2,4,6-Tribromophenol disposition and kinetics in female Sprague Dawley Rats

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Introduction

2,4,6-tribromophenol (TBP, CAS No. 118-79-6) is a widely used as a brominated flame retardant (BFR), precursor for other BFRs, and as a wood antifungal agent [1-3]. TBP is found as a naturally-occurring bromophenol in seafood [4, 5] but may also be encountered as a degradation product of tetrabromobisphenol A and polybrominated diphenyl ethers. As a result of these sources, TBP is a frequently detected contaminant in environmental matrices and biota, including humans.

TBP is a suspected endocrine disrupter, neuro-, reproductive-, and developmental toxicant. TBP has been shown to impair development in both rats and zebrafish [6-9]. Pregnant rats exposed to aerosolized TBP gave birth to offspring that exhibited skeletal malformations while zebrafish exposed to TBP had negatively altered gonad morphology and reduced fertility. *In vitro*, TBP lowered the transcriptional activity of both estrogen and androgen receptor at low micromolar (IC_{50} = 4-14 µM) concentrations [10]. TBP binds the thyroid hormone transport protein, transthyretin, potentially altering thyroid hormone signaling [11]. TBP also inhibits estrogen sulfotransferase activity [12], possibly via the same mechanism as that employed by tetrabromobisphenol A [13].

In exposed workers, circuit board producers and electronics disassemblers had blood concentrations that ranged from 14.2-244.9 pmol TBP/g lipid [1]. Sawmill workers exposed to TBP were found to have urinary concentrations of 5.7-37.2 µmol TBP/g creatinine [3]. In non-occupational exposures, serum levels of TBP had a positive correlation with PBDEs, suggesting similar source of exposure, or that TBP may result from metabolism of PBDEs [14]. In addition to exposure through dust, TBP is likely to be consumed in a diet rich in wild-caught fish [4]. TBP may accumulate in human placenta which is likely to contribute to prenatal exposures [15]. However, no studies have assessed overall TBP disposition or kinetics; therefore, we assessed the dose-, route- and time-dependent disposition of TBP in female Sprague Dawley (SD) rats.

Materials and methods

MODEL ORGANISM Female SD rats (10 weeks, 200-250 g; Envigo, 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 (NIH 31 rat chow, Zeigler, Gardners, PA) 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 TBP by gavage (PO) or intravenous (IV) bolus. PO doses were: 0.1 (6.5 µCi/kg), 10 (100 µCi/kg), or 1,000 (100 µCi/kg) µmol/kg (4 mL/kg). IV dose was 10 µmol/kg (1 mL/kg, 100 µCi/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).

SAMPLE COLLECTIONS Following administration of the compound, excreta and cage rinses (reverse-osmosis water) were collected at 4, 8, 12, and 24 h. Euthanasia was by CO_2 asphyxiation. Tissues (pooled adipose, adrenals, brain, heart, kidneys, large intestine & contents, liver, lung, muscle, ovaries, pancreas, 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 a 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 spectrometry. TBP was quantified by UV/Vis absorbance and radiochemical detection following HPLC separation. The HPLC system was composed of a Agilent 1100 HPLC system, Agilent Eclipse Plus C18 column, and an in-line IN/US β-RAM model 3 Flow Scintillation Analyzer (LabLogic, Inc., Brandon FL). 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 gradient methods with a flow rate of 1 ml/min. Instrument control and analysis software were Laura4 (LabLogic). HPLC-mass spectral analyses were carried out using an Ultimate 3000 HPLC, Agilent Eclipse Plus C18 column, and a Thermo LTQXL

ion trap mass spectrometer with electrospray ionization operated in negative ion mode. Mobile phases and gradient was the same as above.

Results and discussion

TBP appeared to be widely distributed, readily metabolized, and excreted primarily in urine at all doses and routes tested (Figure 1). Systemically available (IV-administered) TBP was rapidly excreted primarily via urine, with ~61% of the dose recovered in urine after 4 h. By 24 h, 89-94% of the dose was recovered in urine with an additional 5% recovered in feces; the balance (1-2%) was recovered in blood and tissues. Orally administered TBP was well absorbed from the gut, with ~25% eliminated via urine in the first 4 hours after dosing at all dose levels. Urinary excretion was essentially unchanged when elimination curves for 0.1 and 10 µmol/kg doses were compared; 1,000 µmol/kg urinary elimination appeared to be slightly delayed but was indistinguishable from that of the other doses by 24 h. Fecal recoveries were not significantly different and varied only slightly by dose and route. A summary of dose recoveries after 24 h is shown in Table 1.

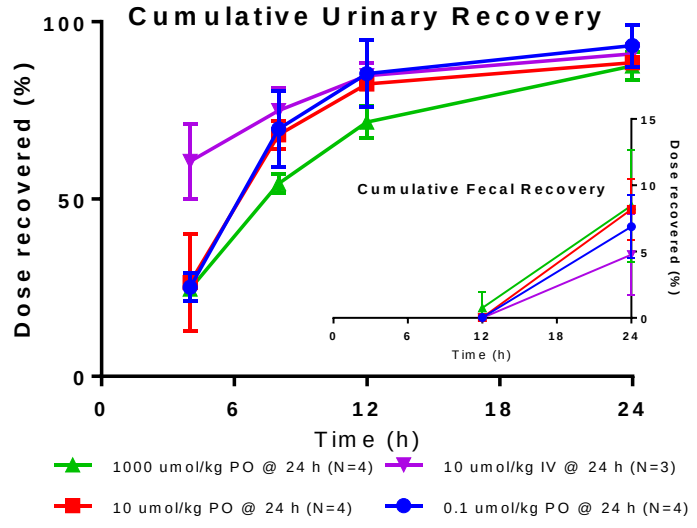

Figure 1. Cumulative dose recoveries in urine and feces after administration of TBP to female SD rats.

	0.1 umol/kg PO			*10 umol/kg PO*			*1000 µmol/kg PO*			*10 umol/kg IV*		
	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.
Feces	6.5	±	2.0	8.2	±	2.3	8.5	±	4.2	4.8	±	3.1
Urine	88.9	±	4.0	88.5	±	1.8	87.6	±	4.1	91.0	±	2.7
Blood	0.1	±	0.03	0.2	±	0.02	0.2	±	0.04	0.8	±	0.03
Non-GI Tissues	0.3	±	0.5	0.7	±	0.5	1.0	±	0.4	1.0	±	0.1
GI tract & content	0.5	±	0.4	0.8	±	0.5	1.3	±	0.6	1.3	±	0.7
Total Recovery	96.2	±	2.0	98.0	±	1.2	98.4	±	0.9	98.8	±	0.8

Table 1. Summary of dose recovery at 24 h following administration of TBP.

HPLC-radiometric analyses of urine found two clear peaks: one that co-eluted with the parent TBP standard and a second more hydrophilic peak (Figure *2*). The relative distributions of peak areas did not markedly change over time or with dose (data not shown). Mass spectral analyses of urine determined the major hydrophilic peak had a mass consistent with that of TBP-glucuronide (Figure 3). In addition, mass spectral analyses found a third compound that eluted immediately before the TBP standard that had a mass consistent with TBP-sulfate. Fecal extracts contained a single peak that coeluted with parent TBP.

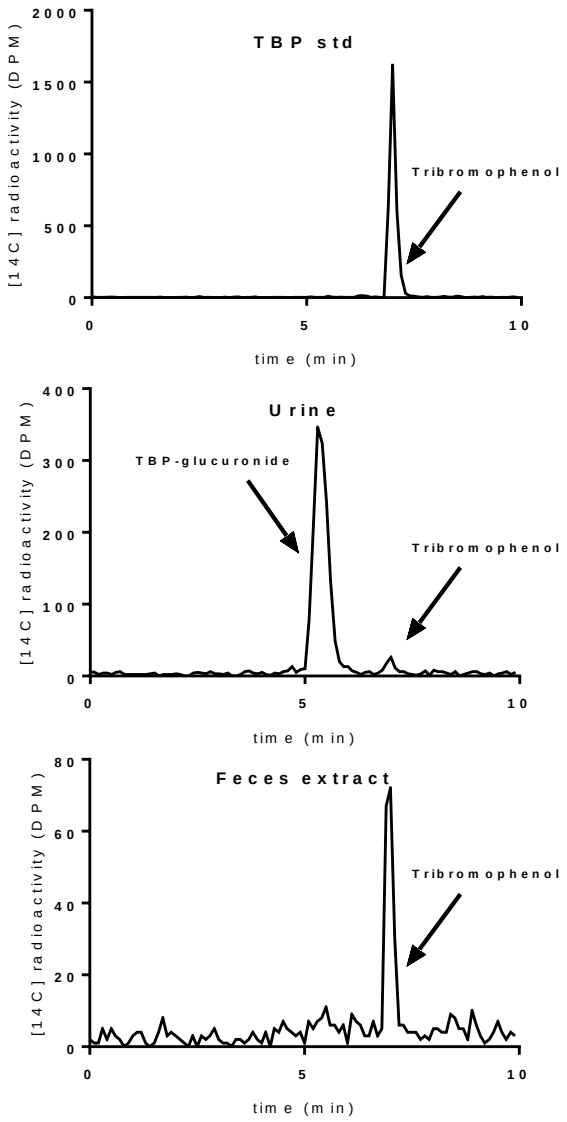

Figure 2. Representative HPLC-radiochromatograms of urine and fecal extracts following administration of TBP (1000 µmol/kg).

Discussion & Conclusions

This is the first comprehensive report of the disposition of TBP. TBP is classified as a high-volume chemical by the US EPA although exact production volumes are not publicly available. TBP is a legacy flame retardant contaminants in home and environmental exposure, especially to small children who are prone to ingesting dust through hand-mouth contact [16]. US households have the highest reported concentrations of brominated flame retardants in dust [16]. However, unlike other legacy BFRs (polybrominated diphenyl ethers, tetrabromobisphenol A, etc.) and novel BFRs (2-ethylhexyl tetrabromobenzoate, bis(2-ethylhexyl) tetrabromophthalate, etc.), TBP dust concentrations are infrequently reported.

TBP was extensively absorbed, readily metabolized, and eliminated by both urinary and fecal routes. At least 2 metabolites were present in urine. Urinary metabolite profiles differed little for doses above or below the 10 µmol/kg dose, indicating negligible saturation of one or more metabolic pathways. TBP 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 TBP or its metabolites was observed and would indicate a low likelihood of bioaccumulation. More work is needed to determine the nature of metabolic processes induced by repeated exposures to TBP. Future studies will include analysis of TBP biliary excretion following oral or IV dosing as well as bioaccumulation and enzyme induction or inhibition potential following repeated administration.

Acknowledgements

The authors would like to thank Ms. Sherry Coulter for technical assistance. This research was supported in part by the Intramural Research Program of NIH/NCI; this abstract may not reflect official NIH policy.

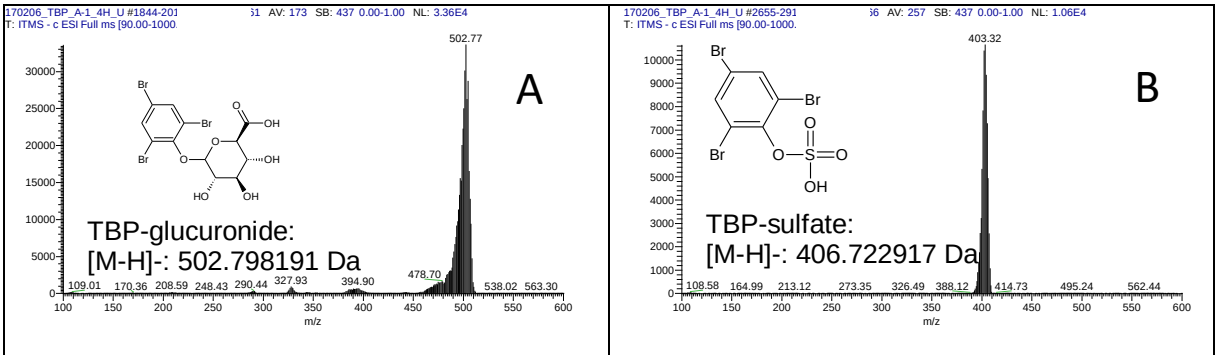

Figure 3. Mass spectra of urine collected after administration of TBP (1000 µmol/kg). A. Extracted ion chromatogram for peak 1 (Rt = 4.2-4.6 min). B. Extracted ion chromatogram for peak 2 (Rt = 6-6.5 min)

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