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Estimation of tetrabromobisphenol A (TBBPA) percutaneous uptake in humans using the parallelogram method

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ABSTRACT

Tetrabromobisphenol A (TBBPA) is currently the world's highest production volume brominated flame retardant. Humans are frequently exposed to TBBPA by the dermal route. In the present study, a parallelogram approach was used to make predictions of internal dose in exposed humans. Human and rat skin samples received 100 nmol of TBBPA/cm^2 skin and absorption and penetrance were determined using a flow-through *in vitro* system. TBBPA-derived [^{14}C]-radioactivity was determined at 6 h intervals in the media and at 24 h post-dosing in the skin. The human skin and media contained an average of 3.4% and 0.2% of the total dose at the terminal time point, respectively, while the rat skin and media contained 9.3% and 3.5%, respectively. In the intact rat, 14% of a dermally-administered dose of ~100 nmol/cm^2 remained in the skin at the dosing site, with an additional 8% reaching systemic circulation by 24 h post-dosing. Relative absorption and penetrance were less (10% total) at 24 h following dermal administration of a ten-fold higher dose (~1000 nmol/cm^2) to rats. However, by 72 h, 70% of this dose was either absorbed into the dosing-site skin or had reached systemic circulation. It is clear from these results that TBBPA can be absorbed by the skin and dermal contact with TBBPA may represent a small but important route of exposure. Together, these *in vitro* data in human and rat skin and *in vivo* data from rats may be used to predict TBBPA absorption in humans following dermal exposure. Based on this parallelogram calculation, up to 6% of dermally applied TBBPA may be bioavailable to humans exposed to TBBPA.

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1. Introduction

Tetrabromobisphenol A (TBBPA), the world's highest production volume brominated flame retardant (BFR) is used primarily in resins of electronic circuit boards *via* reactive chemical incorporation (BSEF, 2012). Non-reactive use of TBBPA as an additive in consumer products may increase as a result of the phase out of polybrominated diphenyl ether (PBDE) flame retardant mixtures (de Wit et al., 2010). Additive use of TBBPA results in greater potential for leaching from products into the environment (Canada, 2013). TBBPA has been identified in occupational, household and environmental dust samples, thus posing a potential risk from dermal, oral, and inhalation exposures, especially among children *via* hand-to-mouth contact (Stapleton et al., 2008; Abdallah et al., 2015). In a two-year oral bioassay by the National Toxicology Program (NTP), TBBPA was shown to induce the formation of highly malignant uterine tumors in Wistar Han rats (Dunnick et al., 2014). TBBPA exposures elicited decreased rat serum thyroxine levels in our laboratory (Sanders et al., 2015), in the NTP study (Dunnick et al., 2014; NTP, 2013), and in both one-generational (Van der Ven et al., 2008), and two-generational studies (Cope et al., 2015). TBBPA alters the expression of efflux transporters in liver (Hu et al., 2015; Miao et al., 2014), but is not a substrate for these transporters (Dankers et al., 2013). One previous study of human TBBPA *in vitro* dermal uptake commissioned for Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) listing with the European Union concluded that less than 2% of a 2 mg/cm^2 dose of TBBPA was bioavailable, with 0.73% of the dose penetrating the skin and 0.9% of the dose remaining in the skin 24 h after dosing (ECHA, 2005).

The previous work has characterized the bioavailability of TBBPA in rodent models to support risk assessment following oral exposure to humans (Hakk et al., 2000; Kuester et al., 2007; Knudsen et al., 2014). The present work provides data to support assessment of risk of dermal exposure of TBBPA to humans. Here, the parallelogram approach (Sobels, 1977; Ross et al., 2011) has been used to characterize and compare the extent of dermal absorption and penetrance of TBBPA *in vivo* in rats and *in vitro* in rat and human skin for prediction of internal dose to humans following dermal exposure to TBBPA.

In vivo studies were conducted using female Wistar Han rats and *in vitro* studies were conducted using split-thickness skin (*e.g.*, epidermis and upper portion of the dermis) from human donors and female Wistar Han rats exposed to TBBPA in a flow-through system as described below. For the *in vitro* experiments, the term 'absorbed' is

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used to describe the portion of the applied dose found within the skin and 'penetrated' is used to describe chemical that has completely diffused through the skin into the underlying fluid (termed 'receptor fluid', analogous to the amount reaching systemic circulation following *in vivo* exposure) (Demierre et al., 2012). The values for absorption and penetration are combined for an estimation of bioavailability of TBBPA.

2. Methods & materials

2.1. Chemicals

[^{14}C]-labeled TBBPA (ring-labeled; Fig. 1, Lot # 3225-235, Perkin Elmer Life and Analytical Sciences [Boston, MA], re-purified in 2013 by Moravek Biochemicals [Brea, CA]) used in these studies had a radiochemical purity of >98% (specific activity = 90.3 mCi/mmol) and a chemical purity of >98%, as compared to a TBBPA reference standard (Sigma-Aldrich; St. Louis, MO). 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.

2.2. In vitro skin samples

Full-thickness human skin was obtained from the National Disease Research Interchange, (Philadelphia, PA, USA) from three (1 male, 2 female) Caucasian individuals aged 71–77 years old (dorsal/scapular skin, excised ≤12 h post-mortem, shipped at −80 °C). The skin was shipped and stored frozen (−80 °C) until use. Full-thickness female Wistar Han rat skin (N = 4, 10–11 weeks old) was obtained from Charles River Laboratories (Raleigh, NC). 24 h prior to excision, hair on the dorsal surface was clipped; the day of shipment, the rats were humanely euthanized by CO_2 inhalation and skin excised. The skin was shipped on dry ice and stored frozen (−80 °C) until use. *In vitro* dermal absorption tests were conducted according to the OECD Test Guideline 428 (OECD, 2004a). Human skin was sampled in quadruplicate while rat skin was sampled in triplicate.

2.3. In vitro dermal absorption apparatus

A flow-through diffusion cell system (Crown Bio Scientific, Inc., Somerville, NJ, USA) and methodology as described by Bronaugh and Stewart (1985) and Bronaugh and Maibach (1991) were used. Teflon Flo-Thru diffusion cells with a diffusional area of 0.64 cm^2 were used. Each cell was placed in a PosiBloc Diffusion Cell Heater, heated by circulating water at a temperature of 35 °C. A peristaltic pump was used to pump receptor fluid at a rate of 1.8 mL/h from a reservoir through Tygon tubing (R-3603, Fisher Scientific Co., Fair Lawn, NJ, USA) to the flow-through cells. Scintillation vials (20 mL) were placed in a fraction collector to collect the receptor fluid. All components of the diffusional apparatus were sterilized with 70% ethanol solution and rinsed with sterile receptor solution before placing the skin samples in the flow-through cells.

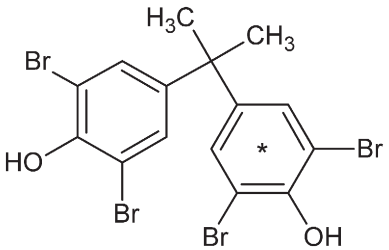

Fig. 1. Chemical structure of TBBPA, asterisk indicates the location of the radiolabel.

2.4. Receptor fluid

HEPES-buffered Hanks' balanced salt solution, pH 7.4, with 10% fetal bovine serum (Sigma Chemical Co., St. Louis, MO, USA), was used as the receptor fluid. This fluid has been shown by Collier et al. (1989) to maintain the viability of rat skin for up to 24 h. Components of the fluid were N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES), fetal bovine serum, and gentamicin sulfate, obtained from GIBCO (Grand Island, NY, USA), and disodium hydrogen phosphate and potassium dihydrogen phosphate, purchased from Scientific Products (McGraw Park, IL, USA) and Matheson Coleman and Bell (Norwood, OH, USA), respectively. The receptor fluid was prepared with distilled water and sterilized by filtration (0.2 μm filter, Nalgene disposable filterware, Sybron Corp., Rochester, NY, USA). A 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.

2.5. In vitro procedures

Human and rat skin experiments were run on separate days. On the day of the experiment, human or rat skin was thawed and dermatomed to approx. 300 μm thicknesses using a Padgett dermatome (Kansas City, MO, USA) and placed in receptor fluid. Four disks were cut from each sample of human skin and 3 from each rat skin with a 0.75-in. diameter bow punch. The thickness of each skin disk was measured along its edge with microcalipers (Model D-1000, The Dyer Co., Lancaster, PA, USA). The mean (±SD) skin thickness of human and rat skin was 294 (±57) and 244 (±25) μm, respectively. The disks were mounted epidermal side up in the flow-through system (described above). Once all of the skin disks were mounted, the receptor fluid pump was started. Each skin disk was rinsed with a small volume of distilled water and dried with Kim Wipe® paper three times prior to TBBPA application.

After an equilibration period of 30 min, the integrity of the human skin was tested as follows: [^{3}H]-H_2O (1 μCi, 100 μL), obtained from Perkin Elmer (Waltham, MA, USA), was applied to the skin with a Pipetman, the pump turned on, and the receptor fluid collected. Residual [^{3}H]-H_2O was removed with Kim Wipe® paper 5 min after it was applied to the skin. The skin was rinsed with a small volume of nonradioactive, distilled water and dried with Kim Wipe® paper three times to remove remaining traces of [^{3}H]-H_2O. Receptor fluid was collected for an additional 1 h. The collected receptor fluid was mixed with 10 mL of scintillation fluid and analyzed for [^{3}H]-radioactivity in a Beckman Instruments (Fullerton, CA) 6000LL liquid scintillation analyzer. The mean percentage of the dose of [^{3}H]-H_2O detected in the receptor fluid for human cadaver skin was less than 0.05%, indicating an intact barrier, analogous to healthy skin.

Human and rat skin disks were treated with 100 nmol/cm^2 of [^{14}C]-TBBPA in 10 μL acetone (~1 μCi). The peristaltic pump was started and fractions were collected every 6 h until 24 h post-dosing, when the peristaltic pump was stopped. The epidermal surface (with the cell top in place) was washed six times with 0.5 mL of a mixture of Joy® liquid soap:water (1:1) to remove unabsorbed chemical. The skin wash fractions were pooled into two vials and mixed with scintillation fluid. The cell top and cell body were individually washed three times with 0.5 mL ethanol. The cell top and body washes and the weigh boat used to wash the cell top were put into separate vials. The skins were allowed to dry overnight. The following day, each skin disk was tape stripped 10 times with clear tape. Each tape strip was placed in a separate vial. Skin washes, cell top and body washes, weigh boats, tape strips and receptor fluid were mixed with scintillation fluid and analyzed for radioactivity in the liquid scintillation analyzer. Washed and stripped skin was then chemically solubilized in 1 mL of Soluene 350 (PerkinElmer) overnight in a water bath set at 37 °C. Hionic Fluor (PerkinElmer) was added to the dissolved skin solution, and absorbed [^{14}C]-radioactivity was quantified.