BIOLOGICAL FATE OF THE EMERGING BROMINATED FLAME RETARDANTS, 2-ETHYLHEXYL TETRABROMOBENZOATE (TBB) AND BIS(2-ETHYLHEXYL)TETRABROMOPHTHALATE (TBPH), IN FEMALE SPRAGUE DAWLEY RATS.

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Introduction

Flame retardants are a common class of pollutants with known and likely deleterious side effects (hazard) and large production volume and wide distribution in consumer, construction, and industrial products (exposure). Flame retardants (FRs) are materials intended to reduce or inhibit the spread of fire.[1] The California residential furniture flammability standard Technical Bulletin 117 (TB117) was adopted in 1975 and became the de facto flame retardant standard in the US although the US Consumer Product Safety Commission has proposed a nationwide standard.[2] One of the simplest and most common methods for complying with TB117 was to add chemical flame retardants to the product.[3] Materials added to decrease flammability can migrate out of the intended depot (e.g., consumer products and building materials) and into people and the environment.[4-7] Since the adoption of TB117, 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 regulations and improve fire safety, ranging from aluminum salts to halogenated organic monomers and polymers.

Halogenated flame retardants are generally classified as chlorinated or brominated although several mixed-halogen flame retardants are now in use. Brominated flame retardants comprise the other commonly used halogenated flame retardant class with polybrominated diphenyl ethers (PBDEs; penta-, octa-, or deca- mixtures), hexabromocyclododecanes, and tetrabromobisphenol A and its derivatives dominating the market as 'conventional' brominated flame retardants.[1,8,9] However, penta- octa- and deca-PBDEs bioaccumulate and have undesirable toxicity profiles. Firemaster 550, BZ-54, and DP-45 were introduced for use in polyurethane foams (PUFs) in the lead up to the pentaBDE phase-out as 'alternative' flame retardant mixtures.[3,10] TBPH production volumes are approx. 450-4,500 tons/year.[11] TBB is a high production volume chemical although production and import volumes are not currently available for TBB.[12] When introduced to the market in 2003, FM550 was described as a proprietary mixture by its manufacturer, Chemtura Corp. (formerly Great Lakes Chemical Corp.), although the approximate mixture was later deduced to contain triaryl phosphate isomers, triphenyl phosphate and a 1:4 mixture of TBPH and TBB.[13] BZ-54 is composed of a 5:2 mixture of TBB & TBPH.[14] DP-45 is composed entirely of TBPH and is marketed primarily as a flame retardant but also as a plasticizer for flexible PVC and neoprene.[15] Samples of couch foam have shown FM550 present in quantities up to 4.2% by weight.[16] TBB & TBPH have been found in couch foam, baby products (mattresses and high-chair foam), house dust, outdoor dust and sediment, and animals.[3,4,17-22] Structurally, TBB and TBPH are brominated phthalates and as such, are listed as two of 8 structurally similar chemicals to undergo full risk assessment under the Toxic Substances Control Act (TSCA) Work Plan and Action Plan.[23] In addition, the US EPA has stated it will begin data collection activities to better understand the environmental fates of TBB and TBPH.[9]

TBB and TBPH are highly lipophilic (logP = 8.07 and 11, respectively) but little is known about their individual disposition. Repeated exposures to FM550 had toxic effects in rodents including increased serum thyroxine and decreased hepatic esterase activity in dams treated with 1 mg/kg/day of FM-550.[24] Furthermore, exposed offspring displayed high anxiety phenotypes, especially in the exposed females group. Male offspring had significantly impaired glucose tolerance. Commercial formulations of flame retardants that contain TBB & TBPH may be complex mixtures and the biological effects ascribed to exposures in the models utilized thus far have been diverse. These studies were designed to determine the disposition of the major brominated components of these novel flame retardant mixtures, TBB & TBPH. Female Sprague-Dawley (SD) rats were administered a range of doses by gavage

and a single dose intravenously and the routes of elimination were described. In addition, the tissue distribution and metabolite profiles were investigated.

Materials and Methods

MODEL ORGANISM Female SD rats (10 weeks, ~200 g; Harlan Laboratories, Raleigh, NC) were used in these studies. Animals were maintained in an AAALAC-approved animal care facility. Animals were housed individually in metabolism cages for collection of urine and feces. Food and water were provided for *ad libitum* consumption. All procedures were approved by the NIEHS Institutional Care and Use committee.

DOSING Animals were administered a single dose of TBB or TBPH by gavage (PO) or intravenous (IV) bolus through an indwelling catheter. PO doses were: 0.1, 1, 10, or 100 µmol/kg (4 mL/kg). IV dose was 0.1 µmol/kg (1 mL/kg). Dosing solutions were composed of corn oil (PO) or ethanol, water, and an emulsifying agent (Cremophore EL) in a 1:3:1 ratio (IV). IV and PO dosing solutions provided 6 or 25 µCi/kg of [^{14}C]-TBB/TBPH. The dispositional effects of repeated exposures were assessed by administering 5 daily doses of TBB (0.1 µmol/kg; 4 non-radiolabeled doses followed by a single [^{14}C]-radiolabeled dose) and determining quantitative elimination of [^{14}C] radioactivity in excreta over 72 h.

SAMPLE COLLECTIONS Following administration of the compound, excreta and cage rinses (reverse-osmosis water) were collected at 6, 12, 24, 48, and 72h. Euthanasia was by CO_2 asphyxiation. Tissues (pooled adipose, adrenals, brain, heart, kidneys, large intestine & contents, liver, lung, muscle, pancreas, ovaries, skin, small intestine & contents, spleen, stomach & contents, thymus, thyroid, urinary bladder, and uterus) were collected at necropsy and stored at -80ºC until analysis. Blood samples were collected via cardiac puncture into heparinized syringes at termination of the experiment. Samples were placed in labeled pre-weighed vials after all collections and maintained at -80ºC until analyses. Plasma was isolated from heparinized blood by centrifugation (5 min at 3,000 RPM).

ANALYTICAL METHODS Samples were analyzed in parallel for quantitative and qualitative analyses. 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 urine and cage rinses was assayed in triplicate by liquid scintillation counting. Fecal samples were dried in a fume hood, weighed and ground to powder using a mortar and pestle. Aliquots of feces and tissues were weighed and [^{14}C]-radioactivity was quantified by combustion in a Packard 307 Biological Sample Oxidizer followed by LSC counting. TBB and TBPH were quantified by UV/Vis absorbance and radiochemical detection following HPLC separation. . The HPLC system was composed of a Waters 1525 binary HPLC pump, C18 column, and a Waters 2487 dual λ absorbance detector with an in-line IN/US β-RAM model 3 Flow Scintillation Analyzer (LabLogic, Inc., Brandon FL). Mobile phases consisted of 0.1% trifluoroacetic acid in water (mobile phase A) and 0.1% trifluoroacetic acid in acetonitrile (mobile phase B). Sample separations were performed using gradient methods with a flow rate of 1 ml/min. Instrument control and analysis software were Breeze (Waters Corp.) and Laura-Lite (LabLogic).

Results

More than 80% of administered radioactivity was recovered after 24 h at doses up to 10 µmol/kg, with fecal recoveries increasing with dose as shown in Figure 1 (24 h: 33→45→50%; 72h: 39→51→60%). Urinary recovery peaked between 8 and 24 h at these doses. Greater than 90% of administered [^{14}C]-radioactivity was eliminated by 48 h at all the dose levels. At the four lowest doses examined, maximal excretion was achieved by 24 h by both urinary and fecal routes. IV administration of TBB resulted in the highest recoveries in urine and lowest recoveries in feces. Oral administration of a 100 µmol/kg dose resulted in a marked delay in maximal elimination in both urinary and fecal recoveries, indicating saturation of elimination pathways. [^{14}C]-radioactivity recovery exceeded 93% by 72 h after all doses and retention of [^{14}C]-radioactivity in tissues was minimal. Urine was found to contain approx. 6 metabolite peaks and the number and relative proportion of each metabolite present in urine were time and dose-dependent. No parent compound was detected in any urine samples. The principal moieties present in fecal extracts corresponded to parent TBB and tetrabromobenzoic acid (TBBA). Fecal samples collected from IV-dosed animals contained no extractable parent TBB.

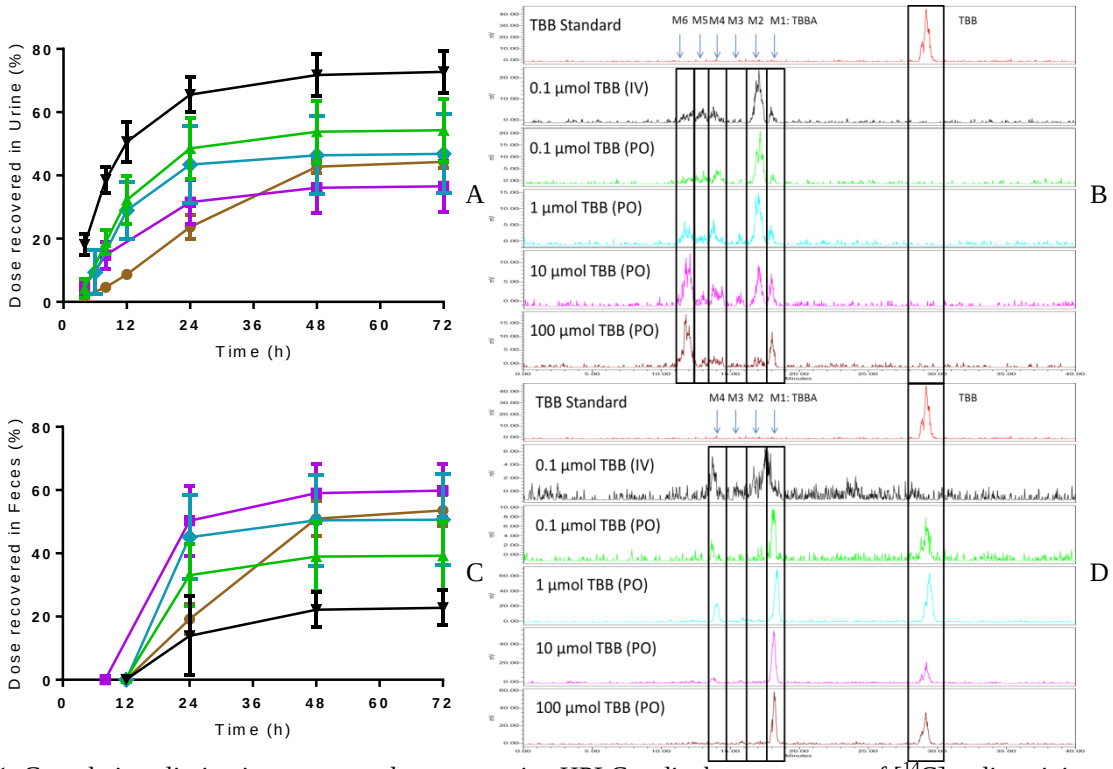

Figure 1. Cumulative elimination curves and representative HPLC-radiochromatograms of [^{14}C]-radioactivity following a single administration of TBB. N=4 per dose group, mean ± S.D. (▼: 0.1 umol/kg, IV; ▲:0.1 umol/kg, oral; ♦: 1 µmol/kg, oral; ■: 10 µmol/kg, oral; ●: 100 µmol/kg, oral). A: Recovery in urine, B: Metabolites in urine, C: Recovery in feces, D: Metabolites in feces.

When TBPH was given by gavage, approximately 75% of administered radioactivity was recovered after 24 h, with negligible difference in fecal recoveries despite a 100-fold difference in dose. Less than 0.3% of the TBPH dose was recovered in urine after oral administrations. [^{14}C]-radioactivity recovery exceeded 98% by 72 h after all oral dosing but reached only 78% after IV administration. Retention of [^{14}C]-radioactivity in tissues after 72 h was significant after IV administration. Feces collected between 0-24 and 24-48 h were extracted and subjected to HPLC-radiometric analyses. A single [^{14}C]-radiolabeled peak that corresponded to the parent compound was detected in samples taken from animals administered TBPH by gavage. Feces collected after IV dosing contained a mixture of parent and metabolites. ~20% of IV-administered [^{14}C]-radioactivity was retained in liver (7%), muscle (4%), and skin, fat and gastrointestinal tract contents containing 2% of the dose, respectively, 72 h after dosing.

Discussion & Conclusions

This is the first report of the disposition of TBB and TBPH independent of the proprietary flame retardant mixtures shown to contain these brominated flame retardants. TBB was extensively absorbed, readily metabolized, and eliminated by both urinary and fecal routes. At least 6 metabolites were present in urine. Urinary metabolite profiles differed markedly for doses above and at or below the 10 µmol/kg dose, indicating a saturation of one or more metabolic pathways which was also evident in the dose-dependent increases in elimination via feces and the radical departure in the rate of elimination at the highest dose. TBPH was poorly absorbed, minimally metabolized, and eliminated almost exclusively by the fecal route after oral administration.

Households in the US have the highest reported concentrations in household dust/environment, similar to its predecessor, PentaBDE[21]. Stapleton et al demonstrated PUFs purchased after 2005 contained mostly a mixture of

flame retardants, principally comprised of TDCPP and Firemaster 550.[3] Firemaster 550 itself is composed of a mixture of halogenated and phosphorylated FR flame retardants (TBB & TBPH). TBB is classified as a high volume chemical by the US EPA although exact production volumes are not publicly available.[25] TBB and TBPH are established 'novel' flame retardant contaminants in home and environmental exposure, especially to small children who are prone to ingesting dust through hand-mouth contact.[21] TBB was readily taken up from the gut when administered to female SD rats and is likely to be absorbed when ingested by humans. However, limited tissue retention of TBB or its metabolites was observed and would indicate a low likelihood of bioaccumulation. However, repeated administrations of TBB showed a significant shift toward increased urinary elimination, increasing systemic exposure to TBB or its metabolites. More work is needed to determine the nature of metabolic processes induced by repeated exposures to TBB. In contrast, TBPH is poorly absorbed after oral exposure, decreasing its likelihood of biological effects.

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