Published in final edited form as:

Toxicol Appl Pharmacol. 2013 December 1; 273(2): 335–344. doi:10.1016/j.taap.2013.09.007.

Proposed Mechanistic Description of Dose-Dependent BDE-47 Urinary Elimination in Mice Using a Physiologically Based Pharmacokinetic Model

Claude Emond^{1,2,*}, J. Michael Sanders³, Daniele Wikoff⁴, and Linda S. Birnbaum³

J. Michael Sanders: sander10@mail.nih.gov; Daniele Wikoff: dwikoff@toxstrategies.com; Linda S. Birnbaum: birnbaumls@mail.nih.gov

¹BioSimulation Consulting Inc., Newark, DE, USA

²Departments of Environmental and Occupational Health, Medicine Faculty, University of Montreal, Montreal, Quebec, Canada

³National Cancer Institute, Research Triangle Park, NC, USA

⁴ToxStrategies, Austin, TX, USA

Abstract

Polybrominated diphenyl ethers (PBDEs) have been used in a wide variety of consumer applications as additive flame retardants. In North America, scientists have noted continuing increases in the levels of PBDE congeners measured in human serum. Some recent studies have found that PBDEs are associated with adverse health effects in humans, in experimental animals, and wildlife. This laboratory previously demonstrated that urinary elimination of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) is saturable at high doses in mice; however, this dose-dependent urinary elimination has not been observed in adult rats or immature mice. *Thus, the primary objective of this study was to examine the mechanism of urinary elimination of BDE-47 in adult mice using a physiologically based pharmacokinetic (PBPK) model.* To support this objective, additional laboratory data were collected to evaluate the predictions of the PBPK model using novel information from adult multi-drug resistance 1a/b knockout mice. Using the PBPK model, the roles of mouse major urinary protein (a blood protein carrier) and P-glycoprotein (an apical membrane transporter in proximal tubule cells in the kidneys, brain, intestines, and liver) were investigated in BDE-47 elimination. The resulting model and new data supported the major role of m-MUP in excretion of BDE-47 in the urine of adult mice, and a lesser role of P-gp as a transporter of BDE-47 in mice. This work expands the knowledge of BDE-47 kinetics between species and provides information for determining the relevancy of these data for human risk assessment purposes.

Keywords

PBPK; pharmacokinetics; PBDE; BDE-47; m-MUP; P-gp; mouse

© 2013 Elsevier Inc. All rights reserved.

*Corresponding Author: Claude Emond, Department of Environmental and Occupational Health, Medicine Faculty, University of Montreal, Montreal, Quebec, Canada, 2375 Chemin de la Côte Ste-Catherine, Bureau 7085, Montreal, Quebec, Canada, H3T 1A8, Telephone: (514) 343-6111, extension 0822, claud.emond@umontreal.ca.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are a persistent and ubiquitous class of environmental pollutants. These compounds have been used in a wide variety of consumer applications as additive flame retardants in the form of three commercial mixtures known as “penta,” “octa,” and “deca” (Chen et al. 2009). PBDEs are composed of 209 congeners, but only approximately 10 to 15 congeners are commonly measured in the environment and biota (Hites 2004). 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) is not the congener of greatest production or presence in the environment, but is often the most dominant PBDE in biota, including human serum and breast milk (Kim et al. 2011; Koh et al. 2010; Mazdai et al. 2003; Schechter et al. 2006). This finding has been attributed to differences in types of exposures, physiochemical properties, and *in vivo* fates of the different congeners (Birnbaum and Cohen Hubal 2006; Sanders et al. 2006b; Staskal et al. 2006c).

Kinetic parameters play a central role in toxicity because they quantify the dose at the target tissues associated with adverse health effects. Available kinetic studies in rodents indicate that BDE-47 causes species-, sex-, age-, and dose-dependent effects (Sanders et al. 2006a; Staskal et al. 2005, Staskal et al. 2006a and c). When comparing the disposition and elimination of BDE-47 in rats and mice, Sanders et al. (2006a) noted that internal dose and excretion patterns were different. Emond et al. (2010) recently described the kinetic behavior of BDE-47 in rats using a physiologically based pharmacokinetic (PBPK) model. The distribution of BDE-47 is dose dependent in rats and driven by the compound's high lipophilicity. Fecal excretion is the primary route of elimination in rats, but both urinary and fecal excretions are prominent in the mouse, with a greater amount of BDE-47 excreted in the urine of male mice compared to female C57BL/6J mice (Sanders et al. 2006a). Staskal et al. (2005) examined the effects of dose, route, and time on the kinetics of BDE-47 in mice and found that BDE-47 was rapidly eliminated in the urine as parent compound and that elimination was dose dependent indicating saturation of the urinary elimination process. Excretion patterns were different in developing and adult mice suggesting that a mechanism involving transport proteins was active in adult mice, but not in young mice (Staskal et al. 2006a).

Previous research has indicated that BDE-47 and other PBDE congeners bind isoforms of a mouse major urinary protein (m-MUP) localized in the blood (Sanders et al. 2006b; Staskal et al. 2006c). The m-MUP is a heterogenous member of the lipocalin family (MW $\approx$ 19 KDa) synthesized in the liver, rapidly secreted into the bloodstream, and eliminated through the kidneys (Berger 1983; Nukaya et al. 2004; Robertson et al. 1997). Janotova et al. (2011) reported that m-MUP is also expressed in the mammary, salivary, and lachrymal glands. This protein transports pheromones in mice and is the major protein component of mouse urine (Flower 1996). Production of m-MUP in female mice is approximately 30% that of males (Beynon and Hurst 2003). The m-MUP appears to be strain dependent, is under hormonal control, and becomes detectable during puberty (Janotova and Stopka 2011; Kwak et al. 2012). Many exogenous substances are bound by m-MUP, including various classes of volatile organic compounds (Kwak et al. 2012). The m-MUP contains an internal cavity that is used as a binding site for predominantly hydrophobic ligands (Beynon and Hurst 2003). Putative binding at this site solubilizes BDE-47 in mouse urine at concentrations much greater than are chemically possible (Sanders et al. 2006b). Staskal et al. (2006b) indicated that BDE-47 binds to the m-MUP-1 isoform.

Several researchers have evaluated the PBDE-induced disruption of thyroid homeostasis via interference with transporters, protein binding, gene regulation, and/or alteration of elimination processes (Dallaire et al. 2009; Dunnick and Nyska 2009). Richardson et al. (2008) reported that decreases of thyroxine (T4) after BDE-47 exposure may be mediated by

proteins that could be involved in urinary elimination of BDE-47 in mice. Specifically, these data suggest that T4 changes, which are involved in the thyroid hormone disruption, might be a result of competition with a key transporter, P-glycoprotein (P-gp) (Richardson *et al.* 2008). As a member of the adenosine triphosphate (ATP)–binding cassette (ABC) transporter superfamily, P-gp is capable of active efflux of a wide variety of compounds across biological membranes (Bain and LeBlanc 1996; Leslie *et al.* 2005). P-gp transports triiodothyronine (T3), structurally-similar to BDE-47, and has been shown to bind, but not necessarily transport, some lipophilic pesticides with molecular weights similar to that of BDE-47 (Bain and LeBlanc 1996; Mitchell *et al.* 2005). P-gp is found in the gastrointestinal tract (GIT), brain, liver, intestines, and in the brush border of the proximal tubular region of kidney nephrons (Al-Shawi and Omote 2005; Kondratov *et al.* 2001; Lin and Yamazaki 2003; Sergent *et al.* 2008). Therefore, it is plausible that P-gp plays a role in excretion of BDE-47 in the urine of mice.

The objective of the current study was to develop a PBPK model in mice to describe the distribution of BDE-47 in tissues and excretion in feces and from the urinary tract. The model evaluates the prominent role of m-MUP in elimination of BDE-47 in urine of mice. Additionally, new data are reported for evaluation of a potential role of P-gp in excretion of BDE-47. This work represents the first step of an initiative to describe the mode of action associated with BDE-47–induced toxicity, as it aids in the characterization of the potential dose to the target tissue.

MATERIALS AND METHODS

Modeling Approach

This section describes the methodology used to develop the PBPK model.

PBPK Model Description and Conceptual Representation—An existing PBPK model developed for the rat did not accurately describe the urinary elimination of BDE-47 in mice (Emond et al. 2010). As such, this model was used as the basis for the model applied in the current study, but was modified to evaluate two transporters (i.e., m-MUP and P-gp) in the excretion of BDE-47 in mice. The pharmacodynamic descriptions of the interaction of m-MUP (in blood) and P-gp (in the brain, liver, kidneys, and blood/GIT), with BDE-47 were detailed as a Michaelis-Menten kinetic equation for the blood or tissue compartment, respectively, using differential equations. Additionally, the gestational component was removed and a pseudo-GIT compartment facilitating the exchange between liver, blood, and GIT lumen has been described. The anatomical, physiological, and biochemical parameters were interspecies-scaled to describe the time course of the absorption, distribution, biotransformation, and elimination of BDE-47 for adult mice. As shown in Figure 1, the model structure contains six compartments (i.e., blood, brain, adipose tissue, kidney, liver, and rest of the body) and a pseudo-compartment (i.e., the GIT). Systemic circulation was described by venous and arterial blood circulation.

Using this model, we also evaluated experimental observations of urinary elimination in immature mice by altering (turning off) the parameters for m-MUP and P-gp; this specifically tested a hypothesis that these proteins do not play a major role in urinary excretion in young mice.

Mathematical Representation—As discussed by Emond et al. (2010), each compartment (i.e., blood, brain, adipose tissue, kidney, liver, and rest of the body) was mathematically described by a mass-balance differential equation. All compartments having minor impacts on the pharmacokinetics and pharmacodynamics of BDE-47 were lumped into the rest of the body compartment. Brain, adipose tissue, and rest of the body are

described as diffusion limited, and the liver and kidneys are described as flow limited. It is important to note that the liver compartment was described as diffusion limited in the rat model (Emond et al. 2010). The mathematical descriptions of m-MUP in blood and P-gp in tissues are further detailed below.

The first order description used for oral absorption intrinsically considers P-gp because this route competes with GIT transit elimination, also described by first order kinetics. Oral absorption ranged from 75% to 85% (EPA 2008). The intravenous route (simulating 100% absorption) was considered and was mathematically described in the present PBPK model code. Fecal excretion pathways represented the contribution of biliary elimination, GIT transit fraction during oral intake, P-gp efflux, and the enterohepatic reabsorption accounted for and described in the codes. A detailed description of the relevant mass-balance differential equations is provided in Appendix A of the Supplementary Materials.

Model Parameters—To develop the PBPK mouse model, several parameters were required to describe the pharmacokinetic behavior of BDE-47 in mice. The measurements, calculations, and references used for these parameters are presented in Tables 1 and 2. The anatomical, physiological, and physicochemical parameters were extracted from literature (Emond *et al.* 2010; Hudachek and Gustafson 2013; ILSI 1994; Krishnan and Andersen 2008) or calibrated during the optimization phase. The tissue distribution of BDE-47 in mice would be assumed to be similar to other persistent non-dioxin-like lipophilic compounds that concentrate predominately in adipose tissue and do not induce CYP1A. However, a description of binding of BDE-47 to transport proteins, must be added to the model to account for the effect on internal dose in mice by the rapid and saturable urinary elimination processes.

m-MUP Carrier Protein—m-MUP binds small, hydrophobic ligands (Robertson et al. 1996), including BDE 47 (Staskal *et al.* 2006c). The present PBPK model takes into account the role of m-MUP in rapid urinary elimination of BDE-47 in mice as a consequence of binding to the protein in blood, filtration in the kidney, and excretion of the complex in the urine. This binding process has been observed and described for a number of other lipophilic xenobiotics (Hakk et al. 2002; Larsen et al. 1990; Larsen et al. 1991). The apparent partition coefficient, calculated by the ratio of concentration of tissue to the blood ($C_{\text{tissue}}:C_{\text{blood}}$) at equilibrium is lower than would be expected for the highly lipophilic chemicals in experimental data (Sanders *et al.* 2006a; Staskal *et al.* 2005), thus supporting the hypothesis that BDE-47 is bound to m-MUP in the blood.

A Michaelis-Menten equation, as shown in Equation 1, was used to mathematically describe the m-MUP component in the PBPK model.

$$CBlood_{bound} = \frac{Bmax_{m-MUP} \times Cb_{free}}{Km1_{m-MUP} + Cb_{free}} \quad (\text{Eq. 1})$$

Where

$CBlood_{bound}$ = Concentration of BDE-47 bound to m-MUP in blood (mg/mL)

$Bmax_{m-MUP}$ = Maximum concentration of m-MUP in the blood (mg/mL)

$Km1_{m-MUP}$ = Affinity constant of m-MUP for BDE-47 (mg/mL)

C_{free} = BDE-47 free in the blood compartment (mg/mL).

According to Equation 1, the binding capacity is saturable; thus, at a high blood concentration, the maximum concentration of the complex (m-MUP BDE-47) will be equal to $B_{\max}1_{m-MUP}$. Thus, at a low-exposure dose, the complex concentration will be equal to

$$\frac{B_{\max}1_{m-MUP} \times C_{free}}{K_{m1_{m-MUP}}}$$

The amount of BDE-47 eliminated directly by glomerular filtration can be determined by using Equation 2. In the equation, total blood (C_b) rather than C_{bfree} is used because literature reports that a 79-kDa protein-BDE-99 complex was filtrated in the nephrons and excreted in the urine of rats (Hakk et al. 2002).

$$\frac{dA_{BDE-47}}{dt} = Q_{kidney} \times EUI \times C_b \quad (\text{Eq. 2})$$

Where

dA_{BDE-47}/dt = Urinary elimination rate of BDE-47 (mg/hr)

Q_{kidney} = Kidney blood flow (mL/hr)

EUI = Blood flow fraction of urinary filtration clearance (varies from 0 to 1; unitless)

C_b = Blood concentration, which includes free and bound concentrations (mg/mL).

P-glycoprotein Tubular Kidney Membrane Transporters—The PBPK model assumes that P-gp is involved with m-MUP in contributing to the elimination of BDE-47 in urine, by effluxing some of the compound from the proximal tubule cells into the urine. A Michaelis-Menten equation, as shown in Equation 3, was used to mathematically describe this potential component. The dynamic parameter values of P-gp were optimized and are presented in Table 2.

$$\frac{dA_{P-gp}}{dt} = \frac{V_{\max P-gp} \times C_{free}}{K_{m P-gp} + C_{free}} \quad (\text{Eq. 3})$$

Where

dA_{P-gp}/dt = Rate of change of BDE-47 bound to P-gp (mg/hr)

$V_{\max}$ = Maximum rate of transfer for P-gp for BDE-47 (mg/hr)

K_m = Affinity constant of P-gp for BDE-47 (mg/mL)

C_{free} = Free blood concentration for BDE-47 (mg/mL).

Optimization and Predictive Accuracy—For the optimization step, a dataset published by Staskal et al. (2005) was used in which adult female C57BL/6J mice received one single oral dose (1.0 mg of BDE-47/kg of body weight). The ^{14}C collected daily for up to 5 days in urine and feces contained mostly parent compound; therefore, the ^{14}C measured (as ng and % dose/g) in different tissues (i.e., blood, adipose tissue, liver, brain, skin, kidney, muscle, and lung) from mice sacrificed at selected timepoints over 21 days was presented as BDE-47 equivalents (see Figure S1 in the Supplemental Materials). These data were used to calibrate the apparent diffusion coefficient, the m-MUP and P-gp protein constants, the absorption, the transit elimination in the GIT, the urinary filtration constants of BDE-47 for liver and urine, and the apparent partition coefficient for different tissues described in the model.

Data from the following studies were used to evaluate the predictive accuracy of the PBPK model:

- Staskal et al. (2005; 2006c): Determined tissue concentration of BDE-47 equivalents and % total dose excreted in urine and feces of adult female mice receiving single oral doses of 0.1 mg, 1.0 mg, 10.0 mg, or 100.0 mg/kg body weight or a single intravenous (iv) dose of 1.0 mg/kg body weight.
- Sanders et al. (2006a): Determined tissue concentrations of BDE-47 equivalents and % total dose excreted in urine and feces of adult male and female B6C3F1 mice 24 hours following a single oral or iv dose of 1 µmol (0.49 mg)/kg of body weight.
- Staskal et al. (2006a): Determined tissue concentrations of BDE-47 equivalents at various timepoints in developing male and female C57BL/6J mice receiving a single oral dose of 1.0 mg/kg body weight on Postnatal Day (PND) 10 or 21. The % total dose excreted in urine and feces was calculated over time in mice dosed on PND 21.
- Investigation of the effect of P-gp on excretion of BDE-47-derived radioactivity in urine and feces of adult FVB mice (FVB-WT) and age-matched adult FVB mice with disrupted P-gp protein (FVB-KO) following single oral administration of 1 or 100 mg/kg body weight (described below).

Software, Algorithms, and Sensitivity Analysis—All simulations and parameters optimization were conducted using acslX™ version 2.5 (Aegis Technologies, Huntsville, AL). The Gear algorithm was used for integrating the double precision variables. The relative-error model estimation was used to drive the parameter fitting. Maximization of the log likelihood function was the decisive factor for predicting accuracy. A limitation of using this approach is that even if the maximum likelihood estimates are reasonable, the error estimates might be incorrect, resulting from the assumption of independent data. Starting values for parameter fitting in acslX parameters estimation subroutine version 3.0.2.1 were determined from parameters obtained by visual fit.

Sensitivity analysis (SA) reveals the ways in which a model response changes under the influence of individual parameter changes (Easterling et al. 2000), and the results are expressed as a magnitude of change in the endpoint of interest (Krishnan and Andersen 2008). To understand the model and the parameters that drive the dose metrics, each parameter in the mouse PBPK model was tested for sensitivity. To perform the tests, each parameter was varied by $\pm 2\%$ of the optimized value to determine the influence of changes in the blood concentration. SA was performed using data from the exposure of 1.0 mg of BDE-47/kg of body weight followed by the blood concentration variation 120 hours (5 days) post exposure. This SA recorded the influence of individual parameter-driven variations of blood concentration affecting the mouse PBPK model.

The variation of concentration in blood was selected as the measure of sensitivity for the variability for BDE-47 because of the rapid urinary elimination in mice. To examine the variability, Equation 4 was used as follows.

$$SA(\% \text{ blood change}) = \left[\frac{\text{Concentration}_{\text{post}} - \text{Concentration}_{\text{opt}}}{\text{Concentration}_{\text{opt}}} \times 100 \right] \quad (\text{Eq. 4})$$

Where

$SA(\% \text{ blood change})$ = Sensitivity analysis variation following a $\pm 2\%$ variation of each parameters (unitless)

$Concentration_{post}$ = Concentration using a variation by 2% of one parameter at a time (mg/mL)

$Concentration_{opt}$ = Variation of concentration using an optimized parameter (mg/mL).

Experimental Approach

This section describes the methodology used for the *in vivo* kinetic study for evaluation of interaction of BDE-47 with P-gp in the PBPK model.

Chemicals and dosing solutions—The [^{14}C]-labeled BDE-47 (26.7 mCi/mmol) used in the present study was generously donated by Great Lakes Chemical Corporation (Indianapolis, IN) for use in the experiments conducted by Staskal et al. (2005, 2006a, b, and c). The remaining radiolabel from those studies was transferred from cold storage (-80°C) at the U.S. Environmental Protection Agency (RTP, NC) to the National Institute of Environmental Health Sciences (NIEHS) (RTP, NC) for use in the current work. The radiolabeled purity of the BDE-47 was determined by HPLC analysis (see below) to be approximately 85% and was deemed acceptable for the purposes of the present work. Unlabeled BDE-47 was obtained from Cerilliant Corporation (Round Rock, Texas). [^{14}C]-Labeled and unlabeled BDE-47 (in toluene) were added to corn oil to formulate oral dosing solutions of 1 and 100 mg/50 $\mu\text{Ci/kg}$ in a dose volume of 10 ml/kg. The toluene was evaporated from the dosing solutions under a steady stream of N_2 .

Animals and Treatment—The goal of the study was to determine if the fate of BDE-47 differed in Friend virus B-type (FVB) mice possessing functional P-gp protein (mice are identified as $\text{Mdr1a/b}^{+/+}$ or FVB-WT in the text and figures) and age-matched adult FVB mice, genetically altered to disrupt genes *Abcb1a* (*Mdr1*) and *Abcb1b* (*Mdr1b*) encoding P-gp 3 and P-gp 1, respectively. The knockout mice are identified as $\text{Mdr1a/b}^{-/-}$ or FVB-KO in this work. All mice were obtained from Taconic Laboratories, Germantown, NY) and were dosed at approximately 10 weeks of age and mean weight of 24 grams. The animal procedures were approved by the Institutional Animal Care and Use Committee of the NIEHS. Following administration of a single dose of BDE-47 by gavage ($n = 4\text{--}8/\text{treatment group}$) using a #20 ball-tipped stainless steel feeding needle, each mouse was housed in a metabolism cage for daily collection of urine and feces for five days. Water and food (NIH #31) were provided for *ad libitum* consumption. The mice were euthanized by carbon dioxide asphyxiation at the end of the study. Blood was collected by cardiac puncture at the time of death and adipose tissue (the major depot for BDE-47-derived radioactivity) was collected at necropsy.

Analysis of BDE-47 radiochemical purity and determination of the amount of BDE-47-derived radioactivity in urine, feces, blood, and adipose tissue—The radiochemical purity of BDE-47 was determined by high performance liquid chromatography (HPLC) analysis. The Waters (Milford, MA) model 1525 pump system utilized a $\mu\text{Bondapak } ^{18}\text{C}$ column with a gradient of a 1:1 ratio of A (0.1% TFA) to B (CH_3CN containing 0.1% TFA) to 100 % B in 15 min at a flow rate of 1.5 ml/min. The R_t of BDE-47 was approximately 10 min in the system. The radiochemical detector was a US β -Ram model 3 (LabLogic, Brandon, FL). The identity of [^{14}C]-labeled BDE-47 was verified by co-injection with unlabeled BDE-47 using a Waters model 2487 UV detector (254 nm). The daily and cumulative amounts of [^{14}C] excreted in urine were determined by counting triple aliquots (10 μl) of urine in vials of Ecolume (MP Biomedicals, Salon, OH) in a Beckman Coulter (Brea, CA) liquid scintillation counter. The amount of [^{14}C] in daily cage

rinses (triple 25 μ l aliquots, processed similarly to urine) were added to those obtained for urine at each timepoint. Triple aliquots of feces, blood and adipose tissue (approximately 25 mg each) were combusted in a Packard model 307 biological sample oxidizer (Perkin Elmer, Waltham, MA) prior to analysis by liquid scintillation spectrometry. Feces were air-dried, weighed, and ground to a fine powder, prior to combustion in the oxidizer.

Data Analysis—Data from this study are presented as the mean $\pm$ standard deviation (SD) and were analyzed by ANOVA and the Tukey-Kramer test using JMP statistical software (SAS Institute, Inc, Cary, NC). Differences between treatment groups were considered to be significant at $p < 0.05$.

RESULTS

PBPK Model Development

As described previously, the conceptual representation of the PBPK model contains six major compartments based on the experimental observation of the kinetic measurements and biological effects of BDE-47 and refers to the framework developed by Emond et al. (2010) (Figure 1). All PBPK parameters used for adult female C57BL/6J mice remained constant during the simulation, except for the variation of body weight over time. All available datasets were used to evaluate the prediction of the distribution or elimination of BDE-47 in mice. After the mouse PBPK model was optimized and calibrated (see Figures S1a–h in the Supplementary Materials) it was then evaluated for different exposure pathways and age groups as described below.

PBPK Model for Adult Mice

In adults the m-MUP and P-gp are functional and were activated in the model. Four oral exposure doses 0.1, 1, 10, and 100 mg BDE-47/kg were administered. The parameters used were the ones obtained from the optimization. The simulations corresponded to the timepoints measured. The modeling output were plotted relative to the measured excretion data described in Staskal et. al (2005) as shown in Figures 2a and 2b. Comparison of the urine simulation profile to the experimental data following oral administration showed an increase in the percentage of dose excreted over time. At doses of 0.1 mg and 1.0 mg of BDE-47/kg of body weight, the curves are superimposed. However, at 10.0 mg and 100.0 mg of BDE-47/kg of body weight, net diminutions of the urinary excretion are noted. For fecal excretion, the elimination is similar within Day 1 for all doses. After Day 1, a higher fecal excretion is correlated with higher exposure doses with a maximum of 50% (Figure 2b). Further, a comparison of the PBPK model simulation to intravenous exposure data (see Figure S2 in the Supplemental Materials) collected by Staskal et al. (2005) demonstrated that excretion of dose in urine was overestimated by a range of 30 to 40% while the feces excretion was well predicted by the model (Figure 3).

PBPK Model for Young Mice

To test the speculation that the role of m-MUP and/or P-gp was minor in infantile mice, the PBPK model was run using four simulations: 1) both m-MUP and P-gp were activated (Figures 4A), 2) both m-MUP and P-gp were inactivated (Figure 4B), 3) only P-gp was inactivated (Figure 4C), and 4) only m-MUP was inactivated (Figure 4D). The first simulation showed a faster elimination than the two other profiles (Figures 4B and C). Figure 4B seemed to predict a slightly better fit than Figure 4C, which corresponded to only the inactivation of P-gp.

The adipose tissue, liver, kidney, rest of the body, and kidney compartments correlated well to the experimental data from developing animals (Staskal *et al.* 2006a). However, the blood

compartment was underestimated by factors ranging from 4 to approximately 10 (see Figures S3a–c in the Supplemental Materials). It is important to note that for each pup's exposure time, the only parameter adapted was the initial age when protein transporters were activated.

Investigation of the effects of P-gp on excretion of BDE-47 in adult mice

No adverse effects were observed from any of the BDE-47 treatments used in this protocol. At time of dosing and at study termination, there were no appreciable differences in weight within and between treatment groups.

As shown in Figure 5, at a low oral dose of 1.0 mg/kg the cumulative excretion of BDE-47 derived radioactivity in the urine of FVB-KO mice without functional P-gp (*Mdra/b^{-/-}*) was statistically lower than observed for FVB-WT mice with functional P-gp (*Mdra/b^{+/+}*) (34 ± 11 vs. 50 ± 10 % total dose; $p < 0.01$). Conversely, the mean amount of [^{14}C] excreted in the feces of the FVB-KO mice was greater (43 ± 6 % total dose) than observed for the FVB-WT mice (37 ± 10 % total dose). However, the difference in fecal excretion of dose was not statistically significant ($p < 0.07$). At five days, post-dosing, FVB-KO mice contained more of the total dose in blood (0.12 ± 0.02 %) than did FVB-WT mice (0.07 ± 0.01 %) and the difference of the mean was highly significant at ($p < 0.001$). There was no difference in the amount of [^{14}C] remaining in the adipose tissue of the two treatment groups (7–8 % total dose). No statistically significant differences were detected in the disposition of BDE-47 in FVB-WT and FVB-KO mice administered a 10-fold higher oral dose. As previously reported for female C57BL/6J mice (Staskal et al. 2005), the cumulative amount of [^{14}C] excreted in urine decreased dramatically (to 14–15 % total dose) over that observed at the lower dose in both FVB-WT and FVB-KO mice following oral administration of 100 mg/kg (Figure 5). The remainder of the dose was excreted in feces (approximately 70 % of the total dose) or was sequestered predominately in the adipose tissue (approximately 15 % of the total dose). Blood contained 0.2 % of the total dose at the terminal timepoint.

Sensitivity Analysis

The sensitivity analyses (SA) for each parameter were evaluated in the model. The percentage of variation was estimated at the end of the simulation (Figure 6). To determine the influence of variation on each parameter (less than 2 % on the metrics concentrations), 32 parameters were evaluated. The most sensitive parameters were the affinity constant for P-gp (K_m), the maximal velocity of P-gp in kidney ($V_{\max k}$), the cardiac output (q_{cc}), the fraction of kidney blood flow (q_{k0}), the urinary extraction constant (EUI), and the feces excretion (K_{st}).

DISCUSSION AND CONCLUSIONS

The objective of this current study was to develop a mouse PBPK model to describe the distribution of BDE-47 in tissues, its elimination in feces and urine, and to evaluate the role of transporters for BDE-47 in these processes. The mouse PBPK model presented in this study represents a tool for toxicologists for use in simulating kinetic behavior, understanding the mode of action, and generating hypotheses. Compared to the rat, mice seem to have a mechanism of urinary elimination that amplifies the limited urinary elimination usually observed with highly lipophilic chemicals.

The framework developed for a study of the rat by Emond et al. (2010), without the gestational sub-model, was used in the present study (Figure 1). The m-MUP (in blood) and P-gp efflux transporters (in the brain, liver, intestines, and kidneys) were added to the model. Previous experimental data indicate that m-MUP facilitates the rapid urinary

excretion of BDE-47 (Sanders *et al.* 2006b; Staskal *et al.* 2006c). New data presented here tested the hypothesis that P-gp is also involved in transport of BDE-47. The behaviors of these two transporters were described based on the Michaelis-Menten equation (Equations 1 and 3). The saturation process was based on the experimental data of Staskal *et al.* (2005). Vmax and Km for both proteins were optimized to reproduce the kinetic urinary profile at low and high doses (Figures 2b and 5). The resulting mouse PBPK model and new data provided here, support the major role of m-MUP in excretion of BDE-47 in the urine of adult mice following exposure to the congener; and indicate that P-gp plays a minor role as a transporter of BDE-47 in mice.

The m-MUP is the major protein component of adult mouse urine (Flower 1996) and is found bound to BDE-47 in the urine of exposed adult mice (Staskal *et al.* 2006c). A blood-binding protein such as m-MUP traps the BDE-47, and thus increases the amount eliminated by glomerular filtration via the kidneys. Previous results for other highly lipophilic chemicals (Béliveau and Krishnan 2000; Emond *et al.* 2005; Proost *et al.* 1996; Schoenborn 1976) indicated that the actual BDE-47 blood concentration should be lower than what was observed by Staskal *et al.* (2005) and Sanders *et al.* (2006a) if lipophilicity is the only parameter responsible for the distribution of BDE-47. This observation supports protein binding to BDE-47 by itself; therefore, the observation was instrumental in driving the selection of this description. Further, this process of dose elimination in mice is supported by data in BDE-47-exposed rats lacking the protein, in which less than 1% of a comparable dose was excreted in urine (Sanders *et al.* 2006a). The m-MUP involvement (and potential involvement of P-gp) in elimination of BDE-47 in mice is further supported by the pattern of dose excretion in developing mice (Janotova and Stopka 2011; Staskal *et al.* 2006b; Staskal *et al.* 2006c). The difference observed in the urinary elimination profile between pups and adult mice may be partially explained by a developmental delay in the expression of both P-gp and m-MUP (Aschengrau *et al.* 2003; Cheng *et al.* 2005; Maher *et al.* 2005). The current PBPK model supports the hypothesis that in young mice, the role of m-MUP and P-gp are significantly less important than in adult animals. It must be noted that urinary elimination of 1 $\mu\text{mol/kg}$ dose of BDE-47 may be greater in adult male mice than in adult female mice (Sanders *et al.* 2006a), putatively as a result of greater expression of m-MUP in males (Janotova and Stopka 2011). However, the goal of the current PBPK model was to determine general m-MUP and P-gp behaviors and limitations and does not address sex-differences in these parameters.

The effect of the absence of P-gp (in FVB-KO mice) on the excretion of a BDE-47 dose was investigated in the current study. The experimental data, suggests that P-gp plays a minor role in excretion of BDE-47 in the urine following oral administration of 1 mg/kg. There was a statistically significant decrease in urinary excretion of [^{14}C] in the FVB-KO mice, accompanied by an increase in the concentration of [^{14}C] in blood at five days post-dosing. It is presumed that concentrations of BDE-47 in blood would increase if P-gp efflux of BDE-47 from kidney were disrupted.

An important issue related to m-MUP and P-gp is that these proteins are saturable, which results in a dose-dependent elimination of BDE-47 as was observed in female C57BL/6J wild-type mice (Staskal *et al.* 2006b). Therefore, it is plausible to assume that the impact of transporters on the elimination of BDE-47 would be more important at a low dose than at a high dose, when saturation is present. The present study demonstrated saturation of excretion of a high BDE-47 dose (100 mg/kg) in urine of both FVB-WT and FVB-KO mice (Figure 5), but failed to establish any correlation of P-gp renal transport at the high dose. Further, no significant effect of P-gp transport was observed in fecal elimination of BDE-47-derived [^{14}C] at either the high or low dose. These results indicate that the binding affinity of P-gp for BDE-47 may be low.

The current study increases the evidence, based on the pharmacokinetic and mechanistic description that m-MUP and to a lesser extent, P-gp contribute to the early and rapid elimination of BDE-47. The current PBPK model framework can describe such mechanisms without experimentally determining all of the required kinetics. However, to fully understand the kinetics of BDE-47, additional experimental data are needed to target the interaction of this chemical within the kidney and its transporters. Other protein carrier may be involved in blood sequestration and excretion of BDE-47 in mice. Many transporters exhibit substrate overlap and thus it is possible other transporters have affinity for BDE-47. Further, many transporters have defined expression profiles during ontogeny; several of these profiles have been characterized in murine renal tissue (Cheng *et al.* 2005; Li *et al.* 2002; Maher *et al.* 2005). Consequently, the amount of BDE-47 excreted in the urine as a result of renal transport would be partially dependent upon age (compared to early stage to the juvenile and adult), and further, evaluation of ontological transporter expression may provide additional insight in understanding the role of transport in the kinetics of BDE-47 in the developing mice. Based on the ontological profiles of other transporters (e.g., organic anion transporters [OATs] 1–3), organic cation transporters (OCTs) 1 and 2 and multi-drug resistance-associated proteins (MRPs) 2 and 3 may also be involved.

Additional information is required to improve the current PBPK model. This information includes further identification and characterization of the transporters (i.e., affinity and specificity constant), the concentrations of transporters in blood, and the relative significance of the transporters within various tissues throughout the body. Similarly, additional investigations to characterize the differences in kinetics between species are needed in the future. The rodent data modeled in this paper, represent doses that are higher than those of environmental exposure to humans. However, these data are considered relevant to assessment of exposure risk to humans because of the bioaccumulative potential of the compound and evidence of PBDE-induced toxicity at low exposures to sensitive populations (Fitzgerald *et al.* 2012). The median blood concentration of BDE-47 in the general U.S. population was calculated by Sjodin *et al.* (2008) to be approximately 52 ng/g lipid (Sjodin *et al.* 2008); however, much higher amounts of BDE-47 have been observed in some individuals (Fitzgerald *et al.* 2012).

In the current study, a PBPK modeling approach was discussed for generating hypotheses and understanding the mode of action of BDE-47 dose elimination in mice. This work expands our knowledge of BDE-47 kinetics between rodent species and provides information for determining the relevancy of these data for human risk assessment purposes. To the best of our knowledge, the current model is the first PBPK model designed for PBDEs in mice and describes the dynamic protein excretion system. Refinement of this model may require more specific and mechanistic data to help improve understanding and characterize the elimination of BDE-47 in adult mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Intramural Research Program of the NIH/National Cancer Institute [Project # ZIA BC 011476]. Any mention of trade names or commercial products in this manuscript does not constitute endorsement or recommendation for use.

ABBREVIATIONS

ABC	adenosine triphosphate-binding cassette
ANOVA	analysis of variance
ATP	adenosine triphosphate
BDE-47	2,2',4,4'-tetrabromodiphenyl ether
FVB-KO	Friend virus B-type Mdr1a/b ^{-/-} -deficient knockout (mice)
FVB-WT	Friend virus B-type Mdr1a/b ^{+/+} wild-type (mice)
GIT	gastrointestinal tract
m-MUP	mouse major urinary protein
Mdr	multi-drug resistance
MRP	multi-drug resistance-associated protein
NIH	National Institutes of Health
OAT	organic anion transporter
OCT	organic cation transporter
ODS	octadecylsilane
P-gp	P-glycoprotein
PBPK	physiologically based pharmacokinetic
PBDE	polybrominated diphenyl ethers
PND	Postnatal Day
R_t	Retention time
SA	sensitivity analysis
SD	standard deviation
T3	triiodothyronine
T4	thyroxine

References

- Al-Shawi MK, Omote H. The remarkable transport mechanism of P-glycoprotein: a multidrug transporter. *J Bioenerg Biomembr*. 2005; 37(6):489–496. [PubMed: 16691488]
- Aschengrau A, Rogers S, Ozonoff D. Perchloroethylene-contaminated drinking water and the risk of breast cancer: additional results from cape cod, massachusetts, USA. *Environ Health Perspect*. 2003; 111(2):167–174. [PubMed: 12573900]
- Bain LJ, LeBlanc GA. Interaction of structurally diverse pesticides with the human MDR1 gene product P-glycoprotein. *Toxicol Appl Pharmacol*. 1996; 141(1):288–298. [PubMed: 8917702]
- Béliveau M, Krishnan K. Concentration dependency of rat blood: air partition coefficients of some volatile organic chemicals. *J Toxicol Environ Health A*. 2000; 60(6):377–389. [PubMed: 10933755]
- Berger FG. Studies on genetic variation in major urinary protein synthesis in mouse liver. *Biochem Genet*. 1983; 21(1–2):15–23. [PubMed: 6838486]
- Beynon RJ, Hurst JL. Multiple roles of major urinary proteins in the house mouse, *Mus domesticus*. *Biochem Soc Trans*. 2003; 31(Pt 1):142–146. [PubMed: 12546672]
- Birnbaum LS, Cohen Hubal EA. Polybrominated diphenyl ethers: a case study for using biomonitoring data to address risk assessment questions. *Environ Health Perspect*. 2006; 114(11):1770–1775. [PubMed: 17107866]

- Chen SJ, Ma YJ, Wang J, Chen D, Luo XJ, Mai BX. Brominated Flame Retardants in Children's Toys: Concentration, Composition, and Children's Exposure and Risk Assessment. *Environmental science & technology*. 2009; 43(11):4200–4206. [PubMed: 19569352]
- Cheng X, Maher J, Chen C, Klaassen CD. Tissue distribution and ontogeny of mouse organic anion transporting polypeptides (Oatps). *Drug Metab Dispos*. 2005; 33(7):1062–1073. [PubMed: 15843488]
- Dallaire R, Dewailly E, Pereg D, Dery S, Ayotte P. Thyroid function and plasma concentrations of polyhalogenated compounds in Inuit adults. *Environ Health Perspect*. 2009; 117(9):1380–1386. [PubMed: 19750101]
- Dunnick JK, Nyska A. Characterization of liver toxicity in F344/N rats and B6C3F1 mice after exposure to a flame retardant containing lower molecular weight polybrominated diphenyl ethers. *Exp Toxicol Pathol*. 2009; 61(1):1–12. [PubMed: 18774282]
- Easterling MR, Evans MV, Kenyon EM. Comparative analysis of software for physiological based pharmacokinetic modeling: Simulation, optimization and sensibility analysis. *Toxicology Mechanisms and Methods*. 2000; 10(3):203–229.
- Emond C, Charbonneau M, Krishnan K. Physiologically-based modeling of the accumulation in plasma and tissue lipids of a mixture of PCB congeners in female Sprague-Dawley rats. *Journal of Toxicology and Environmental Health Part A*. 2005; 68(16):1393–1412. [PubMed: 16009653]
- Emond C, Raymer JH, Studabaker WB, Garner CE, Birnbaum LS. A physiologically based pharmacokinetic model for developmental exposure to BDE-47 in rats. *Toxicol Appl Pharmacol*. 2010; 242(3):290–298. [PubMed: 19883674]
- EPA. Toxicological review of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) In Support of Summary Information on the Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency; 2008. p. 1-85. EPA/635/R-07/005F
- Fitzgerald EF, Shrestha S, Gomez MI, McCaffrey RJ, Zimmerman EA, Kannan K, Hwang SA. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and neuropsychological status among older adults in New York. *Neurotoxicology*. 2012; 33(1):8–15. [PubMed: 22079442]
- Flower DR. The lipocalin protein family: structure and function. *Biochem J*. 1996; 318 (Pt 1):1–14. [PubMed: 8761444]
- Hakk H, Larsen G, Bergman A, Orn U. Binding of brominated diphenyl ethers to male rat carrier proteins. *Xenobiotica*. 2002; 32(12):1079–1091. [PubMed: 12593757]
- Hites RA. Polybrominated diphenyl ethers in the environment and in people: a meta-analysis of concentrations. *Environ Sci Technol*. 2004; 38(4):945–956. [PubMed: 14998004]
- Hudachek SF, Gustafson DL. Incorporation of ABCB1-mediated transport into a physiologically-based pharmacokinetic model of docetaxel in mice. *J Pharmacokinet Pharmacodyn*. 2013
- ILSI. Physiological parameter values for PBPK models. USEPA; 1994. p. 1-120.
- Janotova K, Stopka P. The level of major urinary proteins is socially regulated in wild *Mus musculus musculus*. *J Chem Ecol*. 2011; 37(6):647–656. [PubMed: 21594616]
- Kim UJ, Lee IS, Kim HS, Oh JE. Monitoring of PBDEs concentration in umbilical cord blood and breast milk from Korean population and estimating the effects of various parameters on accumulation in humans. *Chemosphere*. 2011 (0).
- Koh TW, Chih-Cheng Chen S, Chang-Chien GP, Lin DY, Chen FA, Chao HR. Breast-milk levels of polybrominated diphenyl ether flame retardants in relation to women's age and pre-pregnant body mass index. *International Journal of Hygiene and Environmental Health*. 2010; 213(1):59–65. [PubMed: 19781992]
- Kondratov RV, Komarov PG, Becker Y, Ewenson A, Gudkov AV. Small molecules that dramatically alter multidrug resistance phenotype by modulating the substrate specificity of P-glycoprotein. *Proc Natl Acad Sci U S A*. 2001; 98(24):14078–14083. [PubMed: 11707575]
- Krishnan, K.; Andersen, M. Physiologically based pharmacokinetic modelling in toxicology. In: Hayes, AW., editor. *Principles and methods of toxicology*. 5. CRC Press; New York: 2008. p. 231-291.

- Kwak J, Grigsby CC, Rizki MM, Preti G, Koksai M, Josue J, Yamazaki K, Beauchamp GK. Differential binding between volatile ligands and major urinary proteins due to genetic variation in mice. *Physiol Behav.* 2012; 107(1):112–120. [PubMed: 22728785]
- Larsen GL, Bergman A, Klasson-Wehler E. A methylsulphonyl metabolite of a polychlorinated biphenyl can serve as a ligand for alpha 2mu-globulin in rat and major-urinary-protein in mice. *Xenobiotica.* 1990; 20(12):1343–1352. [PubMed: 1706122]
- Larsen GL, Bergman A, Wehler EK, Bass NM. A methylsulfonyl metabolite of a polychlorinated biphenyl can serve as a ligand for liver fatty acid binding protein in rat intestinal mucosa. *Chem Biol Interact.* 1991; 77(3):315–323. [PubMed: 1901246]
- Leslie EM, Deeley RG, Cole SP. Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. *Toxicol Appl Pharmacol.* 2005; 204(3):216–237. [PubMed: 15845415]
- Li N, Hartley DP, Cherrington NJ, Klaassen CD. Tissue expression, ontogeny, and inducibility of rat organic anion transporting polypeptide 4. *J Pharmacol Exp Ther.* 2002; 301(2):551–560. [PubMed: 11961056]
- Lin JH, Yamazaki M. Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin Pharmacokinet.* 2003; 42(1):59–98. [PubMed: 12489979]
- Maher JM, Slitt AL, Cherrington NJ, Cheng X, Klaassen CD. Tissue distribution and hepatic and renal ontogeny of the multidrug resistance-associated protein (Mrp) family in mice. *Drug Metab Dispos.* 2005; 33(7):947–955. [PubMed: 15802388]
- Mazda A, Dodder NG, Abernathy MP, Hites RA, Bigsby RM. Polybrominated diphenyl ethers in maternal and fetal blood samples. *Environ Health Perspect.* 2003; 111(9):1249–1252. [PubMed: 12842781]
- Mitchell AM, Tom M, Mortimer RH. Thyroid hormone export from cells: contribution of P-glycoprotein. *Journal of Endocrinology.* 2005; 185(1):93–98. [PubMed: 15817830]
- Nukaya M, Takahashi Y, Gonzalez FJ, Kamataki T. Aryl hydrocarbon receptor-mediated suppression of GH receptor and Janus kinase 2 expression in mice. *FEBS Lett.* 2004; 558(1–3):96–100. [PubMed: 14759523]
- Proost JH, Wierda JM, Meijer DK. An extended pharmacokinetic/pharmacodynamic model describing quantitatively the influence of plasma protein binding, tissue binding, and receptor binding on the potency and time course of action of drugs. *J Pharmacokinet Biopharm.* 1996; 24(1):45–77. [PubMed: 8827583]
- Richardson VM, Staskal DF, Ross DG, Diliberto JJ, DeVito MJ, Birnbaum LS. Possible mechanisms of thyroid hormone disruption in mice by BDE 47, a major polybrominated diphenyl ether congener. *Toxicology and Applied Pharmacology.* 2008; 226(3):244–250. [PubMed: 17964624]
- Robertson DH, Cox KA, Gaskell SJ, Evershed RP, Beynon RJ. Molecular heterogeneity in the Major Urinary Proteins of the house mouse *Mus musculus*. *Biochem J.* 1996; 316 (Pt 1):265–272. [PubMed: 8645216]
- Robertson DH, Hurst JL, Bolgar MS, Gaskell SJ, Beynon RJ. Molecular heterogeneity of urinary proteins in wild house mouse populations. *Rapid Commun Mass Spectrom.* 1997; 11(7):786–790. [PubMed: 9161047]
- Sanders JM, Chen LJ, Lebetkin EH, Burka LT. Metabolism and disposition of 2,2',4,4'-tetrabromodiphenyl ether following administration of single or multiple doses to rats and mice. *Xenobiotica.* 2006a; 36(1):103–117. [PubMed: 16507516]
- Sanders JM, Lebetkin EH, Chen LJ, Burka LT. Disposition of 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE153) and its interaction with other polybrominated diphenyl ethers (PBDEs) in rodents. *Xenobiotica.* 2006b; 36(9):824–837. [PubMed: 16971346]
- Schecter A, Pöpke O, Harris TR, Tung KC. Partitioning of polybrominated diphenyl ether (PBDE) congeners in human blood and milk. *Toxicological & Environmental Chemistry.* 2006; 88(2):319–324.
- Schoenborn BP. Dichloromethane as an antisickling agent in sickle cell hemoglobin. *Proc Natl Acad Sci U S A.* 1976; 73(11):4195–4199. [PubMed: 1069309]
- Sergent T, Ribonnet L, Kolosova A, Garsou S, Schaut A, De SS, Van PC, Larondelle Y, Pussemier L, Schneider YJ. Molecular and cellular effects of food contaminants and secondary plant

components and their plausible interactions at the intestinal level. *Food Chem Toxicol.* 2008; 46(3):813–841. [PubMed: 18226851]

Sjodin A, Wong LY, Jones RS, Park A, Zhang Y, Hodge C, DiPietro E, McClure C, Turner W, Needham LL, Patterson DG Jr. Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003–2004. *Environ Sci Technol.* 2008; 42(4):1377–1384. [PubMed: 18351120]

Staskal DF, Diliberto JJ, Birnbaum LS. Disposition of BDE 47 in Developing Mice. *Toxicol Sci.* 2006a; 90(2):309–316. [PubMed: 16407092]

Staskal DF, Diliberto JJ, Birnbaum LS. Impact of Repeated Exposure on the Toxicokinetics of BDE 47 in Mice. *Toxicol Sci.* 2006b; 89(2):380–385. [PubMed: 16280385]

Staskal DF, Diliberto JJ, Devito MJ, Birnbaum LS. Toxicokinetics of BDE 47 in female mice: effect of dose, route of exposure, and time. *Toxicol Sci.* 2005; 83(2):215–223. [PubMed: 15509665]

Staskal DF, Hakk H, Bauer D, Diliberto JJ, Birnbaum LS. Toxicokinetics of Polybrominated Diphenyl Ether Congeners 47, 99, 100, and 153 in Mice. *Toxicol Sci.* 2006c; 94(1):28–37. [PubMed: 16936226]

- We report the first study on PBPK model on flame retardant in mice for BDE-47.
- We examine mechanism of urinary elimination of BDE-47 in mice using a PBPK model.
- We investigated roles of m-MUP and P-gp as transporters in urinary elimination.

Fig. 1.
A conceptual representation of the mouse PBPK model for BDE-47.

Figure 2a

Figure 2b

Fig. 2.

The cumulative profile followed single oral doses of 0.1, 1.0, 10.0, and, 100.0 mg of BDE-47/kg of body weight in female C57BL/6J mice (Staskal et al. 2005). Figure 2A shows the cumulative urinary excretion of BDE-47 during 5 days. The percentage urinary (PRCT_urinary) represents urinary excretion media for different doses and is expressed in terms as percentage of dose of BDE-47. Figure 2B shows the cumulative fecal excretion of BDE-47 during the first 5 days of excretion. The cumulative excretion includes a no-absorption fraction and eliminates the mother fraction and metabolism fraction of BDE-47. The percentage feces (PRCT_feces) represent the fecal excretion media for different doses.

Fig. 3.

The prediction profile of the PBPK model's kinetic profiles of BDE-47 of blood concentration following a single intravenous exposure dose of 1.0 mg of BDE-47/kg of body weight (Staskal *et al.* 2006c). This figure shows the percentage of dose in urine (PTAUIX) and feces: PRCTFECES excretion. The symbols represent experimental data 5 days post-exposure, and the lines represent the simulation obtained using the mouse PBPK model. Tissue compartments were sampled only on Day 5, whereas urinary and fecal samples were measured daily from Day 1 through Day 5.

Fig. 4.

The prediction profile of the PBPK model's kinetic profiles (the full line) of BDE-47 of adipose tissue, liver, brain, kidney, rest of the body, and blood concentrations 10 days following a single intravenous exposure dose of 1.0 mg of BDE-47/kg of body weight. The symbols represent experimental data at 3 and 8 hours and at 1, 5, and 10 days post-exposure in mice. Figure 4A represents the situation in which m-MUP and P-gp were activated. Figure 4B shows when m-MUP and P-gp were inactivated. Figure 4C shows when P-gp was inactivated and m-MUP was activated. Figure 4D shows when m-MUP is inactivated and P-gp is activated.

Fig. 5.

The percentage dose of BDE-47 excreted by FVB-KO mice compared to FVB-WT mice (control group) following a single oral exposure of 1.0 mg of BDE-47/kg of body weight or 100.0 mg/kg of body weight and a sample for 5 days post-exposure. The lines represent the simulation using the PBPK model for the four experimental conditions. The symbols represent the following: (■) FVB-KO mice for 1.0 mg of BDE-47/kg of body weight, (◆) FVB-KO mice for 100 mg/kg of body weight, (▲) FVB-WT mice for 1.0 mg/kg of body weight, and (○) FVB-WT mice for 100 mg/kg of body weight. Figure 5A represents the simulation using the optimized parameters. Figure 5B is the simulation after re-optimizing the following elimination parameters: urinary extraction constant (EUI), elimination (E), the affinity constant for P-gp (Km), the maximal velocity of P-gp (Vmax), and the feces excretion (Kst).

Fig. 6. Sensitivity analysis was performed for each parameter after a single oral dose of 1.0 mg of BDE-47/kg of body weight, and the concentration was calculated 21 days after the exposure. This sensitivity analysis recorded the percentage of variation of each parameter one at a time for $\pm 2\%$ of BDE-47, which resulted in a concentration variation in the blood compartment.

Table 1

Parameters Used in the PBPK Mouse Model

Parameters	Symbols	Parameter Values
Body weight (g)	Bw	25
Cardiac output (mL/hr/kg)	Qcc	16 223 ^a
Tissue volume (fraction of body weight, unitless)		
Blood	Vb0	0.049 ^b
Brain	Vbr0	0.017 ^b
Liver	Vli0	0.055 ^b
Adipose tissue	Vf0	0.070 ^b
Kidney	Vk0	0.017 ^b
Rest of the body	Vre0	Calculated
Tissue blood volume (fraction tissue volume, unitless)		
Brain	Vbrb	0.0034 ^c
Adipose tissue	Vfb	0.0035 ^c
Rest of the body	Vreb	0.1000 ^c
Tissue blood flow Qt (fraction Qc, unitless)		
Brain	Qbr0	0.033 ^a
Liver	Qli0	0.161 ^a
Adipose tissue	Qf0	0.100 ^a
Kidney	Qk0	0.090 ^a
Rest of the body	Qre0	Calculated
Tissue permeability (fraction of Qt, unitless)		
Brain	PAbrF	0.02 ^d
Adipose tissue	PAfF	0.04 ^c
Rest of the body	PAreF	0.01 ^c
Apparent partition coefficient (unitless)		
Brain	Pbr	70.0 ^c
Liver	Pli	23.0 ^c
Adipose tissue	Pf	200.0 ^c
Kidney	Pk	4.0 ^c
Rest of the body	Pre	5.0 ^c

^a (Krishnan and Andersen 2008)^b (ILSI 1994)^c Optimized^d Calculated

Table 2

Kinetic Parameters Used in the PBPK Mouse Model

Parameters	Symbols	Parameter Values
Metabolic constant		
Kidney tubular constant (P-gp)		
Maximum velocity (mg/hr)		
Brain	V _{maxbr}	0.003 ^a
Kidney	V _{maxk}	0.01 ^a
Intestine	V _{maxint}	0.00001 ^a
Liver	V _{maxli}	0.00001 ^a
Affinity constant (mg/mL) (P-gp)	K _m	0.1 ^a
Blood-binding m-MUP		
Protein concentration (mg)	B _{maxl}	50 ^a
Affinity constant (mg/mL)	K _{ml}	30 ^a
Elimination extraction fraction constant		
Urinary elimination extraction constant (unitless)	E _{UI}	0.015 ^a
Hepatic extraction constant (unitless)	E	0.005 ^a
Oral absorption constant (hr ⁻¹)	K _a	0.08 ^a
GI tract transit (hr ⁻¹)	K _{st}	0.035 ^a

^aOptimized