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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.

1. 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 t in 2004 to 91,000 t 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 t in 2005 and 2010, respectively. The annual yield of PFRs reached ~70,000 t 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, 2014; Leonards et al., 2011; 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

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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 (Fig. 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.

2. 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.

2.1. 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).

2.2. Test matrices

2.2.1. 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.

2.2.2. 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 13th 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.

2.3. 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 transepidermal 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