Chemosphere 192 (2018) 5–13

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

TBBPA disposition and kinetics in pregnant and nursing Wistar Han IGS rats

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HIGHLIGHTS

- Systemic exposure was unchanged between 1 & 8 h post dose in pregnant rats after a single oral dose.
- Maximal blood concentrations were observed at 30 min in nursing rats and fell steadily through 8 h.
- TBBPA is available to both the developing fetus and nursing pup following maternal exposure.
- Nursing pups are continuously exposed via contaminated milk produced by their mother.

ARTICLE INFO

Article history:
Received 18 July 2017
Received in revised form
16 October 2017
Accepted 23 October 2017

Keywords:
Brominated flame retardant
Toxicokinetics
Disposition
Perinatal exposure
Postnatal exposure
Pregnant and nursing dams

ABSTRACT

Tetrabromobisphenol-A (TBBPA) is a brominated flame retardant (BFR) commonly used in electronics to meet fire safety standards and has the largest worldwide production of any BFR. TBBPA has been detected in human breast milk and maternal/cord serum, indicating exposure to mothers, fetuses, and breastfeeding newborns although exposure to fetuses and newborns is poorly understood. Pregnant or nursing Wistar Han IGS rats were administered [^{14}C]-TBBPA in a single dose (25 mg/kg, 2.5 μCi/kg) and euthanized between 0.5&24 h post dose to determine disposition in pregnant and nursing rats and their pups. Systemic exposure was largely unchanged between 1&8 h post dose in pregnant rats; [^{14}C]-radioactivity in blood varied only slightly between 0.5&8 h ($2.6 \pm 0.6 \rightarrow 2.6 \pm 0.8$ nmol-eq/mL) but was below the limit of detection at 24 h with an absorption half-life of 16min and elimination half-life of 17 h. C_{max} was observed at 30min in lactating rats and concentrations fell steadily through 8 h. Plasma from pregnant rats contained a mixture of TBBPA and TBBPA-conjugates at 30min but only metabolites in subsequent samples. TBBPA was not detected in lactating dam plasma in this study. Placental concentrations increased through 8 h while whole-fetus C_{max} occurred at 2 h post dose. In lactating animals, liver, uterus, and mammary time-concentration curves lagged slightly behind blood-concentration curves. It was clear from these studies that TBBPA is available to both the developing fetus and nursing pup following maternal exposure, and nursing pups are continuously exposed via contaminated milk produced by their mother. This research was supported in part by the Intramural Research Program of NIH/NCI.

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1. Introduction

Tetrabromobisphenol A (TBBPA, CAS No. 79-94-7, Fig. 1) currently represents the highest production volume brominated flame retardant (BFR) and constitutes approximately 60% of worldwide demand for BFRs. TBBPA is used in electronics to meet fire safety standards, with a global market volume of >145,000 metric tons per year (de Wit et al., 2010). TBBPA is most commonly produced as a reactive flame retardant in printed circuit boards, but approximately 10–30% of applications are as an additive FR, most notably in Acrylonitrile-Butadiene-Styrene (ABS) plastic casings (Canada, 2012; BSEF, 2012). Over 90% of printed circuit boards contain TBBPA in the form of 'reactively-bound' flame-retardant although it has been shown that an estimated 0.06% of the total amount used remains unbound (USEPA, 2013). TBBPA is also used in paper, textiles, as a plasticizer, and as an intermediate for the syntheses of other flame retardants (BSEF, 2012).

In single administration studies, TBBPA has an LD_{50} of greater

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https://doi.org/10.1016/j.chemosphere.2017.10.122
0045-6535/Published by Elsevier Ltd.

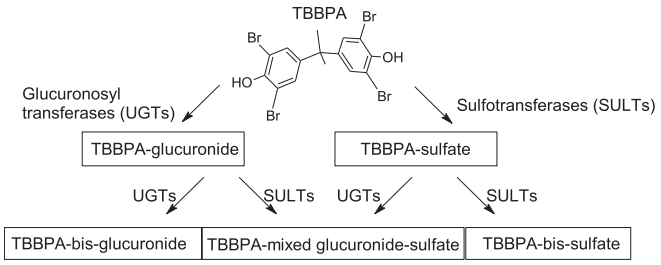

Fig. 1. Chemical structure for TBBPA and metabolism scheme for its conjugative metabolites.

than 5 g/kg when administered by gavage to rats (IPCS/WHO, 1995). Hakk et al. demonstrated that TBBPA (2 mg/kg) is readily absorbed from the gastrointestinal tract of male Sprague Dawley (SD) rats where it undergoes biotransformation to *o*-glucuronide and *o*-sulfate conjugates (Fig. 1) followed by biliary elimination to the intestine (Hakk et al., 2000). Conjugated TBBPA is readily deconjugated in the gut by microflora and absorbed TBBPA is eliminated in the feces as parent chemical. Kuester et al. showed that at a 10-fold higher dose of TBBPA, the systemic bioavailability of the compound (in whole blood) remained low (1.6% available) with a terminal half-life of 95 min in male Fischer-344 (F344) rats (Kuester et al., 2007). We have shown previously that female Wistar Han IGS [Crl:WI(Han)] rats (Knudsen et al., 2014) exhibit similar disposition and kinetic profiles as those seen for male SD and F344 rats. Shauer et al. concluded that the bioavailability of TBBPA in humans following a single exposure is expected to be low (Schauer et al., 2006) but the bioavailability of TBBPA in humans after chronic exposures in pregnant and non-pregnant humans was not explored.

TBBPA is a rodent carcinogen, a suspected carcinogen in humans, and can act as an endocrine disruptor. In repeat-dose subacute and one-generation reproductive studies, TBBPA exposure resulted in decreased thyroxine levels and other endocrine effects (Van der Ven et al., 2008; Sanders et al., 2016). TBBPA interacts with peroxisome proliferator-activated receptors (PPARs) to induce downstream obesogenic effects like adipocyte differentiation via PPAR-gamma (Riu et al., 2011). At very high doses, TBBPA causes hepatotoxicities and heme metabolism disturbances (Szymanska et al., 2000, 2001) that are likely due to the formation of free radicals (Chignell et al., 2008). Repeated and chronic exposures to orally administered TBBPA resulted in the downregulation of gene products implicated in several immunologic pathways in uterine tissues (Sanders et al., 2016; Dunnick et al., 2014; Hall et al., 2017). TBBPA has also been shown to compete with estrogen for conjugation by the estrogen sulfotransferase (SULT1E1), potentially prolonging estrogen signaling in sensitive tissues (e.g., uterus) (Gosavi et al., 2013).

The primary routes of exposure to TBBPA are through ingestion, inhalation, or dermal contact with contaminated dust particles. Recent studies of postpartum mothers found 44–50% of breast milk samples and 30% of maternal/cord serum samples contained detectable levels of TBBPA, demonstrating significant exposure to mothers and fetuses and the risk of exposure of newborns via breastfeeding (Cariou et al., 2008; Fujii et al., 2014; Shi et al., 2013). TBBPA has been consistently detected at low levels in environmental samples (Covaci et al., 2011) but these levels are expected to increase as the production and use of TBBPA in an additive mode increases (BSEF, 2012).

Data from TBBPA chronic exposure studies showed an enhanced susceptibility of female Crl:WI(Han) rats to TBBPA toxicities, including dose-dependent increases in uterine epithelial tumors (NTP, 2014). The presence of TBBPA in human breast milk and serum samples and the chemical's potential for endocrine disruption and tumorigenesis prompted this study into the disposition and kinetics of TBBPA in dams and pups after exposure *in utero* or during nursing. The treatment groups measured the following exposures: disposition of TBBPA in pregnant dams and fetuses immediately prior to parturition (gestation day 20; GD20); the kinetics of TBBPA in nursing pups at the age of maximal milk consumption (postnatal day 12; PND12); and the kinetics of TBBPA immediately prior to weaning (postnatal day 20; PND20). In all cases, the Crl:WI(Han) rat dam was administered a single oral bolus of TBBPA (25 mg/kg).

2. Materials and methods

2.1. Chemicals

[^{14}C]-labeled TBBPA (ring-labeled; Fig. 1, Lot # 3225-235, Perkin Elmer Life and Analytical Sciences [Boston, MA], re-purified in 2013 by Moravek Biochemicals [Brea, CA]) and used in these studies had a radiochemical purity of >98% (specific activity = 90.3 mCi/mmol) and a relative chemical purity of >98%, as compared to a TBBPA reference standard (Sigma-Aldrich; St. Louis, MO). Scintillation cocktails were obtained from MP Biomedicals (Ecolume; Santa Ana, CA) or Perkin-Elmer (Ultima-Flo M & PermaFluor E+; Torrance, CA). All other reagents used in these studies were high performance liquid chromatography (HPLC) or analytical grade.

2.2. Animal model

Timed-pregnant female Crl:WI(Han) rats (Charles River

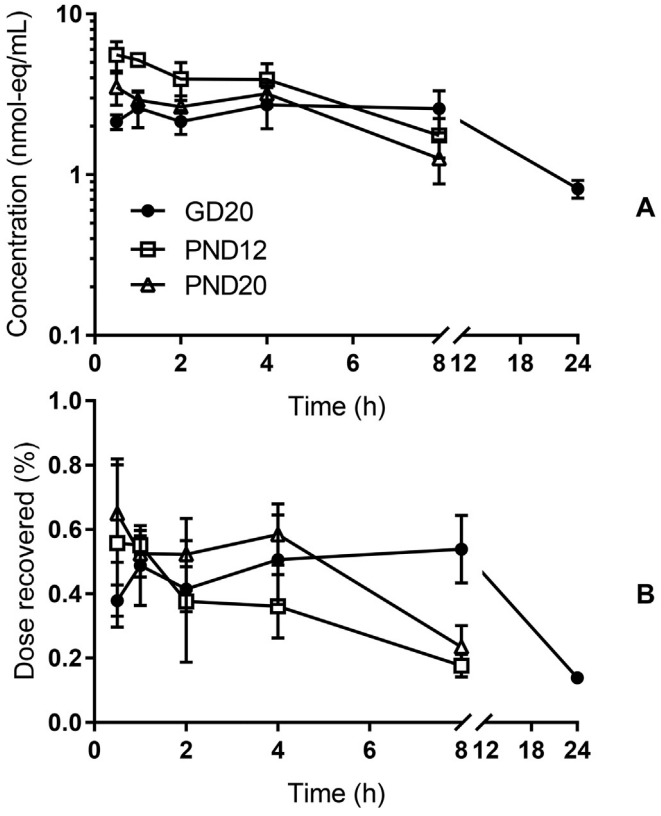

Fig. 2. Time-concentration curves for total chemical (nmol-eq/mL, panel A) in whole blood from rat dams collected at GD20, PND12, and PND20. Panel B: dose fraction recovered in blood. N = 4–5 dams per time-point.