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DOI:
10.1016/j.taap.2015.12.004

Document Version
Peer reviewed version

Citation for published version (Harvard):
Abdallah, M, Pawar, G & Harrad, S 2016, 'Human dermal absorption of chlorinated organophosphate flame retardants; implications for human exposure', *Toxicology and applied pharmacology*, vol. 291, pp. 28-37. https://doi.org/10.1016/j.taap.2015.12.004

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

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Abstract

Tris-2-chloroethyl phosphate (TCEP), tris (1-chloro-2-propyl) phosphate (TCIPP) and tris-1,3-dichloropropyl phosphate(TDCIPP) are organophosphate flame retardants (PFRs) widely applied in a plethora of consumer products despite their carcinogenic potential. Human dermal absorption of these PFRs is investigated for the first time using human *ex vivo* skin and EPISKIN™ models. Results of human *ex vivo* skin experiments revealed 28%, 25% and 13% absorption of the applied dose (500 ng/cm^2, finite dose) of TCEP, TCIPP and TDCIPP, respectively after 24 h exposure. The EPISKIN™ model showed enhanced permeability values (i.e. weaker barrier), that were respectively 16%, 11% and 9% for TCEP, TCIPP and TDCIPP compared to human *ex vivo* skin. However, this difference was not significant ($P>0.05$). Estimated permeability constants (K_p, cm/h) showed a significant negative correlation with log K_{OW} for the studied contaminants. The effect of hand-washing on dermal absorption of PFRs was investigated. Washing reduced overall dermal absorption, albeit to varying degrees depending on the physicochemical properties of the target PFRs. Moreover, slight variations of the absorbed dose were observed upon changing the dosing solution from acetone to 20% Tween 80 in water, indicating the potential influence of the dose vehicle on the dermal absorption of PFRs. Finally, estimated dermal uptake of the studied PFRs via contact with indoor dust was higher in UK toddlers (median ΣPFRs = 36 ng/ kg bw. day) than adults (median ΣPFRs = 4 ng/kg bw. day). More research is required to fully elucidate the toxicological implications of such exposure.

Keywords: Dermal absorption, Organophosphate flame retardants, EPISKIN, PFRs, Human exposure.

Introduction

Organophosphate flame retardants (PFRs) have been associated recently with a variety of applications in a wide range of products (van der Veen and de Boer, 2012). Following the inclusion of tetra- to hepta- brominated diphenyl ether (PBDE) congeners under the Stockholm Convention list of persistent organic pollutants (POPs) (Stockholm convention on POPs, 2013), several flame retardants (FRs) have emerged as alternatives to the banned PBDEs. Among those alternative FRs, the European market demand of PFRs has increased from 83,700 tons in 2004 to 91,000 tons in 2006 accounting for 20% of the EU consumption of FRs in 2006 (EFRA, 2007). In Japan, the production and shipment quantity of PFRs were estimated at 45,400 and 85,700 tons in 2005 and 2010, respectively. The annual yield of PFRs reached ~ 70,000 tons in 2007 and is estimated to increase by 15% annually in China (Wei *et al.*, 2015). 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 flame retardants in flexible and rigid polyurethane foams (PUFs) deployed in furniture, car upholstery and related products (van der Veen and de Boer, 2012). In addition, they are also used as plasticizers in various products including lacquer, paint and glue (Wei *et al.*, 2015).

PFR are not chemically bonded to the polymer matrix (i.e. additive FRs). Therefore, they are likely to leach out from treated products by abrasion and/or volatilization to contaminate the surrounding environment in a similar scenario to PBDEs (Reemtsma *et al.*, 2008). PFRs have been recently detected in both indoor and outdoor environments (Reemtsma *et al.*, 2008; van der Veen and de Boer, 2012). Several studies have reported on levels of various PFRs in soil, sediment, water and air (Martinez-Carballo *et al.*, 2007; Reemtsma *et al.*, 2008; van der Veen and de Boer, 2012; Cristale *et al.*, 2013). Moreover, PFRs were recently reported in biota and

human breast milk indicating their bioavailability to humans and wildlife (Sundkvist *et al.*, 2010; Kim *et al.*, 2011; Leonards *et al.*, 2011; Kim *et al.*, 2014; Brandsma *et al.*, 2015).

Current understanding of the toxicological properties of PFRs is not complete. Few studies have reported on adverse effects of PFRs including liver toxicity, reproductive toxicity, neurotoxicity and interference with normal growth upon long-term exposure in laboratory animals (Regnery *et al.*, 2011; van der Veen and de Boer, 2012). Other studies have reported various toxic effects of TDCIPP including immunotoxicity and disturbance of lipid metabolism in chicken embryos (Farhat *et al.*, 2014), as well as neurodevelopmental defects in embryonic zebrafish (Noyes *et al.*, 2015). TDCIPP was also reported to cause reduced thyroid hormone levels in humans (Meeker and Stapleton, 2010). In addition, TCEP, TCIPP and TDCIPP were subject to an EU risk assessment process under an Existing Substances Regulation (EEC 793/93) and were classified as persistent in the aquatic environment (Regnery *et al.*, 2011). 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" (ECHA, 2010).

Currently, little is known about the sources, magnitude and pathways of human exposure to PFRs. Recent studies have provided estimates of external human exposure to PFRs via inhalation (Cequier *et al.*, 2014), ingestion of indoor dust (Abdallah and Covaci, 2014) and diet (Malarvannan *et al.*, 2015). However, very little is known about the relative contribution of different exposure pathways to the overall human body burdens of these contaminants. More recently, Hoffman et al. reported that concentrations of TDCIPP in indoor dust were not associated with those in hand wipes. However, hand wipe levels were associated with urinary metabolites indicating that hand-to-mouth contact or dermal absorption may be important pathways of human exposure to PFRs (Hoffman *et al.*, 2015). Furthermore,

pharmacokinetic modelling of the extensively studied PBDEs revealed the significance of dermal contact with indoor dust as a pathway of human exposure to these FRs (Lorber, 2008; Trudel *et al.*, 2011). To illustrate, dermal uptake was reported as the 2nd most important contributor (following dust ingestion) to PBDE body burdens of Americans (Lorber, 2008). For Europeans, ingestion of diet and dust, as well as dermal exposure to dust constituted the major factors influencing human body burdens of PBDEs (Trudel *et al.*, 2011). To our knowledge, there is -*to date*- no available information on human uptake of PFRs following dermal contact. This may be attributed to ethical issues associated with both *in vivo* and *in vitro* studies using human tissues. In addition, uncertainties arise from interspecies variation and allometric scaling of dermatokinetic data from animals to humans (Abdallah *et al.*, 2015a). These challenges further support the need for alternative *in vitro* methods to study dermal availability of hazardous chemicals present in indoor dust to humans. To overcome these challenges, our research group recently reported on the application of *in vitro* 3D-human skin equivalents (e.g. EPISKIN™ and EpiDerm™ models) as an alternative approach to study human dermal absorption of various brominated flame retardants. 3D-human skin equivalents (3D-HSE) are cultured from primary human cells to produce fully differentiated, multi-layer tissues that mimic the original human skin both histologically and physiologically (Figure SI-1). They were initially developed as alternatives to animal testing by the pharmaceutical industry and were successfully applied to study the dermal absorption of various topically applied chemicals (Schaefer-Korting *et al.*, 2008a; Ackermann *et al.*, 2010). The paucity of data on human dermal absorption of PFRs represents a research gap that can hinder the accurate risk assessment of this class of emerging contaminants. Therefore, the aims of this paper 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 primary assessment of adult and toddler exposure to the target PFRs via dermal contact with indoor dust.

Materials and Methods

In vitro dermal exposure experiments were performed along the principles of good laboratory practice and in compliance with the OECD guidelines for *in vitro* dermal absorption testing (OECD, 2004). The handling instructions and performance characteristics of EPISKIN™ 3D-human skin equivalent (3D-HSE) model were also taken into consideration. The study protocol received the required ethical approval *(# ERN_12-1502*) from the University of Birmingham's Medical, Engineering and Mathematics Ethical Review Committee.

Chemicals and standards

All solvents and reagents used for preparation, extraction, clean-up and instrumental analysis of samples were of HPLC grade and were obtained from Fisher Scientific (Loughborough, UK). Neat standards (purity > 98%) of tris (2-chloroethyl) phosphate (TCEP), tris (2-chloroisopropyl) phosphate (TCPP), tris (1,3-dichloro-2-propyl) phosphate (TDCIPP), were purchased from Sigma-Aldrich (Gillingham, Dorset, UK). Isotopically labelled d_{15}-triphenyl phosphate (d_{15}-TPhP) and d_{27}-tri-n-butyl phosphate (d_{27}-TnBP) (50 µg/mL in toluene, purity >99%) were obtained from Wellington Laboratories (Guelph, ON, Canada). Florisil® SPE cartridges were purchased from Supelco™ (Bellefonte, Pennsylvania, USA). All culture medium components (Table SI-1) were purchased from Sigma-Aldrich UK (Gillingham, Dorset, UK).

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. Selection criteria included: Caucasian, no stretchmarks, no scars, no hair and full thickness skin without adipose tissue. Skin was kept on ice for no longer than 4 h prior to the onset of the *ex-vivo* skin absorption studies. Upon receipt, the *ex vivo* skin samples were equilibrated for 1 h with 3 mL of DMEM (Dulbecco's Modified Eagle's Medium)-based (Sigma-Aldrich, UK) culture medium (Table SI-1) 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 RHE/L/13 tissue constructs are 1.07 cm^2 tissues shipped on the 13^{th} day of culture required for acceptable tissue differentiation (www.episkin.com). The kit includes maintenance medium (MM) - which is a proprietary DMEM-based medium that allows acceptable differentiated morphology of the tissue for ~ 5 days upon receipt by end users. 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/cm^2 (infinite dose application) of dosing solution II, respectively. Acetone was selected as the dosing vehicle based on its ability to dissolve the test compounds at the desired levels and its minimal effect on skin barrier function. A previous study on the effect of organic solvents on the trans-epidermal water loss (TEWL) as indicator of skin barrier revealed both acetone and hexane to not behave significantly differently in this context to water, while a mixture of chloroform:methanol (2:1 v/v) caused the greatest significant increase in TEWL (Abrams *et al.*, 1993).

To study the possible effect of the dosing vehicle on the percutaneous penetration of the tested chemicals, target PFRs were dissolved in 2 different dosing vehicles of: (A) acetone, and (B) 20% Tween 80 (Sigma-Aldrich, UK) in water at a concentration of (III) 10 ng/µL. For this strand of experiments, *in vitro* skin tissues were dosed with 50 µL/cm^2 (infinite dose application) of dosing solution II and III for comparison. Preparation of the higher dosing level (i.e. 50 ng/µL) was not possible due to limited solubility of target PFRs in vehicle (B).

Percutaneous penetration assay protocol

The permeation experiments were performed using the static set-up approach (Figure SI-1). Skin tissues were mounted in standard Franz-type permeation devices with the *stratum corneum* facing up. Based on the recommendation of the 3D-HSE providers, the EPISKIN™ tissues were mounted in special inserts constructed for this model (SkinEthic Laboratories, Lyon, France), while excised human skin tissues were mounted in standard glass Franz cells. All experiments were performed in triplicate. Following 30 minutes equilibration, the tested chemicals were applied onto the skin surface in the donor compartment. A DMEM-based culture medium (Table SI-1) was used as receptor fluid, maintained at 32 ± 1 °C and magnetically stirred. At fixed time points, aliquots of the receptor fluid (2 mL) were collected from the receptor compartment and immediately replaced with fresh fluid. After 24 hours, the entire receptor fluid was collected and the skin surface washed thoroughly with cotton buds impregnated in (1:1) hexane:ethyl acetate (5 times). The tissues were removed from the permeation devices and both the donor and receptor compartments were washed separately (5 x 2 mL) with (1:1) hexane:ethyl acetate. All samples were stored at -20 ˚C until chemical analysis.

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/cm^2 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).

Sample extraction and chemical analysis

Each permeation assay generated five different types of samples comprising: receptor fluid at various time points, skin tissue, cotton buds (used to thoroughly wipe the skin surface), donor and receptor compartment washes.

The receptor fluid, skin tissue and cotton bud samples were spiked with 30 ng of d_{15}-TPhP used as internal standard prior to extraction according to a previously reported QuEChERs based method (Abdallah *et al.*, 2015c) (more details provided in the SI section).

Skin tissue samples were subject to an extra clean-up step to remove potentially interfering macromolecules. This involved evaporating the extract under a gentle stream of nitrogen prior to solvent exchange to 1 mL of hexane. The crude extract was then loaded onto a Florisil® SPE cartridge (pre-conditioned with 6 mL of hexane). Fractionation was achieved by eluting with 8 mL of hexane (F1, discarded) followed by 10 mL of ethyl acetate (F2). F2 was evaporated to incipient dryness under N_2 (Abdallah and Covaci, 2014). The donor and receptor compartment washes were spiked with 30 ng of d_{15}-TPhP prior to direct evaporation under a gentle stream of N_2.

Target analytes were reconstituted in 100 µL of isooctane containing 250 pg/µL d_{27}-TnBP used as recovery determination (syringe) standard for QA/QC purposes.

Quantification of target PFRs was performed using a TRACE 1310™ GC coupled to a ISQ™ single quadrupole mass spectrometer (Thermo Fisher Scientific, Austin, TX, USA) operated in electron ionization (EI) mode according to the previously described method (Van den Eede *et al.*, 2012; Abdallah and Covaci, 2014). Separation of target PFRs was performed on Agilent DB-5 capillary column (30 m x 0.25 mm; 0.25 µm) using helium as the carrier gas.

The mass spectrometer was run in selected ion monitoring (SIM) with ion source, quadrupole and mass transfer line temperatures set at 230, 150 and 300 °C, respectively. Further details of the GC-EI/MS method are provided in the SI section.

Dermal exposure estimation

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

$$DED = C \times BSA \times DAS \times FA \times IEF BW \times \quad \ldots$$

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).

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 (Niedorf *et al.*, 2008):

$$Jss = \Delta m \Delta t . A = D . K . \Delta C \Delta x$$

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 [cm^2]; Δc = concentration difference [ng/cm^3]; Δx: thickness of membrane [cm].

When using infinite-dose configurations, i.e. in which the donor concentration far exceeds the concentration in the receptor compartment ($C_D >> C_A$), ΔC can be replaced by the known donor concentration, C_D, and the permeated mass per time assumed constant. Therefore, the apparent permeability constant (*Kp, cm/h*), which represents an independent measure of the

membrane resistance against permeation of the examined substance, can be calculated as:

$$Kp = JssCD$$

Absorption data were plotted as a cumulative absorption - time curve and the flux (J_{ss}) and lag time (t_{lag}) were determined from the linear portion ($R^2 \geq 0.9$) of the curve. For infinite dose applications, the permeability constant (K_p; cm/h) was calculated by dividing the steady state flux (ng/cm^2.h) by the concentration of applied chemical (ng/cm^3) (Moore *et al.*, 2014). Determination of the start and upper boundary of the linear range (i.e. steady state conditions) was achieved according to the method described by Niedorf et al.(Niedorf *et al.*, 2008) (a summary flow chart is provided in figure SI-2).

Results are presented as the arithmetic mean of three replicates ± standard deviation (SD). Statistical analysis was performed using SPSS 13.0 software package. Differences in skin permeation were evaluated by the paired student t-test between two datasets. A Games-Howell test was used for analysis of variance (ANOVA) among several datasets with equal variances not assumed; $p < 0.05$ was regarded to indicate a statistically significant difference.

QA/QC

Several stages of QA/QC measurements were performed to check the performance of the percutaneous penetration assay protocol. A "field" blank, comprising a skin tissue exposed to solvents only and treated as a sample, was performed with each sample batch (n= 9). None of the studied compounds were above the limit of detection (LOD) in the field blank samples. Good recoveries of the d_{15}-TPhP labeled internal standard (> 85%) in all sample types were obtained indicating high efficiency of the extraction method. The accuracy and precision of the analytical method was tested via replicate analysis of matrix spikes of EPISKIN™, human *ex vivo* skin and receptor fluid samples at three different concentration levels of the target PFRs. Good results were obtained (Table SI-2) indicating the suitability of the applied analytical protocol for quantification of target PFRs in the studied samples.

Based on the guidelines of the EPISKIN™ model, the viability of the tissue was tested by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay using a standard kit purchased from each provider. Acceptable MTT results (i.e. Formazan concentration ≥ 1.5 mg/mL) were achieved following 24 h of exposure under the specified test conditions, prior to dropping below the recommended level of Formazan at longer times. Both positive and negative control experiments were carried out alongside each sample batch. Positive controls involved the exposure of the test tissue to Triton-X-100 which showed ~ 100% permeation (n=5; 97 ± 4%), while negative controls showed 0% penetration of decabromodiphenyl ethane after 24 h exposure. The integrity of the skin membrane was tested using the standard trans-epidermal electrical resistance (TEER) and methylene blue (BLUE) standard methods (Guth *et al.*, 2015). All skin tissues reported in this study passed all the above QA/QC tests.

Results and Discussion

Percutaneous absorption of chlorinated PFRs applied as a finite dose.

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). Interestingly, mass balance studies showed the reverse trend with the mass of each target PFR accumulated within the skin (Figure 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 (Table SI-3), while a significant negative correlation was established between the cumulative 24 h absorption of target compounds and their log K_{ow} (Table SI-3).

Similar results were obtained using the EPISKIN™ human skin equivalent model (Table 1 and Figure 1). No statistically significant differences ($P > 0.05$) were observed between the results obtained from the investigated *in vitro* skin models. However, it was evident that EPISKIN™ tissues were more permeable (i.e. less barrier function) to all the studied compounds than human *ex vivo* skin (Figure 1). In particular, TCEP, TCIPP and TDCIPP showed 16%, 11% and 9% enhanced absorption in EPISKIN™ model compared to human *ex vivo* skin model, respectively. This is in line with our previous findings for the flame retardants hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBP-A), which achieved higher percutaneous penetration levels through EPISKIN™ compared to human *ex vivo* skin at two different dose levels (Abdallah *et al.*, 2015b).

To our knowledge, this is the first study to report on human dermal absorption of PFRs which precludes comparison with previous similar studies. However, our results are generally in agreement with those of an earlier study of human dermal absorption of two structurally-related organophosphorus pesticides using *ex vivo* human abdominal skin (Moore *et al.*, 2014). The absorbed fractions of the more hydrophilic dichlorvos were higher than those of chlorpyrifos following 24 h dermal exposure (finite dose) in three different vehicles. An *in vivo* study of the dermal absorption of structurally-related polychlorinated biphenyls (PCBs) with various degrees of chlorination applied as finite dose in male rats reported similar results. This rat skin model favoured the rapid absorption of the more hydrophilic, mono- and di-chlorinated PCBs, while the lipophilic hexachlorinated PCB studied, achieved lower absorbed fractions, yet higher skin accumulation levels following 48 h exposure (Garner and Matthews, 1998). This was mainly attributed to the physicochemical parameters of the studied PCBs, which allowed the more polar mono-PCBs to penetrate faster through the water-rich viable epidermis. More lipophilic hexa-PCBs were hypothesized to accumulate for longer in the lipid-rich *stratum corneum* prior to diffusion through the viable epidermis at a

slower rate (Garner and Matthews, 1998). This can also explain our results for the studied PFRs where the more hydrophilic TCEP (Table SI-3) displays the highest absorbed fraction, while the more lipophilic TDCIPP achieves the highest accumulation level within the skin following 24 h exposure (Figure 1).

Percutaneous absorption of chlorinated PFRs applied as infinite dose.

Infinite dose application maximizes the concentration gradient and diffusion/penetration through the skin becomes the rate-limiting step. Therefore, this application mode permits the calculation of the permeability constant (*Kp*) for each investigated compound under the test conditiona (OECD, 2004). Careful inspection of the cumulative absorption curves of the studied compounds over 24 h exposure, reveals a different profile for TDCIPP compared to TCEP and TCIPP (Figure 2). 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*. In a previous study, we observed a comparable absorption profile to that observed here for TDCIPP, for the flame retardant TBBP-A (log K_{OW} = 4.5) applied to both human ex vivo skin and EPISKIN™ in acetone (Abdallah *et al.*, 2015b). Moreover, a similar difference in the cumulative absorption profile was also observed between the lipophilic pesticide Chlorpyrifos (log K_{ow} = 4.9) and the more hydrophilic Dichlorvos (log K_{ow} = 1.4) applied to *ex vivo* human skin in isopropanol (Moore *et al.*, 2014).

The infinite dose application results were used to estimate various dermal absorption parameters for each studied PFR using a previously reported model (Niedorf *et al.*, 2008). Results revealed a significant negative correlation between the estimated permeability

constant (K_p, *cm/h*) of the studied compounds (Table 2) and their log K_{OW} values (Table SI-3). This in agreement with previous reports for BFRs (Abdallah *et al.*, 2015b) and PCBs (Garner and Matthews, 1998). Estimated K_p values for the PFRs in this study are higher than those reported for the flame retardants HBCD and TBBP-A (Abdallah *et al.*, 2015b). This may also be attributed to the more lipophilic nature of HBCD (log K_{OW} = 5.6) and TBBP-A (log K_{OW} = 4.5) compared to the studied PFRs.

Interestingly, comparison between the results obtained using human *ex vivo* skin and EPISKIN™ model (Table 2) revealed that differences in the barrier function (ΔK_p) decreased with decreasing polarity in the order: TCEP (ΔK_p = 0.8) > TCIPP (ΔK_p = 0.6) > TDCIPP (ΔK_p = 0.2). However, more comparative studies with more chemicals covering a wide variety of physicochemical properties are required to confirm this observation and further our understanding of the differences between the barrier function of 3D-HSE and real human skin.

Effect of hand-washing

Following 6 h of finite dose application (500 ng/cm^2) of target PFRs to human *ex vivo* skin, the skin surface was washed thoroughly with a neutral detergent solution, while monitoring the absorbed dose in the receptor fluid continued until 24 h. Results show that while the absorption rate decreases markedly after washing, percutaneous penetration of the studied PFRs continues (Figure 3), which 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 ($P = 0.12$). Nielsen reported on the effect of handwashing on human dermal absorption of four different chemicals using a similar human *ex vivo* skin model (Nielsen, 2010). The effect of skin wash after 6 h dermal exposure on reducing subsequent extent of skin penetration (over 48 h) was more substantial

for hydrophilic caffeine (log K_{OW} = -0.07) compared to the relatively lipophilic organophosphate insecticide malathion (log K_{OW} = 2.75). This was explained by the higher affinity of the lipophilic malathion to deposit within the skin, resulting in a reservoir which can then release the insecticide slowly to the receptor fluid even after washing the skin surface (Nielsen, 2010). In two separate papers, Fenske et al. studied the efficiency of hand-washing for removal of the chlorinated pesticides chlopyrifos (Fenske and Lu, 1994) and captan (Fenske *et al.*, 1998) from the hands of occupationally exposed adults. Results revealed 78% of captan was successfully removed in the group who washed their hands immediately; whereas removal efficiency was reduced to 68 % after 1 h residing on hands (Fenske *et al.*, 1998).

Compared to the results by the same group for chlorpyrifos, removal of this pesticide by hand-washing was substantially lower (43 % at time = 0 and 23 % at time = 1 h) (Fenske and Lu, 1994). These variations were attributed to the differences in physicochemical properties of the two pesticides. In particular, chlorpyriphos (log K_{OW} = 4.9, water solublility 2 mg/L) is more lipophilic and less water soluble than captan (log K_{OW} = 2.4, water solublility 5 mg/L). Moreover, the differences in the applied commercial formulations of the two pesticides were hypothesized to influence their dermal permeation (Fenske *et al.*, 1998).

In general, our results (Figure 3) indicate 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.

Effect of exposure vehicle

Several dermal absorption studies have reported on the influence of the vehicle on the percutaneous penetration of various chemicals including different pharmaceuticals (Karadzovska and Riviere, 2013), organophosphate pesticides (Moore *et al.*, 2014) and brominated flame retardants (Abdallah *et al.*, 2015b). To investigate the potential effect of

vehicle on the dermal absorption of target PFRs, human *ex vivo* skin was exposed to 500 ng/cm^2 of each of TCEP, TCIPP and TDCIPP in 2 different vehicles. After 24 h exposure, results revealed an increase in the absorbed dose of the 3 target compounds from 20 % Tween 80 solution in water compared to acetone (Figure 4). While the effect of vehicle on the absorbed fraction varied for the studied PFRs, none showed a statistically significant difference ($P > 0.05$) between the studied exposure vehicles. In general, a vehicle may hydrate the stratum corneum (SC), extract critical barrier components out of the skin, or damage the skin because it is a strong acid or base. Removing SC lipids may increase percutaneous absorption of drugs. Many organic solvents (e.g. chloroform and methanol) are employed to delipidize the skin, which increases the permeability of hydrophilic - but not lipophilic – compounds (Chiang *et al.*, 2012). Surfactants like Tween 80 and polyethylene glycol were previously reported as permeation enhancers for hydrophilic drugs (Duracher *et al.*, 2009) and steroids (Schaefer-Korting *et al.*, 2008b). Therefore, despite the lack of statistical significance, the enhanced dermal permeation of the studied PFRs (Figure 4) in the presence of Tween 80 is potentially relevant in the context of human exposure. This is due to the presence of natural surface active agents in human skin surface film (sweat/sebum mixture) (Stefaniak *et al.*, 2010), which may influence the dermal absorption of these PFRs. More studies are required to fully characterise the effect of human skin surface film on the dermal uptake of various FRs.

Implications for human exposure

While a recent study highlighted the importance of dermal exposure via contact with indoor dust as a potential contributor to the overall body burdens of TDCIPP and triphenyl phosphate flame retardants in American adults (Hoffman *et al.*, 2015), there is a lack of information on the magnitude of human dermal exposure to PFRs. Therefore, results of dermal absorption of PFRs obtained in this study (Table 1) were used to obtain a preliminary

assessment of the internal dose of target PFRs arising from human dermal exposure to contaminated indoor dust.

Using equation (3), 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 (Brommer and Harrad, 2015) on the minimum, median and maximum concentrations (Table SI-4) 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 (USEPA, 2011) and summarized in Table 3.

Dermal exposure estimates 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. The estimated median intakes of an average UK adult (70 Kg) via dust ingestion were 0.03, 0.92 and 0.07 ng/kg bw.day for TCEP, TCIPP and TDCIPP respectively (Brommer and Harrad, 2015). These are less than the estimated median intakes of the UK adult via dermal absorption in this study (Table 3). For a UK child (20 kg), the median intakes of target PFRs via dust ingestion were 1.7, 43.0 and 4.0 ng/kg bw.day for TCEP, TCIPP and TDCIPP respectively (Brommer and Harrad, 2015). These are slightly higher than the estimated dermal uptake by a UK toddler (15 kg) of 1.5, 32.9, 1.6 ng/kg bw.day for TCEP, TCIPP and TDCIPP in this study. Currently, there are no data available on inhalational exposure of UK population to PFRs. However, Cequier et al. have reported lower average exposure of Norway Women and children to the target PFRs via air inhalation than dust ingestion (Cequier *et al.*, 2014). Collectively, these data highlight the significance of

dermal uptake of PFRs via contact with indoor dust as a pathway of human exposure these contaminants.

It should be noted that our dermal exposure estimates assume a fixed body area undergoing constant exposure to FRs in indoor dust for a constant period daily. While such rigid assumptions may introduce uncertainty to our estimates of dermal exposure, more research is required to fully elucidate the toxicological implications of such exposure in both adults and toddlers.

In a risk assessment context, a No Significant Risk Level (NSRL) of 5.4 µg/day for TDCIPP listed as a carcinogen under the State of California safe drinking water and toxic enforcement act of 1986, PROPOSITION 65 (OEHHA, 2015). No other health based limit values (HBLVs) of legislative standing for our target FRs were found in the literature. However, Ali et al. estimated HBLVs of 22,000, 80,000 and 15,000 ng/kg bw/day for TCEP, TCIPP and TDCIPP, respectively based on a chronic no observed adverse effect level (NOAEL) divided by an uncertainty factor of 1,000, (Ali *et al.*, 2012). Our worst-case scenario exposure estimates for dermal exposure of adults and toddlers (Table 3) fall far below these HBLV values. However, as noted by Ali et al. (Ali *et al.*, 2012), the HBLV values cited here were based on relatively old toxicological studies and it is possible that future research may erode the margin of safety.

Acknowledgement

The research leading to these results has received funding from the European Union Seventh Framework Programme FP7/2007-2013 under grant agreements 327232 (ADAPT project) and 316665 (A-TEAM project). Further support was provided by Restek Corporation.

Supplementary data

Further details of the analytical methodology, quality assurance/quality control parameters, cumulative absorbed doses of PFRs over time and concentrations of target PFRs in dust from different UK indoor microenvironments are available as supplementary data.

References

Abdallah, M.A., Pawar, G., Harrad, S., 2015a. Evaluation of in vitro vs. in vivo methods for assessment of dermal absorption of organic flame retardants: a review. Environ Int **74**, 13-22.

Abdallah, M.A., Covaci, A., 2014. Organophosphate flame retardants in indoor dust from Egypt: implications for human exposure. Environ Sci Technol **48**, 4782-4789.

Abdallah, M.A., Harrad, S., Covaci, A., 2008. Hexabromocyclododecanes and tetrabromobisphenol-A in indoor air and dust in Birmingham, U.K: implications for human exposure. Environ Sci Technol **42**, 6855-6861.

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

Abdallah, M.A., Zhang, J., Pawar, G., Viant, M.R., Chipman, J.K., D'Silva, K., Bromirski, M., Harrad, S., 2015c. High resolution mass spectrometry provides novel insights into products of human metabolism of organophosphate and brominated flame retardants Anal Bioanal Chem **407**, 1871-1883.

Abrams, K., Harvell, J.D., Shriner, D., Wertz, P., Maibach, H., Maibach, H.I., Rehfeld, S.J., 1993. Effect of organic-solvents on in-vitro human skin water barrier function. J Invest Dermatol **101**, 609-613.

Ackermann, K., Borgia, S.L., Korting, H.C., Mewes, K.R., Schafer-Korting, M., 2010. The Phenion full-thickness skin model for percutaneous absorption testing. Skin Pharmacol Physiol **23**, 105-112.

Ali, N., Dirtu, A.C., Eede, N.V., Goosey, E., Harrad, S., Neels, H., t Mannetje, A., Coakley, J., Douwes, J., Covaci, A., 2012. Occurrence of alternative flame retardants in indoor dust

from New Zealand: Indoor sources and human exposure assessment. Chemosphere **88**, 1276-1282.

Brandsma, S.H., Leonards, P.E.G., Leslie, H.A., de Boer, J., 2015. Tracing organophosphorus and brominated flame retardants and plasticizers in an estuarine food web. Sci Total Environ **505**, 22-31.

Brommer, S., Harrad, S., 2015. Sources and human exposure implications of concentrations of organophosphate flame retardants in dust from UK cars, classrooms, living rooms, and offices. Environ Int **83**, 202-207.

Cequier, E., Ionas, A.C., Covaci, A., Marce, R.M., Becher, G., Thomsen, C., 2014. Occurrence of a Broad Range of Legacy and Emerging Flame Retardants in Indoor Environments in Norway. Environ Sci Technol **48**, 6827-6835.

Chiang, A., Tudela, E., Maibach, H.I., 2012. Percutaneous absorption in diseased skin: an overview. J. Appl. Toxicol. **32**, 537-563.

Cristale, J., Vazquez, A.G., Barata, C., Lacorte, S., 2013. Priority and emerging flame retardants in rivers: Occurrence in water and sediment, Daphnia magna toxicity and risk assessment. Environ Int **59**, 232-243.

Duracher, L., Blasco, L., Hubaud, J.-C., Vian, L., Marti-Mestres, G., 2009. The influence of alcohol, propylene glycol and 1,2-pentanediol on the permeability of hydrophilic model drug through excised pig skin. Int J Pharm **374**, 39-45.

ECHA, 2010. European Chemicals Agency: Annex1-Document to RAC opinion on TDCP. http://echa.europa.eu/documents/10162/0410f4e3-7838-4819-b321-f9d75d3a9cce.

EFRA, 2007. (European Flame Retardants Association), Market statistics. http://www.flameretardants.eu/DocShareNoFrame/docs/6/KHAIJIBBHOBJOKBOHNNFGAJL53V443HA4YW3PDB348BT/EFRA/docs/DLS/EFRA_web_11-2007_Market_statistics-1_.pdf.

Farhat, A., Buick, J.K., Williams, A., Yauk, C.L., O'Brien, J.M., Crump, D., Williams, K.L., Chiu, S., Kennedy, S.W., 2014. Tris(1,3-dichloro-2-propyl) phosphate perturbs the expression of genes involved in immune response and lipid and steroid metabolism in chicken embryos. Toxicol Appl Pharm **275**, 104-112.

Fenske, R.A., Lu, C.S., 1994. Determination of handwash removal efficiency - incomplete removal of the pesticide chlorpyrifos from skin by standard handwash techniques. Am Ind Hyg Assoc J **55**, 425-432.

Fenske, R.A., Schulter, C., Lu, C., Allen, E.H., 1998. Incomplete removal of the pesticide captan from skin by standard handwash exposure assessment procedures. B Environ Contam Tox **61**, 194-201.

Garner, C.E., Matthews, H.B., 1998. The effect of chlorine substitution on the dermal absorption of polychlorinated biphenyls. Toxicol Appl Pharm **149**, 150-158.

Guth, K., Schaefer-Korting, M., Fabian, E., Landsiedel, R., van Ravenzwaay, B., 2015. Suitability of skin integrity tests for dermal absorption studies in vitro. Toxicol in Vitro **29**, 113-123.

Hoffman, K., Garantziotis, S., Birnbaum, L.S., Stapleton, H.M., 2015. Monitoring Indoor Exposure to Organophosphate Flame Retardants: Hand Wipes and House Dust. Environ Health Persp **123**, 160-165.

Karadzovska, D., Riviere, J.E., 2013. Assessing vehicle effects on skin absorption using artificial membrane assays. Eur J Pharm Sci **50**, 569-576.

Kim, J.W., Isobe, T., Chang, K.H., Amano, A., Maneja, R.H., Zamora, P.B., Siringan, F.P., Tanabe, S., 2011. Levels and distribution of organophosphorus flame retardants and plasticizers in fishes from Manila Bay, the Philippines. Environ Pollut **159**, 3653-3659.

Kim, J.W., Isobe, T., Muto, M., Tue, N.M., Katsura, K., Malarvannan, G., Sudaryanto, A., Chang, K.H., Prudente, M., Viet, P.H., Takahashi, S., Tanabe, S., 2014.

Organophosphorus flame retardants (PFRs) in human breast milk from several Asian countries. Chemosphere **116**, 91-97.

Leonards, P., Steindal, E.H., Van der Veen, I., Berg, V., Bustnes, J.O., Van Leeuwen, S., 2011. Screening of Organophosphor Flame Retardants 2010. . SPFO-Report 1091/2011. TA-2786/2011. http://www.miljodirektoratet.no/old/klif/publikasjoner/2786/ta2786.pdf.

Lorber, M., 2008. Exposure of Americans to polybrominated diphenyl ethers. J Expo Sci Env Epid **18**, 2-19.

Malarvannan, G., Belpaire, C., Geeraerts, C., Eulaers, I., Neels, H., Covaci, A., 2015. Organophosphorus flame retardants in the European eel in Flanders, Belgium: Occurrence, fate and human health risk. Environ Res **140**, 604-610.

Martinez-Carballo, E., Gonzalez-Barreiro, C., Sitka, A., Scharf, S., Gans, O., 2007. Determination of selected organophosphate esters in the aquatic environment of Austria. Sci Total Environ **388**, 290-299.

Meeker, J.D., Stapleton, H.M., 2010. House Dust Concentrations of Organophosphate Flame Retardants in Relation to Hormone Levels and Semen Quality Parameters. Environ Health Persp **118**, 318-323.

Moore, C.A., Wilkinson, S.C., Blain, P.G., Dunn, M., Aust, G.A., Williams, F.M., 2014. Percutaneous absorption and distribution of organophosphates (chlorpyrifos and dichlorvos) following dermal exposure and decontamination scenarios using in vitro human skin model. Toxicol Lett **229**, 66-72.

Niedorf, F., Schmidt, E., Kietzmann, M., 2008. The automated, accurate and reproducible determination of steady-state permeation parameters from percutaneous permeation data. Altern Lab Anim: ATLA **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.

Noyes, P.D., Haggard, D.E., Gonnerman, G.D., Tanguay, R.L., 2015. Advanced Morphological - Behavioral Test Platform Reveals Neurodevelopmental Defects in Embryonic Zebrafish Exposed to Comprehensive Suite of Halogenated and Organophosphate Flame Retardants. Toxicol Sci **145**, 177-195.

OECD, 2004. Guideline for the testing of chemicals. Skin absorption: in vitro method. Organisation for Economic Cooperation and Development TG 428.

OEHHA, 2015. Office of Environmental Health Hazard Assessment, State of California, Environmental Protection Agency, Safe drinking water and toxic enforcement act of 1986safe drinking water and toxic enforcement act of 1986, PROPOSITION 65. http://oehha.ca.gov/prop65/prop65_list/files/P65single082515.pdf.

Reemtsma, T., Benito Quintana, J., Rodil, R., Garcia-Lopez, M., Rodriguez, I., 2008. Organophosphorus flame retardants and plasticizers in water and air I. Occurrence and fate. Trac-Trend Anal Chem **27**, 727-737.

Regnery, J., Puettmann, W., Merz, C., Berthold, G., 2011. Occurrence and distribution of organophosphorus flame retardants and plasticizers in anthropogenically affected groundwater. J Environ Monitor **13**, 347-354.

Schaefer-Korting, M., Bock, U., Diembeck, W., Duesing, H.-J., Gamer, A., Haltner-Ukomadu, E., Hoffmann, C., Kaca, M., Kamp, H., Kersen, S., Kietzmann, M., Korting, H.C., Kraechter, H.-U., Lehr, C.-M., Liebsch, M., Mehling, A., Mueller-Goymann, C., Netzlaff, F., Niedorf, F., Ruebbelke, M.K., Schaefer, U., Schmidt, E., Schreiber, S., Spielmann, H., Vuia, A., Weimer, M., 2008a. The use of reconstructed human epidermis for skin absorption testing: results of the validation study. Altern Lab Anim: ATLA **36**, 161-187.

Schaefer-Korting, M., Mahmoud, A., Borgia, S.L., Brueggener, B., Kleuser, B., Schreiber, S., Mehnert, W., 2008b. Reconstructed epidermis and full-thickness skin for absorption

testing: Influence of the vehicles used on steroid permeation. Altern Lab Anim: ATLA **36**, 441-452.

Stefaniak, A.B., Harvey, C.J., Wertz, P.W., 2010. Formulation and stability of a novel artificial sebum under conditions of storage and use. Int J Cosmet Sci **32**, 347-355.

Stockholm convention on POPs, 2013. Persistent Organic Pollutants Review Committee (POPRC). http://chm.pops.int/Convention/POPsReviewCommittee/Overview/tabid/2806/Default.aspx.

Sundkvist, A.M., Olofsson, U., Haglund, P., 2010. Organophosphorus flame retardants and plasticizers in marine and fresh water biota and in human milk. J Environ Monitor **12**, 943-951.

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

USEPA, 2011. Exposure factors handbook. www.epa.gov/ncea/efh/pdfs/efh-complete.pdf.

Van den Eede, N., Dirtu, A.C., Ali, N., Neels, H., Covaci, A., 2012. Multi-residue method for the determination of brominated and organophosphate flame retardants in indoor dust. Talanta **89**, 292-300.

van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. Chemosphere **88**, 1119-1153.

Wei, G.L., Li, D.Q., Zhuo, M.N., Liao, Y.S., Xie, Z.Y., Guo, T.L., Li, J.J., Zhang, S.Y., Liang, Z.Q., 2015. Organophosphorus flame retardants and plasticizers: Sources, occurrence, toxicity and human exposure. Environ Pollut **196**, 29-46.

Tables

Table 1: Distribution of target PFRs (expressed as average percentage ± standard deviation of exposure dose) in different fractions of the *in vitro* diffusion system 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
Unabsorbed#	6.8 ± 1.1	10.8 ± 1.2	14.8 ± 1.4
Skin	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
Unabsorbed#	6.8 ± 1.4	10.8 ± 1.0	14.8 ± 1.3
Skin	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

* Comprises cumulative concentrations in the receptor fluid over 24 h + receptor compartment rinse.

Comprises concentrations in the skin surface wipes after 24 h + donor compartment rinse.

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	R^{2*}	J_{ss}	K_p x 10^{-2}	t_{lag}	Range	R^2
TCEP	21.9	2.2	0.28	0.5 - 8	0.97	30.1	3.0	0.21	0.5 - 8	0.98
TCIPP	15.5	1.6	0.29	0.5 - 10	0.98	21.7	2.2	0.23	0.5 - 10	0.96
TDCIPP	5.4	0.5	2.9	4 - 22	0.96	7.4	0.7	2.9	4 - 22	0.98

* R^2 is the linearity coefficient. A minimum value of 0.9 combined with a *P*-value < 0.05 was required to express linearity (Niedorf *et al.*, 2008).

Table 3: Exposure parameters (USEPA, 2011) and estimated dermal exposure (ng/kg bw.day) of UK adults and toddlers to the studied PFRs via contact with indoor dust.

Parameter	Adult			Toddler		
Age	>18 years			2-3 years		
Body weight	70 kg			15 kg		
Body surface area	1.94 m^2			0.6 m^2		
Skin surface exposed	4615 cm^2 (head, forearms, hands and feet)			2564 cm^2 (head, extremities including hands and feet)		
Dust adhered to skin	0.01 mg/cm^2			0.04 mg/cm^2		
Indoor exposure fraction (Abdallah *et al.*, 2008)						
House	63.8%			63.8%		
Office	22.3%			-		
Classroom	-			22.3%		
Car	4.1%			4.1%		
Dermal exposure scenario	**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

Figures

Figure 1: Distribution of the studied PFRs applied as finite dose (500 ng/cm^2) to (a) *ex vivo* human skin and (b) EPISKIN™ tissues following 24 h exposure. Error bars represent one standard deviation (n=3).

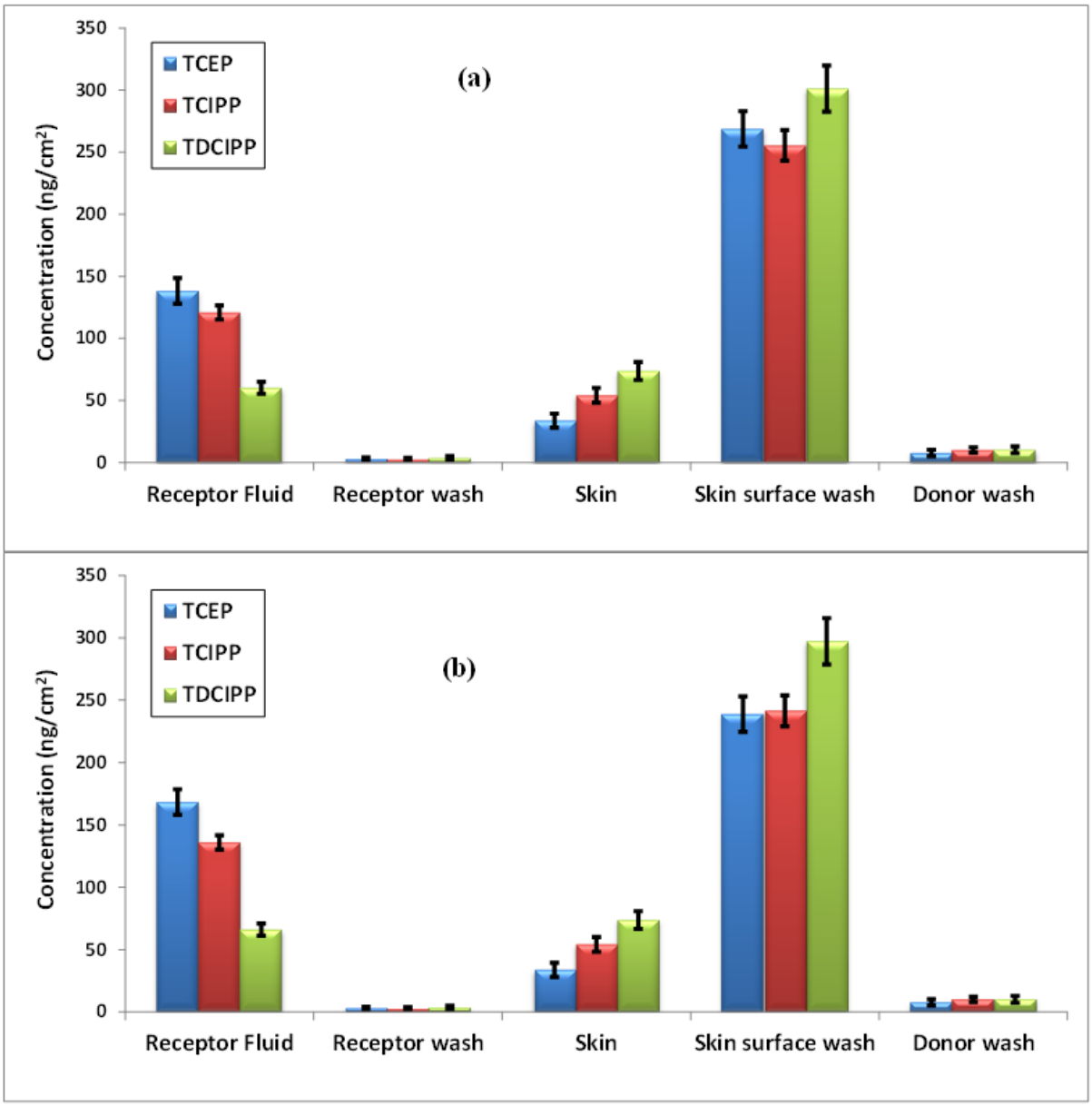

Figure 2: Cumulative absorbed dose of the target PFRs following 24 h exposure of (a) human *ex vivo* skin and (b) EPISKIN™ to 1000 ng/cm^2 of the tested compounds (infinite dose).

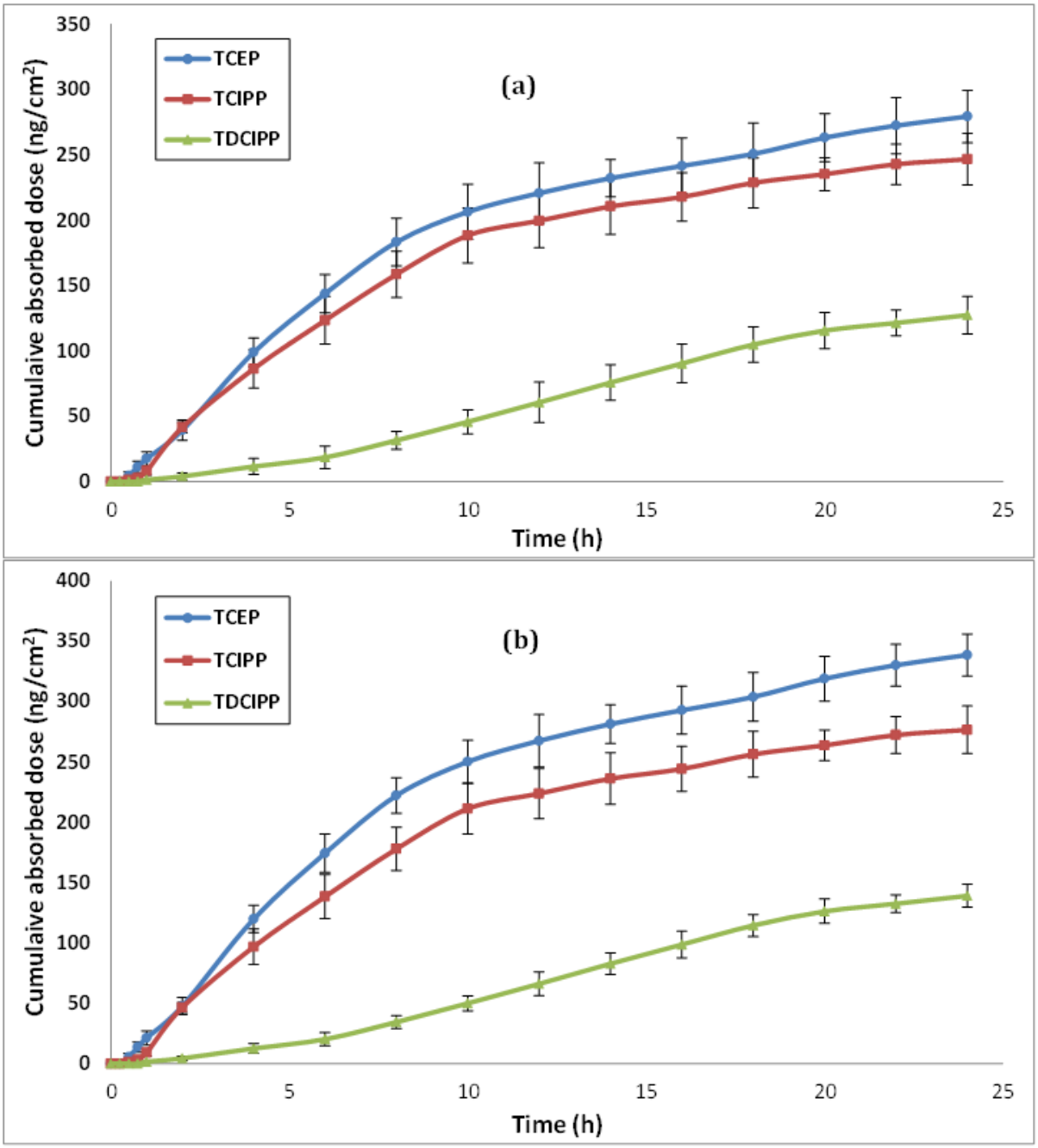

Figure 3: Cumulative absorbed dose of (a) TCEP, (b) TCIPP and (c) TDCIPP applied to *ex vivo* human skin at 500 ng/cm^2 each (finite dose). The skin surface in 3 cells was washed with detergent after 6 h (red line), while the other 3 cells were not washed (blue line).

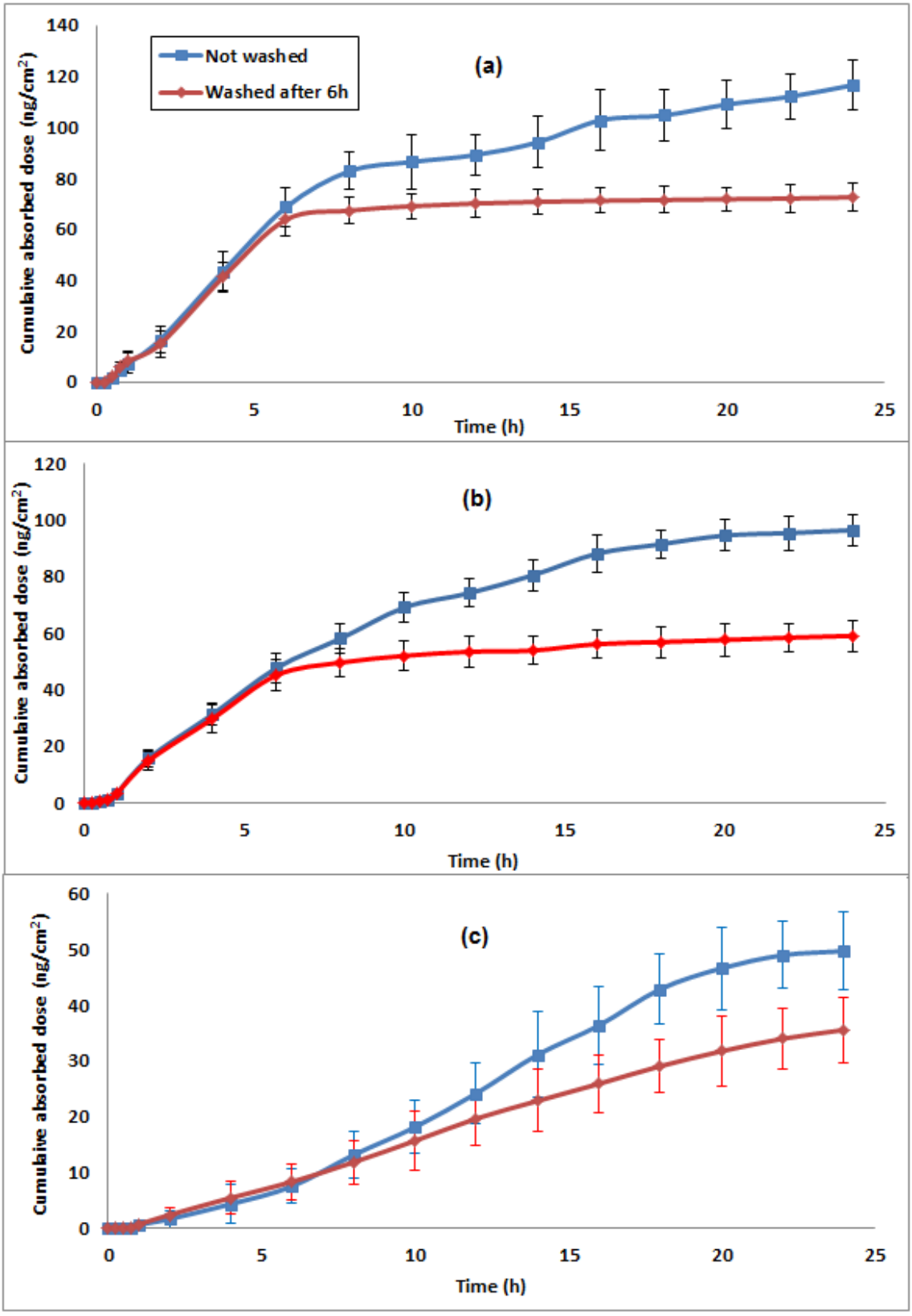

Figure 4: Distribution of (a) TCEP, (b) TCIPP and (c) TDCIPP following 24 h exposure of human *ex vivo* skin to 500 ng/cm^2 of each compound in (i) acetone and (ii) 20% Tween 80 solution in water. Error bars represent one standard deviation (n=3).