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Risk assessment and Biomonitoring Equivalent for 2-ethylhexyl-2,3,4,5 tetrabromobenzoate (TBB) and tetrabromobenzoic acid (TBBA)

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ABSTRACT

2-ethylhexyl-2,3,4,5 tetrabromobenzoate (TBB) is used as a flame retardant. Biomonitoring for TBB exposures include the metabolite, tetrabromobenzoic acid (TBBA), in urine. We derived a Reference Dose (RfD) for TBB and a Biomonitoring Equivalent (BE) for TBBA in urine. Three longer-term studies of oral gavage dosing of a commercial mixture BZ-54 (which includes 70% TBB) in rats were evaluated for deriving the RfD. The 95% lower confidence limits on the BMD associated with a 1 SD change from the mean ($BDML_{SD}$) values ranged from 77 to 134 mg/kg-day. The mean $BMDL_{SD}$ value of 91 mg/kg-day for maternal body weight changes was selected as the appropriate point of departure (POD), corresponding to a human equivalent dose (POD_{HEC}) of 25 mg/kg-day. A total composite uncertainty factor (UF) of 300 yields an RfD of 0.08 mg/kg-day. A urinary mass excretion fraction (Fue) of 0.6 for TBBA following oral doses of TBB in rats was used to calculate BEs for TBBA in urine of 2.5 mg/L and 2.5 mg/g cr. Mean (5.3×10^{-6} mg/L) and maximum (340×10^{-6} mg/L) levels of TBBA measured in urine from human volunteers reported in the literature indicates margins of safety (MOS) are approximately 450,000 and 7,000, respectively.

1. Introduction

The chemical 2-ethylhexyl-2,3,4,5 tetrabromobenzoate (CAS # 183,658-27-7, mw = 549.92, $C_{15}H_{18}Br_4O_2$) is used as a brominated flame retardant. Brominated flame retardants have received increased scrutiny, since the late 1990s, when polybrominated diphenyl ethers were found in samples of human blood (Sjödin et al., 1999) and milk (Meironyté et al., 1999). Because biomonitoring brought about early detection of brominated flame retardants in these sample types, biomonitoring samples of today for brominated flame retardants is often included during exposure assessments. As a result, there is a need for methods to interpret the biomonitoring levels reported for brominated flame retardants in a public health risk context. This paper provides such an approach for interpreting biomonitoring data for 2-ethylhexyl-2,3,4,5 tetrabromobenzoate (TBB) and its metabolite tetrabromobenzoic acid (TBBA; CAS # 27,581-13-1, mw = 437.7, $C_7H_2Br_4O_2$).

Since there is a lack of guidance values for interpreting biomonitoring data for most environmental chemicals, these data are typically presented without any interpretation in the context of potential health risks. Interpretation of biomonitoring data in the context of potential health risks would ideally be done using guidance values based on robust datasets that relate potential adverse effects to biomarker concentrations in human populations (see, for example, the US Centers for Disease Control and Prevention (CDC) blood lead level of concern; http://www.cdc.gov/nceh/lead/). However, development of such epidemiologically-based guidance values is a resource- and time-intensive effort, and in practice, data to support such assessments exist for only a few chemicals. As an interim approach, the concept of Biomonitoring Equivalents (BEs) has been developed (Hays et al., 2007), and guidelines for the derivation (Hays et al., 2008) and communication (LaKind et al., 2008) of these values have been prepared.

A BE is defined as the concentration or range of concentrations of a chemical or its metabolites in a biological medium (blood, urine, or other medium, including tissue biopsies) that is consistent with an existing health-based exposure guidance value such as a reference dose (RfD) or Tolerable Daily Intake (TDI). Existing chemical-specific pharmacokinetic data are used to estimate biomarker concentrations that are consistent with the Point of Departure (POD) used in the derivation of an exposure guidance value (such as the RfD or TDI), and with the exposure guidance

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value itself. BEs can also be derived for other types of exposure guidance factors, including recommended intakes of nutritionally essential elements (Hays et al., 2014). BEs can be estimated using available human or animal pharmacokinetic data (Hays et al., 2008), and BEs have been derived for over 100 compounds (most recently reviewed in Aylward and Hays, 2011; www.biomonitoringequivalents.net).

BEs are intended to be used in the same way that the underlying exposure guidance values are used and upon which they are based. Thus, BEs carry the same functional definition as the underlying guidance value for which they are derived. BE values for multiple chemicals have been used to evaluate nationally representative biomonitoring data in Canada and the United States across chemicals to examine relative levels of exposure in the context of risk assessment-derived exposure guidance values (St-Amand et al., 2014; Aylward et al., 2013).

The BEs derived here for TBB and TBBA will allow for the interpretation of important biomonitoring data currently available and those that become available in the future.

2. Methods

Derivation of a BE requires the selection of the most appropriate biomarker(s) available for the compound, exposure guidance values (or points of departure) of interest, and pharmacokinetic data/model(s) required to convert the exposure guidance value(s) into the chosen biomarkers (Hays et al., 2007). The following, outlines how each of these factors are determined to offer the best fit for use.

2.1. Biomonitoring data

TBB is rapidly metabolized to TBBA in vitro by the liver and intestinal subcellular fractions in both rat and humans via cleavage of the 2-ethylhexyl chain without requiring any added cofactors (Roberts et al., 2012). In the rodent, TBBA is rapidly excreted via urine following oral dosing of TBB (Hoffman et al., 2014; Knudsen et al., 2014). Biomonitoring studies are available that have measured both TBBA in human urine samples (Hoffman et al., 2014; Butt et al., 2014) and TBB in human blood and milk samples (Zhou et al., 2014) (Table 1). TBBA in urine appears to be specific to TBB exposures because TBBA is not produced by any other known parent compounds and TBBA is not known to exist as an environmental degradant of TBB. As a result, TBBA is identified as a specific biomarker for TBB exposures. TBB in blood is likewise specific to exposures to TBB, since TBB is not known to be a metabolite of any currently known compound. TBBA in urine has the advantage of being collected with relatively non-invasive techniques, while TBB in blood is more invasive since blood must be collected via venipuncture. However, both TBBA in urine and TBB in blood are reasonable biomarkers for assessing exposures to TBB. Since the mode of action for the toxicity of TBB is not clearly known, it is likewise unknown whether TBB and/or TBBA is actually a proximate toxicant. Therefore, at this time, neither biomarker has an advantage with respect to these noted factors.

2.2. Risk assessment

No regulatory agencies have developed a risk assessment for TBB to date. Unpublished toxicology studies for TBB (via dosing of BZ-54; 70% TBB and 30% TBPH) were provided to the authors for the purpose of conducting an independent risk assessment (WIL, 1997; MPI, 2008a,b). The process and outcome of the risk assessment follows:

An oral reference dose (RfD) was derived for TBB using the following equation:

$$RfD = POD_{HED} / UF_T$$

Where,

RfD = Reference dose (mg/kg-day); POD_{HED} = Human equivalent dose for the point of departure; and
UF_T = Total uncertainty factor, calculated as the product of individual uncertainty factors for interspecies variation (UFa), intraspecies variation (UFh), LOAEL-to-NOAEL (UFl), subchronic-to-chronic extrapolation (UFs), and database deficiencies (UFd).

A dose-response assessment analysis was conducted for TBB using methods consistent with USEPA guidelines (USEPA, 2002; 2011, 2012), and consists of a number of steps, including: (1) selection of data sets; (2) selection of a dose measurement; (3) dose-response modeling; (4) selection of a point of departure; and (5) selection of uncertainty factors. Each of these individual five steps is summarized below:

2.2.1. Data sets

Toxicity studies available for TBB include three unpublished reports: (1) a 28-day toxicity study in rats (WIL, 1997); (2) a developmental toxicity study in rats (MPI, 2008a); and (3) a two-generation reproductive toxicity study in rats (MPI, 2008b) all of which were exposed to BZ-54, which is 70% TBB and 30% TBPH.

2.2.1.1. 28-Day toxicity study (WIL, 1997). Groups of 6 rats per sex were administered oral (gavage) doses of 0, 160, 400, or 1,000 mg/kg-day BZ-54- via corn oil for 28 days (WIL, 1997). The authors noted clinical findings of a relaxed vaginal opening in all treated female groups, and salivation in both sexes in the highest dose group. Food consumption and body weights were reduced in the highest dose group males, and in the low, mid and high dose group females. Renal tubule epithelial regeneration was reported in all male treatment groups in a dose response manner, including 100% of exposed female animals. The study authors concluded that this study identified a LOEL of 160 mg/kg-day.

2.2.1.2. Developmental toxicity study (MPI, 2008a). Groups of 25 female rats were administered oral (gavage) doses of 0, 50, 100, or 300 mg/kg-day BZ-54 via peanut oil on gestation days 6 through 19 (MPI, 2008a). The authors noted maternal toxicity (lower gestational food consumption and body weight gains, sparse abdominal hair) in animals from the mid and high dose groups. Fetal body

Table 1
Available biomonitoring data for TBB and TBBA in humans.

Study	Population	Analyte	Matrix	units	mean	75th	95th	Maximum
Zhou et al., 2014	nursing mothers	TBB	serum	ng/g lw	1.6		22	
Zhou et al., 2014	nursing mothers	TBB	milk	ng/g lw	0.41		5.3	
Hoffman et al 2014	general population	TBBA	urine	ng/L	5.3	10.8		340.6
Butt et al., 2014	nursing mothers	TBBA	urine	ng/L	NA			62.2
Butt et al., 2014	children	TBBA	urine	ng/L	7.4			84.9