Cod: 4.4010

HUMAN DERMAL ABSORPTION OF CHLORINATED ORGANOPHOSPHATE FLAME RETARDANTS; IMPLICATIONS FOR HUMAN EXPOSURE

S. Harrad[1], G. Pawar[1], M.A. Abdallah[1]

[1]*School of Geography, Earth & Environmental Sciences, University of Birmingham*

Introduction

Chlorinated PFRs include tris-2-chloroethyl phosphate (TCEP), tris (1-chloro-2-propyl) phosphate (TCIPP) and tris-1,3-dichloropropyl phosphate(TDCIPP). They are used as alternatives to the banned penta-BDE mixture for flame-retarding flexible and rigid polyurethane foams (PUFs) deployed in furniture, car upholstery and related products.[1] In addition, they are also used as plasticizers in various products including lacquer, paint and glue.[2] Several studies have reported on levels of PFRs in various biotic and abiotic matrices indicating the ubiquitous nature of these contaminants.[1] Current understanding of the toxicological properties of PFRs is not complete. Few studies have reported on various toxic effects of TDCIPP including immunotoxicity and disturbance of lipid metabolism in chicken embryos [3], as well as Neurodevelopmental defects in embryonic zebrafish [4]. TDCIPP was also reported to cause reduced thyroid hormone levels in humans.[5] Furthermore, TCEP is classified by the EU as a "potential human carcinogen" (carcinogen category 3), while TDCIPP is classified under regulation EC 1272/2008 as a category 2 carcinogen with hazard statement H351 "suspected of causing cancer".[6] While recent studies have provided estimates of external human exposure to PFRs via inhalation [7,] ingestion of indoor dust [8] and diet [9], very little is known about the relative contribution of different exposure pathways to the overall human body burdens of these contaminants. Moreover, there is #to date# no available information on human uptake of PFRs following dermal contact, which can hinder the accurate risk assessment of this class of emerging contaminants. To address this research gap, the aims of the current study are: (a) to investigate the human dermal absorption of TCEP, TCIPP and TDCIPP using two in vitro dermal models, namely human ex vivo skin and EPISKIN™ human skin equivalent, (b) to study the effect of hand washing on the dermal absorption of the studied PFRs and (c) to provide a preliminary assessment of adult and toddler exposure to the target PFRs via dermal contact with indoor dust.

Materials and Methods

Test matrices

Human skin: Freshly excised, healthy human upper breast skin was obtained via Caltag Medsystems Ltd. (Buckingham, UK) from three consented female adults (aged 35, 37 and 34 years) following plastic surgery. Upon receipt, the ex vivo skin samples were equilibrated for 1 h with 3 mL of DMEM (Dulbecco's Modified Eagle's Medium)-based culture medium at 5% CO_2 and 37 °C before use in permeation experiments.

EPISKIN™: The EPISKIN™ RHE/L/13 human skin equivalent kit was purchased from SkinEthic Laboratories (Lyon, France).The kit includes maintenance medium (MM) that allows acceptable differentiated morphology of the tissue for ~ 5 days upon receipt by end users (Figure 1). Upon receipt, the EPISKIN™ tissues were equilibrated overnight with their MM at 5% CO_2 and 37 °C before use in the permeation experiments.

Dosing Solutions: Two different concentration levels of (I) 50 ng/µL and (II) 10 ng/µL of each of TCEP, TCIPP and TDCIPP were prepared in acetone by serial dilution. Based on the exposed surface area, a net dose of 500 ng/cm^2 and 1000 ng/cm^2 was applied to each of the investigated skin tissues using 10 µL/ cm^2 (finite dose application) of dosing solutions I and 100 µL/cm2 (infinite dose application) of dosing solution II, respectively.

Dosing experiments: Experiments were performed in triplicate according to a standardized protocol (Figure 1).[10]

To investigate the potential effect of hand washing on the dermal absorption of PFRs, a separate strand of experiments were performed in triplicate. In these, human ex vivo skin exposed to 500 ng/cm2 of target

PFRs (dosing solution I, finite dose application) was washed after 6 h of exposure, while monitoring the absorbed dose continued until 24 hours. The washing procedure involved wiping the skin surface gently (5 times) with cotton buds presoaked in a detergent solution (5% neutral hand soap in isotonic water, pH = 7.2 ± 0.1).

Chemical analysis

Target compounds were spiked with isotopically labelled internal standard, extracted using ethyl acetate:hexane (1:1 v/v) mixture according to a QUECHERS-based method using successive steps of vortex-mixing, ultra-sonication and centrifugation. Target compounds were quantified using GC-EI/MS.[8,11]

Data analysis and statistical methods

A quantitative description of test compound permeation through the skin barrier is obtained from Fick's first law of diffusion as follows.[12]

$$J_{ss}=\Delta m/(\Delta t.A)= (D.K.\Delta C)/\Delta x \quad (1)$$

Where J_{ss} = steady-state flux [ng/cm^2.h]; Δm = permeated mass [ng]; Δt = time interval [h]; D = diffusion coefficient [cm^2/h]; K = partition coefficient; A = area [cm2]; Δc = concentration difference [ng/cm^3]; Δx: thickness of membrane [cm].

When using infinite-dose configurations, ΔC can be replaced by the known donor concentration, CD, and the apparent permeability constant (Kp, cm/h) can be calculated as:

$$K_p= J_{ss}/C_D \quad (2)$$

Daily exposure to the studied PFRs via dermal contact with indoor dust was estimated using the general equation:

$$DED=(C \times BSA \times DAS \times F_A \times IEF)/(BW \times 1000) \quad \ldots(3)$$

Where DED = Daily exposure dose (ng/kg bw/day), C = PFR concentration in dust (ng/g), BSA =Body surface area exposed (cm^2), DAS = Dust adhered to skin (mg/cm^2), F_A = fraction absorbed by the skin (unitless), IEF = indoor exposure fraction (hours spent over a day in a certain indoor environment) (unitless), BW = Body weight (kg).

Results and Discussion

Percutaneous penetration and mass balance

Following 24 h exposure of human ex vivo skin to a finite dose of 500 ng/cm^2 in 10 µL of acetone, TCEP showed the highest cumulative absorption with 28% of the applied dose detected in the receptor fluid. Lower absorbed fractions of 25% and 13% were observed for TCIPP and TDCIPP, respectively (Table 1). Analysis of the skin tissue resulted in recovery of 15%, 11% and 7% of the applied dose of TDCIPP, TCIPP and TCEP, respectively after 24 h exposure. Statistical analysis revealed a significant ($P < 0.05$) positive correlation between the absorbed fractions of PFRs and their water solubility, while a significant negative correlation was established between the cumulative 24 h absorption of target compounds and their log K_{OW}.

Although not statistically significant ($P > 0.05$), it was evident that EPISKIN™ tissues were more permeable (i.e. less barrier function) to all the studied compounds than human ex vivo skin. In particular, TCEP, TCIPP and TDCIPP showed 16%, 11% and 9% enhanced absorption in EPISKIN™ model compared to human ex vivo skin model.

Careful inspection of the cumulative absorption curves of the studied compounds revealed a different profile for TDCIPP compared to TCEP and TCIPP. Both TCEP and TCIPP showed a rapid increase in the absorbed dose in the first 8 h of exposure, before the absorption rate declined until 24 h. However, TDCIPP showed a slower, yet more consistent rate of absorption throughout the 24 h exposure period (Figure 2). This may be attributed to the higher lipophilicity of TDCIPP (log K_{OW} = 3.8), compared to TCIPP (log K_{OW} = 2.6) and TCEP (log K_{OW} = 1.4), resulting in a slower mass transfer rate of this PFR across the lipophilic stratum corneum. Estimated K_p values for the studied PFRs (Table 2) were

negatively correlated with their K_{OW} values. Interestingly, comparison between the results obtained using human ex vivo skin and EPISKIN™
model revealed that differences in the barrier function (ΔK_p) decreased with decreasing polarity in the order: TCEP ($\Delta K_p = 0.8$) > TCIPP ($\Delta K_p = 0.6$) > TDCIPP ($\Delta K_p = 0.2$).

Effect of hand-washing

While the absorption rate of the studied PFRs decreased markedly after washing, percutaneous penetration continued (Figure 3). This may be attributed to diffusion from the contaminant reservoir within the skin tissue. While statistical analysis revealed a significant difference ($P < 0.05$) in the absorption rates of TCEP and TCIPP with and without washing over a 24 h exposure period, the difference for TDCIPP was not significant ($P = 0.12$). Our results show that hand-washing can reduce the overall dermal absorption of the studied PFRs, albeit to varying degrees depending on the physicochemical properties of the PFRs.

Implications for human exposure

We estimated the dermal exposure of two age groups (UK adults and toddlers) using three exposure scenarios. We used data recently reported by our research group [13] on the minimum, median and maximum concentrations of target PFRs in indoor dust from several UK microenvironments to estimate low, average and high exposure scenarios, respectively. The parameter F_A in equation 3 was replaced by the experimental values obtained in this study for each target PFR using human ex vivo skin model (Table 1). Values for other parameters in equation 3 were obtained from the USEPA exposure factors handbook 14.

Results revealed higher uptake by UK toddlers compared to adults (Table 3). This may be attributed to more dust adhering to the toddlers' skin and higher exposed skin surface area to body weight ratio compared to adults. Higher concentrations of TCIPP in UK indoor dust resulted in higher dermal uptake of UK adults and toddlers to this PFR than for TCEP and TDCIPP combined.

Collectively, these data highlight the significance of dermal uptake of PFRs via contact with indoor dust as a pathway of human exposure these contaminants.

Acknowledgement

The research leading to these results received funding from the European Union Seventh Framework Programme FP7/2007-2013 under grant agreement no. 327232 (ADAPT) and under grant agreement no. 316665 (A-TEAM).

References

1. I. van der Veen, J. de Boer, Chemosphere 88, 1119 (2012).
2. G. L. Wei, D. Q. Li, M. N. Zhuo et al., Environ Pollut 196, 29 (2015).
3. A. Farhat, J. K. Buick, A. Williams et al., Toxicol Appl Pharm 275, 104 (2014).
4. P. D. Noyes, D. E. Haggard, G. D. Gonnerman, R. L. Tanguay, Toxicol Sci 145, 177 (2015).
5. J. D. Meeker, H. M. Stapleton, Environmental Health Perspectives 118, 318 (2010).
6. ECHA, http://echa.europa.eu/documents/10162/0410f4e3-7838-4819-b321-f9d75d3a9cce (accessed 19-6-2012), (2010).
7. E. Cequier, A. C. Ionas, A. Covaci et al., Environ Sci Technol 48, 6827 (2014).
8. M. A. Abdallah, A. Covaci, Environ Sci Technol 48, 4782 (2014).
9. G. Malarvannan, C. Belpaire, C. Geeraerts et al., Environ Res 140, 604 (2015).
10. M. A.-E. Abdallah, G. Pawar, S. Harrad, Environ Int 84, 64 (2015).
11. M. A.-E. Abdallah, J. Zhang, G. Pawar et al., Anal Bioanal Chem 407, 1871 (2015).
12. F. Niedorf, E. Schmidt, M. Kietzmann, Alternatives to laboratory animals : ATLA 36, 201 (2008).
13. S. Brommer, S. Harrad, Environ Int 83, 202 (2015).
14. USEPA, www.epa.gov/ncea/efh/pdfs/efh-complete.pdf, (2011).

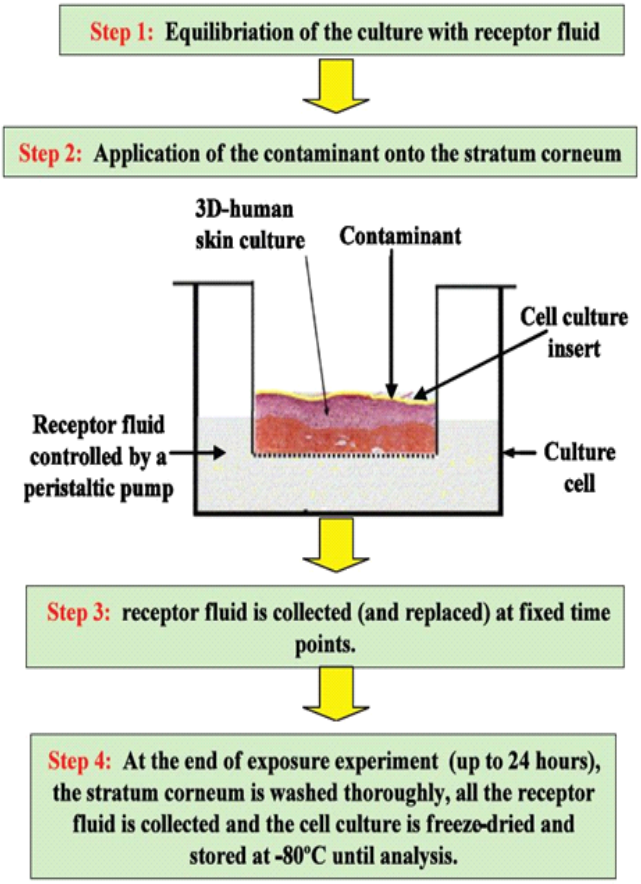

Figure 1: Schematic representation of the dosing experiments

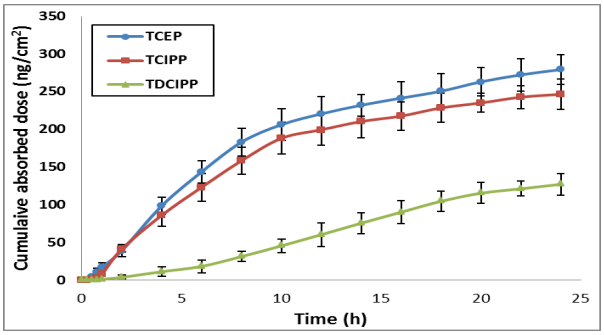

Figure 2: Cumulative absorbed dose through human *ex vivo* skin following exposure to 1000 ng/cm^2 of PFRs.

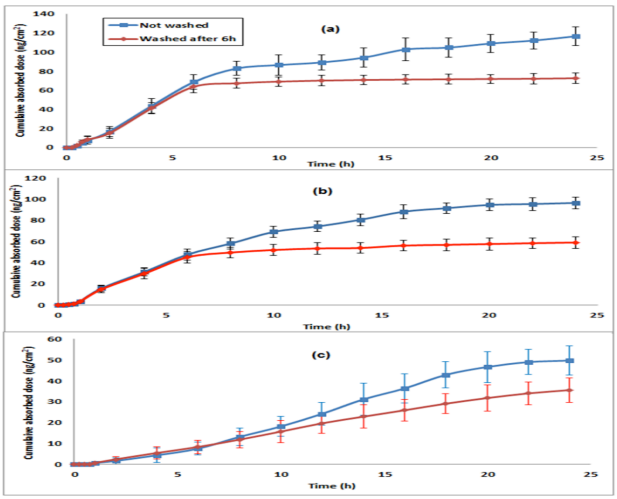

Figure 3: Cumulative absorbed dose of (a) TCEP, (b) TCIPP and (c) TDCIPP applied to *ex vivo* skin.

Table 1: Distribution of target PFRs (expressed as percentage of exposure dose) in different fractions of the *in vitro* model following 24 h exposure to 500 ng/cm^2 (finite dose) of the studied compounds.

Human *ex vivo* skin	TCEP	TCIPP	TDCIPP
Absorbed	28.3 ± 2.3	24.7 ± 1.4	12.7 ± 1.2
Skin	6.8 ± 1.1	10.8 ± 1.2	14.8 ± 1.4
Unabsorbed	55.3 ± 3.5	53.1 ± 2.9	62.3 ± 4.3
Sum	90.3 ± 6.9	88.6 ± 5.5	89.8 ± 6.7
EPISKIN™	TCEP	TCIPP	TDCIPP
Absorbed	33.7 ± 2.5	27.7 ± 1.9	13.9 ± 1.5
Skin	6.8 ± 1.4	10.8 ± 1.0	14.8 ± 1.3
Unabsorbed	49.3 ± 3.9	50.3 ± 3.2	61.5 ± 4.6
Sum	89.7 ± 7.8	88.8 ± 6.1	90.2 ± 7.5

Table 2: Flux rates (J_{ss}, ng/cm^2.h), permeability constants (K_p, cm/h), lag times (t_{lag}, h) and linear ranges (h) estimated from infinite exposure of human *ex vivo* skin and EPISKIN™ to 1000 ng/cm^2 of target PFRs for 24 h.

	Human *ex vivo* skin				EPISKIN™			
	J_{ss}	K_p x 10^{-2}	t_{lag}	Range	J_{ss}	K_p x 10^{-2}	t_{lag}	Range
TCEP	21.9	2.2	0.28	0.5 – 8	30.1	3.0	0.21	0.5 - 8
TCIPP	15.5	1.6	0.29	0.5 - 10	21.7	2.2	0.23	0.5 - 10
TDCIPP	5.4	0.5	2.9	4 – 22	7.4	0.7	2.9	4 - 22

Table 3: Estimated dermal exposure (ng/kg bw.day) of UK adults and toddlers to PFRs via contact with dust.

Dermal exposure scenario	Adult			Toddler		
	Low	Median	High	Low	Median	High
TCEP	<0.1	0.1	10.0	0.1	1.5	38.6
TCIPP	0.5	3.8	22.6	4.9	32.9	217.8
TDCIPP	<0.1	0.2	4.3	<0.1	1.6	37.0