MECHANISTIC DESCRIPTION OF DOSE-DEPENDENT URINARY ELIMINATION OF PBDE-47 IN ADULT MICE USING A PHYSIOLOGICAL BASED PHARMACOKINETIC MODEL

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Abstract
Polybrominated diphenyl ethers (PBDEs) are used as additive flame-retardants. In North America, scientists have noted continuing increases in human body burdens. Our laboratory has previously described urinary elimination of parent compound in adult mice for at least 4 of the major BDE congeners measured in biota (i.e. BDEs 47, 99, 100, and 153). We have also demonstrated that urinary elimination saturates at high doses for BDE-47; however, this dose-dependent urinary elimination has not been observed in rats or immature mice. The objective of this study was to investigate the mechanism of BDE-47 urinary elimination in mice using a physiologically based pharmacokinetic model (PBPK). Specifically, we investigated two hypotheses: The first hypothesis investigated the extent to which mMUP drives the elimination of BDE-47and the second evaluated a disruption of glomerular filtration at high dose of PBDEs. To evaluate these hypotheses, we developed a PBPK model contained 6 compartments built and validated using data from rats and extrapolated to the mouse. At low doses, the model showed increased elimination as compared to higher doses, consistent with the experimental data. This work helps to explain the physiological observations of urinary transport in mice and should be considered when assessing human health risk.

Introduction
Polybrominated diphenyl ethers (PBDE) are used as additive flame-retardants. It is important to understand the risk from their exposure given recent results of toxicological studies in combination with increased measurements of these compounds in humans[1]. Our laboratory has previously reported data related to the rapid excretion of unmetabolized BDE-47 in urine. While initial hypothesis involved an active transport mechanism, further studies demonstrated that mouse Major Urinary Protein (mMUP) was primarily responsible for facilitating urinary excretion[2]. This protein belongs to the same superfamily of proteins which includes FABP, retinol-binding protein, and α2-globulin, known and documented to bind polyhalogenated aromatic hydrocarbons[3-6]. This binding was not found in rats, thus the difference between rodents species for the elimination of BDE-47 raises the question of which species is a better model for human risk assessment[7]. The objective of this study was to investigate the pharmacokinetic implications of urinary elimination of BDE-47 in mice and to specifically assess the extent to which mMUP drives the elimination of BDE 47 and to evaluate the potential role of glomerular filtration diminution. A physiologically based pharmacokinetic (PBPK) platform was used for this analysis and compared to experimental measurements.

Materials and Methods
The physiologically based pharmacokinetic (PBPK) model contains 6 compartments which include blood, brain, adipose tissue, kidney, liver and rest of the body (Figure 1). The last compartment, rest of the body, represents all other tissues lumped into a unique compartment. Liver, brain and adipose tissue were described as diffusion limited (i.e., the distribution in cellular matrices was slower than tissue blood flow). Parameters such as tissue volumes, cardiac output, and blood flows were extracted from previously reported studies [8-9]. The apparent partition coefficients and tissue permeability described for liver, brain, and adipose tissue were optimized with ACSLMath™. The optimization was performed using adult female rat data and neonatal mouse data determined

in our laboratory [9,10]. The PBPK model was extrapolated to mice and validated using a new experimental data set consisting of data from acute exposure data[11]. The mathematical model description was written using ACSL™ Aegis.

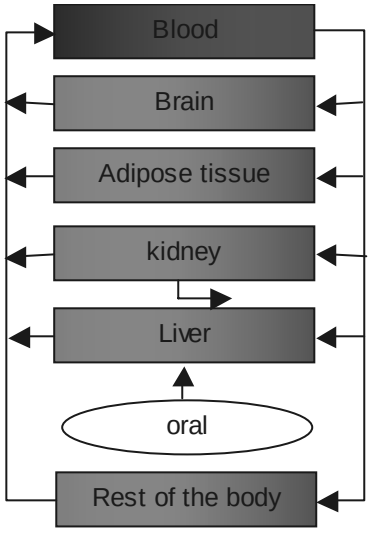

Figure 1: Conceptual representation of PBPK model for rodent

Previous studies in our laboratory have demonstrated that BDE-47 binding to mMUP and subsequent elimination is dose-dependent. Two hypotheses were formulated using PBPK models to understand the behavior of this experimental observation. The first hypothesis investigated the extent to which mMUP drives the elimination of BDE-47. Therefore, we have modeled a scenario in which mMUP bindng follows Michaelis-Menten behaviour (Equation 1).

$$A_{bound} = \frac{B_{\max} \times Ca}{K_m + Ca} \qquad \text{eq. 1}$$

Where,

A_{bound}	=	amount of BDE-47 bound to mMUP (ng)
B_{max}	=	mMUP total protein site (ng)
Ca	=	arterial concentration (ng/ml)
K_m	=	affinity constant of mMUP (ng/ml)

Using this model structure which accounts for a saturation capacity described by the Michaelis-Menten equation, the urine clearance will be dose-dependent. In addition, at higher doses, the amount of BDE-47 found in the urine will be lower in percentage of dose of what we should find if there was no saturation. However, at low doses, the % of dose should be substantial. Simulation was compared to the published mouse data in which both measured urinary elimination of BDE-47.

The second hypothesis evaluated diminution in glomerular filtration. It is plausible that an exposure dose higher than 10 mg/kg of body weight (BW) can alter glomerular filtration. Subsequently, altered filtration could result in a lower % per dose of BDE-47 found in the urine. Our findings indicate that below 10 mg BDE-47/kg of BW, the behavior follows the Michaelis-Menten equation profile. However, at higher doses, the simulations with the PBPK model suggest a discrepancy in the observed excretion compared to the experimental data. In our PBPK model, we assumed the filtration was about 30% of the total BDE-47 bound to mMUP at low doses, but around 10% at higher exposure dose. This approach assumed that only BDE-47 bound to mMUP may be filtered by the

glomerulus and that the free unbound fraction of BDE-47 was available for storage in adipose tissue. The equation utilized to describe filtration is provided below (Eq. 2):;

$$\frac{dA}{dt} = Q_{kidney} \times E_{fraction} \times C_{BDE-47\ bound\ \text{mMUP}} \qquad \text{eq. 2}$$

Where,

A_{bound} = amount of BDE-47 eliminated (ng)
Q_{kidney} = kidney blood flow (ml/hr)
$E_{fraction}$ = filtration fraction of BDE-47 bind to mMUP (unitless)
$C_{BDE\text{-}47\ bound\ mMUP}$ = arterial concentration (ng/ml)

Table 1 Parameters used in the PBPK model in mouse

Parameters	Mouse parameters
Body weight (g) (BW)	25
Cardiac output ml/min/kg	680
Tissue volume (fraction of BW)	
Blood	0.080
Brain	0.006
Liver	0.040
Fat	0.070
Kidney	0.007
Rest of the body	0.707
Tissue blood volume (fraction tissue volume)	
Brain	0.200
Liver	0.266
Fat	0.050
Tissue blood flow Qt (fraction Qc)	
Brain	0.02
Liver	0.18
Fat	0.069
Kidney	0.140
Rest of the body	0.591
Tissue permeability (fraction of Qt)	
brain	0.20
Liver	0.35
Fat	0.08
Apparent partition coefficient	
Brain	3
Liver	10
Fat	100
Kidney	5
Rest of the body	2

Results and Discussion

The first hypothesis was that protein binding to mMUP drives the elimination of BDE-47 and that this binding should be saturable. A simulation for 0.1 1 and 10 mg of BDE-47/kg of BW showed good prediction of experimental data. Nevertheless, at high doses, it seems that the carrier protein did not behave like a saturation profile described with PBPK model, except, for a decrease in urinary elimination. Both the modeling and experimental data showed similar profiles but with less accuracy than what we observed at low dose. This observation suggests that other mechanisms may explain the experimental observation. Our group observed also that at higher doses, more BDE-47 was sequestrated in blood as compared to lower doses[7]. This suggests an inducible phenomenon of mMUP. If this were true, we should see also an increase of BDE-47 elimination in urine; however, this was not observed[7]. Results of PBPK modeling for the second hypothesis suggest that a disruption in glomerular filtration may contribute to the reduction in the elimination, but not to the extent of BDE-47 found in blood observed in the experimental studies. Together, these results suggest that multiple

mechanisms are likely involved in the saturable urinary excretion observed in mice for BDE-47.

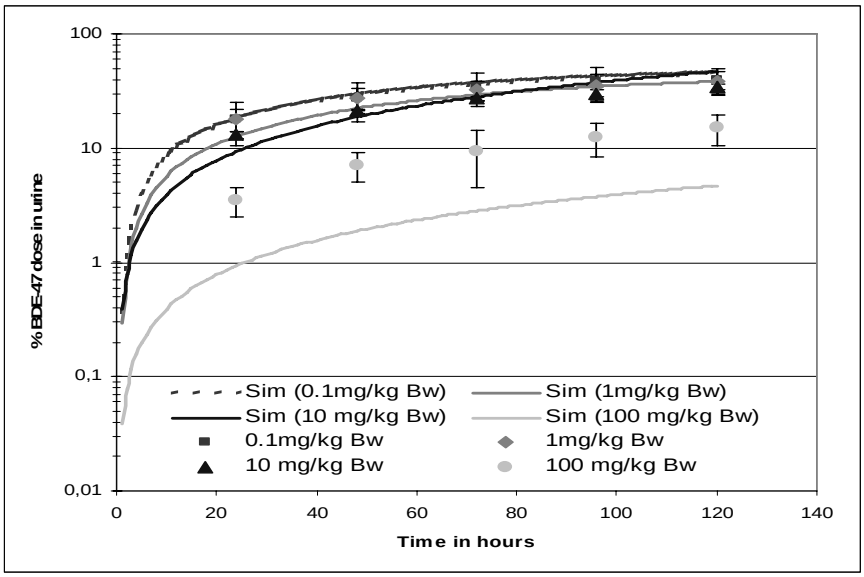

Figure 2
Comparison between cumulative excretions followed a single oral exposure of DBE-47 at 0.1, 1, 10, 100 mg/kg of BW. Symbols represent experimental data and full lines, the simulation obtained using the PBPK model in mice.

This works represents an initiative to more fully understand the urinary excretion of BDE-47 in mice. Application of a PBPK model suggests that protein transport plays a primary role in the elimination process, but that glomerular filtration may be also affected by BDE-47 which may play a role in the saturation observed at high doses in experimental studies. To fully understand the kinetics, additional experimental data are needed to target the interaction of BDE-47 in the kidney. However, further development of a PBPK model and subsequent application to human scenarios may help to understand BDE-47 kinetics in humans.

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