Cod: 8.2005

USING THE PARALLELOGRAM APPROACH TO ESTIMATE HUMAN PERCUTANEOUS BIOAVAILABILITY FOR NOVEL & LEGACY BROMINATED FLAME RETARDANTS

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Introduction

Flame retardants (FRs) are materials intended to reduce or inhibit the spread of fire.(1) A wide range of flame retardants, either additive (added at the time of polymerization) or reactive (chemically bound to the polymer backbone) have been utilized to comply with fire safety regulations. Types of FRs range from aluminum salts to halogenated organic monomers and polymers. Unfortunately, FRs added to consumer products and building materials can migrate into the environment and pose risk of exposure to humans.(2-5)

Tetrabromobisphenol A (TBBPA, Figure 1A) is a conventional flame retardant typically used as a reactive FR but is increasingly used additively.(6, 7) 2-ethylhexyl-2,3,4,5-tetrabromo- benzoate (EH-TBB, Figure 1B), bis (2-ethylhexyl) tetrabromophthalate (BEH-TEBP, Figure 1C), and decabromodiphenyl ethane (DBDPE, Figure 1D) are novel brominated flame retardants used to replace banned polybrominated diphenyl ether FRs.(8) EH-TBB and BEH-TEBP are used alone or in combination. One such combination is FM550, a product that contains EH-TBB, BEH-TEBP, and phosphate FRs and comprises up to 4.2% by weight of some lots of couch foam.(9) In addition to furniture foam, EH-TBB & BEH-TEBP have been found in baby products, dust, sediment, and animals.(2, 10-16) DBDPE is a high production volume FR marketed as a replacement for decabromodiphenyl ether.(17)

Dermal contact with FRs is strongly associated with systemic exposures but very little is known about the dermal disposition of FRs.(2, 18-20) Dermal exposure to FRs likely occurs via contact with contaminated dust. It is likely that dermal exposure to FRs by humans occurs primarily in the home via contact with contaminated dust. In support of future risk assessments, the present work was conducted to predict bioavailability of EH-TBB, BEH-TEBP, TBBPA, or DBDPE in dermally-exposed humans. The calculations are based on the parallelogram method in which dermal bioavailability is first determined in vivo in the rat and in vitro in both rat and human skin.

Materials and Methods

MODEL In vivo studies were conducted using female Wistar Han (TBBPA; Charles River Laboratories, Raleigh, NC) or Sprague Dawley (EH-TBB, BEH-TEBP, DBDPE; Harlan Laboratories, Raleigh, NC) rats and in vitro studies were conducted using split-thickness skin from human donors and female Wistar Han or Sprague Dawley rats.

In vivo: Rats (10 weeks, ~200 g) were maintained in an AAALAC-approved animal care facility. Food (NIH-31 chow) and water were provided for ad libitum consumption. All procedures were approved by the NIEHS Institutional Animal Care and Use committee.

In vitro: Split-thickness human (N=3 individuals/chemical, National Disease Research Interchange, Philadelphia, PA, USA) or rat (N=4-8 individuals/chemical, Harlan Bioproducts for Science, Indianapolis, IN, USA) scapular skin in a flow-through diffusion cell system (Crown Bio Scientific, Inc., Somerville, NJ, USA).

DOSING [^{14}C]-TBBPA,-EH-TBB, -BEH-TEBP were dosed at ~100 nmol/cm^2 (~5 μCi). [^{14}C]-DBDPE was dosed at 2.7 nmol/cm^2 (~0.1 μCi). [^{14}C]-TBBPA was a gift from I. Glenn Sipes, University of Arizona (Tucson, AZ, USA). [^{14}C]-EH-TBB, -BEH-TEBP, and –DBDPE were purchased from Moravek Biochemicals, (Brea, CA, USA). All chemicals were >98% chemically- and radiochemically-pure.

In vivo: Rats received a single dose applied to dorsal skin (1 cm^2, clipped 24 h prior), and were placed in a metabolism cage. Dosed skin was covered with a perforated steel cap to minimize ingestion of the test article. Excreta and cage rinses were collected at 24 h. Tissues & blood were collected at necropsy and maintained at -80ºC.

In vitro: Skin was prepared as described previously.(21) On the day of dosing, skin was split to approx. 300 μm using a Padgett dermatome and placed in the diffusion cell system. A single dose was applied

and fractions of perfusion media were collected at 6 h intervals. After 24 h, the skin was washed and tape stripped to remove unabsorbed BFR. Remaining skin was chemically solubilized in Soluene 350 (Perkin Elmer, Waltham, MA).

ANALYTICAL METHODS Total [^{14}C]-radioactivity content was determined using liquid scintillation counting (LSC). Media, urine, and cage rinses were assayed directly while tissues and feces were weighed and burned in a Packard 307 Biological Sample Oxidizer followed by LSC. Qualitative analyses were performed using HPLC radiometric detection (System 1: Waters Alliance 2695/Packard Radiomatic 500TR/Phenomenex 250 mm Luna C18 column. System 2: Agilent 1100 HPLC/INUS betaRAM3B radiochemical detector/Restek 50 mm Raptor biphenyl column). Water and acetonitrile were used as mobile phases.

PARALLELOGRAM CALCULATION Dermal exposure assessments were used to estimate bioavailability following in vivo human systemic exposures to a relevant dose of dermally-applied chemical ($Human_{in\ vivo}$ = ($Rat_{in\ vivo}$/$Rat_{in\ vitro}$) x $Human_{in\ vitro}$) as described previously.[22] 'Absorbed' describes the fraction recovered in skin; 'penetrated' describes the fraction recovered in media, tissues or excreta; 'unabsorbed' describes the fraction recovered in washes and tape strips.[21, 23] Absorption and penetration were combined to estimate bioavailability for each BFR. Bioavailability was estimated as the sum of the absorbed and penetrated fractions.

STATISTICAL ANALYSIS The data were subjected to statistical analysis using two-way ANOVA followed by the Tukey-Kramer test for pairwise comparisons (GraphPad Prism 6, GraphPad Software, Inc., La Jolla CA). Values were considered to be significantly different at $p < 0.05$.

Results

TBBPA and EH-TBB were well absorbed into and penetrated through skin. Estimated human dermal bioavailability of BEH-TEBP was lower compared to EH-TBB & TBBPA, while DBDPE dermal bioavailability appeared to be highest (Table 1).

In vitro, penetration was significantly lower in human than rat skin when TBBPA, EH-TBB, or BEH-TEBP, and TBBPA were tested ($p<0.05$, Figure 2). No significant differences were found between human and rat skin following application of DBDPE.

In vivo, approx. 11% of EH-TBB was recovered in the skin at the dosing site (absorbed) and 10% was present in tissues or excreta (penetrated). In this same group, 6% of the dose was recovered in urine while 1% of the dose was recovered in feces through 24 h. Blood and other tissues contained 4-5% of the administered dose. As observed during in vitro studies, most of the administered [^{14}C]-radioactivity was recovered unabsorbed from the dosing site after 24 h.

HPLC-radiometric analyses of media, extracts, and excreta from EH-TBB studies found EH-TBB was metabolized to tetrabromobenzoic acid (TBBA) in both human and rat skin (Figure 3). In vivo, dermally applied EH-TBB was primarily excreted in the urine as TBBA; extractable [^{14}C]-radioactivity from feces resolved as a small peak that also co-eluted with TBBA. Dosed skin contained a mixture of EH-TBB and TBBA. Only TBBA was detected in the in vitro media from human and rat skin. No metabolites of TBBPA, BEH-TEBP, or DBDPE were detected in perfusion media, dosed skin or excreta.

Discussion & Conclusions

Humans are frequently exposed to brominated FRs, especially via dermal contact with contaminated dust. Human and rat skin data were integrated using a parallelogram method to predict human absorption of three novel BFRs, EH-TBB, BEH-TEBP, and DBDPE, and one conventional BFR (TBBPA) that are being used as alternatives to banned FRs.

TBBPA & EH-TBB were well absorbed while BEH-TEBP percutaneous absorption was low. The apparently large bioavailability for DBDPE was largely dependent on retention in the skin. Skin contact with these BFRs may represent an important route of exposure, especially for small children. Good hygiene practices may aid in decreasing residence time on the skin, which in turn could limit bioavailability and systemic exposure.

We anticipate these data will be useful in estimating human exposure risk, especially to small children who are exposed to higher levels of household dust [24]. This is of particular importance because, coupled with their increased surface area to volume ratio and immature detoxification pathways [25], early-life exposure to endocrine disrupting chemicals like EH-TBB & BEH-TEBP enhances susceptibility to obesity, diabetes, cancer, and other chronic pathologies. [26, 27]

Acknowledgements

The authors thank Ms. Brenda Edwards, Mr. Ethan Hull, Ms. Katelyn McIntosh and Mr. Vivek Miyani, for technical assistance. This abstract has been reviewed in accordance with the policy of the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. This research was supported in part by the Intramural Research Program of NIH/NCI.

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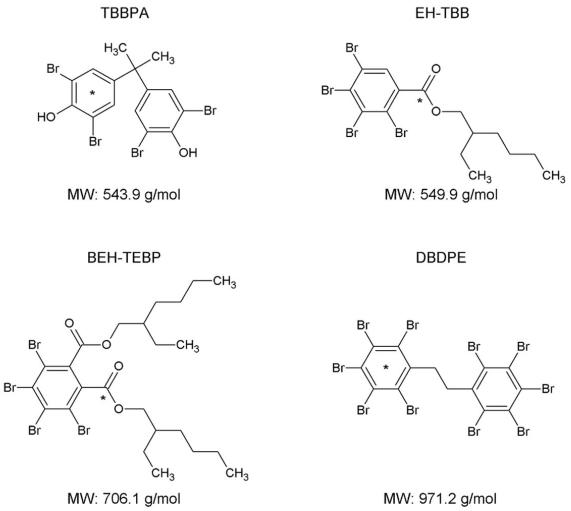

Figure 1. Chemical structures of tested BFRs (*: [^{14}C]-radiolabel)

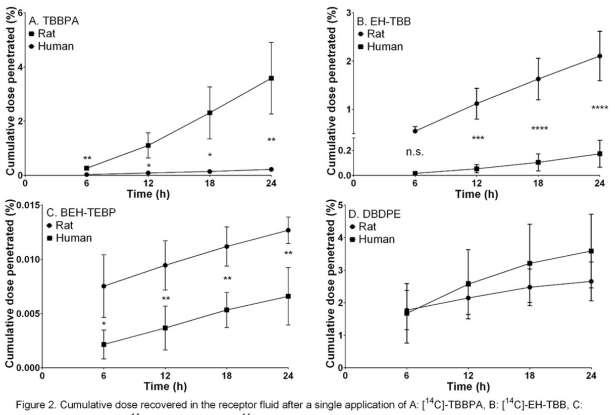

Figure 2. Cumulative dose recovered in the receptor fluid after a single application of A: [^{14}C]-TBBPA, B: [^{14}C]-EH-TBB, C: [^{14}C]-BEH-TEBP, or D: [^{14}C]-DBDPE to rat (●) or human (■) skin in vitro.

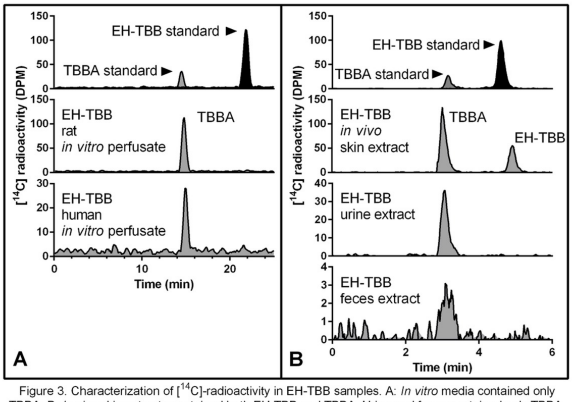

Figure 3. Characterization of [^{14}C]-radioactivity in EH-TBB samples. A: *In vitro* media contained only TBBA. B: *In vivo* skin extracts contained both EH-TBB and TBBA. Urine and feces contained only TBBA.

Table 1. Measured and calculated BFR bioavailable fractions (%).

		Rat (*in vitro*)	Rat (*in vivo*)	Human (*in vitro*)	Estimated Human bioavailability (*in vivo*)
TBBPA	Absorbed (%)	9 ± 2	14 ± 3	3 ± 2	*6 ± 3*
	Penetrated (%)	4 ± 1	8 ± 2	0.2 ± 0.02	
EH-TBB	Absorbed (%)	36 ± 6	10 ± 3	12 ± 9	*7 ± 3*
	Penetrated (%)	2 ± 0.5	11 ± 1	0.2 ± 0.1	
BEH-TEBP	Absorbed (%)	29 ± 2	8 ± 3	2 ± 0.2	*1 ± 0.2*
	Penetrated (%)	0.01 ± 0.002	1 ± 0.3	0.005 ± 0.001	
DBDPE	Absorbed (%)	17 ± 2	17 ± 9	12 ± 4	*18 ± 10*
	Penetrated (%)	3 ± 0.6	5 ± 2	4 ± 1	