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Dermal disposition of Tetrabromobisphenol A Bis(2,3-dibromopropyl) ether (TBBPA-BDBPE) using rat and human skin.

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Abstract

Tetrabromobisphenol A Bis(2,3-dibromopropyl) ether (TBBPA-BDBPE) is a high production volume brominated flame retardant (BFR) used in consumer products, resulting in ubiquitous human exposure. Although the major route of exposure for this chemical is believed to be via ingestion, dermal contact is likely via contaminated dust. Independent trials of a single dose of 100 nmol/cm² (~1 μ Ci [¹⁴C]/cm²) of [¹⁴C]-radiolabeled TBBPA-BDBPE was applied to whole rat skin (*in vivo*) or split-thickness human and rat skin (*ex vivo*) to estimate *in vivo* human percutaneous uptake. [¹⁴C]-radioactivity was quantified to determine dermal absorption (dose retained in dosed skin) and penetrance (dose recovered in receptor fluid [*ex vivo*] or tissues/excreta [*in vivo*]) over 24 h. *In vivo* absorption and penetration for rat skin was 26% and 1%, with a maximum flux of 44 $\pm$ 9 pmol/cm²/h. In *ex vivo* rat skin, absorption and penetration and absorption values were 23% and 0.3% (flux = 26 $\pm$ 8 pmol/cm²/h). In *ex vivo* human skin, 53% was absorbed and penetration was 0.2% with a maximal flux of 16 $\pm$ 12 pmol/cm²/h. Computed maximal flux for *in vivo* human skin was 21 $\pm$ 9 pmol/cm²/h with expected total absorption of ~80% and a penetration of <1%. HPLC-radiometric analyses of samples showed that TBBPA-BDBPE was not metabolized in *ex vivo* or *in vivo* studies. These studies indicate that TBBPA-BDBPE is likely to be dermally bioavailable even after washing and dermal contact with this chemical should be considered an important route of exposure.

Graphical Abstract

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Keywords

dermal bioavailability; bioaccumulation; brominated flame retardant; persistent organic pollutant; lipophilic

Introduction

TBBPA-BDBPE uses and biological effects

Tetrabromobisphenol A Bis(2,3-dibromopropyl) ether (TBBPA-BDBPE, CAS No. 21850-44-2, EC No. 244-617-5, Figure 1) is an additive brominated flame retardant and is widely used in polyolefin, high impact polystyrene, acrylobutadiene styrene plastic and other products [1]. As an additive flame retardant rather than reactive flame retardant, it is expected to migrate more readily out of the products it is used in and into dust [2]. US domestic production and import volumes for TBBPA-BDBPE was reported to be between 1,000,000–10,000,000 lb per year in 2011 but data for subsequent years has been withheld for confidential business reasons [3]. Manufacture and/or import into the European Economic Area (European Union, Iceland, Liechtenstein, and Norway) was last reported to be 1,000–10,000 metric tons per year [4]. TBBPA-BDBPE is highly lipophilic (XLogP3= 10.6, Log Kow = 12) and large (943.621 g/mol) [5, 6].

In environmental assessments, TBBPA-BDBPE has been consistently measured in air [7], soil [8], aquatic mesocosms [9, 10], and indoor dust [11–13], demonstrating evidence of consistent use and the likelihood of environmental persistence [7]. In the US, indoor dust was found to contain up to 0.6 nmol/g TBBPA-BDBPE, while air samples from sites around the Great Lakes reported geometric means of 0.2–1.3 fmol/m³ [7, 14]. In Belgium and the UK indoor dust was found to contain up to 11 nmol/g TBBPA-BDBPE [11]. Sampling in China found TBBPA-BDBPE concentrations of 1.3 nmol/g in soil and 2,400 fmol/m³ in air [12]. Differences in soil, dust, and air concentrations likely arise from differences in product use and proximity to manufacturing facilities. TBBPA-BDBPE is a known replacement flame retardant for polybrominated diphenyl ethers (PBDE) which may explain its higher levels in European samples compared to those from the US, likely reflecting the earlier switch to alternative flame retardants following the 2004 ban of penta- and octa-BDE in the European Union [15]. Analysis of testing sites around the Great Lakes region of North America found there is a significant relationship between population density and TBBPA-BDBPE concentrations in air, indicating its release from consumer and household products and subsequent human exposure [7].

TBBPA-BDBPE is used in a wide variety of consumer products which is expected to result in human exposures. As with exposure to other BFRs, biological consequences for human health are expected. However, few studies have investigated the disposition or biological effects of TBBPA-BDBPE. TBBPA-BDBPE has endocrine disrupting potential, as it has been shown to have moderate or high potency for binding to transthyretin (TTR) and the sulfotransferase responsible for conjugation of estradiol (SULT1E1) [16]. When TBBPA-BDBPE was orally administered to male Fischer 344 rats (2 mg/kg), it was poorly absorbed from the gut and had very limited oral bioavailability ($f = 2.2\%$) [17]. From these studies it was deduced that the limited amounts of TBBPA-BDBPE that are absorbed from the gastrointestinal tract are mostly retained in the liver and only slowly excreted in the bile after oral administration.

Dermal uptake of chemicals in dust is a common route of exposure, especially in young children [18] but little is known about the absorption of TBBPA-BDBPE. This is the first study to investigate its dermal disposition using methods described previously [19–21]. *Ex vivo* studies were conducted using skin from the epidermis and upper portion of the dermis (i.e., split-thickness) from human donors and female SD rats.

Materials and Methods

Chemicals

[^{14}C]-labeled TBBPA-BDBPE (ring- ^{14}C (U)-labeled) was custom synthesized by Moravek Biochemicals (Brea, CA; Figure 1). [^{14}C]-TBBPA-BDBPE had radiochemical purity of 98%, as determined by radiochemical HPLC, with a specific activity of 45.2 mCi/mmol. [^{14}C]-TBBPA-BDBPE was diluted in acetone to a stock concentration of 500 $\mu\text{Ci/mL}$. Scintillation cocktails were obtained from MP Biomedicals (Ecolume; Santa Ana, CA), Perkin-Elmer (Ultima Gold, Hionic Fluor & PermaFluor E+; Torrance, CA) or Lablogic Inc. (Flow Logic U; Brandon, FL). All other reagents used in these studies were HPLC or analytical grade.

Model system

Female Sprague Dawley Rats (SD; 11 weeks, ~200 g; Harlan Laboratories, Raleigh, NC) were dosed with [^{14}C]-TBBPA-BDBPE. Animals were maintained in an AAALAC-accredited animal care facility (humidity: ~49%, room temperature: ~72°F, 12 h light/dark cycle). Animals were acclimated to the facility for one week prior to dermal dosing (Techniplast [West Chester, PA, USA] polycarbonate shoebox cages with Sani-Chip bedding [PJ Murphy Forest Products, Montville NJ, USA]). Feed (NIH #31, Zeigler Brothers, Gardners, PA) and tap water (City of Durham, NC) were provided for *ad libitum* consumption. The tap water was treated by reverse-osmosis in the animal facility before use. 24 h prior to dosing, the dorsal scapular region of anaesthetized (isoflurane) SD rats was clipped with an electric hair clipper (Wahl, Sterling, IL) and animals recovered in plastic metabolism cages (Techniplast) under investigator observation. On the day of dosing, rats ($N=4$) were again anaesthetized with isoflurane and administered a single dose (100 nmol/cm², 6 μL applied to 0.6 cm²) on the dorsal scapular surface. The dosing area was covered with a perforated steel cap, which was glued onto the skin using cyanoacrylate

adhesive, and rats were placed back in the plastic metabolism cages for collection of urine and feces at 6, 12, and 24 h and analyzed as described previously [22]. All procedures were approved by the NIEHS Institutional Animal Care and Use Committee. Aliquots of dosing solutions were assayed in triplicate using liquid scintillation counting to determine final concentrations of [^{14}C]-radioactivity (LS6500 Scintillation Counter; Beckman Coulter, Brea, CA) and were subjected to HPLC-radiochemical analysis to confirm test material purity and concentration.

Animals were euthanized by CO₂ inhalation after 24 h. Following euthanasia, blood was collected by cardiac puncture, treated skin was excised, and complete necropsies were performed as described previously [21]. Skin from the application area was treated in accordance with the OECD 427 method for *in vivo* testing of chemicals [23]. Briefly, the skin was washed 3 times using a 1:1 mixture of Joy® liquid soap: water, 6 times using distilled water, and air dried overnight. Dried skin was tape stripped 10 times using clear packing tape (anTech Industries, Inc., Johnson City, TN, USA). Feces were air-dried and ground to a powder using a mortar and pestle. Tissues (including dosed skin) and feces were sampled in triplicate (approximately 25 mg/sample), combusted in a Packard (Waltham, MA, USA) 307 Biological Sample Oxidizer, and [^{14}C]-radioactivity content was quantified by liquid scintillation counting (LSC) analysis. Triplicate samples of urine, cage washes, skin swabs, and tape strips were analyzed by direct LSC. [^{14}C]-radioactivity in cage washes and urine were combined in the summary urinary recoveries.

Ex vivo absorption and penetration of [^{14}C]-labeled TBBPA-BDBPE through SD rat dorsal skin (Harlan Bioproducts for Science, Indianapolis, IN, USA, hair clipped 24 h prior to excision, N=4, female, 11 weeks old) or human dorsal scapular skin (National Disease Research Interchange, Philadelphia, PA, USA, N=3, 1 male, 2 females, 67–87 years old) was determined using a Bronaugh flow-through system. Previous studies using similar methods refer to these studies as ‘*in vitro*’ assays but given the tissues in use are derived from a living individual and not cell culture, it is more accurate to use the term ‘*ex vivo*’ to describe the assays. Rat and human skin were stored at –80° until used in the experiment. The skins were thawed to room temperature before processing. single dose of 100 nmol/cm² of [^{14}C]-labeled TBBPA-BDBPE (acetone vehicle, 6.4 μL , 0.64 cm²) was applied to split-thickness female SD rat skin (back, n=4 $\times$ 3 replicates, 11 weeks) or human skin (back, n=3 $\times$ 4 replicates) with thicknesses of 337 $\pm$ 40 and 386 $\pm$ 31 μm for rat and human skin, respectively. After dosing, perfusion media was collected for 24 h at 6 h intervals. Perfusion media was composed of HEPES-buffered Hanks’ balanced salt solution, pH 7.4, with 10% fetal bovine serum. This fluid has been shown to maintain the viability of skin for up to 24 h [24]. The chemical composition of the receptor fluid included sodium chloride, potassium chloride, calcium chloride (dihydrate), magnesium sulfate (heptahydrate), dextrose, sodium bicarbonate, and sodium hydroxide (Sigma, St. Louis, MO, USA). Additional components were N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid, sodium salt) (HEPES), fetal bovine serum, and gentamicin sulfate (GIBCO, Grand Island, NY, USA), disodium hydrogen phosphate (Scientific Products, McGraw Park, IL, USA) and potassium dihydrogen phosphate (Matheson Coleman and Bell, Norwood, OH, USA). The receptor fluid was prepared with distilled water and sterilized by filtration (0.2 μm filter, Nalgene disposable filterware, Sybron Corp., Rochester, NY, USA). 10% solution (v/v) of fetal

bovine serum was prepared with the sterile receptor fluid. The receptor fluid was continuously gassed with 100% oxygen throughout the experiment. Dosing of human skin samples followed the same procedure as those described for rat skin, except for the addition of a tritiated water test (5 min exposure, 1 h perfusion media collection) to determine water permeability of the samples. This test gives an indication of the barrier integrity of the skin. Penetrance of <0.05% of applied radioactivity ($[^3\text{H}]\text{-H}_2\text{O}$, 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. After application of TBBPA-BDBPE, solvent evaporation was facilitated by gentle airflow over the dosing area and samples remained open to the air for the duration of the studies while perfusion media was maintained at 37°C.

After 24 h, skin samples were removed from the flow-through system, washed in triplicate (500 μL) with a 1:1 mixture of Joy® liquid soap: water followed by 3 washes with water (500 μL), 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 using clear packing tape to remove the stratum corneum then chemically solubilized (Soluene 350, PerkinElmer). Aliquots of the perfusates, wash and tape strip samples were mixed with Ultima Gold. The dissolved skin samples were mixed with Hionic-Fluor. Amounts of $[^{14}\text{C}]$ -labeled radioactivity in dissolved skin and tapes strips (maximum absorbed dose) and $[^{14}\text{C}]$ -labeled radioactivity in collected receptor fluid fractions (penetrated dose) were counted by liquid scintillation spectrometry as the percentage of dose absorbed and penetrated. Any $[^{14}\text{C}]$ -labeled radioactivity recovered in the washes were recorded as unabsorbed dose.

A parallelogram approach to the dermal exposure assessments was used to estimate bioavailability following *in vivo* human systemic exposure to dermally-applied TBBPA-BDBPE as described previously [21, 25] where *in vivo* human bioavailability was estimated as a function of *ex vivo* human exposure multiplied by a normalization factor based on the same dose applied to rat skin *in vivo* and *ex vivo*. Flux (J_{ss}) and standard deviation were calculated as described previously [26, 27].

Analytical methods

Samples were analyzed in parallel for quantitative and qualitative differences. Quantitative analyses of total $[^{14}\text{C}]$ -radioactivity content was determined using a Beckman Coulter LS6500 Multi-Purpose Scintillation Counter (Brea, CA). Total $[^{14}\text{C}]$ -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 $[^{14}\text{C}]$ -radioactivity was quantified by combustion in a Packard Model 307 Biological Sample Oxidizer (PerkinElmer; Torrance, CA) 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 $[^{14}\text{C}]$ -radioactivity was quantified by combustion. Limits of detection (0.6 fmol) and limits of quantitation (1 fmol) were calculated as 3 and 5 times sample background, respectively.

TBBPA-BDBPE in perfusates and extracts of tissues and feces was quantified by radiochemical detection following HPLC separation (HPLC/FSA). The HPLC system used for analysis was composed of a Waters (Milford, MA, USA) 2695 Separations Module,

Restek (Bellefonte, PA, USA) Raptor Biphenyl column (3.5 μ m, 4.6 mm $\times$ 50 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 an isocratic method; initial conditions (1% A:99% B) maintained over 4 min with a flow rate of 1 mL/min. Laura 4 software package (Lablogic, Brandon, FL) was used for instrument control and analysis. Radiochemical flow cell was 500 μ L and scintillant flow rate was 2 mL/min. Injection volumes were 100 μ L.

Excreta and tissues containing [14 C]-radioactivity were sampled and subjected to extractions using a previously described method [28]. Briefly, aliquots (~250 mg) of ground, dried feces or minced skin were placed in labeled pre-weighed glass screw-top tubes and re-suspended in 5 mL acetone 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; supernatants were transferred before repeating the process twice. When recoveries were less than 95% of expected values, the pellet was further extracted with ethyl acetate (3 $\times$ 5 mL) and 1% HCl in 95% ethanol (3 $\times$ 5 mL). At each step, the samples were centrifuged, and the supernatants transferred and pooled. Extracts were concentrated to approx. 100 μ L 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 sample combustion 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 significantly different at $p < 0.05$.

Results

Dermal uptake of TBBPA-BDBPE was tested *ex vivo* using female SD rat and human skin and *in vivo* using female SD rats. Recoveries over time showed the dose penetrating the skin increased slowly throughout the experiment with similar flux rates for all matrices between 0 and 12 h; between 12 and 24 h, greater amounts were recovered after *in vivo* dosing (Figure 2A). There was no evidence that rats were able to access the dosing site to ingest any of the test article, as the steel mesh cap was still well affixed to the skin at the termination of the experiment, but the difference in recoveries between *in vivo* and *ex vivo* skin was reflected in the uncertainty calculated for *in vivo* human skin. Over a 24-hour period, $0.2 \pm 0.1\%$ (mean $\pm$.D.) and $0.3 \pm 0.1\%$ of the TBBPA-BDBPE that was administered penetrated the *ex vivo* human and rat skin, respectively (Figure captions

Table

Table 1). The maximum absorbed fractions for *ex vivo* human and rat skin were $53 \pm 17\%$ and $26 \pm 6\%$, respectively; dose remaining in the human and rat skin samples after tape stripping amounted to $31 \pm 18\%$ and $14 \pm 5\%$ of the dose, respectively. *In vivo* dermal exposure to TBBPA-BDBPE showed a similar result, with penetrated and absorbed fractions containing similar amounts of [^{14}C]-radioactivity (1.7 ± 0.3 and $38 \pm 20\%$, respectively). Of the small percentage of applied TBBPA-BDBPE that penetrated the skin *in vivo*, only a small amount of the dose remained in the tissues, with the greatest proportion attributed to retention in muscle ($0.1 \pm 0.1\%$) and large intestine/cecum contents ($0.3 \pm 0.1\%$); whole blood and non-dosed skin contained approx. 0.1% of the applied dose. TBBPA-BDBPE was resistant to metabolism or biological degradation in these studies. HPLC separation and radiochemical analysis of perfusate media and extracts from skin and feces detected only a single peak that co-eluted with the parent TBBPA-BDBPE (Figure 2B). Maximal state TBBPA-BDBPE flux (slopes of the penetrated mass across each barrier plotted versus sampling period) through rat skin occurred between 0 and 6 h post-application was 26 ± 8 pmol/cm²/h *ex vivo*; *in vivo* the flux and was 79 ± 19 pmol/cm²/h and occurred between 12 and 24 h post dose. Maximal flux for *ex vivo* human skin was 16 ± 12 pmol/cm²/h and was reached between 0 and 6 h. Computed maximal flux for *in vivo* human skin was 38 ± 16 pmol/cm²/h and was driven by the dose recovered in excreta recovered between 12 and 24 h from the *in vivo* rat study. Based on the parallelogram calculation, a highly conservative estimate indicates approx. $78 \pm 51\%$ of TBBPA-BDBPE may be absorbed into human skin *in vivo* but less than 1% of the parent chemical is expected to reach systemic circulation after 24 h of continuous exposure.

Discussion and Conclusions

TBBPA-BDBPE is used in multiple applications resulting in its presence in dust and air [7, 8]. Because TBBPA-BDBPE is such a large and lipophilic molecule, when it was applied to skin only a small amount fully penetrated the barrier; however, a large fraction was found in the skin and was not removed by washing. It has been reported that tape stripping may not be sufficient to completely remove the stratum corneum [29], and to be risk conservative, radioactivity recovered in tape strips as well as compound in skin that was tape stripped was considered “absorbed”. Therefore, these absorption values represent ‘worst-case’ or risk-averse scenario.

In vivo penetration in rat skin was limited to less than 2% of the dose but absorption into the skin was 38% while 0.3% penetrated *ex vivo* rat skin. Similarly, only 0.2% of the dose penetrated split-thickness human skin but 53% was retained even after washing and tape stripping. For consistency, the unabsorbed fraction was calculated using the same scaling formula, which, as an academic exercise results in a >100% mass balance. Calculated a second way (assuming similar recovery in *in vivo* human as was recovered in the *in vivo* rat study), the unabsorbed fraction for *in vivo* human skin would be 14%. Total recovery in rat skin approached, but was less than 95% on average, adding to the uncertainty in the predictions for *in vivo* human skin. From these data, it was concluded that TBBPA-BDBPE is likely to have a dermal bioavailability of around 80%, far exceeding its oral bioavailability

of 2% [17]. Exposure in this study was limited to 24 h; given the near continuous exposure of human skin to dust in homes, schools, and offices, longer exposures are expected to have increased penetrance and subsequent systemic exposure, as was seen in a previous study of the structurally related chemical Tetrabromobisphenol in our lab [19]. While the dose used in this study is likely to be an order of magnitude or more greater than that experienced by the general population, occupational exposure is expected to be higher for TBBPA-BDBPE [30]. A recent risk assessment determined there is a strong increasing trend in potential for exposure, especially in an occupational settings and at wastewater treatment plants [31].

To represent a risk-averse scenario, we assumed complete bioaccessibility for exposures to TBBPA-BDBPE from dust. By using a highly volatile vehicle, we could establish effectively instantaneous delivery to the stratum corneum while minimizing permeation. The low surface tension of the vehicle helped maximize uniform distribution to the dosed skin. The efficiency by which chemical is transferred from contaminated dust to skin is likely variable, depending on a range of factors from the composition of the dust, ambient environment, personal care product use, along with age and relative health of the exposed skin. In studies of hexabromocyclododecane isomers and Tetrabromobisphenol A, dermal bioaccessibility of analyte of interest adsorbed to dust was roughly 50% in a “normal” synthetic/sebum mixture but was increased in the presence of shower gel or sunscreen lotion [32].

While percutaneous penetration was generally low, TBBPA-BDBPE accumulated in both the stratum corneum and lower layers of the skin; this fraction of the chemical is expected to reach systemic circulation over time even after surface washing and removal [33]. These data are expected to be useful in assessing the contribution of dermal exposure to aggregate exposures to susceptible populations. We anticipate these data will be useful in estimating human exposure risk for TBBPA-BDBPE, especially in pregnant women and small children who are exposed to higher levels of household dust [34]. This is of particular importance because, coupled with their increased surface area to volume ratio and immature detoxification pathways [35], early-life exposure to endocrine disrupting chemicals like TBBPA-BDBPE enhances susceptibility to diseases like obesity and other chronic pathologies as the high dermal bioavailability of the chemical increases its likelihood for low, continuous systemic exposure to occur with a highly environmentally persistent chemical like TBBPA-BDBPE [6, 36, 37].

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Highlights

- It is important to investigate the disposition of Tetrabromobisphenol A Bis(2,3-dibromopropyl) ether (TBBPA -BDBPE) based on concerns over its high potential for environmental persistence and bioaccumulation and high production volume.
- In the present study, a single dose of [^{14}C]-TBBPA-BDBPE was applied to skin from human donors or female Sprague Dawley rats to assess potential dermal uptake of TBBPA-BDBPE.
- The epidermis was a depot for dermally applied TBBPA-BDBPE; risk-averse estimates predict $\sim 78 \pm 51\%$ of TBBPA-BDBPE may be absorbed into human skin *in vivo*; $\sim 0.7 \pm 0.4\%$ of the parent chemical is expected to reach systemic circulation over a 24 h period following continuous exposure.

Figure 1.
TBBPA-BDBPE chemical structure

Figure 2. TBBPA-BDBPE recovery and analyses. A: Cumulative penetration of [^{14}C] TBBPA-BDBPE through rat or human skin. Estimated penetrance for *in vivo* human skin was calculated using the parallelogram method described above. B: Representative HPLC-radiochromatograms of [^{14}C]-radioactivity in perfusate, skin, and excreta samples. single peak that co-eluted with the TBBPA-BDBPE standard was detected in all samples.

Table 1.

Summary of measured and calculated fractional recoveries for unabsorbed, absorbed, and penetrated TBBPA-BDBPE from *ex vivo* and *in vivo* studies. Dermal bioavailability was calculated as the sum of absorbed and penetrated fractions (N = 3–4, mean $\pm$ S.D.).

			Rat	Rat	Human	Human	
			(<i>ex vivo</i>)	(<i>in vivo</i>)	(<i>ex vivo</i>)	(<i>in vivo</i>)	
			measured	measured	measured	calculated	
Unabsorbed	Dosing cell	%	22 ± 11	0.1 ± 0.1	12 ± 13	<u>0.1 ± 0.1</u>	
		(nmol)	(14 ± 7)	(0.1 ± 0.1)	(7 ± 7)	<u>(0.04 ± 1)</u>	
	Wash	%	46 ± 11	53 ± 19	30 ± 3	<u>34 ± 15</u>	
		(nmol)	(29 ± 7)	(34 ± 11)	(17 ± 2)	<u>(19 ± 8)</u>	
	Total Unabsorbed	%	68 ± 7	53 ± 19	42 ± 15	32 ± 17	
Absorbed	Tape strips	%	12 ± 2	12 ± 4	22 ± 21	<u>22 ± 22</u>	
		(nmol)	(8 ± 1)	(7 ± 3)	(13 ± 12)	<u>(12 ± 12)</u>	
	Skin	%	14 ± 5	26 ± 16	31 ± 18	<u>58 ± 54</u>	
		(nmol)	(9 ± 3)	(16 ± 9)	(18 ± 10)	<u>(33 ± 29)</u>	
	Total Absorbed	%	26 ± 6	38 ± 20	53 ± 17	78 ± 51	
Penetrated	Perfusate/Excreta & tissues	0–6 h	%	0.2 ± 0.05	0.1 ± 0.02	0.1 ± 0.1	<u>0.1 ± 0.1</u>
		(nmol)	(0.1 ± 0.03)	(0.1 ± 0.01)	(0.1 ± 0.05)	<u>(0.1 ± 0.04)</u>	
		6–12 h	%	0.1 ± 0.01	0.2 ± 0.04	0.05 ± 0.03	<u>0.2 ± 0.1</u>
			(nmol)	(0.05 ± 0.01)	(0.1 ± 0.02)	(0.03 ± 0.02)	<u>(0.1 ± 0.1)</u>
		12–18 h	%	0.04 ± 0.01	0.2 ± 0.04	0.03 ± 0.01	<u>0.2 ± 0.1</u>
			(nmol)	(0.03 ± 0.01)	(0.1 ± 0.02)	(0.02 ± 0.01)	<u>(0.1 ± 0.04)</u>
		18–24 h	%	0.04 ± 0.01	1.5 ± 0.3	0.02 ± 0.1	<u>0.8 ± 0.3</u>
			(nmol)	(0.02 ± 0.01)	(1 ± 0.1)	(0.01 ± 0.003)	<u>(0.4 ± 0.2)</u>
		Total Penetrated	%	0.3 ± 0.1	1.7 ± 0.3	0.2 ± 0.1	1.2 ± 0.7
		Bioavailable		%	26 ± 6	39 ± 20	54 ± 17
Total recovery		%	94 ± 1	92 ± 5	95 ± 2	<u>111 ± 1</u>	