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DERMAL UPTAKE OF NOVEL BROMINATED FLAME RETARDANTS (NBFRs) AND HBCD USING AN *EX VIVO* HUMAN SKIN MODEL

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Introduction

Since the ban of most polybrominated diphenyl ethers (PBDEs) the production pattern of flame retardants has changed and alternatives are increasingly being used. These are generally described as novel brominated flame retardants (NBFRs) and include e.g. EH-TBB and BEH-TEBP in Firemaster 550®, which is one of the PentaBDE replacement products. However, little is known about exposure pathways, not least dermal absorption, for NBFRs and other POPs. Several studies have shown that for PBDEs dust is a significant exposure pathway[1,2], similar can be expected for NBFRs. Positive correlations between PBDEs in dust, serum and handwipes have been reported[3], but whether it is due to hand-to-mouth behavior or dermal absorption is unknown. Similarly, a positive correlation of EHTBB and BEH-TEBP in dust and hand-wipes of children was found[4].
The aim of the current study was to estimate the extent of dermal transport of NBFRs and determine the rate of the transport, which can be used in exposure scenarios. This is done using an *ex vivo* human skin model.

Materials and methods

Franz' diffusion cells were used to study the percutaneous penetration of NBFRs. The system has previously been used for pesticide evaluation[5] and consists of two half-cells as shown in Figure 1. A full thickness human skin sample dividing the two cells was mounted horizontally on a metal grid on the receptor chamber. A clamp kept the two half-cells together and held the skin in place at the same time. The cells were kept in a water bath at 34-38ºC ensuring a skin surface temperature close to 32ºC. The mean diffusion area was 2.64 cm^2/cell and the mean receptor chamber volume 16.6 ml. Human skin was sampled from three female donors (age 38-41y) that underwent plastic surgery. The donors were given complete anonymity and only registered according to age, gender, date of operation, skin region, and size of skin patch. Skin samples were kept at -20 ºC for periods not exceeding nine months. This has proven to keep the barrier properties of the skin and no significant change in the water permeability[6]. The skin was allowed to thaw at room temperature before removal of subcutaneous fat and mounting in the diffusion cells. Full-thickness skin with an average thickness of 0.4 - 0.8 mm was used. 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 (pH 7.4); and a worst-case receptor fluid (WOC) consisting of 50% ethanol in water, which is known to increase skin permeability significantly[7]. After mounting, 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 55 nF. After ensuring the integrity of the skin, the saline was removed and the NBFR were added to the donor chamber in 500 µl ethanol (with 20% isooctane residue). The cells were covered with parafilm and left in the waterbath with individual magnetic stirring for 72 hr.

The experiment was terminated, and the residue in the donor chamber was collected by gently drying the skin using cotton swabs, followed by a gentle wash of skin and donor chamber with hexane soaked cotton swabs, and then again gentle wiping of the skin with dry cotton swabs. Afterwards saline was again added and the capacitance measured once again, it should not exceed 100 nF. The saline was discarded and the cells were dismounted. The

entire volume of receptor fluid was sampled, and the chamber was rinsed with approximately 1 ml of fresh receptor fluid. The epidermis was scraped off the skin using a surgery knife, and of the remaining dermis-fraction the exposed part was separated from the surrounding tissue using scissors.

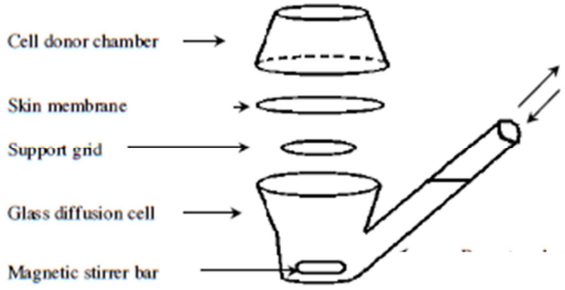

Figure 1. Schematic of the experimental setup. Modified from OECD test guideline 428[8].

The samples were analysed for decabromodiphenyl ethane (DBDPE), 1,2-bis(4,2,4-tribromophenoxy) ethane (BTBPE), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), 2-ethylhehexyl-2,3,4,5-tetrabromobenzoate (EH-TBB also known as TBB), bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (BEH-TEBP also known as TBPH) as well as α, β and γ-HBCD in the following way. Donor chamber (D), epidermis (E) and dermis (H)-samples were extracted using ultrasonication with 10ml hexane:dichloromethane (1:1) two times for 30min. The extracts were evaporated and cleaned up on a glass column packed with 2g Al_2O_3 (10% H_2O), 2g silica and Na_2SO_4 and eluted with 60ml hexane: dichloromethane (1:1). However, some dermis samples contained lipid residues and required further clean-up. This followed the H_2SO_4 containing column clean-up previously used for NBFRs in biota[9], with the exception that the alternative gel permeation chromatography clean-up was not applied and therefore, BEH-TEBP was lost. The receptor fluid was extracted using Soxhlet extraction as described for PBDEs[10], followed by the simple column clean-up described above. DBDPE, BTBPE, DPTE, EH-TBB and BEH-TEBP were analysed by GC-MS (ECNI) while HBCDs were analysed by LC-MS-MS.
The distribution in the different compartments was calculated as the measured mass in the compartment relative to the total mass measured in the cell, the average of n cells is given in Table 1 and 2. The total absorbable is the sum of E, H, and R relative to the total mass in each cell. The mass recovery was calculated as the total mass measured in each cell relative to "archive spikes" (triplicates), which were merely 1 ml flasks spiked with the test solution at the same time as the cells, internal standards were added and they were analysed along with the samples. The flux was calculated for the individual cells as:

$$Time\ averaged\ flux\ [\frac{ng}{cm^2\ hr}] = \frac{Total\ absorbable\ [ng]}{Area\ of\ cell\ [cm^2] \times duration\ of\ experiment\ [hr]}$$

Since the flux in depending on concentration a compound specific a pseudo permeability coefficient, $K_{p,pse}$, which is based on the time averaged flux, was calculated as:

$$K_{p,pse}[\frac{cm}{hr}] = \frac{Time\ average\ flux\ [ng/cm^2\ hr]}{Concentration\ in\ test\ solution\ [ng/cm^3]}$$

Results and discussion
The preliminary results of the distribution of NBFRs and HBCDs between the compartments for physiological and worst-case receptor fluids are shown in Table 1 and 2, respectively. The overall mass recoveries in the experiments were close to 100% (±20%). The majority of the added amount was recovered in the donor chamber (76-92%) after 72 hr in both experiments, and only very little or nothing was found in the receptor fluid. Within the skin, the majority was found in the epidermis and only a smaller fraction in dermis. However, when using the worst-case receptor fluid a larger fraction seem to reach dermis, at least for the smaller compounds like DPTE (Table 2). Based on the fractional absorption the observed difference between the compounds is relatively small but with

DPTE having the highest fraction absorbed. However, fractional absorption can depend on the applied dose (if the transport is flux-limited), resulting in larger fractional absorption at lower doses[11]; thus with the different doses of the compounds it can be misleading to compare in this way (Table 1 and 2). However, the flux is depending on concentration in the test solution. Therefore, the compound-specific pseudo permeability coefficient, $K_{p,pse}$ is more convenient for comparing compounds (Table 3 and Figure 2).

Table 1. Average distribution of NBFRs and HBCDs between compartments in skin penetration experiments using a physiological receptor fluid (PHY) (n=2).

	Load (ng/cm^2)	**Receptor**	**Dermis**	**Epi-dermis**	**Donor**	**Total absorbable**	**Mass recovery**	**Flux$_{72\ hr}$** ($ng\ cm^{-2}\ hr^{-1}$)
DPTE	3.79	0.52%	1.5%	11%	87%	15%	111%	0.0066
EHTBB	15.0	0.24%	0.8%	11%	88%	11%	88%	0.0224
BTBPE	14.9	0.10%	0.8%	11%	89%	11%	94%	0.0212
BEH-TEBP	37.2	0.08%	0.6%	12%	88%	12%	98%	0.0500
DBDPE	18.6	0.00%	0.4%	7%	92%	9%	116%	0.0154
α-HBCD	4.46	0.15%	1.5%	12%	87%	13%	97%	0.0068
β-HBCD	4.46	0.17%	1.3%	12%	86%	13%	96%	0.0066
ϒ-HBCD	4.42	0.13%	1.3%	11%	87%	11%	88%	0.0058

Table 2. Average distribution of NBFRs between compartments in skin penetration experiment using a "worst-case" (WOC) receptor fluid of 50% ethanol in water (n=6). Analysis of HBCDs in progress.

	Load (ng/cm^2)	**Receptor**	**Dermis**	**Epi-dermis**	**Donor**	**Total absorbable**	**Mass recovery**	**Flux$_{72\ hr}$** ($ng\ cm^{-2}\ hr^{-1}$)
DPTE	18.6	0.29%	11%	12%	76%	23%	97%	0.056
EHTBB	44.7	0%	3%	11%	85%	13%	91%	0.074
BTBPE	44.6	0.05%	3%	10%	88%	11%	94%	0.064
BEH-TEBP	112	0%	n.a.	9%	91%	$\geq$ 7%	86%	$\geq$ 0.122
DBDPE	55.7	0%	2%	11%	87%	11%	83%	0.045

n.a. Not available (see Materials and methods), if estimated from the mass recovery this fraction can be up to 14%. As a result, total absorbable and flux may be higher.

While DPTE was observed to have the highest fractional absorption it had one of the lowest fluxes in the experiment and the largest flux was observed for BEH-TEBP followed by DBDPE, which had the lowest fractional absorptions. When comparing $K_{p,pse}$, DPTE had the highest value, indicating faster transport.

The correlation between log K_{ow} (Table 3) and $K_{p,pse}$ is shown in Figure 2. For both types of receptor fluid, the rate of permeation is clearly decreasing with increasing log K_{ow}. The effect of the worst case receptor fluid seems only to have an effect on the compounds with the lowest log K_{ow} (DPTE).

Table 3. Physical-chemical properties and pseudo permeability coefficients $K_{p,pse}$ of NBFRs.

	MW (g/mol)	**log K_{ow}** [12]	**$K_{p,pse}$ (PHY)** (µm/h)	**$K_{p,pse}$ (WOC)** (µm/h)
DPTE	530.7	5.8	3.3	5.7
EHTBB	549.9	7.7	2.8	3.1
BTBPE	687.6	8.3	2.5	2.1
BEH-TEBP	706.2	9.3	2.7	2.7
DBDPE	971.2	11.1	1.6	1.5
α-HBCD	641.7	7.9	2.9	n.a.
β-HBCD	641.7	7.9	2.8	n.a.
ϒ-HBCD	641.7	7.9	2.5	n.a.

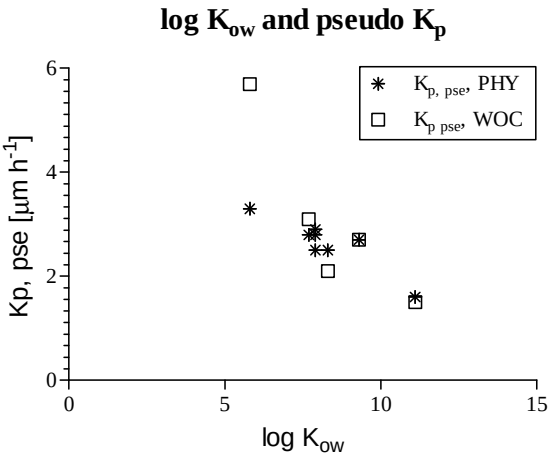

Figure 2. Pseudo permeability coefficient, $K_{p,pse}$ as a function of log K_{ow}.

Only a few other studies on dermal uptake of BFRs have been published. Hughes et al (2001)[13] measured dermal absorption of BDE-209 in mouse skin mounted in flow-through cells, and found that the fractional absorption depended on the dose. Pawar et al (2014)[14] used reconstructed epidermis to estimate the dermal permeability of EHTBB and BEH-TEBP. In agreement with our study, they did not find BEH-TEBP in the receptor fluid within the duration of the experiment in spite of doses up to 200 times higher than in the present study.

The human *ex vivo* skin model can be used to estimate dermal uptake of NBFRs. We have shown that for lipophilic compounds like NBFRs the skin depot is more important than the transfer to the receptor fluid. The skin depot has the potential for delayed systemic uptake *in vivo*. One of the advantages of the model is that the skin is stable for longer periods allowing experiments to run up to 72 hr, which is important for heavy, lipophilic compounds with long lag times.

Acknowledgements
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