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The biological fate of decabromodiphenyl ethane following oral, dermal or intravenous administration

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Abstract

1. The disposition of decabromodiphenyl ethane (DBDPE) was investigated based on concerns over its structural similarities to decaBDE, high potential for environmental persistence & bioaccumulation, and high production volume.
2. In the present study, female Sprague Dawley rats were administered a single dose of [¹⁴C]-DBDPE by oral, topical, or IV routes. Another set of rats were administered 10 daily oral doses of [¹⁴C]-DBDPE. Male B6C3F1/Tac mice were administered a single oral dose.
3. DBDPE was poorly absorbed following oral dosing, with 95% of administered [¹⁴C]-radioactivity recovered in the feces, 1% recovered in the urine and less than 3% in the tissues at 72 h. DBDPE excretion was similar in male mice and female rats. Accumulation of [¹⁴C]-DBDPE was observed in liver and the adrenal gland after 10 daily oral doses.
4. The dermis was a depot for dermally applied DBDPE; conservative estimates predict ~14±8% of DBDPE may be absorbed into human skin *in vivo*; ~7±4% of the parent chemical is expected to reach systemic circulation following continuous exposure (24 h).
5. Following intravenous administration, 6% of the dose was recovered in urine and 28% in the feces, while ~70% of the dose remained in tissues after 72h, with the highest concentrations found in the liver (42%) and lung (17%).

Keywords

ADME; bioaccumulation; brominated flame retardant; persistent organic pollutant; lipophilic

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Declaration of interests

The authors report no declarations of interest.

Introduction

Decabromodiphenyl ethane (DBDPE, Bis[pentabromophenyl] ethane, CAS: 84852-53-9, MW: 971.2 g/mol) is an additive brominated flame retardant (BFR) used in products marketed under the Saytex® 8010 and Firemaster® 2100 trade names. DBDPE has a similar structure to decabrominodiphenyl ether (decaBDE, BDE-209) and is marketed as an alternative BFR to be used in place of decaBDE (Vorkamp and Riget, 2014). Reported US import and production of DBDPE exceeded 700,000 lb. in 2011 although four out of five reporting companies withheld or classified their import or manufacture volumes as “confidential business information” (USEPA, 2016a). Reported production in China was 11,000 tons in 2006 (Hu et al., 2010) while European imports were estimated to be between 1 and 5,000 tons in 2001 (Covaci et al., 2011). DBDPE has been found in indoor air, sewage sludge, sediment, fish, and worms (Kierkegaard et al., 2004, Law et al., 2006, Zhang et al., 2013). In addition, the use of DBDPE in electronics casings has increased the exposure to DBDPE in electronic waste (Shi et al., 2009). Reported median levels of DBDPE in house dust sampled worldwide ranged from 0.01 nmol/g in Pakistan (Ali et al., 2012) to 2.8 nmol/g in China (Wang et al., 2010b). Estimated maximum intake via dust exposure might be expected to be 0.1–35 pmol/kg/d for adults and 2–807 pmol/kg/d for toddlers (Harrad et al., 2008). Exposures may be even higher as elevated surface dust tends to contain higher concentrations of BFRs than floor dust, the matrix most often sampled (Al-Omran and Harrad, 2016).

DecaBDE exhibits less bioavailability and potential toxicity than lower brominated polybrominated diphenyl ether (PBDE) flame retardants. However, it remains a part of the highly regulated polybrominated diphenyl ether (PBDE) chemical group and concerns remain over possible health effects, including the debromination of decaBDE *in vivo* (el Dareer et al., 1987). DecaBDE was partially absorbed and readily eliminated following oral administration in Sprague Dawley rats (Sandholm et al., 2003, Morck et al., 2003). Like decaBDE, DBDPE is considered less toxic than lower brominated flame retardants, but has been linked to oxidative stress in freshwater fish (Feng et al., 2013). With its similar structure and physico-chemical characteristics, DBDPE is expected to have similar disposition as decaBDE. With a high production volume, along with a high molecular weight and low water solubility (estimated K_{ow} : 13.62 (USEPA, 2016b)) comparable to that of decaBDE, DBDPE is also suspected to exhibit similar environmental accumulation, persistence, and potential toxicity upon debromination (He et al., 2012, La Guardia et al., 2012, Wang et al., 2010a).

Previous disposition studies of DBDPE have focused on high dose exposures (Hardy et al., 2002, Hardy et al., 2010, Wang et al., 2010a). However, the effects seen at high doses may not accurately reflect disposition of DBDPE after much lower occupational or environmental exposures. In the present study, DBDPE was administered orally, dermally, or intravenously at lower levels than previously published. Doses were administered to female Sprague Dawley rats to determine the extent of its absorption, distribution, metabolism, and elimination in a mammalian model. An exploratory assessment of possible species- or sex-related differences in the disposition of DBDPE was performed using male B6C3F1/Tac

mice in a prior paradigm (Buchanan et al., 1997). In addition, *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 DBDPE. For these studies, the term ‘absorbed’ is used to describe the portion of the applied dose found within the skin, ‘penetrated’ is used to describe chemical that has completely diffused through the skin into the underlying media, analogous to the amount reaching systemic circulation following *in vivo* exposure (Demierre et al., 2012, Knudsen et al., 2015). 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 absorption and penetration were combined to estimate dermal bioavailability for DBDPE.

Materials and Methods

Chemicals

[^{14}C]-labeled DBDPE (1,2-Bis(pentabromophenyl)ethane, [phenyl- $^{14}\text{C}(\text{U})$]-) was custom synthesized by Moravek Biochemicals (Brea, CA; Lot 268-009-026-A-20131024-ZXI; Figure 1). [^{14}C]-DBDPE had radiochemical purity of 97.6%, as determined by radiochemical HPLC, with a specific activity of 26 mCi/mmol. [^{14}C]-DBDPE was diluted in toluene to a stock concentration of 0.1 mCi/mL. Food-grade corn oil (Mazola, ACH Food Companies, Inc., Memphis, TN) was purchased from a local grocery store. 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 ultra-high performance liquid chromatography (UPLC), HPLC, or analytical grade.

Test models

Female Sprague Dawley Rats (SD; 10 weeks, ~ 200 g; Harlan Laboratories, Raleigh, NC) or male B6C3F1/Tac mice (10 weeks; ~20 g; Taconic Farms, Germantown, NY) were dosed with [^{14}C]-DBDPE by gavage orally (4 ml/kg) at a concentration of 100 nmol/kg. Rats were also administered 0.1 this same dose intravenously (1 ml/kg). SD rat skin (*in vivo* and *in vitro*, [Harlan Bioproducts for Science, Indianapolis, IN, USA]) or human skin (*in vitro*, [National Disease Research Interchange, Philadelphia, PA, USA]) were used for single dermal dose studies. Animals were maintained in an AAALAC-approved animal care facility (humidity: ~49%, room temperature: ~72°F, 12 h light/dark cycle). Animals were acclimated for 1 week prior to oral administration or 24 h prior to IV dosing. Food (NIH #31, Zeigler Brothers, Gardners, PA) and water (City of Durham, NC) were provided for *ad libitum* consumption. Immediately following dosing, animals were housed individually in metabolism cages for collection of urine and feces. All procedures were approved by the NIEHS Institutional Care and Use committee.

Oral dosing studies

A single dose was administered orally at a concentration of 100 nmol/kg (0.02 mg/kg; N=4) and tissue distribution was determined 72 h post-dose. DBDPE bioaccumulation potential in female SD rats was assessed by examining [^{14}C]-radioactivity recoveries in excreta and tissues collected 24 h after 10 daily oral administrations of DBDPE (100 nmol/kg/day, N=4).

Male mice were administered a single dose of DBDPE (100 nmol/kg, N=4) by gavage to determine 72 h elimination and tissue disposition. Doses provided approximately 6 $\mu\text{Ci/kg}$ of [^{14}C]-DBDPE. Dosing solutions were prepared by adding appropriate volumes of [^{14}C]-DBDPE dissolved in toluene to corn oil at a dose volume of 4 mL/kg. Toluene was removed by evaporation under a steady stream of nitrogen. Oral doses were administered using a #16 and #20 ball-tipped stainless steel feeding needle to rats and mice, respectively.

Dermal dosing studies

Dermal studies were conducted as described previously (Knudsen et al., 2015). A single dermal dose of [^{14}C]-DBDPE (5 μL , 2.7 nmol/cm²) dissolved in toluene was applied to dorsal scapular skin and samples were collected for 24 h. Toluene was used as a vehicle due to limited solubility of the test chemical in more traditional solvents such as ethanol or acetone in this laboratory. The small dose volume combined with the rapid volatilization of the solvent (dosing cells and skin *in vivo* were open to the air and performed in a fume hood) likely minimized physiological effects (e.g., skin delipidation by toluene). For *in vivo* studies, dorsal scapular region of anaesthetized (isoflurane) SD rats was clipped 24 h prior to dosing. On the day of dosing, rats were anaesthetized with isoflurane, administered a single dose (approx. 5 μL) on the dorsal scapular surface, dosing area covered using a perforated steel cap, and rats were placed in plastic metabolism cages with free access to food and water. For *in vitro* studies, a single dose of 2.7 nmol/cm² of [^{14}C]-labeled DBDPE was administered to split-thickness rat skin (back, n=4 with 3 replicates) or human skin (back, n=3 with 4 replicates; 2 male, 1 female, ages: 67–74 years) with thicknesses of 322 ± 32 and 387 ± 38 μm for rat and human skin, respectively. After application of DBDPE, solvent evaporation was facilitated by gentle airflow over the dosing area and samples remained open to the air for the duration of the studies.

The *in vitro* absorption and penetration of [^{14}C]-labeled DBDPE was determined using a flow-through system with receptor fluid collections at 6 hour intervals for 24 hours. Skin samples were then washed in triplicate (500 μL , 1:1 mixture of Joy® liquid soap: water), and dried for 24 hours. The flow-through system cells were rinsed in triplicate with 500 μL of ethanol. After drying, skin samples were tape stripped ten times to remove the *stratum corneum* and chemically solubilized (Soluene 350, PerkinElmer). The amounts of [^{14}C]-labeled radioactivity in dissolved skin and tapes strips (maximum absorbed dose) and [^{14}C]-labeled radioactivity in collected receptor fluid fractions (penetrated dose) were counted by liquid scintillation spectrometry as the percentage of dose absorbed and penetrated, respectively. Any [^{14}C]-labeled radioactivity recovered in the washes were recorded as unabsorbed dose. Dosing of human skin samples followed the same procedure as rat skin but the human skins were subjected to a tritiated water test before loading of [^{14}C]-labeled DBDPE to determine water permeability of the samples. Penetration of <0.05% of applied radioactivity ([^3H]-H₂O, 1 μCi , 100 μL ; Perkin Elmer, Waltham, MA, USA) was indicative of an intact barrier and the human skin sample was used in the study.

The parallelogram approach to the dermal exposure assessments was used to estimate bioavailability following *in vivo* human systemic exposure to dermally-applied DBDPE (Equation 1) as described previously (van Ravenzwaay and Leibold, 2004). Briefly, *in vivo*

human bioavailability was estimated as a function of *in vitro* human exposure multiplied by a normalization factor based on the same dose applied to rat skin *in vivo* and *in vitro*. Maximal flux (J_{ss}) was calculated as described by Hughes et al. (Hughes et al., 2001) and derived from Fick's first law of diffusion (Niedorf et al., 2008). 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 (Equation 2). Flux was not dose-limited, as the experimental duration was expected to be insufficient to produce significant depletion of the applied DBDPE. Error was calculated as described previously (Caldwell and Vahidsafa, 2015).

IV dosing studies

To determine the fate of systemically available DBDPE, a single intravenous (IV) bolus was injected into the rat lateral tail vein (100 nmol/kg, N=4). For IV dosing, [^{14}C]-DBDPE was added to Cremophore EL[®] with toluene removed by nitrogen evaporation followed by the addition of 1 part ethanol and 3 parts water. Final concentrations of administered [^{14}C]-radioactivity were determined by assaying aliquots of dosing solution in triplicate by liquid scintillation spectrometry at the time of dosing using a LS6500 Multi-Purpose Scintillation Counter (Beckman Coulter, Waltham, MA).

Sample Collections

Following administration of the compound, excreta and cage rinses were collected at 4, 8, 12, 24, 48, and 72 h in single-dose studies and at 24 h intervals in dermal and oral repeat-dose studies. Initial studies included the collection of [^{14}C]-radioactivity in expired air, either as volatile organic compounds (VOC) or $^{14}\text{CO}_2$, using the method described by Sanders et al. (2000) at 1, 4, 8, and 24 h. Euthanasia was realized by CO_2 asphyxiation. Tissues (adipose, adrenals, brain, heart, kidneys, large intestine and contents, liver, lung, muscle, pancreas, ovaries [testes in male animals], skin, small intestine and contents, spleen, stomach and contents, thymus, thyroid, urinary bladder, and uterus) were dissected and placed in labeled pre-weighed vials. Blood samples were collected into heparinized syringes via cardiac puncture immediately following euthanasia. A pilot study was conducted where bile samples were collected from rats administered DBDPE by gavage via an indwelling common bile-duct cannula at 1 h intervals up to 6 h post-dose (N=2). Samples were placed in labeled pre-weighed vials after all collections and maintained at -80°C until analyses. Bile samples ($\sim 100\ \mu\text{L}$) were diluted with 1 mL water prior to analyses. Total mass of incompletely collected tissues were calculated as a fraction of body weight (adipose: 11%, muscle: 50%, skin: 16%, blood: 8%) using previously published values (Birnbaum et al., 1980). All other tissues were completely dissected and their weight determined gravimetrically. Samples were maintained at -80°C until further analyses.

Analytical methods

Samples were analyzed in parallel for quantitative and qualitative differences. Quantitative analyses of total [^{14}C]-radioactivity content was determined using a Beckman Coulter LS6500 Multi-Purpose Scintillation Counter. Total [^{14}C]-radioactivity content of plasma (50 μL), urine (10–100 μL) and cage rinses (1 mL) was assayed in triplicate by liquid scintillation counting. Triplicate aliquots of whole blood were prepared and [^{14}C]-

radioactivity was quantified by combustion in a Packard 307 Biological Sample Oxidizer followed by LSC counting. Fecal samples were dried in a fume hood, weighed and ground to a powder using a mortar and pestle. Triplicate aliquots of feces and tissues (~25 mg) were weighed and [^{14}C]-radioactivity was quantified by combustion. [^{14}C]-radioactivity values for urine and cage rinses were combined for reporting purposes.

DBDPE in extracts of tissues and feces was quantified by UV/Vis absorbance and radiochemical detection following HPLC separation (HPLC/FSA). The HPLC system used for analysis was composed of a Waters (Milford, MA, USA) 2695 Separations Module, Agilent (Santa Clara, CA, USA) Eclipse Plus C18 column (3.5 μm , 4.6 mm $\times$ 150 mm), and a Waters 996 Photodiode Array with an in-line Packard Radiomatic 500TR Flow Scintillation Analyzer (FSA). Mobile phases consisted of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). Sample separations were performed using a gradient; initial conditions (50% A:50% B) were changed to 0% A and 100% B over 20 min. The column was returned to initial conditions and allowed to equilibrate for 5 min before re-use. Flow rates were 1 ml/min. Instrument control and analysis software were Empower Pro (Waters Corp., Milford MA) and FLO-ONE for Windows (PerkinElmer, Torrance, CA). Radiochemical flow cell was 500 μL and scintillant flow rate was 2 mL/min. Injection volumes were 100 μL .

Excreta and tissues containing [^{14}C]-radioactivity were sampled and subjected to extractions using a previously described method (Knudsen et al., 2016). Briefly, aliquots (~250 mg) of ground, dried feces or minced tissue samples were placed in labeled pre-weighed glass screw-top tubes and re-suspended in 5 mL toluene and mixed by vortexing for 1 minute. Samples were then centrifuged at $830 \times g$ for 10 min using a Sorvall RC6+ centrifuge (Thermo Scientific, Inc., Waltham, MA) with a SH-3000 swing bucket rotor to sediment solids. This process was repeated twice, with the supernatants transferred and pooled. When recoveries were less than 95% of expected values, the pellet was further extracted with ethyl acetate ($3 \times 5\text{ mL}$) and 1% HCl in 95% ethanol ($3 \times 5\text{ mL}$). At each step, the samples were centrifuged and the supernatants transferred and pooled. Extracts were concentrated to approx. 100 μL using a Savant SPD1010 SpeedVac (Thermo Scientific, Inc., Waltham, MA) without heating, and aliquots were analyzed by UV/radiometric HPLC. Remaining sample pellets were air dried, weighed, and [^{14}C]-radioactivity remaining in the sample (unextracted fraction) was quantified by combustion in a Model 307 Biological Sample Oxidizer (PerkinElmer; Torrance, CA) followed by LSC counting. Fecal extraction efficiencies approached 100%.

Statistical methods

The data were subjected to statistical analysis using paired t-tests or two-way ANOVA followed by the Tukey-Kramer test for pairwise comparisons (Graphpad Prism 6, Version 6.07, Graphpad Software, Inc., La Jolla CA). Values were considered to be significantly different at $p < 0.05$. Linear regressions were generated using Graphpad Prism 6.

Results

Disposition after single gavage

[¹⁴C]-radioactivity recovered in feces exceeded 98% by 72 h after oral dosing. Feces contained ~75% of the dose after 24 h when DBDPE was administered at the 0.1 µmol/kg dose level by gavage to female SD rats (Figure 2). Approx. 1% of either DBDPE dose was recovered in urine after oral administration to rats (Table 1). Male B6C3F1/Tac mice were observed to eliminate DBDPE-derived radioactivity more rapidly via the fecal route than female rats. Data from a pilot study using rats with an indwelling bile-duct cannula found ~0.1% of the dose was recovered in bile from animals 6 h post-administration of DBDPE by gavage, which suggests orally administered DBDPE has low absorption and radioactivity recovered in feces represents unabsorbed dose. [¹⁴C]-radioactivity extracted from the feces collected after oral administration co-eluted with the [¹⁴C]-DBDPE standard (Figure 3). Selected tissues were surveyed to determine any compound-specific sites of retention (Table 1). [¹⁴C]-radioactivity retained in tissues collected at 72 h following oral administration of DBDPE was low (~1% of total dose in assayed tissues).

Disposition after repeated gavage

Ten daily oral administrations of [¹⁴C]-DBDPE were used to estimate bioaccumulation potential of the chemical. Similar to single-dose studies, repeated administration of DBDPE resulted in a small amount of the total dose excreted in urine, with the overwhelming majority recovered in feces (Figure 4). Total elimination was determined at 24 h intervals and compared to elimination from a group of animals administered a single dose and euthanized 24 h later (Table 2). Concentrations in adrenal tissue increased approximately 8-fold after 10 doses. Significantly more [¹⁴C]-radioactivity was present in liver after 10 doses (10 ± 2 pmol-eq/g) than after one (0.7 ± 0.6 pmol-eq/g).

Disposition after dermal application

Dermal uptake of DBDPE was tested *in vitro* and *in vivo* using rat or human skin samples. Over a 24 hour period, DBDPE administered to human and rat skin *in vitro*, $4 \pm 1\%$ and $3 \pm 0.5\%$ of the dose penetrated the skin, respectively (Table 3). Recoveries over time showed the dose penetrating the skin appeared to increase only marginally between 18 and 24 h for *in vitro* skin samples while smaller amounts were recovered after *in vivo* dosing although flux rates were similar (Figure 5). The maximum absorbed fractions for human and rat skin *in vitro* were $16 \pm 6\%$ and $27 \pm 3\%$, respectively; dose remaining in the human and rat skin samples after tape stripping amounted to $12 \pm 4\%$ and $17 \pm 2\%$ of the dose, respectively. *In vivo* dermal exposure to DBDPE showed a similar result, with penetrated and absorbed fractions containing similar amounts of [¹⁴C]-radioactivity (5 ± 2 and $17 \pm 10\%$, respectively). Of the small percentage of applied DBDPE that penetrated the skin *in vivo*, most of the dose remained in the tissues, with the greatest proportion attributed to retention in muscle ($3 \pm 2\%$) and adipose tissue ($0.8 \pm 0.4\%$); whole blood and non-dosed skin contained approx. 0.5% of the applied dose. From these studies it was determined that maximal DBDPE flux through rat skin *in vitro* occurred between 0 and 6 h post-application and was 9.5 ± 2.1 pmol-eq/cm²/h. *In vivo*, maximum flux for rat skin was 1.8 ± 0.7 fmol-eq/cm²/h and occurred between 12 and 24 h post dose. Maximal flux for human skin (*in*

vitro) was 6.5 ± 3.5 pmol-eq/cm²/h and was reached between 0 and 6 h. Based on the parallelogram methodology, conservative estimates predict approx. $0.4 \pm 0.3\%$ of DBDPE may be absorbed into human skin *in vivo*; approx. $0.03 \pm 0.02\%$ of the parent chemical is expected to reach systemic circulation after 24 h of continuous exposure. Recoveries from individual fractions collected during and after *in vitro* and *in vivo* studies are shown in Table S1 and Table S2, respectively.

Disposition after IV administration

Recovery in feces reached approx. 34% after IV administration by 72 h post-dose with the remainder of the administered [¹⁴C]-radioactivity recovered in tissues (Figure 2, Table 1). [¹⁴C]-radioactivity that was retained in tissues at 72 h after dosing was primarily found in liver, lung, and spleen (Table 1). The gastrointestinal tract and contents retained an additional 2% of the dose. Lung, spleen, adrenal glands and liver retained the highest concentrations (pmol-eq/g tissue) of [¹⁴C]-radioactivity among the tissues analyzed after IV administration of DBDPE. [¹⁴C]-radioactivity extracted from liver collected at 72 h after IV administration co-eluted with the [¹⁴C]-DBDPE standard (Figure 3).

Discussion

DBDPE is a widespread BFR in the environment, likely due to its high production volume combined with its manufacture and use for over 20 years (Luo et al., 2010, Qi et al., 2014). This study presents the dermal disposition of DBDPE in detail, and compares the oral disposition in female rats and male mice at the lowest unpublished dose to date of this BFR. DBDPE was poorly absorbed, minimally metabolized, and almost exclusively eliminated by the fecal route after oral administration. However, that which was absorbed appeared to accumulate in the liver and adrenal glands, with 3–4% of oral doses recovered in urine, blood, or tissues.

Contrary to previous reports where DBDPE was used as a negative control (Abdallah et al., 2015b), we found that a small amount of DBDPE was able to permeate human and rat skin after 24 h of continuous exposure. Dermal uptake and penetrance were greater than expected when compared to previous dermal disposition studies performed in this laboratory (Knudsen et al., 2015, Knudsen et al., submitted 2016). When maximal flux was calculated, DBDPE flux fell between that of 2-ethylhexyl tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP) and greatly exceeded the estimated flux for decabromodiphenyl ether (Abdallah et al., 2015a). However, at later times when any remaining toluene (used as vehicle) had undoubtedly evaporated, DBDPE accumulation in the media was linear between 6 and 18 h, allowing for the calculation of both a maximal flux and steady state flux rate for both human and rat skin (Abdallah et al., 2015c, Frasch et al., 2014, Hughes et al., 2001).

DBDPE exhibited differential routes of elimination following IV and oral administrations. Spleen, lung, liver and adrenal tissues retained the highest concentrations of [¹⁴C]-radioactivity at 72 h after a single IV dose of DBDPE. Considering the highly lipophilic character of DBDPE, the recovery of [¹⁴C]-labeled radioactivity in the lung and spleen is likely due to distribution of the parent molecule to these highly perfused tissues.

Intravenously administered DBDPE was very slowly excreted, indicating a limited hepatic and renal capacity to metabolize or eliminate the chemical, similar to other highly lipophilic BFR (Knudsen et al., 2007, Knudsen et al., 2016). Systemically available DBDPE (e.g., after IV administration) was partially eliminated in the feces, although a large proportion of the dose was retained in well perfused tissues like spleen, lung and liver. [^{14}C]-labeled radioactivity extracted from the livers of rats follow IV administration found the chemical is retained as parent chemical even 72 h after dosing. However, extraction efficiencies of DBDPE from target tissues were low due to the highly hydrophobic character of DBDPE and its reluctance to dissolve in either aqueous or organic solvents. In addition, after successfully extracting DBDPE from feces, the presence of a cloudy precipitate upon concentration of liver extractions suggested that DBDPE binds to lipids. Studies investigating the bioaccumulation potential of DBDPE in environmental samples have described DBDPE as having a relatively high binding affinity (Li et al., 2014). Taken together, we concluded that the precipitation of DBDPE-protein complexes upon concentration of tissue extractions may also contribute to low extraction efficiencies.

Previous investigations of decaBDE found adrenals were a major depot for retained compound (Morck et al., 2003). Furthermore, Seyer et al. (2010) demonstrated more specifically, this distribution is limited to the adrenal cortex. The extreme lipophilicity and planar structure of DBDPE may also result in its preferential distribution to the adrenal gland. Overall, adipose tissue did not appear to be a major depot for DBDPE, a property shared by decaBDE (Morck et al., 2003). Accumulation of xenobiotics in a variety of tissues has been well documented, but physiologic explanations of tissue-specific affinities are scant. At least two PBDEs, 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) and decabromodiphenyl ether (decaBDE), accumulate in liver, adrenal, and other tissues (Morck et al., 2003, Hakk et al., 2002). Apparent liver bioaccumulation likely results from "slow" hepatic metabolism of parent molecule coupled with low biliary elimination.

The structures of decaBDE and DBDPE are similar to that of PCBs, a class of chemicals that are well-documented as endocrine disruptors (Jansen et al., 1993, Cooke et al., 1996). PCBs and some of their hydroxylated metabolites accumulate in adrenal tissues of marine mammals, inducing hyperplasia of the adrenal cortex (Ross et al., 1996). Rats administered PCB-contaminated fish oil exhibited immunotoxicity consistent with the known deregulation of the immune system by corticosteroids (Ross et al., 1997). Cortical hyperplasia of the adrenal glands may be correlated with an increased production of cortisol, which in turn triggers the down regulation of the immune system. Given the structural similarity between PCBs, decaBDE, and DBDPE, it is noteworthy that decaBDE residues mainly target the outer part of the adrenal cortex (Seyer et al., 2010). Why these residues specifically accumulate in this part of the adrenal glands while, in contrast, the medulla is not a target for decaBDE, remains to be better understood. Along with BDE-99 and decaBDE, another BFR with similar lipophilicity, BEH-TEBP, has also been found to accumulate in adrenal tissue after repeated dosing (Knudsen et al., 2016). Other studies of [^{14}C]-labeled xenobiotics found adrenal extracts co-eluted with cholesteryl ester with evidence for their degradation and incorporation into endogenous substances (Darnerud and Brandt, 1985). Systemically available DBDPE appeared to be primarily eliminated by biliary secretion, which may help explain hepatic bioaccumulation. Localization to the adrenal gland is less intuitive, although

the impact of endocrine disrupting chemicals (like DBDPE and other BFRs) on steroid synthesis may eventually yield an explanation of the phenomenon. Further *in vivo* exploration studies of the adrenal function will be necessary to confirm this hypothesis for DBDPE.

Conclusions

DBDPE was poorly absorbed, minimally metabolized, and almost exclusively eliminated unchanged by the fecal route after a single oral administration to female SD rats or male B6C3F1/Tac mice. This conclusion was supported by a pilot study that found less than 0.1% of a 0.1 $\mu\text{mol/kg}$ oral dose was eliminated in bile over the first 24 h. Repeated DBDPE administration showed similar results, although increases in [^{14}C]-radioactivity concentrations in liver and adrenal tissues were noted after 10 daily doses. A study in male B6C3F1 mice dosed with [^{14}C]-DBDPE by gavage indicate a primary recovery of [^{14}C]-labeled radioactivity in the feces following oral administration, which is similar to elimination in female Sprague Dawley rats. This suggests that DBDPE exhibits no major sex-dependent or species-dependent disposition characteristics. Mice appeared to excrete DBDPE more rapidly via feces, a phenomenon seen with other poorly absorbed FRs (Knudsen et al., 2016). Results from HPLC/FSA analysis of extracted feces collected during the 72 hour orally dosed rat study indicated DBDPE was eliminated unchanged in feces after oral administration to female SD rats or male B6C3F1/Tac mice (data not shown). Rat skin is more permeable than human skin for a number of substances, which may explain the differences in dose penetration (Roper et al., 2006, Api et al., 2013, Banks and Birnbaum, 1991, Ross et al., 2011, van Ravenzwaay and Leibold, 2004). These results indicate that dermal exposure to DBDPE contributes a small portion to the total exposure to DBDPE from the environment. Combined, oral and percutaneous exposures to DBDPE contribute to the low, but widespread, serum and milk concentrations detected in human populations (Zhou et al., 2014, Marnett et al., 2013, Karlsson et al., 2007).

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Figure 1.
Chemical structure of DBDPE; *: [^{14}C]-radiolabel.

Figure 2. Cumulative recovery of $[^{14}\text{C}]$ -radioactivity in excreta following a single administration of DBDPE. N=3–4 per dose group, mean $\pm$ S.D. A: Recovery in feces, B: Recovery in urine (◆: 100 nmol/kg, PO, mouse; ▼: 100 nmol/kg, IV, rat; ▲: 100 nmol/kg, PO, rat).

Figure 3. Representative HPLC-radiochromatograms of [¹⁴C]-radioactivity in excreta & liver following DBDPE administration (100 nmol/kg) to female SD rats. A: [¹⁴C]-DBDPE standard. B: [¹⁴C]-radioactivity in fecal extracts after oral administration. C: [¹⁴C]-radioactivity in liver after IV administration.

Figure 4. Cumulative elimination of $[^{14}\text{C}]$ -radioactivity following repeated administration of DBDPE to female SD rats. N=4 per dose group, mean $\pm$ S.D.

Figure 5.

Cumulative total dose recovered in the receptor fluid (*in vitro*) or excreta (*in vivo*) after a single application of 2.7 nmol/cm² [¹⁴C]-DBDPE to rat (●) or human (■) skin. A: Cumulative dose penetrated (%), B: Penetrance (pmol-eq/cm²). Dashed lines show the linear regression of the penetrance data. Data represents mean ± S.D.; N=4 rat; N=3 human.

Equation 1.

Estimation of human *in vivo* systemic exposure relative to the ratio of animal to human penetration of dermally applied chemicals.

$$\text{Human}_{\text{in vivo}} = (\text{Rat}_{\text{in vivo}} / \text{Rat}_{\text{in vitro}}) \times \text{Human}_{\text{in vitro}}$$

Equation 2.

Estimation of percutaneous flux. Delta m is the difference in accumulated mass in the perfusion media (pmol-eq), delta t is the difference in time (h), A is diffusion area (cm²).

$$J = \frac{\Delta m}{\Delta t A}$$

Table 1

Recovery of administered [^{14}C] radioactivity in excreta and selected tissues at 72 h post dose (%). Data given as mean $\pm$ S.D., N=4 animals per study.

	Dose recovered	PO, mouse	PO Rat	IV Rat
Feces	%	94 $\pm$ 11	95 $\pm$ 2	28 $\pm$ 3 ^a
Urine	%	0.3 $\pm$ 0.1	1.4 $\pm$ 0.3	6 $\pm$ 1 ^a
Blood	%	0.1 $\pm$ 0.1	0.7 $\pm$ 0.2	0.7 $\pm$ 0.2
	(pmol eq/g)	(0.06 $\pm$ 0.05)	(0.2 $\pm$ 0.06)	(0.2 $\pm$ 0.04)
Liver	%	0.03 $\pm$ 0.02	0.07 $\pm$ 0.06	42 $\pm$ 3 ^a
	(pmol eq/g)	(0.7 $\pm$ 0.6)	(0.7 $\pm$ 0.6)	(366 $\pm$ 98) ^{β}
Adipose	%	0.2 $\pm$ 0.2	0.3 $\pm$ 0.3	1.2 $\pm$ 0.7
	(pmol eq/g)	(1.7 $\pm$ 1.6)	(1 $\pm$ 1)	(5 $\pm$ 2)
Adrenal	%	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.1 $\pm$ 0.04
	(pmol eq/g)	(18 $\pm$ 6)	(8 $\pm$ 6)	(92 $\pm$ 0.3)
Muscle	%	0.4 $\pm$ 0.3	0.8 $\pm$ 0.3	6 $\pm$ 8 ^{β}
	(pmol eq/g)	(0.8 $\pm$ 0.6)	(0.8 $\pm$ 0.3)	(3 $\pm$ 2)
Kidney	%	0.01 $\pm$ 0.01	0.3 $\pm$ 0.02	0.3 $\pm$ 0.2
	(pmol eq/g)	(1.4 $\pm$ 1.5)	(1.4 $\pm$ 0.09)	(13 $\pm$ 10)
Skin	%	0.1 $\pm$ 0.1	0.4 $\pm$ 0.2	1.7 $\pm$ 0.3
	(pmol eq/g)	(0.5 $\pm$ 0.7)	(1 $\pm$ 0.7)	(4 $\pm$ 2)
Spleen	%	n.d.	0.01 $\pm$ 0.01	6 $\pm$ 4 ^{β}
	(pmol eq/g)	n.d.	(1 $\pm$ 1)	(4 $\pm$ 2)
Lung	%	0.0004 $\pm$ 0.005	0.02 $\pm$ 0.01	17 $\pm$ 6
	(pmol eq/g)	(0.6 $\pm$ 0.7)	(2 $\pm$ 1)	(1223 $\pm$ 723) ^a
Brain	%	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.02
	(pmol eq/g)	(1 $\pm$ 1)	(1 $\pm$ 1)	(1 $\pm$ 1)

n.d.: not determined

^a: significantly different from rat oral dose, p<0.0001

^{β} : significantly different from rat oral dose, p<0.01

Table 2

[¹⁴C] radioactivity recovered in excreta and select major tissues after 1 or 10 oral administrations of DBDPE (100 nmol/kg/day). Tissues were collected 24 h after the final dose.

	Dose recovered	1 dose	10 doses
Feces	%	94 ± 11	94 ± 8
Urine	%	0.3 ± 0.1	2 ± 0.4
Blood	%	0.1 ± 0.1	0.004 ± 0.003
	(pmol eq/g)	(0.06 ± 0.05)	(0.02 ± 0.01)
Liver	%	0.03 ± 0.02	0.06 ± 0.01
	(pmol eq/g)	(0.7 ± 0.5)	(10 ± 2) ^a
Adipose	%	0.2 ± 0.2	0.2 ± 0.1
	(pmol eq/g)	(2 ± 2)	(11 ± 10)
Adrenal	%	0.01 ± 0.01	0.008 ± 0.002
	(pmol eq/g)	(18 ± 6)	(146 ± 21) ^a
Muscle	%	0.4 ± 0.3	0.01 ± 0.06
	(pmol eq/g)	(0.8 ± 0.6)	(1.6 ± 1)
Kidney	%	0.01 ± 0.01	0.005 ± 0.003
	(pmol eq/g)	(1 ± 1)	(6 ± 3)
Skin	%	0.1 ± 0.1	0.07 ± 0.04
	(pmol eq/g)	(0.5 ± 0.7)	(3 ± 2)
Lung	%	0.0004 ± 0.005	0.003 ± 0.002
	(pmol eq/g)	(0.6 ± 0.7)	(3 ± 2)
Brain	%	0.01 ± 0.01	0.002 ± 0.002
	(pmol eq/g)	(1 ± 1)	(2 ± 2)

Data presented as mean ± S.D., N=4 animals per study.

^a: significantly different from single dose, p< 0.0001

Table 3

[¹⁴C]-radioactivity recovery in various fractions at 24 h post-dose after dermal administration of DBDPE (%).

Species		Human (<i>in vitro</i>)	Rat (<i>in vitro</i>)	Rat (<i>in vivo</i>)
Unabsorbed (%)	Washes	73 ± 9	62 ± 9	62 ± 14
	Cell/cap	3 ± 0.5	4 ± 3	1 ± 1
Absorbed (%)	Tape strips	4 ± 2	10 ± 3	7 ± 1
	Skin	12 ± 4	17 ± 2	17 ± 10
Penetrated (%)		4 ± 1	3 ± 0.5	5 ± 2
Recovery (%)		96 ± 8	96 ± 6	93 ± 3

Human *in vitro*; mean ± S.D., N = 3, assayed in quadruplicate; rat *in vitro*; mean ± S.D., N = 4, assayed in triplicate; rat *in vivo*; mean ± S.D., N = 4.