2,4,6-Tribromophenol disposition and kinetics in non-pregnant, pregnant, and nursing Sprague Dawley rats

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Introduction.

2,4,6-Tribromophenol (TBP, CAS No. 118-79-6, Figure 1) is a widely used brominated chemical used as a flame retardant (BFR), precursor for other BFRs, and wood antifungal agent (1-3). TBP is also naturally occurring in some marine fishes, such as Pacific salmon (4). Due to this wide variety of natural and anthropogenic sources, TBP is frequently detected (60-100% detection frequency) in environmental matrices and biota, including human breast milk, placenta, and serum (2, 3, 5-15). High levels of TBP have been reported in indoor air and in household dust, highlighting the potential for TBP exposure via inhalation and ingestion of dust (16, 17). In addition to potential oral or dermal exposure through dust, TBP is likely to be consumed in a diet rich in wild-caught fish (18).

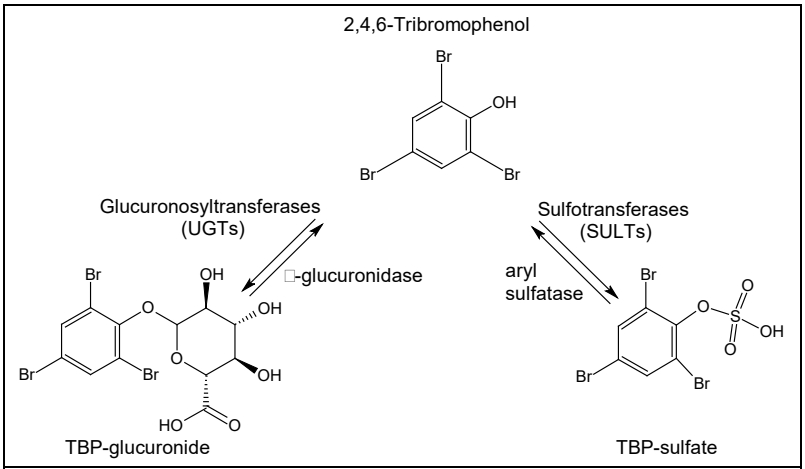

Figure 1. TBP chemical structure and metabolism.

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 correlated positively with those of polybrominated diphenyl ethers (PBDEs), suggesting similar sources of exposure, or that TBP may result from metabolism of PBDEs (5).

Accumulation of TBP in human placenta is expected to contribute to prenatal exposures (10). Recently, our laboratory published a description of the disposition and pharmacokinetics of TBP in Sprague Dawley rats (19). To address the observations of TBP accumulation in placenta and breast milk, studies were therefore conducted to characterize the disposition and toxicokinetic profile of TBP in pregnant (gestation day 12) or nursing (postnatal day 12) Sprague Dawley rats following single oral bolus (10 μmol/kg) administration to the dam to evaluate the disposition and kinetics of TBP *in utero* via gestational exposure and postnatally via lactational exposure.

Materials and methods

MODEL ORGANISM Non—pregnant and **t**imed-pregnant female Sprague Dawley rats (N = 4-5/dose group, 10-14 weeks old) were used in these studies. Animals were maintained in an AAALAC-approved animal care facility. Animals were housed individually or with their respective litters in polycarbonate shoebox cages. Litters were culled and balanced to 4 males and 4 females at 4 days postpartum. Food (NIH-31) 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 [^{14}C]-labeled TBP by gavage (PO; 10 μmol/kg, 25 μCi/kg, 4 mL/kg). Dosing solutions were composed of [^{14}C]-TBP dissolved in a corn oil vehicle.

SAMPLE COLLECTIONS Following administration of the compound, animals were euthanized at 0.25, 0.5, 1, 2, 3, 6, 12 or 24 h post dose and tissues were collected from the dam and fetuses or nursing pups. Blood was drawn from an indwelling jugular vein cannula from non-pregnant animals and the animals were euthanized after 24 h for tissue analyses. 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 -20ºC until analysis. Dam and pup blood samples were collected via cardiac puncture immediately following euthanasia. Samples were placed in labeled pre-weighed vials after all collections and maintained at -20ºC 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. Tissue aliquots were weighed and [^{14}C]-radioactivity was quantified by combustion in a Packard 307 Biological Sample Oxidizer followed by LSC counting. TBP was quantified by UV/Vis absorbance and radiochemical detection following HPLC separation. The HPLC system was composed of a Waters (Watertown, MA) Alliance 2695 separation module with a Waters 2487 dual wavelength detector, Phenomenex (Torrance, CA, USA) Luna 150 x 4.6 mm C18 column and an in-line Radiomatic 610TR Flow Scintillation Analyzer (PerkinElmer). HPLC control and analysis software was Laura4 (LabLogic, Brandon, FL, USA). Mobile phases consisted of (A) 0.2% formic acid in water and (B) 0.2% formic acid in acetonitrile. Sample separations were performed using a gradient from A to B; initial conditions (90% A) were reduced to 0% A over 5 min then held at 100% B for 5 minutes before returning the column to initial conditions and equilibrating the column for 2 minutes before re-use. HPLC flow rates were 1 mL/min and scintillation cocktail flow rates were 2 mL/min.

Results and discussion

The treatment groups (non-pregnant, GD 12, and PND 12) measured the following exposures: the disposition of TBBPA in pregnant and non-pregnant animals, the kinetics of TBP in nursing pups at maximum nursing, and the kinetics of TBP at the final point before weaning. Non-pregnant, pregnant (GD12) or nursing rats (PND12) were administered a single dose of [^{14}C]-labeled TBP and euthanized between 0.25 and 24 h to determine disposition in the dosed rats and their offspring (Figure 2). In non-pregnant rats, there was an observed rapid rise to Cmax, followed by an up-down-up oscillating concentration plateau (15 min-4 h) that may be due to enterohepatic cycling of TBP and its metabolites after oral administration of TBP. Systemic exposure decreased steadily post-dose in pregnant rats with the calculated C_{max} occurring before 15 min. In nursing rats C_{max} was observed at 1 h in lactating rats and concentrations fell steadily through 24 h.

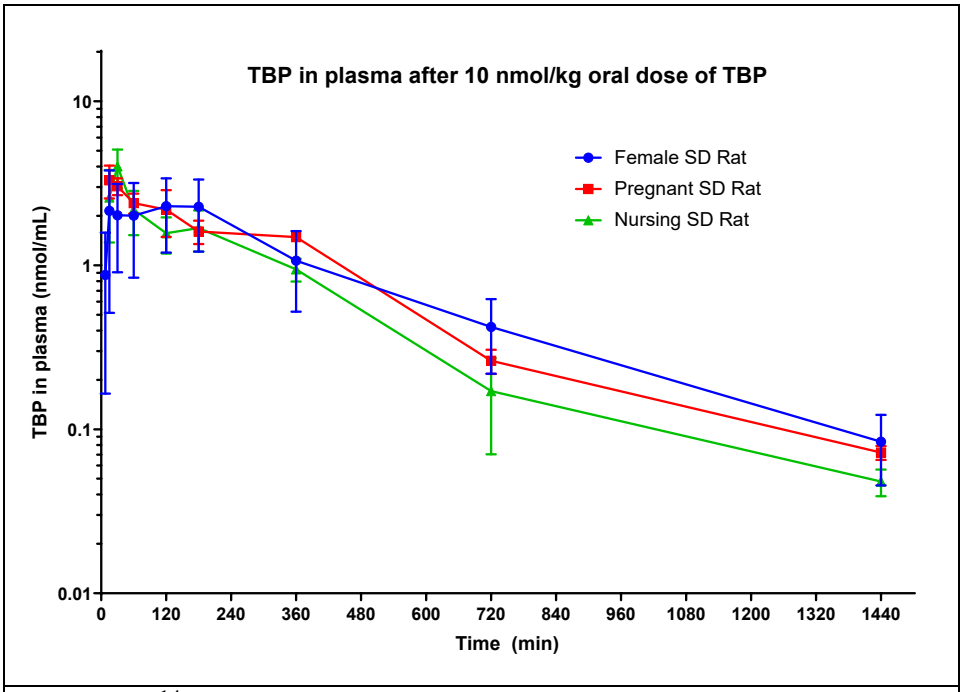

Figure 2. [^{14}C]-TBP in dam plasma following a single dose of TBP

Maternal exposure to TBP is expected to lead to transplacental fetal exposure or lactational exposure to pups. More research is needed to explore at what levels we see adverse effects in offspring and what mechanisms are affected. Research is also needed to determine why TBP is more readily absorbed in pregnant animals and nursing animals, although changes in total body water are expected to play a large role.

Gestation day 12 embryos were removed and quartered to determine disposition. As expected, fetal concentrations followed that of concentrations in the dam plasma. No radioactivity levels above the limit of detection were found in PND12 pup blood, indicating minimal systemic exposure to nursing animals. In lactating animals, liver, uterus, and mammary tissues remain to be assessed for TBP disposition. Fetal sex will be determined using a single-step PCR method (20) to determine whether sex or implantation site alters exposure. Lactational transfer of TBP or its metabolites will be assessed. In addition, concentrations of TBP and its metabolites will be assessed in stomach contents, liver, and kidney.

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