TBBPA disposition and kinetics in pregnant and nursing Wistar Han rats

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Introduction.

Tetrabromobisphenol A (TBBPA; CAS No. 79-94-7; Fig. 1) is a brominated flame retardant (BFR) commonly used in electronics to meet fire safety standards and consequently has the largest production volume of any BFR worldwide. TBBPA use represents nearly 60% of all worldwide demand for brominated flame retardants, with a global market volume of approximately 150,000 tons/year[1]. TBBPA is primarily used in printed circuit boards, Acrylonitrile-Butadiene-Styrene (ABS) plastic casings, and laminates. TBBPA is also used in paper, textiles, as a plasticizer, and as an intermediate for the syntheses of other flame retardants[2].

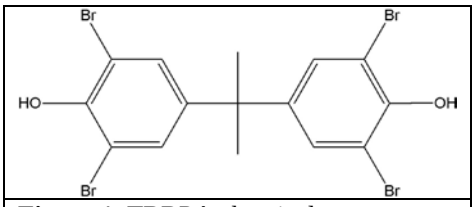

Figure 1. TBBPA chemical structure.

TBBPA has been consistently detected at low levels in environmental samples[3]; expected increases in the production and use of TBBPA rely on its application as an additive flame retardant, especially for use in ABS plastic housing for electronic devices. A study of post-partum mothers found 44% of breast milk samples and 30% of maternal/cord serum samples contained detectable levels of TBBPA, demonstrating significant exposure to mothers & fetuses and the risk of exposure of newborns via breastfeeding[4].

In single administration studies, TBBPA has an established LD50 of greater than 5 g/kg when administered by gavage to rats[5]. Intraperitoneal administration of TBBPA has been shown to cause hepatotoxicity and heme metabolism disturbances[6,7], phenomena that may relate to the formation of free radicals *in vivo*[8]. In repeat-dose subacute and one-generational reproductive studies, TBBPA exposures resulted in decreased thyroxine levels and other endocrine effects[9]. Hakk et al. demonstrated that TBBPA (2 mg/kg) is readily absorbed from the gastrointestinal tract of male Sprague-Dawley rats where it undergoes biotransformation to o-glucuronide and o-sulfate conjugates followed by biliary elimination to the intestine[10]. TBBPA was eliminated in the feces as parent compound. Kuester et al. demonstrated that at a 10-fold higher dose of TBBPA in male Fischer-344 rats, the systemic bioavailability of the compound (in whole blood) remained low (1.6% available) with a terminal half-life of 95 min[11]. We have shown previously that female Wistar-Han rats exhibit similar disposition and kinetic profiles as those seen for male SD and F344 rats[12]. Shauer et al. concluded that the bioavailability of TBBPA in humans following a single exposure is expected to be low but chronic exposures were not explored[13].

Data from TBBPA chronic exposure studies showed an enhanced susceptibility of female Wistar-Han rats to TBBPA toxicity[14].Studies were therefore conducted to characterize the disposition and toxicokinetic profile of TBBPA in pregnant (gestation day 20) or nursing (post natal day 12 or 20) Wistar-Han rats following single oral bolus (25 mg/kg) administration to the dam to evaluate the disposition and kinetics of TBBPA *in utero* via gestational exposure and postnatally via lactational exposure.

Materials and methods

MODEL ORGANISM Timed-pregnant Female Wistar-Han rats 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 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 TBBPA by gavage (PO; 25 mg/kg, 2.5 µCi/kg, 4 mL/kg). Dosing solutions were composed of [^{14}C]-TBBPA dissolved in a sesame oil vehicle.

SAMPLE COLLECTIONS Following administration of the compound, animals were euthanized at 0.5, 1, 2, 4, and 8 h post dose and tissues were collected from the dam and fetuses or nursing pups. 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. Dam blood samples were collected via cardiac puncture immediately following euthanasia. 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. Fetal samples were snap frozen and homogenized using a BioPulverizer Cryogenic Tissue Crusher. Tissue aliquots were weighed and [^{14}C]-radioactivity was quantified by combustion in a Packard 307 Biological Sample Oxidizer followed by LSC counting. TBBPA was quantified by UV/Vis absorbance and radiochemical detection following HPLC separation. High Performance Liquid Chromatography (HPLC) system used for analysis of extracts from dosed plasma was composed of an Agilent 1100 system, an Agilent Eclipse Plus C18 column (3.5µm, 4.6mm×150mm) and an IN/US βRAM-3. 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 a gradient. Initial conditions (100% A) were reduced to 60%A over 5 minutes, 60%A was reduced to 10%A over 2 minutes, then to 0%A over 5 minutes. 0%A was maintained for one minute and then returned to initial conditions over one minute. Flow rates were 1 mL/min. Instrument control and analysis software was LAURA4. Radiochemical flow cell volume was 500 µL and scintillant flow rate was 2 mL/min.

Results and discussion

The treatment groups (GD 20, PND 12, and PND 20) measured the following exposures: the disposition of TBBPA in pregnant animals, the kinetics of TBBPA in nursing pups at maximum nursing, and the kinetics of TBBPA at the final point before weaning. Pregnant (GD20) or nursing rats (PND12 or PND20) were administered a single dose of [^{14}C]-labeled TBBPA and euthanized between 0.5 and 8 h to determine disposition

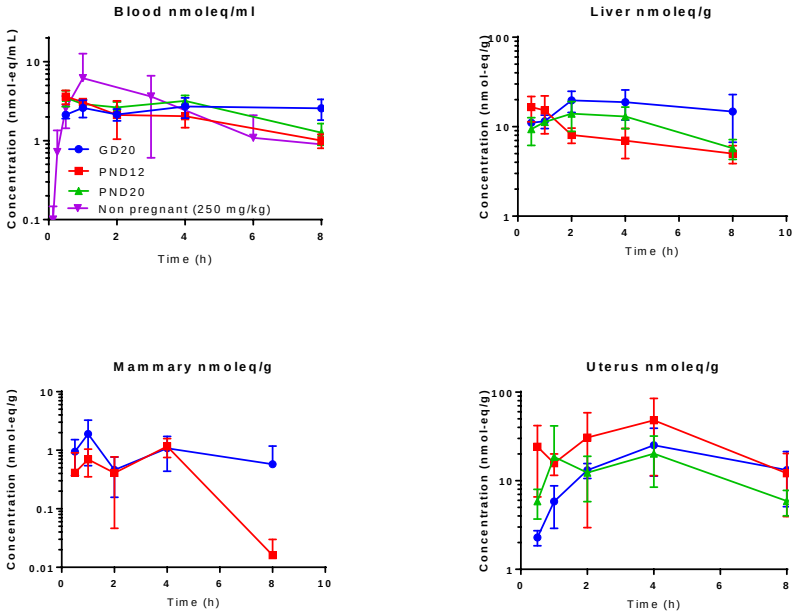

Figure 2. [^{14}C]-radioactivity in dam tissues following exposure

in pregnant or nursing Wistar Han rats and their offspring. Systemic exposure was largely unchanged between 1 and 8 h post-dose in pregnant rats; [^{14}C]-radioactivity concentrations in blood varied only slightly over the time course (2.6±0.6→2.6±0.8 nmol-eq/mL). C_{max} was observed at 30 min in lactating rats and concentrations fell steadily through 8 h. HPLC analyses of [^{14}C]-radioactivity in dam plasma found a mixture of TBBPA and TBBPA-conjugates was detected in the 30 minute time-point sample from pregnant rats while all other samples contained only conjugated TBBPA in the plasma (Fig. 2). Parent TBBPA was not detected in lactating dam plasma.

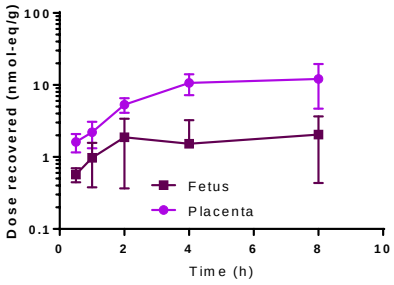

Figure 3. TBBPA dose recovery in fetuses and placenta (GD20)

Placental concentrations increased through 8 h while whole-fetus C_{max} occurred at 2 h post dose (Fig. 3). In lactating animals, liver, uterus, and mammary time-concentration curves lagged slightly behind blood-concentration curves. Dose equivalents in the placentas collected at GD20 increased with time following exposure, and showed levels of TBBPA approximately 10 nmol-eq/g higher than were present in the fetuses. Approx. 0.5% of the dose remained in the placenta at 8 h post dose, with individual litters containing approximately 1% of the dose.

Lactational transfer of TBBPA or its metabolites was detected, with 0.1% of the dose found in mammary tissue and an additional 0.01% of the dose found in pup liver or stomach contents (Fig. 4). TBBPA concentrations in the PND 12 pup livers decreased with time by approximately 0.2 nmol-eq/g. The levels of TBBPA in the PND 20 pup livers show no general trend. The level of TBBPA in the PND 12 pup stomach contents increase from approximately 0.5 nmol-eq/g at 4h to ~1.0 nmol-eq/g at 8h. The levels of TBBPA in the PND 20 pup stomach contents follow a similar trend to the PND 12 pup stomach contents, with a maximum at

~1.0 nmol-eq/g. No statistically significant sex or litter differences were observed. In GD 20 dam plasma, parent compound and metabolites (TBBPA-glucuronide and TBBPA-sulfate) were seen at 0.5 h and 1 h. Only metabolites were found at the later time points. In PND 12 and PND 20 dams' plasma, only metabolites were found at all time points.

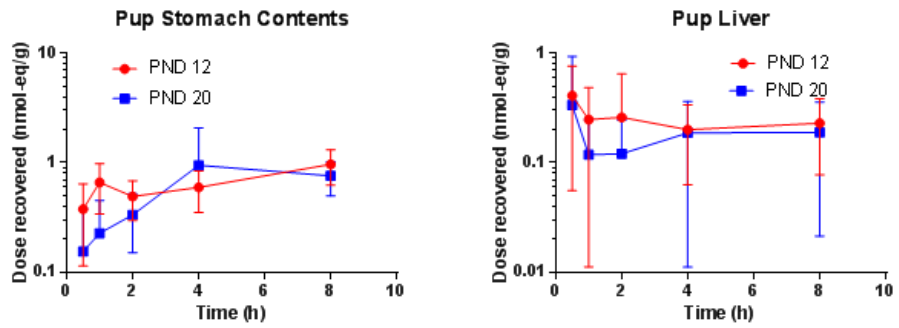

Figure 4. TBBPA dose recovery in PND 12 and PND 20 pup tissues

TBBPA was detected in pup livers and stomach contents following lactational exposure to the chemical and in fetuses following gestational exposure. The presence of metabolite at early time points in the pregnant and nursing animals supports studies that have shown efficient metabolism of TBBPA in fetal and juvenile liver.

Maternal exposure to TBBPA leads 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 TBBPA is less efficiently metabolized in pregnant animals than in nursing animals.

Acknowledgements

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