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Estimation of human percutaneous bioavailability for two novel brominated flame retardants, 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP)

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ABSTRACT

2-Ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl)tetrabromophthalate (BEH-TEBP) are novel brominated flame retardants used in consumer products. A parallelogram approach was used to predict human dermal absorption and flux for EH-TBB and BEH-TEBP. [^{14}C]-EH-TBB or [^{14}C]-BEH-TEBP was applied to human or rat skin at 100 nmol/cm^2 using a flow-through system. Intact rats received analogous dermal doses. Treated skin was washed and tape-stripped to remove "unabsorbed" [^{14}C]-radioactivity after continuous exposure (24 h). "Absorbed" was quantified using dermally retained [^{14}C]-radioactivity; "penetrated" was calculated based on [^{14}C]-radioactivity in media (*in vitro*) or excreta + tissues (*in vivo*). Human skin absorbed EH-TBB (24 ± 1%) while 0.2 ± 0.1% penetrated skin. Rat skin absorbed more (51 ± 10%) and was more permeable (2 ± 0.5%) to EH-TBB *in vitro*; maximal EH-TBB flux was 11 ± 7 and 102 ± 24 pmol-eq/cm^2/h for human and rat skin, respectively. *In vivo*, 27 ± 5% was absorbed and 13% reached systemic circulation after 24 h (maximum flux was 464 ± 65 pmol-eq/cm^2/h). BEH-TEBP *in vitro* penetrance was minimal (<0.01%) for rat or human skin. BEH-TEBP absorption was 12 ± 11% for human skin and 41 ± 3% for rat skin. *In vivo*, total absorption was 27 ± 9%; 1.2% reached systemic circulation. *In vitro* maximal BEH-TEBP flux was 0.3 ± 0.2 and 1 ± 0.3 pmol-eq/cm^2/h for human and rat skin; *in vivo* maximum flux for rat skin was 16 ± 7 pmol-eq/cm^2/h. EH-TBB was metabolized in rat and human skin to tetrabromobenzoic acid. BEH-TEBP-derived [^{14}C]-radioactivity in the perfusion media could not be characterized. <1% of the dose of EH-TBB and BEH-TEHP is estimated to reach the systemic circulation following human dermal exposure under the conditions tested.
Chemical compounds studied in this article: 2-Ethylhexyl 2,3,4,5-tetrabromobenzoate (PubChem CID: 71316600; CAS No. 183658-27-7 FW: 549.92 g/mol logP$_{est}$: 7.73–8.75 (12)) Abdallah et al., 2015a. Other published abbreviations for 2-ethylhexyl-2,3,4,5-tetrabromobenzoate are TBB EHTeBB or EHTBB Abdallah and Harrad, 2011.
bis(2-ethylhexyl) tetrabromophthalate (PubChem CID: 117291; CAS No. 26040-51-7 FW: 706.14 g/mol logP$_{est}$: 9.48-11.95 (12)). Other published abbreviations for bis(2-ethylhexyl)tetrabromophthalate are TeBrDEPH TBPH or BEHTBP.

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1. Introduction

Flame retardant (FR) chemicals are added to consumer products and building materials to decrease the risk of fire Stapleton et al., 2012. However, FRs are also environmental pollutants, especially when incorporated into products as additive agents Ali et al., 2011a; Ali et al., 2011b; Ali et al., 2012; Api et al., 2013. After decades of consumer use it was concluded that pentabrominated diphenyl ether mixtures (pentaBDE), primarily used as FRs in polyurethane foams, bioaccumulate and have undesirable toxicity profiles with evidence for thyroid, liver, neurological, and reproductive toxicities, and cancer endpoints Api et al., 2013; Bearr et al., 2010; Bergman et al., 2012; Birnbaum and Staskal, 2004; Boireau-Adamezyk et al., 2014; Boyce et al., 2009; Boyce et al., 2009; Bronaugh and Stewart, 1985. As such, pentaBDE (and octaBDE) formulations were voluntarily withdrawn from the US marketplace by their manufacturers at the end of 2004 while decaBDE formulations were withdrawn in 2013 Butt et al., 2014. This restriction on the use of pentaBDE has resulted in the utilization of novel brominated FRs as replacements. Penta- and octaBDE congeners are included under the United Nations Environmental Programme (UNEP) Persistent Organic Pollutants (POPs) list Butt et al., 2016. As a result, polyurethane foam

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for soft furnishings produced after 2004 contains a mixture of brominated and chlorinated FRs, including tris(1,3-dichloro-2-propyl) phosphate (TDCPP; "chlorinated tris"), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EH-TBB), and bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP), among others Abdallah et al., 2015b; Carignan et al., 2013. EH-TBB and BEH-TEBP are used in couch foam and baby products (mattresses and high-chair foam). In addition, BEH-TEBP is used as a FR or plasticizer in polyurethane foams, flexible polyvinyl chloride, adhesives, carpet backing, fabric coating, film and sheeting, wire and cable insulation, and wall coverings while the only known application for EH-TBB is in polyurethane foam.

EH-TBB and BEH-TEBP have been found in dust collected in the US, Europe, Oceania, and Asia, indicative of the global distribution of FR foams in consumer products Chemtura, 2016; Chen et al., 2009; Covaci et al., 2011; Davis et al., 2012; de Wit, 2002; Demierre et al., 2012; EFSA, 2012. In addition to household and office dust, EH-TBB and BEH-TEBP are found worldwide in outdoor dust, sediment, and wildlife Abdallah et al., 2015b; Ali et al., 2011a; Chemtura, 2016; Chen et al., 2009; Eilstein et al., 2015; Escobar-Chavez et al., 2008; Fang and Stapleton, 2014; Fluhr et al., 2012. In studies of the Great Lakes atmosphere, both chemicals appear to be increasing with calculated doubling times of 3–6 years Franz et al., 2009. Both EH-TBB and BEH-TEBP are slated to undergo a full risk assessment under the Toxic Substances Control Act (TSCA) Work Plan and Action Plan Frasch et al., 2014. US national production volume for BEH-TEBP in 2012 was 1,000,000–10,000,000 lb./yr. Neither EH-TBB production and import volumes to the US, nor international production volumes are publically available Frederiksen et al., 2016. However, EH-TBB is not listed in the US EPA High Production Volume Information System, indicating its US production and import volumes are less than the threshold of "1 million pounds or more per year". Exact global production volumes for EH-TBB and BEH-TEBP are unavailable; conservative estimates for total novel BFR production is 100,000 tons/year Fromme et al., 2014; Fujiwara et al., 2014. Both EH-TBB and BEH-TEBP have low vapor pressures, high lipophilicity (estimated log P of 7.73–8.75 and 9.48–11.95, respectively (1, 2)), as well as high persistence and bioaccumulation characteristics Chen et al., 2009; Franz et al., 2009. Toxicity profiles for both chemicals are poorly described Fujiwara et al., 2014; Gomes et al., 2016.

Several studies have detected EH-TBB, BEH-TEBP, or their metabolites in human samples Harju et al., 2009; Hays and Pyatt, 2006. Precise routes of exposure are unclear but ingestion and inhalation of FRs in dust has been well documented Hoffman et al., 2014; Hughes and Edwards, 2010; Hughes et al., 2001; Imai et al., 2015. In addition, dermal contact with FRs has been associated with systemic exposures Ali et al., 2011a. Unfortunately, few studies have investigated the role of dermal uptake despite repeated demonstration of strong positive correlations between FR levels in the indoor environment (*e.g.*, dust), on human skin (hand wipe collections), and in the bodies of adults and children (serum concentrations) Ali et al., 2011a; Fang & Stapleton, 2014; Jakasa and Kezic, 2008. Dermal bioavailability of legacy brominated flame retardants (*i.e.*, BDEs) in humans has been investigated Johnson et al., 2013; Jung and Maibach, 2015;King et al., 2013 but very little is known about the dermal disposition of novel brominated flame retardants King et al., 2013.

Previous disposition studies investigating EH-TBB and/or BEH-TEBP alone or in commercial preparations (Firemaster 550, Firemaster BZ-54, Uniplex FRP-45), in mammals Kissel, 2011; Klosterhaus et al., 2012; Knudsen et al., 2014 or fish Knudsen et al., 2015, found EH-TBB was more readily absorbed from the gut and excreted as metabolite(s) while BEH-TEBP was less likely to be absorbed but was more likely to bioaccumulate in liver and other organs after repeated administration. Disposition of newer formulations that contain EH-TBB and BEH-TEBP (*e.g.*, Firemaster 600 Knudsen et al., 2016a) have not been tested.

Here, *in vivo* studies were conducted using female Sprague Dawley (SD) rats and *in vitro* studies were conducted using split-thickness skin (*i.e.*., epidermis and upper portion of the dermis) from human donors and female SD rats exposed to 100 nmol/cm^2 radiolabeled EH-TBB or BEH-TEBP. This dose was selected based on expert opinion Knudsen et al., 2016b and the need to apply enough [^{14}C]-radioactivity to detect the chemicals in the receptor fluid or excreta. Following 24 h exposure, the treated skin was washed and tape stripped. For these studies, the term 'absorbed' is used to describe the portion of the applied dose found within the skin and tape strips. Tape stripping may not be sufficient to completely remove the human stratum corneum Kullak-Ublick et al., 2001, but to provide a conservative estimate for potential bioavailability, chemical recovered in tape strips was included in the 'absorbed' fractions calculations. Similarly, although dose retained within skin ('absorbed') may ultimately be removed by normal desquamation and never reach the bloodstream, amounts recovered in the 'absorbed' fraction were included in the estimations of bioavailability in an effort to provide conservative estimates for uptake. In descriptions of *in vitro* experiments, 'penetrated' is used to describe chemical that has completely diffused through the skin into the underlying fluid (termed 'receptor fluid or perfusion media'), analogous to the amount reaching systemic circulation following *in vivo* exposure La Guardia et al., 2012; Lehman et al., 2011. The sum of excreted and retained [^{14}C]-radioactivity in tissues outside the dosed skin was used to determine the total penetrated fraction *in vivo*. The values for penetration were used to estimate bioavailability and flux for EH-TBB and BEH-TEBP. Finally, the sum of 'absorbed' and 'penetrated' and the absorptive flux calculated for each model.

2. Methods & materials

2.1. Chemicals

[^{14}C]-labeled EH-TBB and BEH-TEBP were custom synthesized by Moravek Biochemicals (Brea, *CA*) with the carboxyl carbon radiolabeled (Fig. 1). [^{14}C]-EH-TBB (Lot # 256-063-055-A-20130423-DJI) had a radiochemical purity of 99.4% (specific activity = 55 mCi/mmol). [^{14}C]-BEH-TEBP (Lot # 256-061-0605-A-20130419-DJI) had a radiochemical purity of 99.9% (specific activity = 60.5 mCi/mmol). Radiochemical purity was confirmed by radio-HPLC using the methods described below (Fig. 3(A) and Fig. 4(A), respectively). Both chemicals had a chemical purity of >99%, as compared to their respective reference standard (Accustandard, New Haven, CT). 2,3,4,5-Tetrabromobenzoic acid (TBBA; >98% pure) was purchased from the Duke University Small Molecule Synthesis Facility (Durham, NC). Scintillation cocktails were obtained from MP Biomedicals (Ecolume; Santa Ana, CA), Perkin-Elmer (Ultima Gold & PermaFluor E+; Torrance, CA), or Lablogic Inc., (Flow Logic U; Brandon, FL). All other reagents used in these studies were high performance liquid chromatography (HPLC) or analytical grade. Chemical structures were drawn using ACD/Labs Chemsketch (Advanced Chemistry Development, Inc., Toronto, Canada).

2.2. Flux calculation

Maximal flux (J_{ss}) was calculated for both *in vitro* and *in vivo* studies using the method described by Hughes et al. Liu et al., 2016 and derived from Fick's first law of diffusion Ma et al., 2012. Mass was calculated from the amounts of chemical recovered in media (*in vitro*) or in excreta (*in vivo*). Briefly, the maximal flux (pmol-eq per square centimeter per hour) was derived from the slopes of the penetrated mass across each barrier plotted *versus* sampling time period (Eq. (1)). The experimental duration was expected to be insufficient to produce significant depletion of the applied chemical, *i.e.*, flux was not dose-limited.

Estimation of percutaneous flux.

$$J = \frac{\Delta m}{\Delta t A} \quad (1)$$