BIOLOGICAL EFFECTS OF TETRABROMOBISPHENOL A (TBBPA) IN FEMALE WISTAR-HAN RATS

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Introduction

Tetrabromobisphenol A (TBBPA; CAS No. 79-94-7) is a brominated flame retardant with a global market volume of approximately 150,000 tons/year.[1] As such, TBBPA use represents nearly 60% of all worldwide demand for brominated flame retardants. TBBPA is primarily used in printed circuit boards, Acrylonitrile-Butadiene-Styrene (ABS) plastic casings, and laminates. TBBPA is used in paper, textiles, as a plasticizer, and as an intermediate for the syntheses of other flame retardants like TBBPA-bis(2,3-dibromopropyl ether), TBBPA-allyl ether, and TBBPA carbonate oligomers.[2] TBBPA is expected to undergo an expansion in application and production as polybrominated diphenyl ethers and hexabromocyclododecanes are withdrawn from the market.

TBBPA has been consistently detected at low levels in environmental samples; 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.[3] A recent 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 hepatotoxicities and heme metabolism disturbances, phenomena that may relate to the formation of free radicals *in vivo*.[6-8] 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] Shauer et al. concluded that the bioavailability of TBBPA in humans following a single exposure is expected to be low; chronic exposures were not explored.[12]

Ongoing analyses of data from TBBPA chronic exposure studies indicate an enhanced susceptibility of female Wistar-Han rats to TBBPA toxicities.[13] Studies were therefore conducted to characterize the disposition and toxicokinetic profile of TBBPA in female Wistar-Han rats following single oral bolus (25, 250 or 1,000 mg/kg) or intravenous (25 mg/kg) administration to determine whether any unique ADME/PK phenomena exist in this strain/sex combination. In addition, studies were conducted to investigate alterations in genes known to regulate thyroid homeostasis, oxidative stress, and perturbations of lipid and endogenous estrogen metabolism. The present work investigated modes-of-action of TBBPA-mediated toxicity in female Wistar Han rats.

Materials and methods

MODEL ORGANISM Female Wistar-Han rats 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. Prior to gene response studies, animals were placed in synchronized estrus by exploiting the Whitten effect.[14] All procedures were approved by the NIEHS Institutional Care and Use committee.

DOSING For disposition and kinetics studies animals were administered a single dose of TBBPA by gavage (PO) or intravenous (IV) bolus through an indwelling catheter. PO doses were: 25, 250, or 1000 mg/kg (4 or 8 mL/kg). IV dose was 25 mg/kg (1 mL/kg). Dosing solutions were composed of ethanol, water, and an emulsifying agent (Cremophore EL) in a 1:3:1 ratio. IV and PO dosing solutions provided 50 μCi/kg [^{14}C]-TBBPA. For gene response studies animals were administered 5 consecutive daily doses of TBBPA or vehicle by gavage (250 mg/kg, 4 mL/kg; N = 10/treatment group) by gavage.

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 or an indwelling jugular vein cannula into heparinized syringes at intervals between 7.5 min and 24 h post-dose. 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). For gene reponse studies, the rats were euthanized twenty-four hours following the final dose and biological changes were assessed in blood, liver, and uterus.
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 counting. TBBPA was quantified by UV/Vis absorbance and radiochemical detection following HPLC separation. The HPLC system was composed of a Waters 2695 Separations Module, Agilent Eclipse Plus C18 column (3.5 µm, 4.6 x 150 mm), and a Waters 996 Photodiode Array with an in-line Radiomatic 500TR Flow Scintillation Analyzer. Mobile phases consisted of 0.1% acetic acid in water (mobile phase A) and 0.1% acetic acid in acetonitrile (mobile phase B). Sample separations were performed using a gradient; initial conditions (60% A) were maintained for 5 minutes then A was reduced to 10% over 2 minutes then to 0% A over 13 minutes. The column was returned to initial conditions and allowed to equilibrate for 5 minutes. Flow rate was 1 ml/min. Instrument control and analysis software were Empower Pro (Waters Corp.) and FLO-ONE for Windows (Packard Instruments Co., Inc.; v. 3.6). TBBPA was quantified based on a 5-point calibration curve.
PK MODEL & SOFTWARE TBBPA time-concentration data were fit to established pharmacokinetic models using the Phoenix WinNonlin (Certara L.P.) software package. Time-concentration data from animals administered TBBPA intravenously were fit to a one-compartment model with bolus input and first order output. Time-concentration data from animals administered TBBPA by gavage were fit to a one-compartment model with first order input and output or a two-compartment model with first order input and output. Goodness of fit was assessed by comparing the sum of squared residuals.
GENE STUDIES Serum concentrations of thyroid hormones (T3, T4, and TSH) and estradiol (E2) were determined. RNA was isolated from liver and from the proximal (nearest the vagina) and the distal (nearest the ovaries) sections of the uterine horn. The purity and quality of the RNA were verified and the RNA was reversed-transcribed and analyzed by quantitative PCR using probe-based assays. Genes of interest were chosen to investigate putative biological pathways affected by TBBPA treatment.

Results and discussion
Toxicokinetic profiles for TBBPA following oral or IV administrations were estimated based on free TBBPA detected in plasma (Figure 2). Oral administration of TBBPA (250 mg/kg) resulted in a rapid absorption of compound, with a C_{max} occurring between 1-3 h post-dose, and the subsequent distribution phase was consistent with a one-compartment model. The absorption and distribution half-lives were approx. 71 and 104 min, respectively. Following IV administration (25 mg/kg), TBBPA concentrations in plasma decreased rapidly with less than 2% of the administered radioactivity remaining at 6 h post dose. The time-concentration profile for free TBBPA following IV administration of [^{14}C]-TBBPA was consistent with a one-compartment model. These results indicate less than 5% systematic bioavailability.
Following oral administration of [^{14}C]-TBBPA, the primary route of elimination of radioactivity was in feces; dose recoveries in 72h were 95.7±3.5%, 94.3±3.6% and 98.8±2.2%, respectively (Figure 1). After a single IV administration of 25 mg/kg of [^{14}C]-TBBPA, ~10% of the administered radioactivity was eliminated in the feces within 6 h. Animals administered 1,000 mg/kg TBBPA showed clear differences in rates of fecal elimination through 24 h. Recoveries in urine ranged from 0.2-2% of dose. Less than 0.1% of the administered [^{14}C]-radioactivity was detected in tissues collected at 72 h following oral doses.

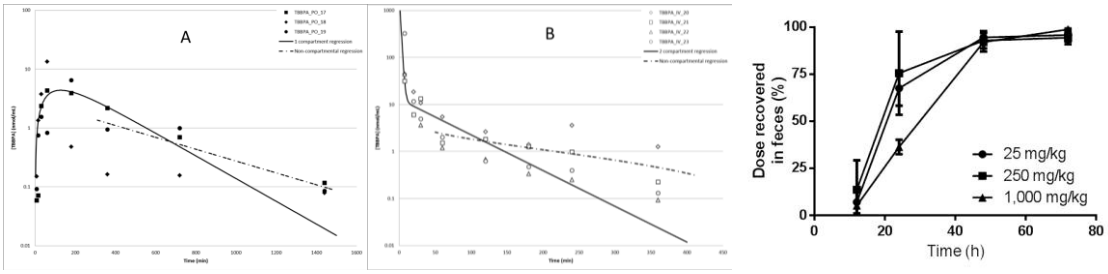

Figure 1. Kinetics and disposition of TBBPA in female Wistar Han rats. A & B: Time-concentration profiles of TBBPA in plasma following B: oral administration (250 mg/kg; N=3); C: IV administration (25 mg/kg; N=4). Regression lines show model-predicted data.C:Cumulative disposition and elimination following oral administration of TBBPA.

TBBPA treatment resulted in changes in the serum concentration of T4 and in expression of genes Thra and Thrb for thyroid receptor alpha (TRα) and beta (TRβ), respectively. Although, the serum concentration of estradiol was not affected, expression of mRNA for estrogen receptor alpha (ERα) or beta (ERβ) was affected in uterine tissue of TBBPA–treated rats. TBBPA treatment also resulted in changes in expression of Ppara, in genes associated with cell division and growth (Ccnb1, Ccnb2, Ccnd3, Cdk4, and Igf1), and in genes associated with metabolism of TBBPA and/or estrogen (Comt, Cyp1b1, Cyp2b1, Cyp2b2, Cyp17a1, Hsd17b2, Hsd17b4, Sult2a1, and Ugt1a1).

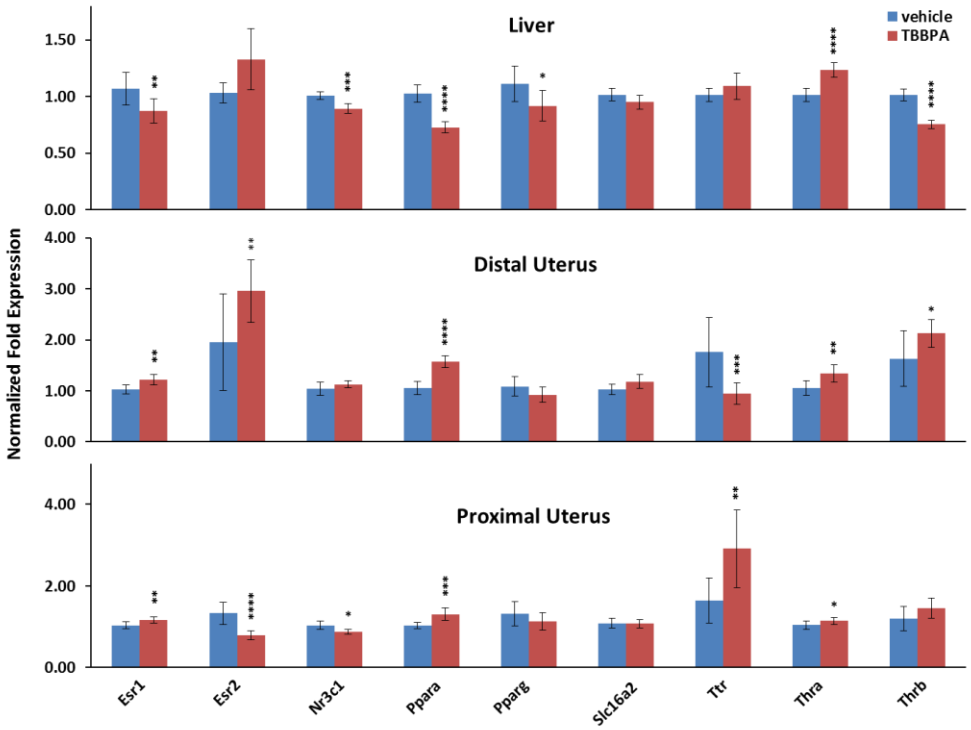

Figure 2. Expression of genes associated with transport or receptors of thyroid hormones and/or estrogen in tissues of rats 24 hours following 5 days oral administration of TBBPA or vehicle. Each value is the mean ± SE of 6-10 rats/group and 3-9 replicates/rat. The value is significantly different from vehicle control at *p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001.

Discussion & Conclusions

Changes in expression of some genes were organ-dependent (liver or uterus) or specific to tissue location in the uterine horn. The biological changes observed in the present study may correlate to specific toxicities, including the carcinogenic response observed in TBBPA-treated female rats.At the 12 and 24 h times low but detectable amounts of TBBPA persisted and could not be accounted for by the model; this persistence of compound in plasma may be due in part to entero-hepatic recirculation of TBBPA. Accumulated evidence suggests biliary elimination is slower in fed female Wistar-Han rats than has been previously reported using other strains. However, these data indicate that TBBPA has a similar ADME-TK profile in female Wistar-Han rats as that previously reported for male Sprague-Dawley or Fischer-344 rats.[10,11,15]

Acknowledgements

The authors would like to thank Dr. June Dunnick (National Toxicology Program) for advice and Mr. Abdella Sadik for technical assistance. This research was supported in part by the Intramural Research Program of NIH/NCI.

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