between 74-95% of the applied dose, except for DBDPE which was 95% and 133% for the two types of receptor fluid. The majority of the recovered FRs was found to still be present in the donor chamber (including the wash of the skin) and on average exceeded 73% of the mass recovered for all compounds in both set-ups (Table 1). This is consistent with the intent of having infinite dosing in the experiment. When the physiological receptor fluid was used, the majority of the absorbable fraction of the FRs was found in the epidermis (7.9 –11% of applied) and only a smaller fraction in the dermis (0.5–1.6%). Using the worst-case receptor fluid resulted in increased deposition in the skin, particularly the transport trough the upper skin layers was increased, thus a larger fraction was generally found both in the epidermis (10–13%) and particularly in the dermis (1.1–14%) (Table 1). Despite the higher uptake still very little (or none) of the applied FR was recovered in the receptor fluid. This was not due to limited solubility, as the recovered concentrations (or LOQ in case of non-detects) were less than 5% of the soluble amount for all compounds in the WOC receptor fluid (Table S3). Due to the BSA present in PHY receptor fluid it was not possible to make a good estimate of the solubility, but it will exceed that of pure water (Table S3).

3.2. Permeability coefficients, k_p

The lower- and upper-bound k_ps for both types of receptor fluid are shown in Table 2. For the physiological receptor fluid, upper-bound k_p -values were generally one order of magnitude higher than the lower-bound estimates. In case of the worst-case receptor fluid the differences were smaller. Figure 2 shows the upper-bound k_p plotted against log K_{ow} at 32°C, a significant decreasing trend ($p<0.0001$) was observed for both

types of receptor fluid, indicating reduced dermal uptake with increasing log K_{ow} for these highly lipophilic compounds. The same trend was observed for the lower bound k_p-values of both receptor fluids, though with larger variation (Figure S1).

Table 1. Mean distribution of FRs in different compartments (percent of total detected amount) after 72h in Franz cells. Mass recovery was calculated relative to the applied dose. For receptor fluids the detection frequency is given in parentheses, for all other compartments the detection frequency was 100%. Values <LOQ were set to LOQ in calculations.

	Physiological receptor fluid (n=5)					**Worst-case receptor fluid (n=13)**				
	Donor chamber	**Epi-dermis**	**Dermis**	**Receptor fluid**	***Mass recovery***	**Donor chamber**	**Epi-dermis**	**Dermis**	**Receptor fluid**	***Mass recovery***
TBP-DBPE	87%	11%	1.6%	< 0.1% (0%)	*95%*	73%	13%	14%	0.2% (15%)	*87%*
EH-TBB	89%	10%	0.6%	0.2% (20%)	*89%*	83%	13%	4.3%	< 0.05% (0%)	*82%*
BTBPE	89%	10%	0.7%	0.1% (20%)	*89%*	84%	12%	3.6%	0.1% (8%)	*83%*
DBDPE	88%	10%	1.0%	< 0.5% (0%)	*133%*	84%	12%	3.2%	< 0.6% (0%)	*95%*
BEH-TEBP	90%	9.5%	0.5%	0.04% (40%)	*89%*	88%	11%	1.1%	0.03% (23%)	*81%*
***syn* DDC-CO**[a]	91%	8.4%	0.5%	< 0.1% (0%)	*87%*	85%	13%	2.2%	0.1% (29%)	*74%*
***anti* DDC-CO**[a]	91%	7.9%	0.8%	0.2% (33%)	*90%*	85%	12%	2.7%	0.2% (57%)	*74%*
α-HBCDD	88%	11%	1.0%	0.1% (40%)	*89%*	85%	12%	2.5%	0.1% (54%)	*79%*
β-HBCDD	88%	11%	0.8%	0.1% (40%)	*90%*	84%	12%	3.7%	0.03% (31%)	*79%*
γ-HBCDD	89%	10%	0.8%	0.1% (40%)	*86%*	87%	10%	2.8%	0.1% (15%)	*76%*

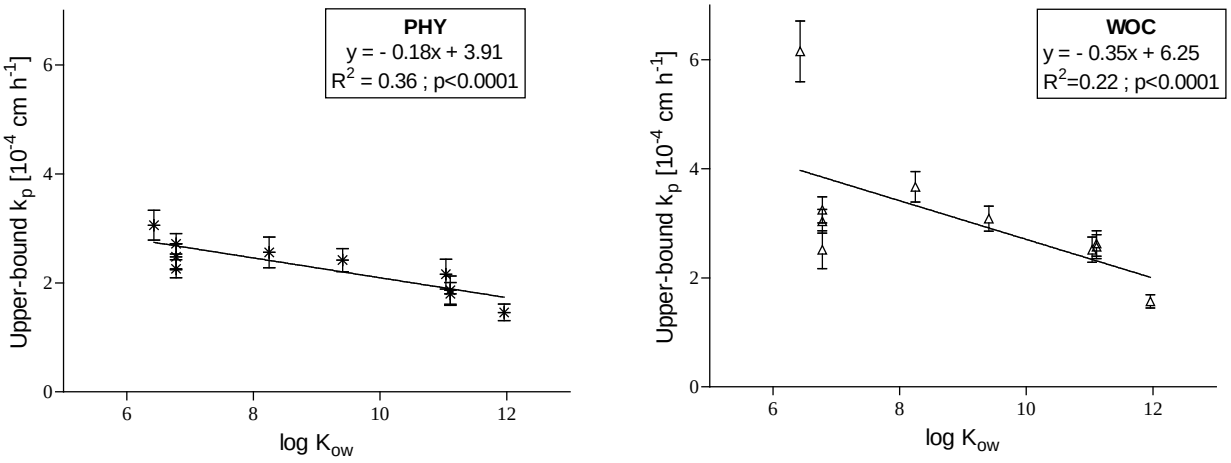
[a]For syn- and anti DDC-CO n=3 for physiological receptor fluid and n=7 for worst case receptor fluid.

Table 2. Lower and upper bound permeability coefficient, kp, for physiological (PHY) (n=5) and worst case (WOC) receptor fluid (n=13)

	Lower bound k_p(PHY) *[10^{-4} cm/h]*	Upper bound k_p (PHY) *[10^{-4} cm/h]*	Lower bound k_p(WOC) *[10^{-4} cm/h]*	Upper bound k_p(WOC) *[10^{-4} cm/h]*
TBP-DBPE	0.38	3.1	3.3	6.2
EH-TBB	0.16	2.5	0.92	3.7
BTBPE	0.16	2.4	0.71	3.1
DBDPE	0.11	2.2	0.42	2.5
BEH-TEBP	0.13	1.4	0.32	1.6
***syn* DDC-CO**[a]	0.11	1.9	0.39	2.6
***anti* DDC-CO**[a]	0.16	1.8	0.46	2.6
α-HBCDD	0.22	2.7	0.52	3.1
β-HBCDD	0.17	2.5	0.76	3.2
γ-HBCDD	0.15	2.3	0.55	2.5

[a]For syn- and anti DDC-CO n=3 for physiological receptor fluid and n=7 for worst case receptor fluid.

Figure 2. Log K_{ow} at 32°C and upper-bound k_p (mean ± SEM) for PHY receptor fluid (n=5) and WOC receptor fluid (n=13) (for DDC-COs n=3 and 7 for PHY and WOC, respectively).



4. Discussion

4.1. Mass distribution and recovery

We estimated skin deposition and permeability for ten organic flame retardants using an *ex-vivo* human skin model. Despite the low levels applied, the mass recovery was >74% and high consistency between cells was observed, proving the strengths of the model and analytical procedure for the purpose of this study.

In our experiments, there were little or no detectable levels of the target compounds in the receptor fluid, even following 72 h. Our results indicated that FRs were absorbed into the skin—about 10% for many compounds—with most of each compound in the epidermis and about an order of magnitude less in the dermis. The effect of the WOC receptor fluid was primarily that the compounds were deposited deeper in the skin (at 72 h) while percutaneous penetration did not increase markedly. This is in line with a study of another highly lipophilic compound (DEHP) on separated epidermis and dermis, finding that transport across the epidermis is greatly increased using 50% ethanol/water compared to a buffered saline receptor fluid while the effect on transport across the dermis is modest (Pelling et al., 1997). Stratum corneum is generally considered the main barrier for percutaneous penetration (Hadgraft, 2004), but for highly lipophilic compounds, like the FRs in the current study, the dermis and viable epidermis may provide a significant barrier and temporary site for deposition and subsequent systemic absorption due to their hydrophilic nature

(Nielsen et al., 2009). Similarly, Zhou et al. (2013) studied the percutaneous penetration di(2-ethylhexyl) adipate, which is structurally very different from the FRs but with similar log K_{ow} (8.1) and found the majority of the absorbable dose present in the skin depot and very little in the receptor fluid.

4.2. Permeability coefficients, k_p

The fraction absorbed may depend on experimental conditions such as skin loading and use of these numbers in risk assessments can be misleading (Kissel, 2011). The physical paradigm for the *ex vivo* skin experiments is that of gradient-driven diffusion across the skin. As a result, we are more interested in flux and the skin permeability coefficient than fraction absorbed. Traditionally, only the fraction in the receptor fluid would be considered when calculating k_p (Niedorf et al., 2008), but for highly lipophilic compounds like these FRs it may not be the most relevant measure, as the compounds may be temporarily retained either in the skin (Díez Sales et al., 1993) or in remaining subcutaneous fat and may therefore not reach the receptor fluid during the time span of the experiment. Slowly absorbed compounds such as our target compounds may be eliminated via desquamation or other mechanisms such as metabolism. Rather than representing steady state, our flux and k_p estimates are instead an average over the experimental time period and do not take the potential lag time into account, which is expected to be substantial for these compounds as lag time has been found to increase with molecular weight (MW) (Nielsen et al., 2009). The average flux into the dermis and receptor fluid will tend to underestimate the steady-state flux, while the average flux into the epidermis may overestimate the steady-state flux (Cleek and Bunge, 1993). Taking these factors into account, we believe that our estimates of skin permeability (computed from average flux) represent lower and upper bounds. However, the water-rich dermis will for the studied compounds provide a significant barrier, whereas *in vivo* blood will also perfuse the lower regions of the epidermis and transport the chemicals to the blood stream, therefore we believe the upper-bound k_p is closer to the true value of k_p than the lower-bound.

There are very few other data on *ex vivo* skin experiments for this type of compounds. Abdallah et al. (2015) reported k_p values of 2.16×10^{-4}, 1.47×10^{-4} and 1.37×10^{-4} cm/hr for α-, β- and γ-HBCDD, respectively, and found similar results using 3D-human skin equivalents. These are within a factor of two of our upper-bound estimates for the same compounds. Often it is difficult to compare absolute numbers from different

laboratories using different setups, but the rank order is expected to be the same (van de Sandt et al., 2004). Abdallah et al. (2015) were able to achieve steady state and computed their estimates based on mass in the receptor fluid. There may be other differences between the two experiments, for example, skin thickness or fat remaining in the skin, which may increase the retention of lipophilic compounds. Although we removed as much underlying fat as we could from our samples before mounting them in the diffusion cells, taking care not to damage the skin, traces of fat may remain. Abdallah et al. state that their skin samples were "full thickness without adipose tissue" with an average thickness of 550 μm compared to 800 μm in the current study. Despite these potential differences in experimental set-ups, the rank order of estimated permeability for the three HBCDDs is the same in both experiments.

Hughes et al. (2001) used mice skin to study the uptake of BDE-209, which has a very similar MW and log K_{ow} as well as structural resemblance to DBDPE tested in the current study. Hughes et al. found that the majority of the absorbable dose was recovered in the skin depot rather than in the receptor fluid after 24 h, which agrees with our findings for DBDPE.

Despite great structural differences of the FRs included in the current study a clear, significant decreasing trend of k_p with increasing log K_{ow} was observed. For the HBCDDs a single value of log K_{ow} (at 32°C) was computed using SPARC, however experimental data suggest that log K_{ow} increases in the order α-HBCDD < β-HBCDD < γ-HBCDD (Hayward et al., 2006). Thus the individual HBCDD isomers also fit the overall trend of decreasing k_p with increasing log K_{ow} (Table 2).

There are a number of models, both biologically and QSAR-based, for predicting skin permeability using the physical chemical properties of the compounds, as the DERMWIN module of the USEPA model EPIsuite (US EPA, 2004). The DERMWIN estimates are based on a modification of the classic Potts and Guy model (Potts and Guy, 1992), a QSAR model using log K_{ow} and MW, though the coefficients of the regression equation have been modified using additional chemical data (US EPA, 2004). There are several reasons not to use the DERMWIN permeability estimates in our case. The Potts and Guy model is designed to model permeability of the stratum corneum, but as discussed above viable epidermis and dermis may provide the

main barrier for highly lipophilic compounds. Thus, the log K_{ow} and MW for the compounds we studied are outside the "effective predictive range" of DERMWIN (US EPA, 2004). Instead, skin can be modeled as resistors in series (with permeability inversely related to resistance) (Díez Sales et al., 1993; Weschler and Nazaroff, 2012). The effect of the skin layers on penetration of highly lipophilic compounds is well illustrated by Diez-Sales et al. showing an optimum k_p at log K_{ow} of approximately 3.5 (Díez Sales et al., 1993). Similarly, Nielsen et al. (2009) found that above a log K_{ow} of 2 no further increase in k_p was observed for a number of pesticides. Another interesting approach to this problem is that of Bunge et al. (1995) who developed methods for estimating the ratio of the permeability in the stratum corneum to that of the viable epidermis. When this ratio (B) is less than about 0.1, the resistance of the stratum corneum dominates. Following the procedure in Weschler and Nazaroff (2012), we calculated values of B for the compounds in our data set: all were above 0.1, some much higher (data not shown). However, these estimates of B also depend on the estimate of the stratum corneum permeability. The latter was computed using the model of Mitragotri (2002), which is mechanistic rather than a QSAR (although its final mathematical form also relies on log K_{ow} and MW); it predicts stratum corneum permeability better than a number of alternatives (Lian et al., 2008). The physical assumptions of the Mitragotri model may break down for MW>400, which includes all of the compounds we studied. Additional research is needed on the applicability of models for predicting skin permeability of the types of compounds in our study.

4.3. Strengths and weaknesses

The current study did not investigate the kinetics of the dermal uptake due to the low levels found in the receptor fluid at termination. Kinetic information could only be obtained by sacrificing entire cells during the experiment, which we did not find feasible. Furthermore, as a consequence of using non-viable skin the effect of metabolism was not included. However, for these very persistent compounds, metabolism is expected to be of minor importance i.e. Abdallah et al. (2015) had a similar mass recovery of the parent HBCDDs using viable skin, indicating that metabolism is negligible. The strength of our study is the use of full-thickness human skin, which means that no extrapolation between species is necessary. In addition, the experimental design and protocol followed those of previous studies (Nielsen et al., 2004; 2009; 2010;

Nielsen and Nielsen, 2000) on skin uptake and penetration of organic chemicals, allowing comparisons between compounds of different physical-chemical properties. Furthermore, the ethical implications of using the skin, which is considered waste, are minimal. In the current study all calculations were done at the relevant temperature rather than the reference temperature.

4.4. Implications for dermal exposure

The current study indicates that the included FRs can be taken up via dermal absorption. Though little of the applied amount was recovered in the receptor fluid, the skin depot, which may eventually become available, was noteworthy. In the skin depot, the largest fraction was found in the upper skin layers – from there it can either penetrate deeper or be eliminated, for example via desquamation or hand washing. The relative importance of the two depends on the time scale on which they occur; in the current study no information on lag time was available, but in real life the exposure is likely to be continuous. Again, more data are available for PBDEs and pesticides. Watkins et al. (2011) showed that increased handwashing frequency is associated with lower serum levels – indicating that PBDEs to some extent can be washed off the skin. Likewise, experimental studies on four model compounds have demonstrated that skin cleansing within 6 h of exposure significantly reduce skin permeation (Nielsen, 2010). Thus, from a preventive point of view, more FRs with potential toxicity may need to be phased out or exposures should be reduced, but in the meantime secondary prevention based on increased hygienic precautions may help. The question how the compound application in the study relates to contact to these compounds in dust, products or air warrants further research.

5. Conclusion

Dermal uptake but little or no percutaneous penetration was observed for all the studied FRs. The permeation was found to decrease linearly with increasing log K_{ow} despite the structural diversity of the compounds. Continuous dermal exposure to FRs at the skin surface may lead to dermal uptake and possibly eventually percutaneous penetration though this would proceed in a time lagged manor.

6. Acknowledgements

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7. References

Abdallah, M.A., Pawar, G., Harrad, S., 2015. Evaluation of 3D-human skin equivalents for assessment of human dermal absorption of some brominated flame retardants. Environ. Int. 84, 64-70.

Bronaugh, R.L., Stewart, R.F., Simon, M., 1986. Methods for in vitro percutaneous absorption studies. VII: Use of excised human skin. J Pharm Sci. 75, 1094-1097.

Bunge, A.L., Cleek, R.L., Vecchia, B.E., 1995. A new method for estimating dermal absorption from chemical exposure. 3. Compared with steady-state methods for prediction and data analysis. Pharm. Res. 12, 972-982.

Carignan, C.C., Heiger-Bernays, W., McClean, M.D., Roberts, S.C., Stapleton, H.M., Sjödin, A., Webster, T.F., 2013. Flame retardant exposure among collegiate United States gymnasts. Environ Sci Technol. 47, 13848-13856.

Chu, I., Dick, D., Bronaugh, R., Tryphonas, L., 1996. Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. Food and Chemical Toxicology. 34, 267-276.

Cleek, R.L.´and Bunge, A.L., 1993. A new method for estimating dermal absorption from chemical exposure. 1. General approach. Pharm. Res. 10, 497-506.

Covaci, A., Harrad, S., Abdallah, M.A., Ali, N., Law, R.J., Herzke, D., de Wit, C.A., 2011. Novel brominated flame retardants: A review of their analysis, environmental fate and behaviour. Environ. Int. 37, 532-556.

de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere. 46, 583-624.

Deng, J., Liu, C., Yu, L., Zhou, B., 2010. Chronic exposure to environmental levels of tribromophenol impairs zebrafish reproduction. Toxicol. Appl. Pharmacol. 243, 87-95.

Díez Sales, O., Pérez Sayas, E., Martín Villodre, A., Herráez Domínguez, M., 1993. The prediction of percutaneous absorption: I. Influence of the dermis on in vitro permeation models. Int. J. Pharm. 100, 1-7.

Frederiksen, M., Thomsen, C., Frøshaug, M., Vorkamp, K., Thomsen, M., Becher, G., Knudsen, L.E., 2010. Polybrominated diphenyl ethers in paired samples of maternal and umbilical cord blood plasma and associations with house dust in a Danish cohort. Int. J. Hyg. Environ. Health. 213, 233-242.

Fromme, H., 2016. Brominated flame retardants – Exposure and risk assessment for the general population. Int. J. Hyg. Environ. Health. 219, 1-23.

Hadgraft, J., 2004. Skin deep. European journal of pharmaceutics and biopharmaceutics. 58, 291-291-299.

Hakk, H., Larsen, G., Bowers, J., 2004. Metabolism, tissue disposition, and excretion of 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) in male Sprague-Dawley rats. Chemosphere. 54, 1367-1374.

Hayward, S.J., Lei, Y.D., Wania, F., 2006. Comparative evaluation of three high-performance liquid chromatography-based Kow estimation methods for highly hydrophobic organic compounds: polybrominated diphenyl ethers and hexabromocyclododecane. Environ Toxicol Chem. 25, 2018-2027.

Hoh, E., Zhu, L.Y., Hites, R.A., 2006. Dechlorane Plus, a Chlorinated Flame Retardant, in the Great Lakes. Environ. Sci. Technol. 40, 1184-1189.

Hoh, E., Zhu, L.Y., Hites, R.A., 2005. Novel Flame Retardants, 1,2-Bis(2,4,6-tribromophenoxy)ethane and 2,3,4,5,6-Pentabromoethylbenzene, in United States' Environmental Samples. Environ. Sci. Technol. 39, 2472-2477.

Holmgaard, R., Benfeldt, E., Sørensen, J.A., Nielsen, J.B., 2013. Chronological Age affects the absorption of fentanyl through human skin in vitro. Skin Pharmacol Physiol. 26, 155-159.

Hughes, M.F., Edwards, B.C., Mitchell, C.T., Bhooshan, B., 2001. In vitro dermal absorption of flame retardant chemicals. Food and Chemical Toxicology. 39, 1263-1270.

Kierkegaard, A., Björklund, J., Fridén, U., 2004. Identification of the Flame Retardant Decabromodiphenyl Ethane in the Environment. Environ. Sci. Technol. 38, 3247-3253.

Kissel, J.C., 2011. The mismeasure of dermal absorption. J Expo Sci Environ Epidemiol. 21, 302-309.

Lian, G., Chen, L., Han, L., 2008. An Evaluation of Mathematical Models for Predicting Skin Permeability. J Pharma. Sci. 97, 584-598.

Linares, V., Bellés, M., Domingo, J.L., 2015. Human exposure to PBDE and critical evaluation of health hazards. Arch. Toxicol. 89, 335-356.

Lorber, M., 2008. Exposure of Americans to polybrominated diphenyl ethers. J Expo Sci Environ Epidemiol. 18, 2-19.

Lyche, J.L., Rosseland, C., Berge, G., Polder, A., 2015. Human health risk associated with brominated flame retardants (BFRs). Environ. Int. 74, 170-180.

Mitragotri, S., 2002. A theoretical analysis of permeation of small hydrophobic solutes across the stratum corneum based on Scaled Particle Theory. J. Pharm. Sci. 91, 744-752.

Niedorf, F., Schmidt, E., Kietzmann, M., 2008. The automated, accurate and reproducible determination of steady-state permeation parameters from percutaneous permeation data. ATLA. Alterna. Lab. Anim. 36, 201-213.

Nielsen, J.B., 2010. Efficacy of skin wash on dermal absorption: an in vitro study on four model compounds of varying solubility. Int Arch Occup Environ Health. 83, 683-690.

Nielsen, J.B., Nielsen, F., Sørensen, J.A., 2004. *In Vitro* Percutaneous Penetration of Five Pesticides—Effects of Molecular Weight and Solubility Characteristics. Ann Occup Hyg. 48, 697-705.

Nielsen, J.B., Sørensen, J.A., Nielsen, F., 2009. The Usual Suspects—Influence of Physicochemical Properties on Lag Time, Skin Deposition, and Percutaneous Penetration of Nine Model Compounds. J Toxicol Environ Health A. 72, 315-323.

Nielsen, J.B.´and Nielsen, F., 2000. Dermal in vitro penetration of methiocarb, paclobutrazol, and pirimicarb. Occup. Environ. Med. 57, 734-737.

OECD, 2004. Test Guideline 428: Skin Absorption: *in vitro* Method. Organisation for Economic Co-operation and Development .

Pelling, D., Phillips, J.C., Cunninghame, M.E., 1997. Absorption of hydrophilic and lipophilic compounds through epidermal and subepidermal strata of rat skin in vitro. Toxicology in Vitro. 12, 47-55.

Potts, R.O.´and Guy, R.H., 1992. Predicting skin permeability. Pharm. Res. 9, 663-669.

Saunders, D.M.V., Higley, E.B., Hecker, M., Mankidy, R., Giesy, J.P., 2013. In vitro endocrine disruption and TCDD-like effects of three novel brominated flame retardants: TBPH, TBB, & TBCO. Toxicol. Lett. 223, 252-259.

Springer, C., Dere, E., Hall, S.J., McDonnell, E.V., Roberts, S.C., Butt, C.M., Stapleton, H.M., Watkins, D.J., McClean, M.D., Webster, T.F., Schlezinger, J.J., Boekelheide, K., 2012. Rodent thyroid, liver, and fetal testis toxicity of the monoester metabolite of bis-(2-ethylhexyl)tetrabromophthalate (TBPH), a novel brominated flame retardant present in indoor dust. Environ Health Perspect. 120, 1711-1719.

Stapleton, H.M., Allen, J.G., Kelly, S.M., Konstantinov, A., Klosterhaus, S., Watkins, D., McClean, M.D., Webster, T.F., 2008. Alternate and new brominated flame retardants detected in U.S. house dust. Environ. Sci. Technol. 42, 6910-6916.

Trudel, D., Scheringer, M., von Goetz, N., Hungerbühler, K., 2011. Total Consumer Exposure to Polybrominated Diphenyl Ethers in North America and Europe. Environ. Sci. Technol. 45, 2391-2391-2397.

UNEP, 2013. Report of the Persistent Organic Pollutants Review Committee on the work of its ninth meeting. Rome, 14-18 October 2013. UNEP/POPS/POPRC.9/13.

United Nations, Stockholm Convention on Persistent Organic Pollutants, Adoption of Amendments to annexes A, B and C, Reference C.N.524.2009.TREATIES-4 (Depositary Notification). 2009.

US EPA, 2004. Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment. EPA/540/R/99/005, Appendix A.

van de Sandt, J.J.M., van Burgsteden, J.A., Cage, S., Carmichael, P.L., Dick, I., Kenyon, S., Korinth, G., Larese, F., Limasset, J.C., Maas, W.J.M., Montomoli, L., Nielsen, J.B., Payan, J.-., Robinson, E., Sartorelli, P., Schaller, K.H., Wilkinson, S.C., Williams, F.M., 2004. In vitro predictions of skin absorption of caffeine, testosterone, and benzoic acid: a multi-centre comparison study. Regul Toxicol Pharma. 39, 271-281.

Vetter, W., von der Recke, R., Ostrowicz, P., Rosenfelder, N., 2010. Liquid chromatographic enantioseparation of the brominated flame retardant 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE) and enantiomer fractions in seal blubber. Chemosphere. 78, 134-138.

Vorkamp, K., Bossi, R., Rigét, F.F., Dietz, R., 2011a. Temporal trends of hexabromocyclododecane, polybrominated diphenyl ethers and polychlorinated biphenyls in ringed seals from East Greenland. Environ Sci Technol. 45, 1243-1249.

Vorkamp, K., Bossi, R., Rigét, F.F., Skov, H., Sonne, C., Dietz, R., 2015. Novel brominated flame retardants and dechlorane plus in Greenland air and biota. Environmental Pollution. 196, 284-291.

Vorkamp, K., Thomsen, M., Frederiksen, M., Pedersen, M., Knudsen, L.E., 2011b. Polybrominated diphenyl ethers (PBDEs) in the indoor environment and associations with prenatal exposure. Environ. Int. 37, 1-10.

Watkins, D.J., McClean, M.D., Fraser, A.J., Weinberg, J., Stapleton, H.M., Sjödin, A., Webster, T.F., 2011. Exposure to PBDEs in the office environment: evaluating the relationships between dust, handwipes, and serum. Environ. Health Perspect. 119, 1247-1252.

Weschler, C.J.´and Nazaroff, W.W., 2012. SVOC exposure indoors: Fresh look at dermal pathways. Indoor Air. 22, 356-377.

Weschler, C.J.´and Nazaroff, W.W., 2014. Dermal Uptake of Organic Vapors Commonly Found in Indoor Air. Environ Sci Technol. 48, 1230-1237.

WHO, 2006. Dermal Absorption. Envirnmental Health Criteria 235.

Wu, N., Herrmann, T., Paepke, O., Tickner, J., Hale, R., Harvey, E., La Guardia, M., McClean, M.D., Webster, T.F., 2007. Human Exposure to PBDEs: Associations of PBDE Body Burdens with Food Consumption and House Dust Concentrations. Environ Sci Technol. 41, 1584-1589.

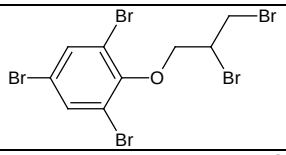
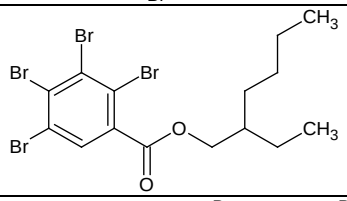
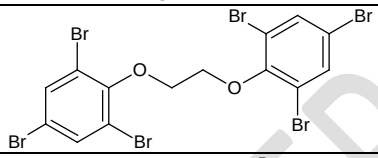
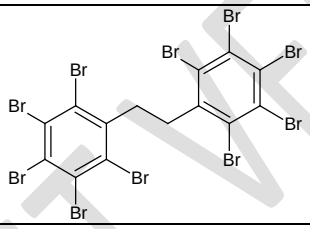
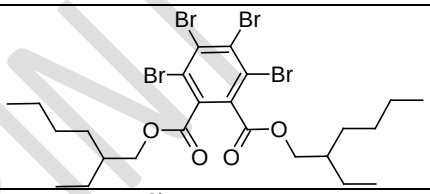
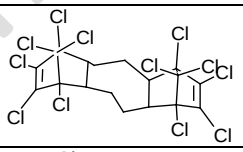
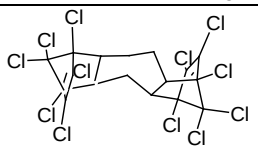
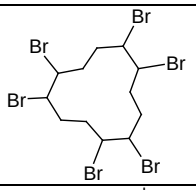
Zhou, S.N., Moody, R.P., Aikawa, B., Yip, A., Wang, B., Zhu, J., 2013. In vitro dermal absorption of Di(2-ethylhexyl) adipate (DEHA) in a roll-on deodorant using human skin. J. Toxicol. Environ. Health Part A Curr. Iss. 76, 157-166.

Supplementary Material

Extraction, clean-up and analysis of FRs

The samples were analysed in the following way: Donor chamber (D), epidermis (E) and dermis (S)-samples were extracted by sonication for 30min with 10ml hexane:dichloromethane (1:1) two times using fresh solvent. Extracts from donor and epidermis samples were evaporated and cleaned up on a glass column packed with 2 g Al_2O_3 (10% H_2O), 2 g silica and Na_2SO_4 and eluted with 60 ml hexane: dichloromethane (1:1). However, some dermis samples contained lipid residues and required better clean-up, on the other hand BEH-TEBP as is not stable in strong acid, therefore in a subset of samples we chose to divide the extracts for two different types of clean-up. 50% of the extract was cleaned up using the column described above and analysed for BEH-TEBP using ^{13}C-BEH-TEBP as internal standard. The other 50% of the extract were cleaned up in a column packed with 5 g Al_2O_3 (10% H_2O), 5 g silica impregnated with 40% sulphuric acid, 2 g silica topped with 1 cm anhydrous Na_2SO_4 and eluted with 250ml hexane: dichloromethane (1:1), which is identical to the H_2SO_4 containing column clean-up previously used for NBFRs in biota (Vorkamp et al., 2015). The receptor fluid was dried with Hydromatrix® and extracted using Soxhlet extraction as described for PBDEs (Vorkamp et al., 2004a; 2004b), followed by the simple column clean-up described above. After clean-up, the extracts were divided for GC-MS and LC-MS-MS analysis in the following way: 50% was evaporated to dryness and reconstituted in 25 0 µl methanol; the other half was evaporated to dryness in silicone coated vials (Vorkamp et al., 2014) and reconstituted in 200 µl isooctane. D-samples were also analysed in dilution. DBDPE, BTBPE, TBP-DBPE, EH-TBB, BEH-TEBP as well as syn- and anti-DDC-CO were analysed by GC-MS (ECNI) while HBCDDs were analysed by LC-MS-MS as previously described (Vorkamp et al., 2015). The LOQs of the method are given in Table S4.

Table S1. Structure, CAS number, molecular weight (MW) and calculated log K_{ow} at 32°C for the flame retardants included in the study.

	CAS number	Structure	MW (g/mol)	Log Kow at 32°C[a]
TBP-DBPE	35109-60-5		530.7	6.43
EH-TBB	183658-27-7		549.9	8.25
BTBPE	37853-59-1		687.6	9.41
DBDPE	84852-53-9		971.2	11.96
BEH-TEBP	26040-51-7		706.21	11.04
***syn*-DDC-CO**	13560-89-9		653.7	11.11[b]
***anti*-DDC-CO**				
α-HBCDD	134237-50-6		641.7	6.78[b]
β-HBCDD	134237-51-7			
γ-HBCDD	134237-52-8			

[a]Log K_{OW} calculated using the SPARC online calculator v.6.1 [b]Calculations in SPARC do not distinguish between isomers.

Table S2. Detailed results of adhesion test. Mean (± standard deviation) as percent of applied dose (n=5).

	Available in solution	Washable from glass surfaces	Remains adhered on glass after washing	Mass recovery (excl. skin depot)
TBP-DBPE	73% ± 3%	8% ± 1%	7% ± 2%	88% ± 3%
EH-TBB	70% ± 3%	10% ± 1%	7% ± 2%	87% ± 5%
BTBPE	67% ± 2%	8% ± 1%	7% ± 2%	82% ± 2%
DBDPE	65% ± 19%	11% ± 11%	9% ± 8%	85% ± 31%
BEH-TEBP	73% ± 5%	10% ± 2%	7% ± 2%	90% ± 8%
***syn* DDC-CO**	65% ± 2%	8% ± 2%	6% ± 2%	79% ± 1%
***anti* DDC-CO**	65% ± 2%	8% ± 2%	6% ± 2%	79% ± 2%
α-HBCDD	73% ± 3%	8% ± 3%	10% ± 2%	91% ± 5%
β-HBCDD	73% ± 2%	7% ± 2%	9% ± 2%	89% ± 3%
γ-HBCDD	76% ± 5%	7% ± 2%	10% ± 2%	93% ± 5%

Table S3. Estimated solubility of FRs in water and WOC receptor fluid (water:ethanol, 1:1 vol) at 32C (SPARC online calculator v.6.1)

	Solubility in water at 32C (mg/L)	Solubility in WOC at 32C (mg/L)	Measured concentration relative to solubility (WOC)[a]
TBP-DBPE	8.1E-03	7.8E-01	0.001%
EH-TBB	5.4E-05	4.1E-02	0.01%
BTBPE	7.1E-07	5.8E-04	1%
DBDPE	2.5E-11	2.8E-04	5%
BEH-TEBP	1.1E-08	8.4E-05	5%
***syn* DDC-CO**	1.7E-07	1.2E-03	0.3%
***anti* DDC-CO**			
α-HBCDD	7.7E-04	2.0E-01	0.001%
β-HBCDD			
γ-HBCDD			

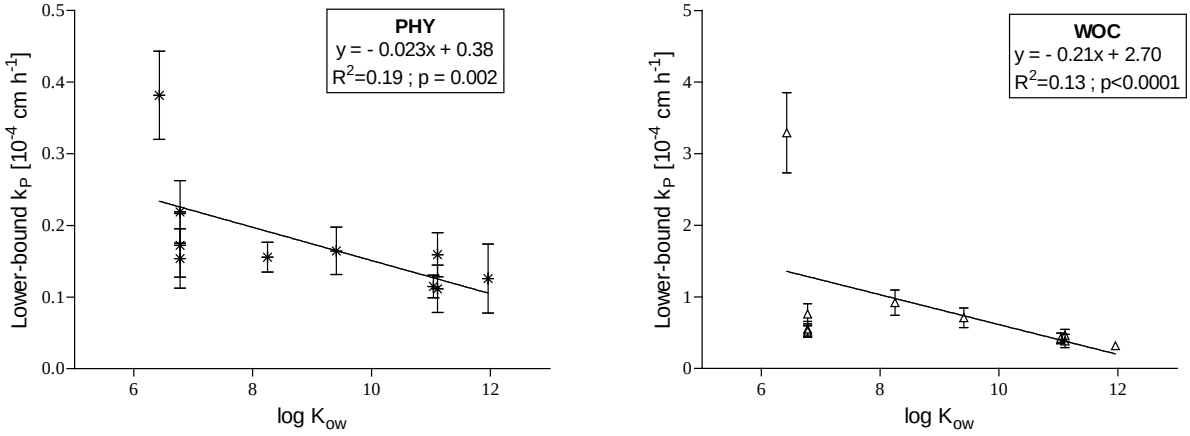
[a]<LOQ was replaced with LOQ

Table S4. Limit of quantification (LOQ) of FRs

	General LOQ (ng/sample)	Corresponding LOQ of receptor fluid (mg/L)[a]
TBP-DBPE	0.1[b]	6,0E-06
EH-TBB	0.1	6,0E-06
BTBPE	0.25	1,5E-05
DBDPE	0.25	1,5E-05
BEH-TEBP	0.1	6,0E-06
***syn* DDC-CO**	0.2[b]	1,5E-05
***anti* DDC-CO**	0.2[b]	1,5E-05
α-HBCDD	0.1	6,0E-06
β-HBCDD	0.1	6,0E-06
γ-HBCDD	0.1	6,0E-06

[a]using average receptor chamber volume [b]elevated above the highest blank.

Figure S1. Log K_{ow} at 32°C and lower-bound k_p (mean ± SEM) for PHY receptor fluid (n=5) and WOC receptor fluid (n=13) (for DDC-COs n=3 and 7, respectively).



References

Vorkamp, K., Christensen, J.H., Rigét, F.F., 2004a. Polybrominated diphenyl ethers and organochlorine compounds in biota from the marine environment of East Greenland. Sci. Total Environ. 331, 143-155.

Vorkamp, K., Bossi, R., Rigét, F.F., Skov, H., Sonne, C., Dietz, R., 2015. Novel brominated flame retardants and dechlorane plus in Greenland air and biota. Environmental Pollution. 196, 284-291.

Vorkamp, K., Christensen, J.H., Glasius, M., Riget, F.F., 2004b. Persistent halogenated compounds in black guillemots (Cepphus grylle) from Greenland—levels, compound patterns and spatial trends. Mar. Pollut. Bull. 48, 111-121.

Vorkamp, K., Nielsen, F., Kyhl, H.B., Husby, S., Nielsen, L.B., Barington, T., Andersson, A., Jensen, T.K., 2014. Polybrominated Diphenyl Ethers and Perfluoroalkyl Substances in Serum of Pregnant Women: Levels, Correlations, and Potential Health Implications. Arch. Environ. Contam. Toxicol. 67, 9-9-20.