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Disposition and kinetics of tetrabromobisphenol A in female Wistar Han rats

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ABSTRACT

Tetrabromobisphenol A (TBBPA) is the brominated flame retardant with the largest production volume worldwide. NTP 2-year bioassays found TBBPA dose-dependent increases in uterine tumors in female Wistar Han rats; evidence of reproductive tissues carcinogenicity was equivocal in male rats. To explain this apparent sex-dependence, the disposition and toxicokinetic profile of TBBPA were investigated using female Wistar Han rats, as no data were available for female rats. In these studies, the primary route of elimination following [^{14}C]-TBBPA administration (25, 250 or 1000 mg/kg) was in feces; recoveries in 72 h were 95.7 ± 3.5%, 94.3 ± 3.6% and 98.8 ± 2.2%, respectively (urine: 0.2–2%; tissues: <0.1). TBBPA was conjugated to mono-glucuronide and -sulfate metabolites and eliminated in the bile. Plasma toxicokinetic parameters for a 250 mg/kg dose were estimated based on free TBBPA, as determined by UV/radiometric-HPLC analyses. Oral dosing by gavage (250 mg/kg) resulted in a rapid absorption of compound into the systemic circulation with an observed C_{max} at 1.5 h post-dose followed by a prolonged terminal phase. TBBPA concentrations in plasma decreased rapidly after an IV dose (25 mg/kg) followed by a long elimination phase. These results indicate low systemic bioavailability ($F < 0.05$), similar to previous reports using male rats. Elimination pathways appeared to become saturated leading to delayed excretion after a single oral administration of the highest dose (1000 mg/kg); no such saturation or delay was detected at lower doses. Chronic high exposures to TBBPA may result in competition for metabolism with endogenous substrates in extrahepatic tissues (*e.g.*, SULT1E1 estrogen sulfation) resulting in endocrine disruption.

1. Introduction

Tetrabromobisphenol A (TBBPA, 2,2′,6,6′-Tetrabromo-4,4′-isopropylidene diphenol; CAS No. 79-94-7) is the largest production volume brominated flame retardant (BFR) with >230,000 tons manufactured per year [13]. This represents ~60% of all worldwide demand for BFR chemicals; TBBPA is used primarily as a reactive (chemically-bound) flame retardant in >90% of printed circuit boards, as well as in laminates, paper, textiles, as a plasticizer, and intermediate for the syntheses of other flame retardants [7]. Although it has been purported to be sequestered in its end-use product [7], TBBPA has been consistently detected in environmental samples [12]. Use of TBBPA as an additive flame retardant (not chemically bound) has been reported in products like Acrylonitrile–Butadiene–Styrene plastic casings and this application is expected to increase as other flame retardants (*e.g.*, high molecular weight polybrominated diphenyl ethers and hexabromocyclododecanes) are

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withdrawn from the market [8]. This additive use increases the potential for its release to the environment. Recent studies of nursing mothers found greater than 50% of breast milk samples and 30% of maternal/cord serum samples contained detectable levels of TBBPA, demonstrating widespread exposure to mothers & fetuses and potentially to newborns *via* breastfeeding [1,3,9,10,35,36].

TBBPA has been shown to be a ligand for several hormone receptors *in vitro*. TBBPA is a T4 competitor in the TTR-binding assay [19,20] and binds to the rat thyroid hormone receptor with high affinity [25]. TBBPA was also reported to bind to human transthyretin [28]. Both TBBPA and TBBPA-sulfate have been shown to be human peroxisome proliferator-activated receptor γ [31,32]. In studies using the estrogen-dependent rat pituitary tumor cell line MtT/E-2, TBBPA enhanced proliferation but to a lower extent than its non-brominated analog, bisphenol A [25]. TBBPA binds to the estrogen receptor, but to a lower degree than bisphenol A [33]. TBBPA was an estrogen receptor subtype alpha (ERα) agonist and a progesterone receptor (PR) antagonist in transfected yeast. TBBPA and two metabolites (2,6-dibromo-4-[2-hydroxypropane-2-yl]-phenol and 2,6-dibromo-4-[2-methoxypropane-2-yl]-phenol) have been shown to have estrogenic activity in MCF-7 cells [26,33,39]. TBBPA has been shown to be an inhibitor of estradiol sulfation *in vitro* [19]. Recent crystallographic studies show that it can be bound with high affinity to the estrogen sulfotransferase SULT1E1 [16].

TBBPA has an LD_{50} of greater than 5 g/kg when administered as a single dose by gavage to rats [21]. Intraperitoneal administration of TBBPA has been shown to cause hepatotoxicity and heme metabolism disturbances [37,38], phenomena that may relate to the formation of free radicals *in vivo* [11]. In repeat-dose subacute and one-generation reproductive studies, TBBPA exposures resulted in decreased thyroxine levels and other endocrine effects [40]. Hakk et al. [18] demonstrated that, at a dose of 2 mg/kg, TBBPA is readily absorbed from the gastrointestinal tract of male Sprague Dawley (SD) rats where it undergoes biotransformation to *o*-glucuronide and *o*-sulfate conjugates followed by biliary elimination. TBBPA was eliminated in the feces as parent compound demonstrating intestinal deconjugation. Similarly, at a 300 mg/kg dose of TBBPA to male SD rats, TBBPA was rapidly absorbed with a plasma C_{max} of 103 μmol/L reached within 3 h; TBBPA-sulfate was the major metabolite detected in plasma along with TBBPA-glucuronide [34]. Kang et al. [24] reported plasma C_{max} values of 23, 34, and 57 μmol/L for oral doses of 200, 500, and 1000 mg/kg TBBPA (T_{max} occurred at 4.2–5 h), respectively for male SD rats; metabolites were not described. Kuester et al. [27] demonstrated that at a 20 mg/kg dose of TBBPA in male Fischer-344 (F-344) rats, the systemic bioavailability of the compound was low (1.6%). The bioavailability of TBBPA in humans is unknown, although it is expected to be low. TBBPA was absorbed from the gut of healthy human volunteers receiving a single oral dose of 0.1 mg/kg but TBBPA was below the limit of detection in all blood samples. However, a glucuronide conjugate of TBBPA was present in samples collected at all of the time points up to 72 h, with peak concentrations detected between 2 and 6 h and traces of

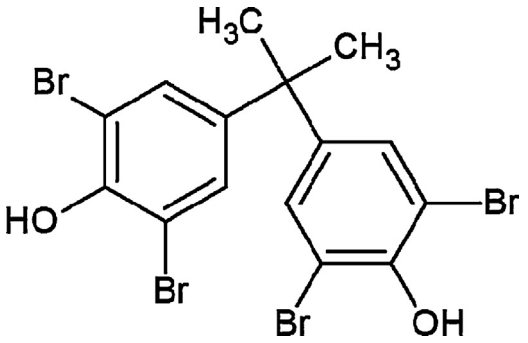

Fig. 1. Chemical structure of TBBPA.

TBBPA-glucuronide were detected in urine [34]. TBBPA was detected at low levels (<1–3.4 pmol/g lipid) in serum of computer workers and workers in an electronics dismantling plant [17,22]. TBBPA was metabolized by human liver subcellular fractions in a similar fashion to those of rats [41].

Ongoing analyses of data from TBBPA chronic exposure studies has demonstrated that TBBPA induced highly malignant uterine tumors in female Wistar Han [Crl:WI(Han)] rats in a dose-dependent manner. Male Crl:WI(Han) rats did not develop a significant tumor burden whereas administration of TBBPA resulted in increased incidences of nonneoplastic lesions of the liver and kidney in male B6C3F1 mice and in the forestomach of male and female mice [30]. However, no disposition, metabolism or kinetics data are available for the female Crl:WI(Han) rat. Therefore, studies were conducted to characterize the disposition of TBBPA in female Crl:WI(Han) rats following single or repeated oral or intravenous administration. The toxicokinetic profile of TBBPA in plasma after a 250 mg/kg dose was also investigated, as this dose was the lowest dose found to be associated with tumor formation in the NTP 2-year bioassay [30].

2. Materials and methods

2.1. Chemicals

[^{14}C]-Radiolabeled TBBPA (uniform ring-labeled; Fig. 1), previously described by Kuester et al. [27] was generously provided by I. Glenn Sipes at the University of Arizona (Tucson, AZ) for use in the present study. Reversed-phase HPLC fractionation (described below) was used to acquire a radiochemical purity of >98% (specific activity = 90.3 mCi/mmol). [^{14}C]-TBBPA was reconstituted in dimethyl sulfoxide. Chemical purity was determined to be >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). Cremophore EL®, dimethylsulfoxide, β-glucuronidase (EC 3.2.1.31, Type B-10), ammonium acetate, D-saccharic acid 1,4-lactone, and aryl sulfatase were purchased from Sigma–Aldrich. All other reagents used in these studies were high performance liquid chromatography (HPLC) or analytical grade.