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Research Article

Physiologically Based Pharmacokinetic Modeling for 1-Bromopropane in F344 Rats Using Gas Uptake Inhalation Experiments

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ABSTRACT

1-Bromopropane (1-BP) was introduced into the workplace as an alternative to ozone-depleting solvents and increasingly used in manufacturing industry. The potential exposure to 1-BP and the current reports of adverse effects associated with occupational exposure to high levels of 1-BP have increased the need to understand the mechanism of 1-BP toxicity in animal models as a mean of understanding risk in workers. Physiologically based pharmacokinetic (PBPK) model for 1-BP has been developed to examine 2 metabolic pathway assumptions for gas-uptake inhalation study. Based on previous gas-uptake experiments in the Fischer 344 rat, the PBPK model was developed by simulating the 1-BP concentration in a closed chamber. In the model, we tested the hypothesis that metabolism responsibilities were shared by the p450 CYP2E1 and glutathione (GSH) conjugation. The results showed that 2 metabolic pathways adequately simulated 1-BP closed chamber concentration. Furthermore, the above model was tested by simulating the gas-uptake data of the female rats pretreated with 1-aminobenzotrizole, a general P450 suicide inhibitor, or D,L-buthionine (S,R)-sulfoximine, an inhibitor of GSH synthesis, prior to exposure to 800 ppm 1-BP. The comparative investigation on the metabolic pathway of 1-BP through the PBPK modeling in both sexes provides critical information for understanding the role of p450 and GSH in the metabolism of 1-BP and eventually helps to quantitatively extrapolate current animal studies to human.

Key words: physiologically based pharmacokinetic modeling; 1-bromopropane; F344 rats; gas uptake inhalation

1-Bromopropane (1-BP), a halogenated alkane, was introduced into the workplace as an alternative to ozone-layer depleting solvents (ODA) after the discovery of the reproductive and hematopoietic toxicities of 2-brompropane (2-BP) in workers (Kim *et al.*, 1997; Yu *et al.*, 1999a, c, 2001a). 2-BP was first used as an ODA in Korea and Japan and discovered to cause reproductive disorders in workers (Kim *et al.*, 1997; Park *et al.*, 1997). Animal studies confirmed its adverse effects, which included reductions in testes weight and sperm counts, atrophy of the seminiferous tubules, a decrease in the number of ovarian follicles and an increase in irregular estrous cycles (Ichihara *et al.*, 1997, 1999, 2000a,b, 2004a, 2005; Kamijima *et al.*, 1997a, b; Nakajima *et al.*, 1997a, 1997b; Wang *et al.*, 2002, 2003; Yu *et al.*, 1999a, b, 2001a, b; Yamada *et al.*, 2003). The isomer 1-BP was thereafter introduced and approved as an ODA by Environmental Protection Agency's (EPA, 2007). Subsequent animal studies identified the potential for 1-BP-mediated reproductive and neurotoxicity (Yu *et al.*, 1998a, 2001a). 1-BP is categorized as a high-production volume chemical (Eisenberg and Ramsey, 2010). Its usage has increased dramatically, rising to around 20 million pounds/year, which could result in a widespread occupational exposure (Anderson *et al.*, 2010; NTP, 2013; OSHA, 1999). 1-BP is demonstrated to be a potent neurotoxic compound compared with 2-BP. In addition, 1-BP has reproductive toxicity, but the target cells are different from those of 2-BP. For example, exposure to 1-BP inhibits spermiation in male rats and disrupts the development of follicles

in female rats, in contrast to 2-BP, which targets spermatogonia and oocytes in primordial follicles. Because the first animal study revealing the neurotoxicity of 1-BP (Yu *et al.*, 1998a), over a dozen human cases of neurotoxicity have been reported with manifestations of 1-BP toxicity. Among 1-BP exposed workers, dose-dependent prolongation of distal latency in the tibial nerve with decreased vibratory sensation in the lower extremities has been shown (Ichihara *et al.*, 2004b).

Species and strain-specific effects of 1-BP were observed in rats and mice (Ichihara *et al.*, 2012; Liu *et al.*, 2009). The majority of toxicological studies were conducted in rats. In a comparative study between the 2 inbred strains of rats, F344 and Wistar, neurotoxicity revealed by distal latency was more significant in F344 than in Wistar rats. Furthermore, mice were reported to be more susceptible than rats to 1-BP-mediated hepatotoxicity and reproductive toxicity (Liu *et al.*, 2009). Hepatotoxicity and male reproductive toxicity were compared among the 3 strains of mice (C57BL/6J, DBA/2J, and BALB/cA) exposed to 1-BP at 0, 50, 110, and 250 ppm for 8 h/day for 28 days by inhalation. Histopathological evaluation of the liver damage showed a significantly larger area of necrosis and more degenerative lobules in BALB/cA in the order of BALB/cA > C57BL/6J > DBA/2J. BALB/cA showed higher CYP2E1 protein level and lower total glutathione (GSH) content and glutathione-S-transferases (GST) activity in the liver than DBA/2J. These results indicate that BALB/cA mice are the most susceptible to the hepatotoxicity of 1-BP among the 3 strains tested, and that CYP2E1 and GSH level/GST activity may contribute to the susceptibility to 1-BP hepatotoxicity. Exposure to $\geq$ 50 ppm of 1-BP also decreased sperm count and sperm motility and increased sperms with abnormal heads in all 3 strain mice, whereas rats exposed to 200 ppm of 1-BP for 12 weeks showed no changes in sperm count and sperm motility (Ichihara *et al.*, 2000b), suggesting mice are far more susceptible than rats. Most recently, a long-term inhalation animal study from the National Toxicological Program (NTP) found that exposure of male and female F344/N rats to 1-BP significantly increased the incidences of adenomas of the large intestine and skin neoplasms (Morgan *et al.*, 2011; NTP, 2013). In male rats, the incidence of malignant mesothelioma was statistically significantly increased at 500 ppm. There was no evidence of carcinogenic activity of 1-BP in male B6C3F1 mice; however, significantly increased incidences of alveolar/bronchiolar neoplasms of the lung were present in female mice. The mechanism of the different incidence of the carcinogenesis observed between rat and mice and male and female is still unclear, which makes it uncertain how to translate these results from animals to humans.

Proposed Occupational Exposure limits (OELs) for 1-BP are diverse in both the selection of critical effects and judgments of remaining uncertainty because its toxicological mechanisms are still poorly known. The OEL values differ by ~10-fold. Toxicology Excellence for Risk Assessment evaluated the underlying basis of existing OELs through critical effect, benchmark dose, and uncertainty factor, and concluded that the critical effect has decreased the live litter size with a benchmark dose lower confidence limit of 190 ppm (Maier *et al.*, 2004). The OEL of 20 ppm is derived using an uncertainty factor of 10-fold, which is composed of 3-fold for extrapolation from an experimental animal study to humans for expected toxicodynamic differences and 3-fold for expected human variability in toxicokinetics and toxicodynamics within the worker population. The American Conference of Industrial Hygienists (ACGIH) recommends an 8-h time weighted average threshold limit values of 0.10 ppm to provide protection against the potential for neurotoxicity, hepatotoxicity, and reproductive and developmental toxicity in 1-BP exposed workers (ACGIH, 2014). Other professional organizations and manufacturers have recommended exposure limits (RELs) ranging from 20 to 100 ppm (Stelljes and Wood, 2004). The acceptable exposure limit of 1-BP from the U.S. EPA is 25 ppm (8-h time-weighted average). Currently, the National Institute for Occupational Safety and Health does not have a REL, nor does the Occupational Safety and Health Administration have a permissible exposure limit for 1-BP.

The potential for human exposure to 1-BP and its adverse effects related with occupation requires understanding of the potential mechanism of these adverse effects in rats and mice as a means of understanding risk in workers. Risk assessment for 1-BP exposure is limited due to lack of animal or human toxicokinetic study. Gas uptake studies analyzed by physiologically based pharmacokinetic (PBPK) models have been used to estimate metabolic parameters for many volatiles (Dobrev *et al.*, 2003, 2008; Lilly *et al.*, 1997). The metabolic constants for a saturable pathway (V_{max} and K_m) and for a first-order process are typically inferred from the decline in chemical concentration observed in closed chamber exposures. The objective of the current study was to develop a physiologically based PBPK model to simulate the concentration of 1-BP in a closed chamber from a gas-uptake experiment in the F-344 rats. The PBPK model tested the hypotheses, including sex-specific metabolism of 1-BP, and investigated the role of the 2 major metabolic pathways, cytochrome P450 CYP2E1 and GSH conjugation, in the metabolism of 1-BP. The results showed that these 2 metabolic pathways adequately simulated the concentration of 1-BP in the closed chamber. Furthermore, the above model was further tested by simulating the gas-uptake data from a female rat pretreated with a general P450 suicide inhibitor 1-aminobenzotrizole (ABT) or GSH biosynthesis inhibitor D,L-buthionine (S,R)-sulfoximine (BSO) prior to the exposure of 800 ppm 1-BP. The comparative investigation on the metabolic pathways of 1-BP through the PBPK modeling in both sexes provides critical information in understanding the role of P450 and GSH conjugation in the metabolism of 1-BP and eventually helps to quantitatively extrapolate animal studies to human.

MATERIALS AND METHODS

Experimental data. The experimental data such as time-course of 1-BP concentrations in a closed chamber were obtained from Garner's gas-uptake study (Garner and Yu, 2014). Briefly, the gas-uptake chamber was constructed based on previous reports (Gargas *et al.*, 1989; Garner *et al.*, 2006). The chamber volume was measured by dividing the total amount of 1-BP injected into a closed chamber by the initial chamber concentration monitored by a detector. The rate of hair adsorption was experimentally determined by placing a rat carcass in the chamber at 800 ppm 1-BP (RTI Internation, 2005). Plots of chamber 1-BP concentrations in an empty chamber and in one that contained a rat carcass were generated. There was an approximate 1.5 $\pm$ 1.5% loss of 1-BP from the empty gas-uptake chamber atmosphere over a 6-h period. The rate of loss of 1-BP to hair and skin was similar between rat and mouse with the estimated adsorption rate constants of 0.0415 h^{-1}. During the gas uptake experiment, 1-BP was injected into a closed chamber at the initial target concentrations of 70, 240, 800, and 2700 ppm with a single male or female rat. Four separate experiments were conducted in male or female rats ($n = 4$). Flame ionization detector (GC/FID) was used to continuously monitor the concentration of 1-BP in the

closed chamber for 6 h after injection. The unit of the concentration "mg/l" was used in the model development and data presentation in the plotted figures after conversion of "ppm" to "mg/l".

Model structure. The current model was constructed as 7-compartment model with blood, lung, fat, rapidly perfused tissue (brain, organs, etc.), slowly perfused tissue (bone, muscle, etc.), kidney and liver based on mass balance and 2 assumptions including a flow-limited distribution and saturable metabolism (Fig. 1). Figure 1 illustrates the elimination of 1-BP in the closed chamber in the gas-uptake study. After injection 1-BP into a closed chamber with 1 rat, 1-BP was rapidly deposited in the lung through inhalation and distributed into 6 compartments through blood circulation and then partially metabolized in liver compartment and excreted through urine (Garner *et al.*, 2006). The major amount of 1-BP was eliminated from the body by exhalation of 1-BP (Garner *et al.*, 2006). A small amount of 1-BP was absorbed by skin and fur. 1-BP is found to be absorbed, rapidly distributed, and predominantly eliminated by exhalation in rodents, but is also excreted in the urine and feces (Garner *et al.*, 2006). In rats and mice, most of the 1-BP administered by iv injection was exhaled unchanged or as carbon dioxide within 4 h of exposure. Urinary metabolites accounted for 13%–23% of the administered dose after 48 h (Garner *et al.*, 2006). As shown in Figure 2, cytochrome P450-mediated oxidation is a major pathway of 1-BP elimination following inhalation and intravenous exposure in both rats and mice (Garner *et al.*, 2006). The principal P450-mediated urinary metabolite is 1-bromo-2-hydroxypropanol (2OHBP), which is subsequently conjugated with GSH or glucuronic acid or further metabolized to highly reactive metabolites such as bromoacetone (Garner *et al.*, 2006). The urinary metabolite profile in rat changed as the dose increased. At low doses, the profile consisted of mercapturates of several P450-mediated metabolites and at very high doses, changed to a single mercapturic acid metabolite derived from direct conjugation of parent compound with GSH. Therefore, we included 2 metabolic pathways P450 enzymes-mediated metabolism of 1-BP (V_{max}, K_m) and GSH-related constant K_{gst} in the current model.

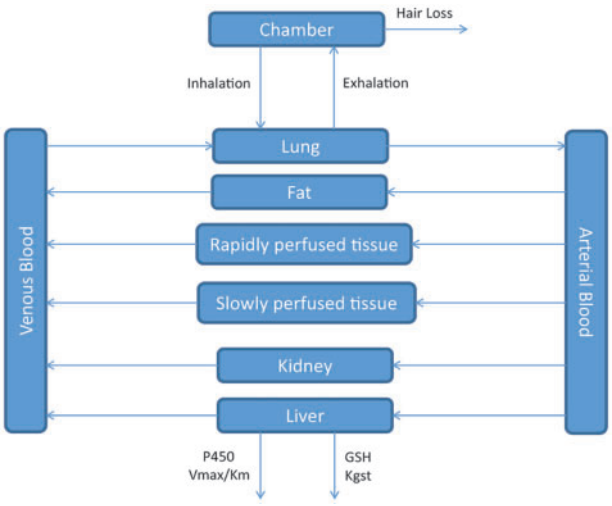

FIG. 1. Physiologically based pharmacokinetic (PBPK) model structure for 1-BP in F344 male and female rats.

Model parameters. The physiological parameters (Table 1) used in the model for the male and female F344 rats are listed in Table 1. The body weight (BW) of individual male and female rat was obtained from the RTI final report (RTI Internation, 2005). The fraction of blood flow for the male rat was obtained directly from the Brown's report (Brown *et al.*, 1997). The fraction of blood flow to slowly perfused tissue (QSC) was set to 0.458 including the blood flow distribution to muscle, bone, and skin. The fraction of blood flow to rapidly perfused tissue (QRC) was calculated based on the following equation:

$$QRC = 1 - QFC - QLC - QSC - QKC,$$

where QFC is the fraction of blood flow to fat tissue, QKC the fraction of blood flow to kidney tissue, and QLC is the fraction of blood flow to liver tissue.

Tissue volumes of liver and kidney were obtained directly from the Brown's report (Brown *et al.*, 1997), in which the tissue volumes of the liver and kidney for the male and female F344 rats were separately calculated based on 10 randomly selected NTP animal studies in rats. The tissue volumes of fat and slowly perfused tissue were obtained from the EPA document (EPA, 2006) and used to develop the male rat model. There are limited data on the tissue volume of fat in the female rats as discussed in the Brown's report (Brown *et al.*, 1997). The fraction of the fat volume in the female rat was assumed to be same across species and 1.5 times of the fraction of the fat volume in the male rats, which is based on the human relative adipose tissue weight as reported by International Commission on Radiological Protection (ICRP) in 1975 (Brown *et al.*, 1997). The fraction of blood volume was set to 0.064 based on the total blood volume as reported by Gearhart *et al.* (1990). The fraction of the rapidly perfused tissue (VRC) is calculated with the following equation:

$$VRC = 1 - VFC - VLC - VBloodC - VSC - VKC,$$

where VFC is the fraction fat tissue, VLC the fraction liver tissue, VBloodC the fraction blood volume, VSC the fraction slowly perfused tissue, and VKC is the fraction kidney tissue.

Alveolar ventilation rate at rest was obtained directly from the Brown's report (Brown *et al.*, 1997). The cardiac output was adapted from Brown's report (Brown *et al.*, 1997), in which the cardiac output was estimated to be 117.6 ml/min for a 366-g rat. The cardiac output is calculated with the following equation:

$$\text{Cardiac Output } (\text{l/h}) = 15.0(\text{l/h/kg}^{0.75}) * \text{BW}^{0.75}$$

Chemical-specific parameters (Table 2) were obtained from the Gargas' study and RTI final report (Gargas *et al.*, 1989; RTI Internation, 2005). The partition coefficients for 1-BP were previously reported by Gargas, where the partition coefficients of a number of low-molecular-weight volatile chemicals including 1-BP were determined (Gargas *et al.*, 1989). Kaneko *et al.* (1997) reported the partition coefficients for 1-BP in a short communication report. The reported values for the blood:air, water:air, muscle:air, and liver:air were significantly larger than those values from Gargas *et al.* (1989), whereas the values for fat:air was about half of the value as reported by Gargas (105 vs 236). By using the same method as reported by Gargas, RTI measured the saline:air and blood:air partition coefficients for 1-BP (Gargas *et al.*, 1989; RTI Internation, 2005). The saline:air and blood:air coefficient were 1.6 ± 0.3 (Mean $\pm$ SD) and 12 ± 1 (Mean $\pm$ SD), respectively. Those values were similar to those

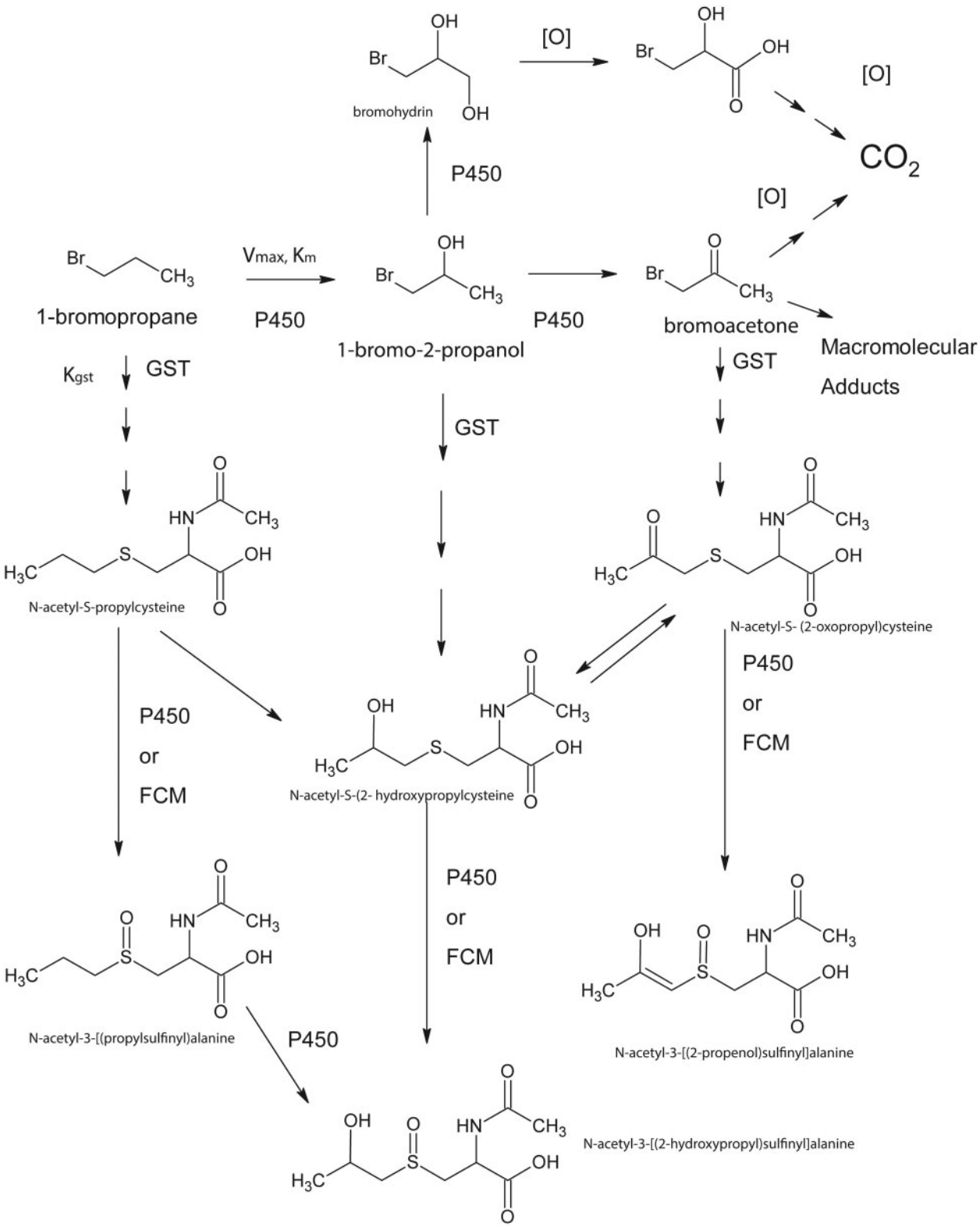

FIG. 2. Proposed metabolism of 1-Bromopropane (1-BP) metabolism in male F344 rats following inhalation exposure (Garner *et al.* 2007, 2006). Both P450-mediated oxidation (V_{max}, K_m) and direct glutathione (GSH) conjugation (K_{gst}) occurred during the metabolism of 1-BP. The principal P450-mediated urinary metabolite is 1-bromo-2-hydroxypropanol (2OHBP), and conjugated with GSH or glucuronic acid or further metabolized to potentially reactive metabolites such as bromoacetone and α-bromohydrin.

TABLE 1. PBPK Model Parameters for Rats and Human

Parameter	Description	Values		
		Male Rat	Female Rat	Human
Blood flows				
QPC	Alveolar ventilation l/h/kg	31.7[a]	31.7[a]	14.57[a]
QCC	Cardiac output l/h/kg$^{0.75}$	15.0[b]	15.0[b]	6.47[i]
QFC	Fraction of blood flow to fat	0.07[a]	0.07[a]	0.052[a]
QLC	Fraction of blood flow to liver	0.183[a]	0.183[a]	0.227[a]
QSC	Fraction of blood flow to slowly perfused tissue	0.458[a]	0.458[a]	0.291[a]
QKC	Fraction of blood flow to kidney	0.141[a]	0.141[a]	0.175[a]
QRC	Fraction of blood flow to rapidly perfused tissue	0.148[c]	0.148[c]	0.255[b]
Tissue volumes				
BW	Body weight (kg)	0.35[d]	0.185[d]	70[a]
VFC	Fraction of fat tissue	0.0723[d]	0.108[h]	0.214[a]
VLC	Fraction of liver tissue	0.0429[a]	0.035[a]	0.0257[a]
VBloodC	Fraction of blood volume	0.064[f]	0.064[f]	0.079[a]
VSC	Fraction of slowly perfused tissue	0.75[e]	0.75[e]	0.58[a]
VKC	Fraction of kidney tissue	0.0068[a]	0.0076[a]	0.0044[a]
VRC	Fraction of rapidly perfused tissue	0.064[g]	0.0354[g]	0.0969[a]

[a]From Brown *et al.* (1997).
[b]Cardiac Output (l/h)=14.9 (l/h /kg$^{0.75}$)*BW$^{0.75}$.
[c]QRC=1-QFC-QLC-QSC-QKC.
[d]From RTI (RTI Internation, 2005).
[e]From EPA (2006).
[f]From Gearhart *et al.* (1990).
[g]VRC=1-VFC-VLC-VBloodC-VSC-VKC.
[h]Assume 1.5 times of male parameter based on ICRP, 1975.
[i]From Yu *et al.* (1998b).

TABLE 2. PBPK Model: 1-BP Specific Parameters for Rats and Human

Parameter	Description	Values		
		Male Rat	Female Rat	Human
Partition coefficient (PCs)				
PB	Blood:air	11.7[a]	11.7[d]	11.7[e]
PLA	Liver:air	8.17[a]	8.17[d]	8.17[e]
PMA	Muscle:air	4.21[a]	4.21[d]	4.21[e]
PFA	Fat:air	236[a]	236[d]	236[e]
PKA	Kidney:air	16.3[b]	16.3[d]	16.3[e]
PVA	Viscera:air	16.3[c]	16.3[d]	16.3[e]

[a]From Gargas *et al.* (1989).
[b]From RTI Internation (2005).
[c]Assume to be same with kidney.
[d]Assume to be same across sexes.
[e]Assume to be same across species.

values in the same rat strain as reported by Gargas (1.44 ± 0.12 and 11.7 ± 0.4, individually). Therefore, we used the partition coefficients as reported by Gargas *et al.* Because only liver:air, muscle:air, and fat:air partition coefficients were included in the Gargas's study, additional tissue:air partition coefficients, including kidney, brain, and skin were measured (Gargas *et al.*, 1989; RTI Internation, 2005). In this model, each organ:blood partition coefficient was calculated based on organ:air and blood:air partition coefficient (PB). Partition coefficients were assumed the same across sexes and species. As an example, the equation of the liver:blood partition coefficient (PL) is:

$$PL = PLA/PB,$$

where PLA is the liver:air partition coefficient.

To simulate the metabolic behavior of 1-BP in rats, we first estimated and optimized the metabolic parameters: V_{max} (maximum velocity of the reaction), K_m (Michaelis constant), and GSH-related constant K_{gst} in the model. GSH-related constant K_{gst} reflects the binding kinetics of GST to GSH as well as other undefined factors that affect GSH consumption and synthesis. These metabolic parameters of the male rat were first optimized in each rat at 240 ppm by adequate visually fitting the curve of the time–concentration. The mean and standard deviation (mean ± SD) were calculated and applied to other concentrations. For the metabolic parameters in the female rats, we started the optimization of these metabolic parameters at the highest dose (2700 ppm) and then applied them to simulate the curves of other exposure levels. The hair absorption rate was experimentally determined, and the adsorption rate constant was estimated to be 0.0415 h^{-1} (RTI Internation, 2005).

The metabolic process was described as a classic Michaelis–Menten Kinetics as follows:

$$\text{Oxidative}_{\text{Metabolism}} = C_{\text{liver}} * V_{\text{max}}/(C_{\text{liver}} + K_m)$$
$$\text{Conjugation} = C_{\text{liver}} * K_{\text{gst}}$$

We applied a general human PBPK model for the volatile compounds which is previously developed by Anderson *et al.* (2008) to predict blood concentration in a repeated exposure scenario to 1-BP. Physiological parameters for the human model were adopted from Brown's report (Brown *et al.*, 1997) to mimic the general human body (Table 1). The same 7-compartment model with blood, lung, fat, rapidly perfused tissue (brain, organs, etc.), slowly perfused tissue (bone, muscle, etc.), kidney and liver based on mass balance and 2 assumptions, including a flow-limited distribution and saturable metabolism, were applied (Brown *et al.*, 1997). In order to mimic a workplace

exposure scenario, the model used cardiac output and alveolar ventilation rates typical of humans at a light physical activity. For metabolic rate, the value of V_{max} for human (V_{max_human}) was scaled from the male rat (V_{max_rat}) based on "3/4 power law" (Brody, 1945; Peters, 1983):

$$V_{max_{human}} = V_{max_{rat}} * (BW_{human}/BW_{rat})^{0.75}$$

K_{gst} for human (K_{gst_human}) was extrapolated from the value of the male rat following the same procedure. Partition coefficients and K_m were assumed the same across rat to human. Hair adsorption rate was not included in the human model.

Simulations at a low dose exposure or impact of the GSH synthesis inhibitor and cytochrome P450s inhibitor. After the optimization of the metabolic parameters, the model was used to simulate a low dose exposure of 1-BP (20 ppm) in rats. To simulate the occupational exposure scenario, the exposure time was set as 8 h per day, 5 days under the exposure of 20 and 800-ppm of 1-BP. Both cytochrome P450s and GSH were identified to be involved in the elimination of 1-BP (Garner *et al.*, 2006). To examine the roles of P450s and GSH in the metabolism of 1-BP, BSO, a GSH synthesis inhibitor, and ABT, an inhibitor of cytochrome P450s (Balani *et al.*, 2002), were used in the experiment. A female rat pretreated with 1000 mg/kg BSO for 3 days was exposed to 800 ppm of 1-BP. Another female rat pretreated with 50 mg/kg ABT for 2 h was exposed to 800 ppm 1-BP. Replication of this experiment was not conducted due to rat death after 5 h exposure to 1-BP in BSO group.

Sensitivity analysis. Sensitivity analysis was conducted to examine the impact of each parameter on the area under the curve (AUC) of 1-BP closed chamber concentration in the male rats. Each parameter was increased by 1% in 4 doses in the male rat model, respectively. The AUC of the closed chamber concentration was calculated. The fractional change in the AUC was calculated by dividing the AUC after a single parameter increase by an initial AUC in the model. Normalized sensitivity coefficients (NCSs) were calculated as follows:

NSC = Fractional change in AUC/fractional change in parameter (1%)

Parameters were identified as low, medium, high influence on the AUC of the closed chamber concentration based on following criteria: low: NSC < 0.2; medium: 0.2 ≤ NSC < 0.5; high: NSC ≥ 0.5 (Yoon *et al.*, 2009).

In addition, a time-dependent sensitivity analysis was conducted based on the results from AUC-based sensitivity analysis. The NSCs was calculated by the change of chamber concentration of 1-BP at multiple time points (0, 0.5, 1, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 h).

NSC = Fractional change in 1-BP chamber concentration/fractional change in parameter (1%)

Software. Berkeley Madonna Version 8.3 (University of California at Berkeley, CA) was used to develop 1-BP gas-uptake PBPK model.

RESULTS

Derivation of Metabolic Rates and Model Verification

As shown in Figure 1, a 7-compartment PBPK model for the gas uptake study including a flow-limited distribution and saturable metabolism was developed. After injection 1-BP into a closed chamber with 1 rat, 1-BP was rapidly deposited in lung through inhalation and a small amount of 1-BP was absorbed by skin and fur (Garner *et al.*, 2006). 1-BP was distributed into compartments through blood circulation and then partially metabolized in liver and excreted through urine. In the liver, CYP2E1 oxidation (V_{max} and K_m) and GSH conjugation (K_{gst}) were the 2 major metabolic pathways of the metabolism of 1-BP (Garner *et al.*, 2006).

PBPK Model for Male F344 Rats

We developed a PBPK model with the physiological parameters as well as the partition coefficient of 1-BP as listed in Tables 1 and 2. We first applied previously estimated metabolic rate into the model to simulate the metabolism of 1-BP by P450 oxidation and GSH conjugation in the male F344 rat (Garner and Yu, 2014). The initial simulated concentrations of 1-BP in the closed chamber were considerably underestimated the metabolic capability for the male rat. By optimizing through the visual fitting of the curve versus the measured concentration of 1-BP in the closed chamber for the individual rat at 240 ppm (Fig. 3B), these metabolic constants, including V_{max}, K_m, and K_{gst} were optimized and the results were summarized in Table 3. The V_{max} was estimated to be 2.72 mg/h at 240 ppm, and the K_m and K_{gst} were estimated to be 0.45 mg/l and 0.2 l/h, respectively. As shown in Figure 3A, the simulated concentration of 1-BP in the closed exposure chamber fit well with the data from the 4 individual rats. The extrapolations with the estimated values of V_{max}, K_m, and K_{gst} to other 3 dose levels were conducted as shown in Figure 3B-D. The simulated curves of 1-BP concentration in the closed exposure chamber with the initial concentration of 70 ppm (A) and 800 ppm (C) visually reflected optimized fitting for those exposure levels. But at the highest dose level of 2700 ppm, the simulated concentration were slightly lower than those measured levels in the closed chamber during the first hour (Fig. 3D). Exposure to high concentration of 1-BP might lead to respiratory depression and reduce the ventilation rate of the rat.

PBPK Model for Female F344 Rats

Because of a significantly different BW of the female rats versus the male rats, the initial PBPK model for the female rats was scaled with the female BW with other parameters unchanged. This model showed good curve fits at 70 and 800 ppm but overestimation at 240 ppm and underestimation of the closed chamber concentration at 2700 ppm (data not shown). These results may be due to the difference in the physiological parameters or metabolic kinetics between the female and male rat. Garner and Yu (2014) used a traditional 2-compartmental model to estimate metabolic rate for male and female rats and found that metabolic constants (V_{max} and K_{gst}) for the male rats were 2 times of female rats after 1-BP exposure. Considering the significant difference of adipose tissue percentage between the male and female humans and the lack of data in rodents, we assumed that female fat volume fraction was 1.5 times of the male fat fraction. Further optimization of metabolic parameters was performed in our PBPK model and these values were listed in Table 3. With the incorporation of the adjusted physiological parameters, metabolic parameters in the female rat were optimized by each rat at 2700 ppm exposure level (Table 3). Using visual fitting, V_{max} was estimated to be 1.92 mg/h at 1-BP 2700 ppm. The K_m and K_{gst} constants were estimated to be 0.2 mg/l and 0.09 l/h, respectively. These values were then applied to simulate the closed chamber concentrations of 1-BP

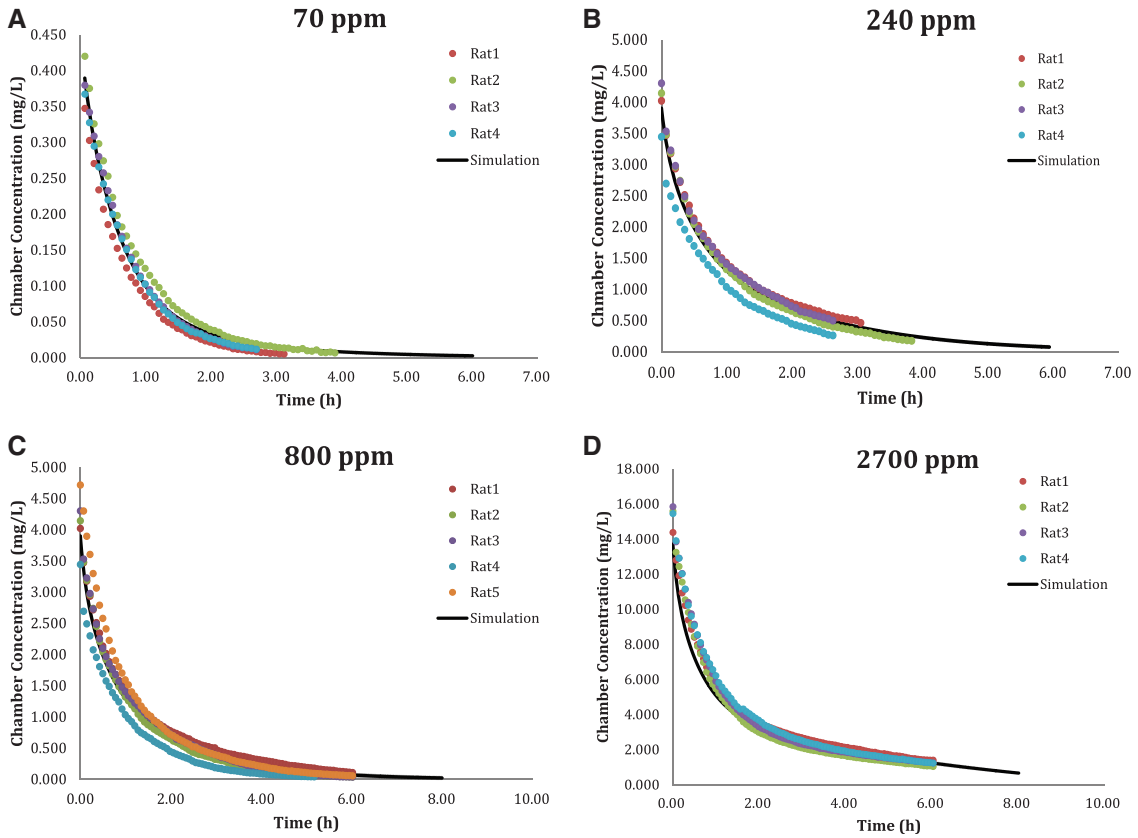

FIG. 3. Time-course of the concentrations of 1-BP in the closed chamber with F344 male rats at doses of 70 ppm (A), 240 ppm (B), 800 ppm (C), and 2700 ppm (D). Solid lines represented the simulated concentration using estimated metabolic parameters and spotted lines represented the measured concentrations in the individual rats.

TABLE 3. Physiologically Based Pharmacokinetic (PBPK) Model: Metabolic Parameters for 1-BP in Rat and Human

Parameter	Male Rat (Mean ± SD, $n = 4$)	Female Rat (Mean ± SD, $n = 4$)	Human
V_{max} (mg/h)	2.72 ± 0.76^a	1.92 ± 0.43^a	145^b
K_m (mg/l)	0.45 ± 0.389^a	0.203 ± 0.005^a	0.45^c
GSH-related constant (K_{gst}) (l/h)	0.2^a	0.09^a	10.6^d
Hair absorption rate (mg/h)	0.04^a	0.04^a	Neglected

[a]From adequately visual fitting.
[b]$V_{max\_human} = V_{max\_male\ rat} * (BW_{human}/BW_{male\ rat})^{0.75}$.
[c]Assume to be same across species.
[d]$K_{gst\_human} = K_{gst\_male\ rat} * (BW_{human}/BW_{male\ rat})^{0.75}$.

at 70 ppm (A), 240 ppm (C) and 800 ppm (D). As shown in Figures 4A–D, the simulation with those optimized metabolic parameters visually adequately fit the curves of the time-concentrations in other exposure levels.

Impact of the GSH Synthesis Inhibitor and Cytochrome P450s Inhibitor

The inhibition of P450 with ABT in liver resulted in significant change in the disposition of 1-BP (Garner *et al.*, 2006). The total amount of metabolites in the urine dramatically decreased 90% after ABT treatment. Recently, it was shown that ABT treatment resulted in an increase of half-life of 1-BP elimination from 2 to 9.6 h in rat gas uptake experiment (Garner and Yu, 2014). These findings suggest that P450 enzymes are the critical enzyme system for 1-BP metabolism. In the same studies, 2OHBP, a major metabolite from the P450 oxidation, was conjugated with GSH for further metabolism. Based on the PBPK model we developed, we simulated the concentration of 1-BP in the closed chamber with the pretreatment of cytochrome P450s inhibitor ABT or GSH synthesis inhibitor BSO. Initial values of V_{max}, K_m, and GSH-related constant K_{gst} from the gas-uptake experiment at 800 ppm in the female rat were applied. Visual fitting of the curve was conducted (Fig. 5A), and the optimized values are listed in Table 4. ABT was a widely used CYP450 inhibitor through the destruction of its enzymes and reduction of enzyme content (Demontellano and Mathews, 1981). K_m was assumed the same with untreated female rat. With the P450 inhibitor ABT pretreatment, a significant reduction of V_{max} was made to fit the measured data, 0.19 mg/h for V_{max}. With the GSH synthesis inhibitor, a simulation of the 1-BP concentration in the closed chamber was conducted with the same metabolic parameters in the female rat except the value of K_{gst} was set at 0 l/h. A slightly lower concentration of 1-BP concentration in the closed chamber was observed in the simulation versus measured concentration, especially during the first 2 h of exposures (Fig. 5B).

Sensitivity Analysis

A total of 24 parameters were included in the sensitivity analysis at 4 doses. Parameters with an absolute value of NSC larger than or equal to 0.2 were presented in Figure 6. In all 4 dosing

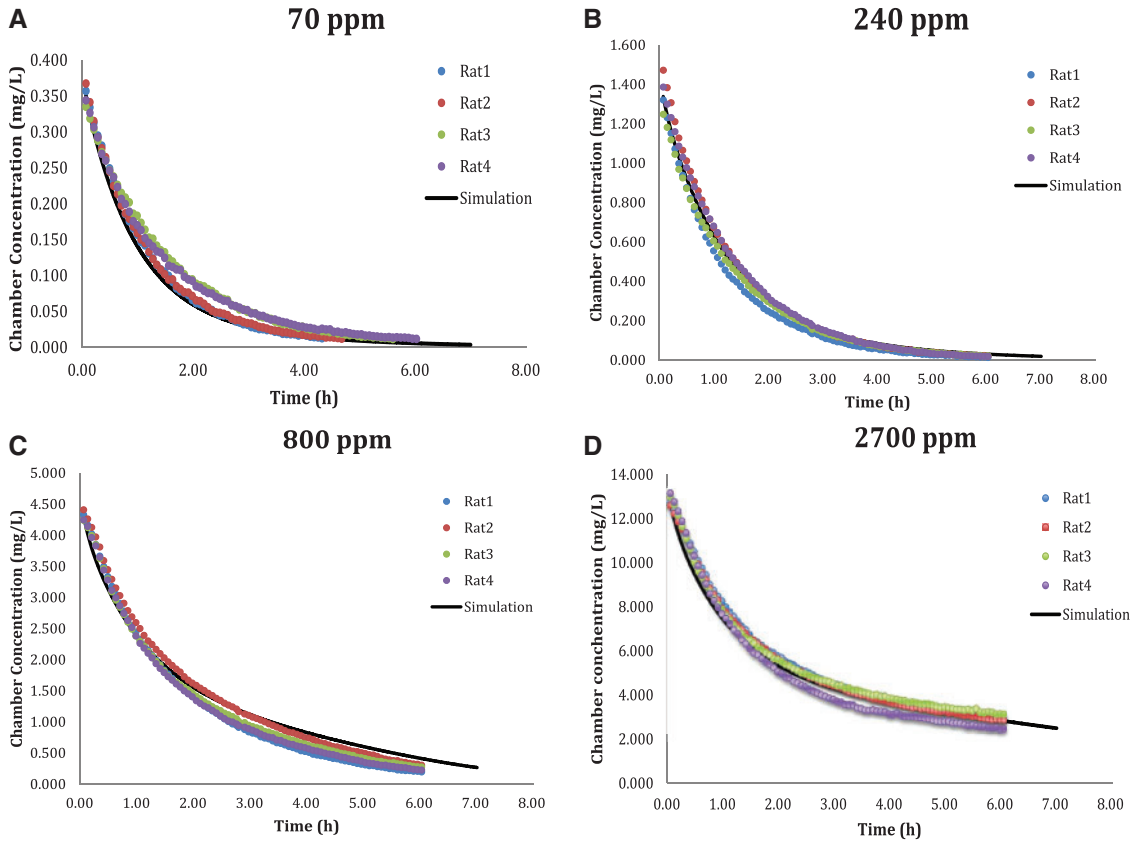

FIG. 4. Time-course of the concentrations of 1-BP in the closed chamber with F344 female rats at doses of 70 ppm (A), 240 ppm (B), 800 ppm (C), and 2700 ppm (D). Solid lines represented the simulated concentration using estimated metabolic parameters and spotted lines represented the measured concentrations in the individual rats.

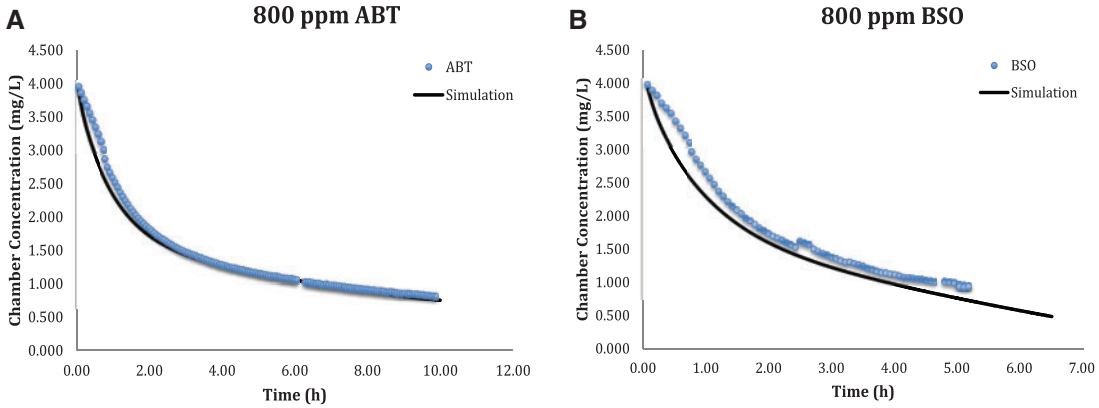

FIG. 5. Time-course of the concentrations of 1-BP in the closed chamber with F344 female rats at the dose of 800 ppm. A female rat was pretreated with a cytochrome P450s inhibitor 1-aminobenzotriazole (ABT, A), or a GSH synthesis inhibitor D,L-buthionine (S,R)-sulfoximine (BSO, B). Solid lines represented the simulated concentrations using estimated metabolic parameters (Table 3) and the spotted lines represented the measured concentrations in the individual rat either pre-treated with ABT or BSO (B) before the inhalation of 1-BP.

TABLE 4. Physiologically Based PBPK Model: Metabolic Parameters for the Female Rats Pretreated With the Cytochrome P450s Inhibitor 1-Aminobenzotriazole, or GSH Synthesis Inhibitor BSO

Parameter	V_{max} (mg/h)	K_m (mg/l)	GSH-Related Constant (K_{gst}) (l/h)
ABT	0.19[a]	0.20[a]	0.09[a]
BSO	1.92[a]	0.20[a]	0.00[a]

[a]From adequately visual fitting.

groups, the AUCs of the 1-BP in the closed chamber were highly negatively sensitive to BW and slightly positively sensitive to K_m. Because the NSC of K_m was less than 0.2 and was not presented in the Figure 6. The AUC at 70 ppm (A) was moderately negatively sensitive to alveolar ventilation (QPC), cardiac output (QCC), fraction of blood flow to the liver (QLC), and blood:air partition coefficient (PB). The AUC at 240 ppm (B) was moderately negatively sensitive to QPC, QCC, QLC PB, and V_{max}. The AUC at 800 ppm (C) was moderately negatively sensitive to QPC and

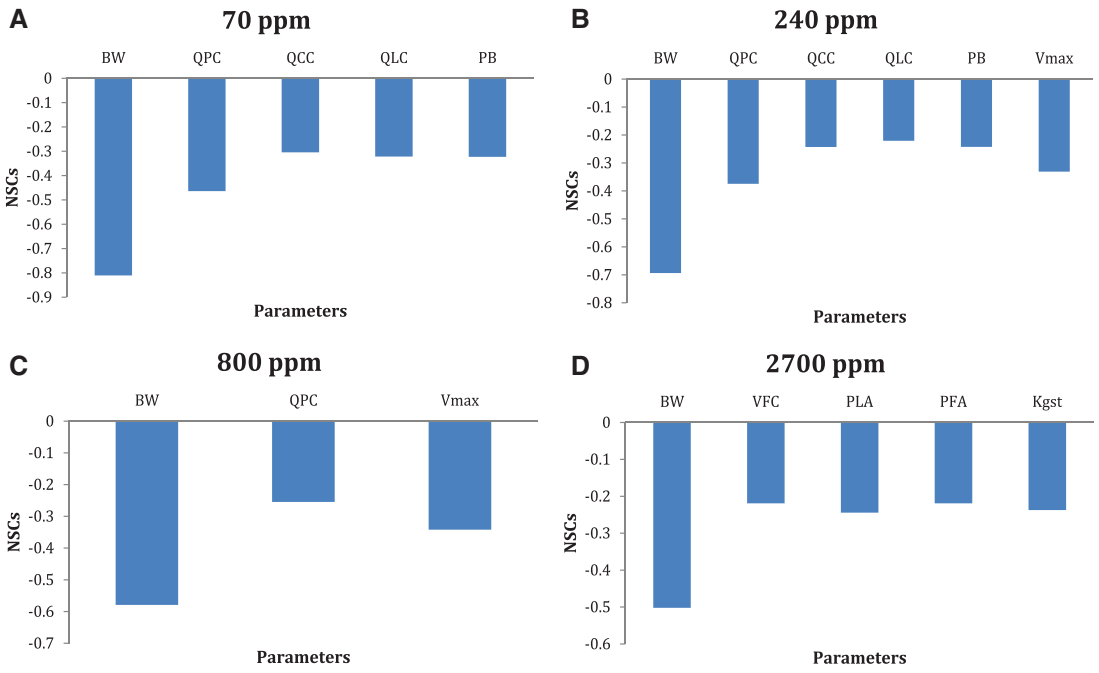

FIG. 6. Normalized sensitivity coefficients (NSCs) for the selected parameters at doses of 70, 240, 800, and 2700 ppm exposure of 1-BP in the male rats. Only the parameters with an absolute value of NSC greater than 0.2 were included in the figure.

V_{max}. The AUC at 2700 ppm (D) was negatively sensitive to the tissue volume of the fat tissue (VFC), liver:air partition coefficient (PLA), fat:air partition coefficient (PFA), and GSH-related constant (K_{gst}). In a time-dependent sensitivity analysis, different parameters showed different impacts on 1-BP chamber concentration at different exposure times (Supplementary Figure 1). For example, BW and QPC had the maximum effects on the chamber concentration within 1–2 h at 70 ppm, and then reduced their effects with increasing impact of QCC and PB (Supplementary Figure 1).

PBPK Model Prediction

Low-Dose Exposure. The development and validation of the PBPK model for 1-BP in rat was based on gas-uptake studies with high exposure levels compared with occupational exposure. We applied this model to predict a low dose inhalation at 20 ppm in male and female rats. As shown in Figure 7, 1-BP concentration in the closed chamber rapidly decreased and was almost eliminated within 6 h. The male rat eliminated the 1-BP at a much faster speed than that in the female rats.

Prediction of Blood Concentration. The blood concentrations of 1-BP were predicted in both male (Fig. 8A) and female rats (Fig. 8B) under the exposure levels of 70, 240, and 800 and 2700 ppm. With 1-BP being inhaled and distributed rapidly into the whole body, the arterial blood concentration of 1-BP rapidly increased and reached the highest concentration within 0.5 h after exposure at the 4 dosing levels. At the highest dose (2700 ppm), the male and female blood concentrations did not decrease to 0 mg/l at 10 h after exposure in simulation (Figure 8), and the female rats retained a higher blood concentration of 1-BP than the male rats.

Repeated Exposure of 20 and 800 ppm for 8 h/day, 5 days/week in Rats and Humans. We predicted the blood concentration of 1-BP at a repeated exposure scenario as observed in the workplace. In

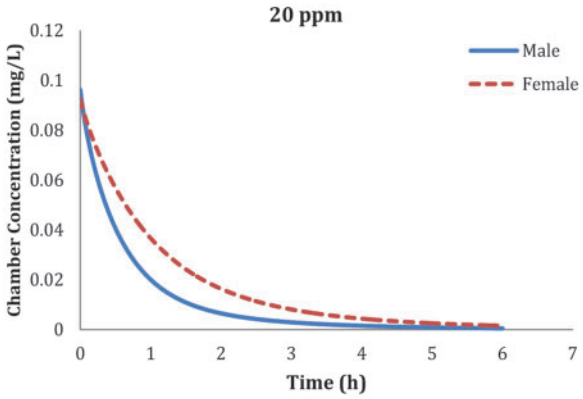

FIG. 7. Simulated chamber concentrations of the male and female F344 rats following 20 ppm exposure of 1-BP. Solid line represented a male rat exposed at 20 ppm 1-BP. Dashed line represented a female rat exposed to 1-BP at 20 ppm.

Figure 9, the arterial blood concentration in the male rat rapidly reached to 0.4 mg/l within 1 h of exposure and continuously increased to 0.5 mg/l at the end of 8 h exposures at 20 ppm of 1-BP (Fig. 9A). The blood concentration dropped quickly to 0.08 mg/l within 30 min after exposure. For exposure at 800 ppm (Fig. 9C), the arterial blood concentration rapidly increased to 27 mg/l within 1 h and continuously increased to 33 mg/l at the end of 8 h exposure. The blood concentration decreased quickly to 7 mg/l within 30 min after exposure. In the female rat, the arterial blood concentration rapidly reached to 0.38 mg/l in 1 h and increased to 0.41 mg/l at the end of 8 h exposure at 20 ppm of 1-BP (Fig. 9B). The blood concentration dropped quickly to 0.07 mg/l within 30 min after exposure. For 800 ppm exposure, arterial blood concentration rapidly increased to 25 mg/l in 1 h and continuously increased to 33 mg/l at the end of 8 h exposure. The blood concentration of 1-BP decreased quickly to 7.8 mg/l within 30 min after exposure. There is no accumulative

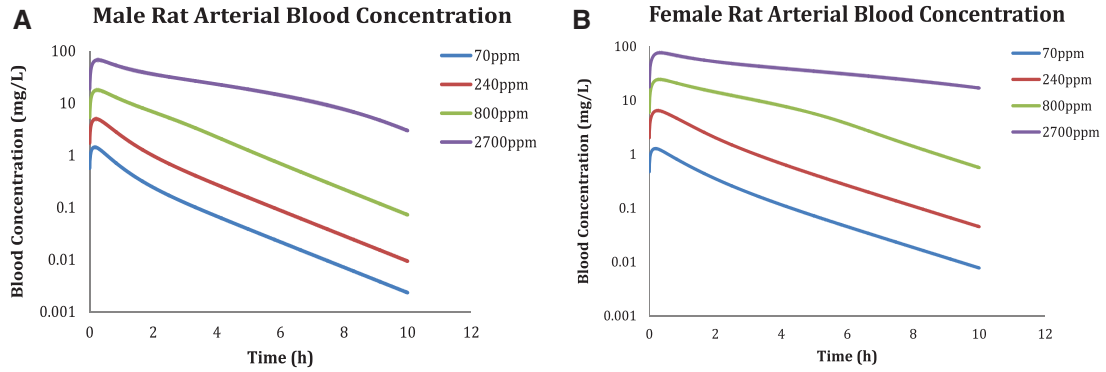

FIG. 8. Simulated arterial blood concentrations of 1-BP in the male (A) and female F344 rat (B) after a single exposure to 1-BP at 70, 240, 800, and 2700pm (log scale).

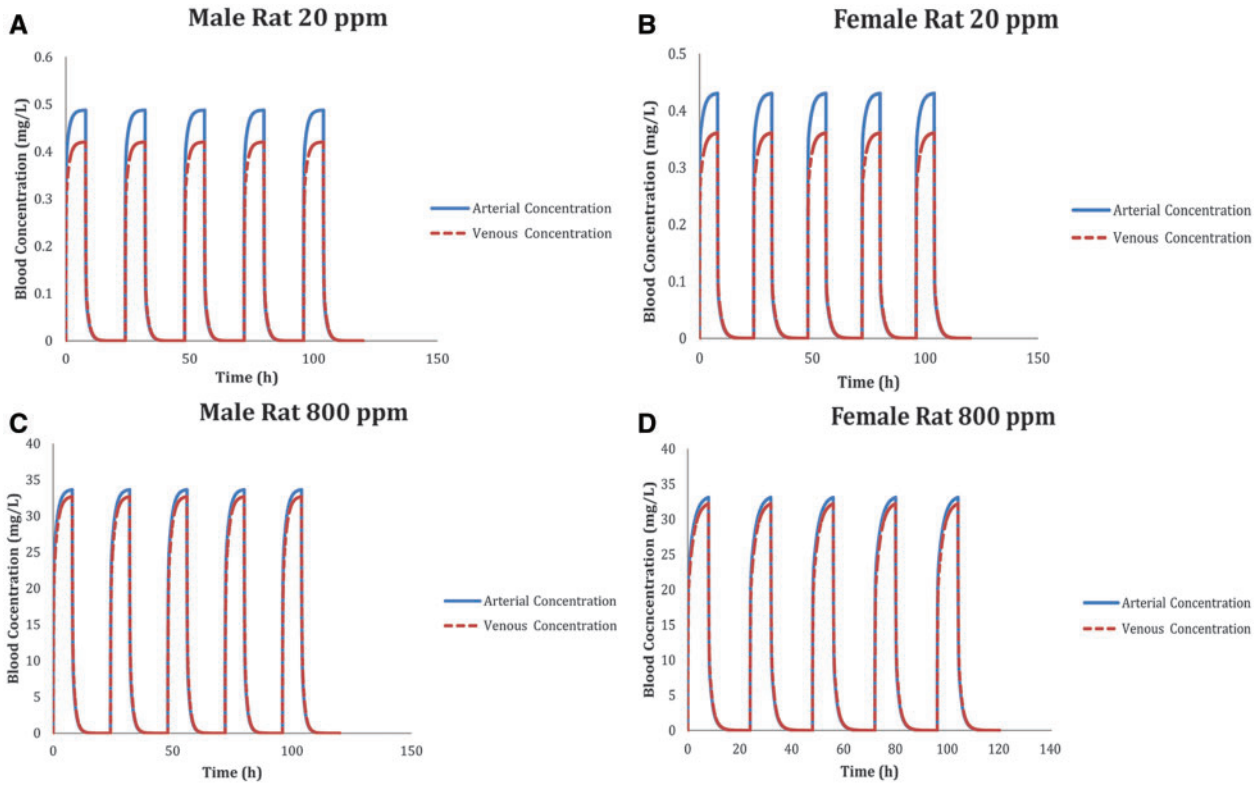

FIG. 9. Simulated concentrations of 1-BP in the arterial and venous blood in the male (A, C) and female (B, D) F344 rat following a repeated exposure scenario (8 h/day, total 5 days) at 20 ppm (A, B) or 800 ppm (C,D). Solid line represented arterial blood concentrations at 20 or 800 ppm. Dashed line represented venous blood concentration at 20 ppm or 800 ppm.

increase of blood concentration under an "8 h/day and 5 days/week" exposure scenario at both 20 and 8oo ppm exposure levels in both sexes.

A human PBPK model for 1-BP was developed by extrapolating the metabolic parameters obtained from the gas-uptake studies in rats. We applied a general human PBPK model for the volatile compounds as previously reported by Anderson *et al.* (2008). Arterial and venous blood concentrations of 1-BP were predicted in a repeated exposure scenario for 8 h/day, and 5 days/week. During an 8-h exposure and after exposure for 16 h, we first simulated 4 exposure levels as used in the animal experiment in the model. The blood concentration in each exposure level rapidly increased within the first hour of exposure and reached a maximum level slowly at the end of exposure (Fig. 10). Blood concentration dropped dramatically within 1 h after exposure. However, both arterial and venous blood concentrations did not reach 0 mg/l at the end of the post-exposure prediction (Fig. 10). A repeated exposure scenario was simulated at either 20 and 200 ppm exposure levels, which was representative of occupational exposure levels at a low or a high level. In these 2 repeated exposure scenarios, the blood concentration of 1-BP increased slightly in each exposure cycle of a day. Compared with the blood concentration at the first 24 h, 1-BP concentration in arterial and venous blood increased by 25% (from 0.012 mg/l to 0.016 mg/l in arterial blood) after a 5-day exposure at 20 ppm. At 200 ppm exposure, the blood concentration after a 5 day repeated exposure increased by 26% (from 0.16 to 0.21 mg/l in arterial blood) as compared with that at the end of first day exposure.

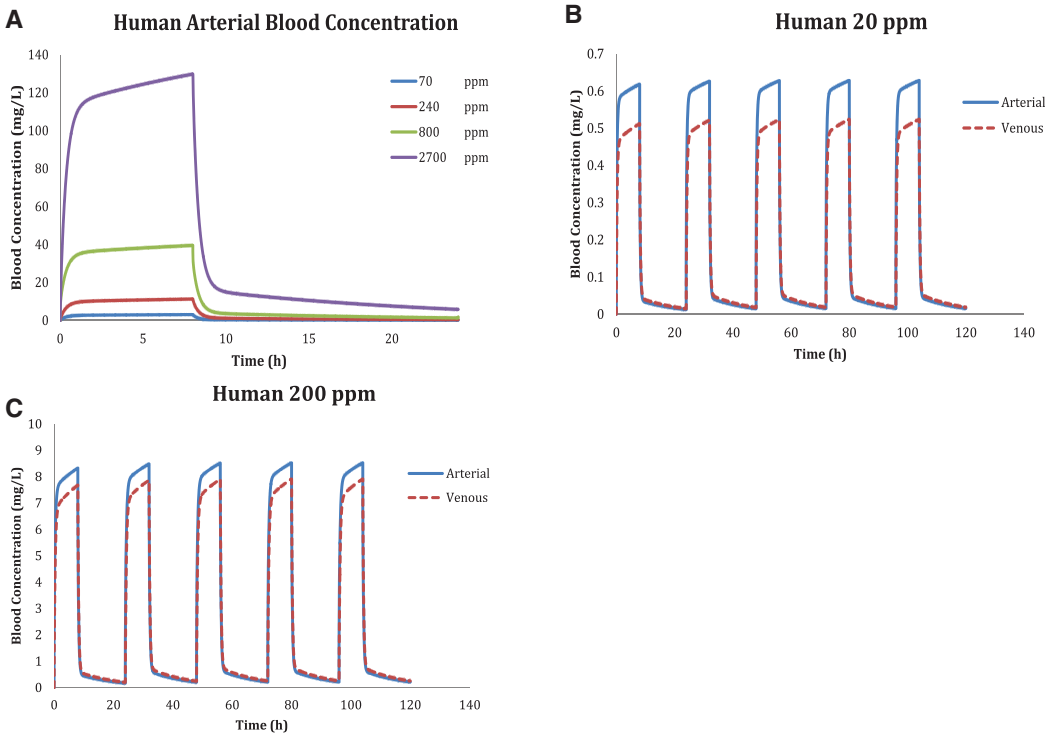

FIG. 10. Simulated arterial blood concentrations of 1-BP in human after exposure to 1-BP at 70, 240, 800, and 2700 pm (A) as well as a simulated concentration of 1-BP in the arterial and venous blood following a repeated exposure scenario (8 h/day, total 5 days) at 20 ppm (B) or 200 ppm (C). Solid line represented arterial blood concentrations at different doses. Dashed line represented venous blood concentrations at different doses.

DISCUSSION

The PBPK model developed for 1-BP in F344 rat in this study effectively demonstrated the time–course of the 1-BP concentrations in the closed chamber at doses ranging from 70 to 2700 ppm in both male and female. Sex-specific metabolic parameters were estimated and extrapolated into different exposure levels in this PBPK model. Another potential use of the current PBPK model is to describe tissue or organ specific dose including blood and organ systems after inhalation exposure. Our model applied multiple compartments to describe the metabolic kinetics of 1-BP through the inhalation exposure experiment. The occupational exposure scenario was simulated with our current PBPK model through a repeated exposure at 8 h per day for 5 days. Those characteristics will provide critical information for further mechanistic study of the toxicity and risk assessment of 1-BP.

As a volatile solvent compound, 1-BP directly merged into systematic circulation and distributed into each compartment through blood. The rapid decrease of 1-BP closed chamber concentration suggested a fast uptake by rat through the inhalation of 1-BP vapor. In the simulation of the time course of the concentration in the closed chamber, 1-BP concentrations were underestimated at 2700 ppm exposure level in both sexes within the first hour of inhalation exposure and slightly over-estimated at the end of female rats' exposure at 800 ppm 1-BP. Those results indicate that different metabolic behaviors exist in the high exposure level. Both models showed adequate simulations at other levels of exposure. As for the target tissue dosimetry, additional experimental data for specific organs, blood, and urinary 1-BP and its metabolites are needed to further validate current model.

Incorporation of the female-specific physiological parameters and partition coefficients greatly improved the simulation of the time course 1-BP concentration in the female rats. Those adjustments suggested that sex-specific differences exist in the toxicokinetics of 1-BP. Due to the huge difference in the BW between male and female rats, BW adjusted physiological parameters, such as cardiac output and alveolar ventilation, were applied as previously established equations (Brown *et al.*, 1997). Species-specific parameter adjustment is necessary to extrapolate these data into other species or human. Previous study reported the different susceptibility of 1-BP in C57BL/6J, DBA/2J, and BALB/cA mice (Liu *et al.*, 2009). Furthermore, faster clearance of 1-BP in the closed chamber was observed in male mice (Garner and Yu, 2014). Further comparative examination of the toxicokinetic among the species will provide additional information to see if the difference in toxicokinetic contributes to the observed difference in the sensitivity of 1-BP induced-toxicity. One of the objectives of this model is to simulate occupational exposure scenario to provide more information for risk assessment. Using a generic inhalation PBPK, Anderson *et al.* (2008) predicted that highly lipophilic volatile compounds with a fat tissue:blood partition coefficient (PFA) over a hundred were found to generally accumulate in fat tissue or blood after a repeated exposure. 1-BP has a high value of fat:air partition coefficient (236), with a moderate fat:blood partition coefficient (20.2), which suggested that 1-BP might be stored and cumulative in the fat tissue after chronic or repeated exposure as other solvents (Yu *et al.*, 1998b). But no elevation of the 1-BP concentration in venous or arterial blood concentrations were observed after a simulation of repeated exposure of 8 h/day up to 5 days at both 20 and 800 ppm exposure levels in the rat model. However, in human repeated exposure model, blood concentration of 1-BP increased by over 20% after 5-day exposure at 20 or 200 ppm. The significant difference of fat tissue volume between rat (7%) and human (21.4%) could contribute to the 1-BP accumulation in human blood combining with moderate

1-BP fat:blood partition coefficient. The results suggest 1-BP concentration in blood might directly indicate the current exposure level in the rodent model, but may also reflect the cumulative exposure in human.

So far, 2 potential metabolic pathways were studied and correlated with 1-BP reproductive toxicity and hepatotoxicity (Garner *et al.*, 2007; Lee *et al.*, 2010). CYP2E1-dependent oxidation and GSH conjugation were the 2 critical metabolic pathways and produced the major 1-BP metabolites detected in the urine samples. Jones and Walsh (1979) first suggested cytochrome P450 was responsible for 1-BP oxidation. B'hymer and Cheever (2004) found P450 enzymes oxidized at C2 or C3 of 1-BP. Further study found that after pretreatment with ABT, the metabolites in urine and $^{14}CO_2$ exhalation and retained in the liver dramatically decreased with increasing exhaled volatile organic chemicals (Garner *et al.*, 2006). Also, CYP450 oxidized metabolite 2OHBP might contribute to 1-BP induced reproductive toxicity (Garner *et al.*, 2007). Therefore, we included P450-dependent oxidation in the liver compartment to simulate the metabolic process. For GSH, Lee *et al.* examined the GSH level and found GSH reduced after exposure to 1-BP in a dose-dependent manner because of the formation of GSH conjugates in mice. GSH conjugation may contribute to hepatotoxicity and immunotoxicity of 1-BP exposure. Garner *et al.* (2006) found that the number of urinary metabolites was reduced dramatically after pretreatment with GSH synthesis inhibitor, which indicated the role of GSH in 1-BP metabolism. In our model, the metabolic rate V_{max} and Km were 2.72 mg/h and 0.45 mg/l, about 1.5 and 2 times larger in the male rat than those in the female (1.92 mg/h and 0.20 mg/l). GSH-related constant (K_{gst}) in the male rat was estimated to be 0.2 l/h, about 2 times of the female constant. These results suggested that sex difference existed in systematic clearance of 1-BP, which followed a similar trend with Garner's parameter estimation results (Garner and Yu, 2014). However, incorporating Garner's estimated metabolic parameters into the PBPK model showed severely underestimation of rat metabolic capacity of 1-BP. The difference might be due to Garner's data was estimated by 2-compartmental model, which simply described the gas exchange between closed chamber and rodents with a classic Michaelis–Menten Kinetics for P450. After adjusting V_{max} by rat's BW, the values were similar among male and female rat, which indicates BW may contribute to sex-difference in metabolic capacity.

The current 7-compartment model revealed more details about the metabolic kinetics of 1-BP and showed sex-specific metabolic behavior or potential species-specific metabolic rate. Different P450 activity (K_m, V_{max}) and GSH conjugation rate K_{gst} considerably contributed to the species and sex-specific metabolic rate.

Pretreatment with P450 inhibitor ABT and GSH biosynthesis inhibitor BSO dramatically prolonged half-life of 1-BP elimination, even though each condition was conducted with only 1 female rat due to significant adverse effects. The observation suggested CYP 450 and GSH were critical for 1-BP metabolism (Garner and Yu, 2014). Our model simulation further validated this finding through the rate changes for each metabolic pathway to fit the plotted data. For P450 inhibitor ABT data, the simulation showed adequate fitting with a 90% reduction in V_{max}. The time for 1-BP in the closed chamber concentration reaching 0.5 mg/l increased dramatically. For GSH biosynthesis inhibitor BSO, after reducing the GSH-related constant K_{gst} to zero, the simulation still under-estimated the clearance capacity of the female rat, which indicated more complex circulation of GSH or unknown potential factors needed to be incorporated into the current PBPK model.

In the sensitivity analysis of the parameters under the different exposure levels, NSC of the QPC was greater than 0.2 at 70, 240, and 800 ppm exposure levels whereas NSC of QCC was greater than 0.2 at 70 and 240 ppm. The analysis indicated alveolar ventilation and cardiac output affected the AUCs of 1-BP chamber concentration greatly. Exposure to high concentration of 1-BP might lead to respiratory depression and affect the ventilation rate. As suggested in the modeling at the highest dose level of 2700 ppm, the simulated concentration was slightly lower than those measured levels in the closed chamber during the first hour (Fig. 3D). In the workplace, these 2 parameters would change considerably due to intensive physical activity, which could lead to a profound change in absorption and distribution of 1-BP. As for the metabolic parameters, V_{max} had a medium effect on the AUC of the closed chamber concentration at 240 and 800 ppm. GSH conjugation-related constant K_{gst} greatly affects the simulation at 2700 ppm, which suggested metabolic pathways might contribute unproportionally to the elimination of 1-BP at different doses.

In conclusion, we developed a PBPK model for 1-BP to simulate the concentration of 1-BP in a closed chamber from a gas-uptake experiment in the F-344 rats. Sex-specific metabolic constants, including cytochrome P450 CYP2E1 and GSH conjugation, in the metabolism of 1-BP were estimated by fitting the time-concentration of 1-BP in the closed chamber with the gas-uptake inhalation study. The comparative investigation on the metabolic pathways of 1-BP through the PBPK modeling in both sexes provides critical information in understanding the role of P450 and GSH conjugation in the metabolism of 1-BP. The calculated metabolic constants are based on systemic concentrations of the 1-BP and can be used for risk assessment and dosimetry for other routes of exposure. Several studies have monitored urine metabolites from animals and humans occupationally exposed to 1-BP (Hanley *et al.*, 2006, 2009, 2010; Valentine *et al.*, 2007). Further development of the PBPK model to include the compartment of the metabolites of 1-BP is undergoing. Incorporation of metabolites of 1-BP in the PBPK model will help to quantitatively extrapolate animal studies to human, providing more information for the risk assessment of 1-BP.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH) (2014). TLVs and BEIs based on the Documentation of the Threshold limit values for chemical substances and physical agents and biological exposure indices. ISBN: 978-1-607260-72-1.

Andersen, M. E., Reddy, M. B., and Plotzke, K. P. (2008). Are highly lipophilic volatile compounds expected to bioaccumulate with repeated exposures? *Toxicol. Lett* **179**, 85–92.

Anderson, S. E., Munson, A. E., Butterworth, L. F., Germolec, D., Morgan, D. L., Roycroft, J. A., Dill, J., and Meade, B. J. (2010). Whole-body inhalation exposure to 1-bromopropane suppresses the IgM response to sheep red blood cells in female B6C3F1 mice and Fisher 344/N rats. *Inhal. Toxicol.* **22**, 125–132.

B'Hymer, C., and Cheever, K. L. (2004). Development of a gas chromatographic test for the quantification of the biomarker 3-bromopropionic acid in human urine. *J. Chromatog. B* **802**, 361–366.

Balani, S. K., Zhu, T., Yang, T. J., Liu, Z., He, B., and Lee, F. W. (2002). Effective dosing regimen of 1-aminobenzotriazole for inhibition of antipyrine clearance in rats, dogs, and monkeys. *Drug Metabol. Dispos.* **30**, 1059–1062.

Brody, S. (1945). Bioenergetics and growth, with special reference to the efficiency complex in domestic animals. Reinhold Publish Coperation. Available at: http://animalsciences.missouri.edu/research/bec/Brody%20Book/2.%20Bioenergetics%20and%20Growth%20-%20Chapters%201%20-%206.pdf. Accessed February 10, 2015.

Brown, R. P., Delp, M. D., Lindstedt, S. L., Rhomberg, L. R., and Beliles, R. P. (1997). Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Indust. Health* **13**, 407–484.

Demontellano, P. R. O., and Mathews, J. M. (1981). Autocatalytic alkylation of the cytochrome-P-450 prosthetic heme group by 1-aminobenzotriazole - isolation of an Nn-bridged benzene-protoporphyrin Ix adduct. *Biochem. J.* **195**, 761–764.

Dobrev, I. D., Nong, A., Liao, K. H., Reddy, M. B., Plotzke, K. P., and Andersen, M. E. (2008). Assessing kinetic determinants for metabolism and oral uptake of octamethylcyclotetrasiloxane (D4) from inhalation chamber studies. *Inhal. Toxicol.* **20**, 361–373.

Dobrev, I. D., Reddy, M. B., Plotzke, K. P., Varaprath, S., McNett, D. A., Durham, J., and Andersen, M. E. (2003). Closed-chamber inhalation pharmacokinetic studies with hexamethyldisiloxane in the rat. *Inhal. Toxicol.* **15**, 589–617.

Eisenberg, J., and Ramsey, J. (2010). Evaluation of 1-Bromopropane Use in Four New Jersey Commercial Dry Cleaning Facilities, Health Hazard Evaluation Report Department of Health and Human Services, Centers for Disease Control and Prevention. Available at: http://www.cdc.gov/niosh/hhe/reports/pdfs/2008-0175-3111.pdf, pp. 1–28. Accessed February 10, 2015.

Environmental Protection Agency (EPA). (2006). Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment. *Federal Register* **71**, 55469–55470.

Environmental Protection Agency (EPA). (2007). Protection of stratospheric ozone: listing of substitutes for ozone-depleting substances-n-propyl bromide in adhesives, coatings, and aerosols. *Federal Register* **72**, 30168–30207. Available at: http://www.gpo.gov/fdsys/pkg/FR-2007-2005-2030/pdf/E2007-9706.pdf. Accessed February 10, 2015.

Gargas, M. L., Burgess, R. J., Voisard, D. E., Cason, G. H., and Andersen, M. E. (1989). Partition-coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* **98**, 87–99.

Garner, C. E., Sloan, C., Sumner, S. C. J., Burgess, J., Davis, J., Etheridge, A., Parham, A., and Ghanayem, B. I. (2007). CYP2E1-catalyzed oxidation contributes to the sperm toxicity of 1-bromopropane in mice. *Biol. Reprod.* **76**, 496–505.

Garner, C. E., Sumner, S. C. J., Davis, J. G., Burgess, J. P., Yueh, Y., Demeter, J., Zhan, Q., Valentine, J., Jeffcoat, A. R., Burka, L. T., and Mathews, J. M. (2006). Metabolism and disposition of 1-bromopropane in rats and mice following inhalation or intravenous administration. *Toxicol. Appl. Pharmacol.* **215**, 23–36.

Garner, C. E., and Yu, X. (2014). Species and sex-dependent toxicokinetics of 1-bromopropane: the role of hepatic cytochrome P450 oxidation and glutathione (GSH). *Xenobiotica.* **44**, 644–656.

Gearhart, J. M., Jepson, G. W., Clewell, H. J., Andersen, M. E., and Conolly, R. B. (1990). Physiologically based pharmacokinetic and pharmacodynamic model for the inhibition of acetylcholinesterase by diisopropylfluorophosphate. *Toxicol. Appl. Pharmacol.* **106**, 295–310.

Hanley, K. W., Petersen, M., Curwin, B. D., and Sanderson, W. T. (2006). Urinary bromide and breathing zone concentrations of 1-bromopropane from workers exposed to flexible foam spray adhesives. *Ann. Occupat. Hygiene* **50**, 599–607.

Hanley, K. W., Petersen, M. R., Cheever, K. L., and Luo, L. (2009). *N*-Acetyl-S-(*n*-propyl)-L-cysteine in urine from workers exposed to 1-bromopropane in foam cushion spray adhesives. *Ann. Occupat. Hygiene* **53**, 759–769.

Hanley, K. W., Petersen, M. R., Cheever, K. L., and Luo, L. (2010). Bromide and *N*-acetyl-S-(*n*-propyl)-L-cysteine in urine from workers exposed to 1-bromopropane solvents from vapor degreasing or adhesive manufacturing. *Int. Archiv. Occupat. Environ. Health* **83**, 571–584.

Ichihara, G. (2005). Neuro-reproductive toxicities of 1-bromopropane and 2-bromopropane. *Int. Archiv. Occupat. Environ. Health* **78**, 79–96.

Ichihara, G., Asaeda, N., Kumazawa, T., Tagawa, Y., Kamijima, M., Yu, X. Z., Kondo, H., Nakajima, T., Kitoh, J., Yu, I. J., *et al* (1997). Testicular and hematopoietic toxicity of 2-bromopropane, a substitute for ozone layer-depleting chlorofluorocarbons. *J. Occupat. Health* **39**, 57–63.

Ichihara, G., Ding, X., Yu, X., Wu, X., Kamijima, M., Peng, S., Jiang, X., and Takeuchi, Y. (1999). Occupational health survey on workers exposed to 2-bromopropane at low concentrations. *Am. J. Indust. Med.* **35**, 523–531.

Ichihara, G., Huang, F., Ichihiara, S., Kitagawa, E., Mizugami, S., Iwahashi, H., Mari, I., and Junzho, K. (2012). Susceptibility to 1-bromopropane exposure and gene expression in two rat strains. *Toxicol. Lett.* **211**, S193–S193.

Ichihara, G., Kitoh, J., Yu, X., Asaeda, N., Iwai, H., Kumazawa, T., Shibata, E., Yamada, T., Wang, H., Xie, Z., *et al* (2000a). 1-Bromopropane, an alternative to ozone layer depleting solvents, is dose-dependently neurotoxic to rats in long-term inhalation exposure. *Toxicol. Sci.* **55**, 116–123.

Ichihara, G., Li, W., Ding, X., Peng, S., Yu, X., Shibata, E., Yamada, T., Wang, H., Itohara, S., Kanno, S., *et al.* (2004a). A survey on exposure level, health status, and biomarkers in workers exposed to 1-bromopropane. *Am. J. Indust. Med.* **45**, 63–75.

Ichihara, G., Li, W., Shibata, E., Ding, X., Wang, H., Liang, Y., Peng, S., Itohara, S., Kamijima, M., Fan, Q., *et al* (2004b). Neurologic abnormalities in workers of a 1-bromopropane factory. *Environ. Health Perspect.* **112**, 1319–1325.

Ichihara, G., Yu, X., Kitoh, J., Asaeda, N., Kumazawa, T., Iwai, H., Shibata, E., Yamada, T., Wang, H., Xie, Z., *et al.* (2000b). Reproductive toxicity of 1-bromopropane, a newly introduced alternative to ozone layer depleting solvents, in male rats. *Toxicol. Sci.* **54**, 416–423.

Jones, A. R., and Walsh, D. A., 1979. Oxidative-metabolism of 1-bromopropane in the rat. *Xenobiotica* **9**, 763–772.

Kamijima, M., Ichihara, G., Kitoh, J., Tsukamura, H., Maeda, K., Yu, X. Z., Xie, Z. L., Nakajima, T., Asaeda, N., Hisanaga, N., *et al.* (1997a). Ovarian toxicity of 2-bromopropane in the non-pregnant female rat. *Jo. Occupat. Health* **39**, 144–149.

Kamijima, M., Ichihara, G., Yu, X. Z., Xie, Z. L., Kitoh, J., Tsukamura, H., Maeda, K., Nakajima, T., Asaeda, N., Hisanaga, N., *et al* (1997b). Disruption in ovarian cyclicity due to 2-bromopropane in the rat. *Journal of Occupational Health* **39**, 3–4.

Kaneko, T., Kim, H. Y., Wang, P. Y., and Sato, A. (1997). Partition coefficients and hepatic metabolism in vitro of 1- and 2-bromopropanes. *J. Occupat. Health* **39**, 341–342.

Kim, Y., Jung, K., Hwang, T., Jung, G., Kim, H., Park, J., Kim, J., Park, J., Park, D., Park, S., *et al.* (1997). Hematopoietic and reproductive hazards of Korean electronic workers exposed to solvents containing 2-bromopropane (vol 22, pg 387, 1996). *Scandinav. J. Work, Environ. & Health* **23**, 80.

Lee, S. K., Kang, M. J., Jeon, T. W., Ha, H. W., Yoo, J. W., Ko, G. S., Kang, W., Jeong, H. G., Lyoo, W. S., and Jeong, T. C. (2010). Role of metabolism In 1-bromopropane-induced hepatotoxicity in mice. *J. Toxicol. Environ. Health A* **73**, 1431–1440.

Lilly, P. D., Andersen, M. E., Ross, T. M., and Pegram, R. A. (1997). Physiologically based estimation of in vivo rates of bromodichloromethane metabolism. *Toxicology* **124**, 141–152.

Liu, F., Ichihara, S., Mohideen, S. S., Sai, U., Kitoh, J., and Ichihara, G. (2009). Comparative study on susceptibility to 1-bromopropane in three mice strains. *Toxicol. Sci.* **112**, 100–110.

Maier, A., Dourson, M., Zhao, J., and Hack, E. (2004). Scientific review of 1-bromopropaneoccupational exposure limit derivations—preliminary thoughts and areas for further analysis. Toxicol. *Excellence Risk Assess.* 1–14. http://www.tera.org/Publications/TERA%20Analysis%20of%20OELs%20for%201-Bromopropane.pdf. Accessed February 10, 2015.

Morgan, D. L., Nyska, A., Harbo, S. J., Grumbein, S. L., Dill, J. A., Roycroft, J. H., Kissling, G. E., and Cesta, M. F. (2011). Multisite carcinogenicity and respiratory toxicity of inhaled 1-bromopropane in rats and mice. *Toxicol. Pathol.* **39**, 938–948.

Nakajima, T., Shimodaira, S., Ichihara, G., Asaeda, N., Kumazawa, T., Iwai, H., Ichikawa, I., Kamijima, M., Yu, X. Z., Xie, Z. L., *et al.* (1997a). 2-Bromopropane-induced hypoplasia of bone marrow in male rats. *J. Occupat. Health* **39**, 228–233.

Nakajima, T., Shimodaira, S., Ichihara, G., Asaeda, N., Kumazawa, T., Iwai, H., Ichikawa, I., Kamijima, M., Yu, X. Z., Xie, *et al.* (1997b). Histopathologic findings of bone marrow induced by 2-bromopropane in male rats. *J. Occupat. Health* **39**, 81

National Toxicology Program (NTP). (2013). Revised Draft Report on Carcinogens Monograph for 1-Bromopropane. http://ntp.niehs.nih.gov/ntp/roc/thirteenth/monograph_drafts/reviseddraftroc1bp_monograph_508.pdf. Accessed February 10, 2015.

OSHA—U.S. Occupational Safety and Health Administration. (1999). Nomination of 1-Bromopropane (1-BP) and 2-Bromopropane (2-BP) for Testing by the National Toxicology Program. In: NIH, (Ed.). Available at: http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/bromopropanes_508.pdf, pp. 1–12. Accessed February 10, 2015.

Park, J. S., Kim, Y. H., Park, D. W., Choi, K. S., Park, S. H., and Moon, Y. H. (1997). An outbreak of hematopoietic and reproductive disorders due to solvents containing 2-bromopropane in an electronic factory, South Korea: Epidemiological survey. *J. Occupat. Health* **39**, 138–143.

Peters, R. H. (1983). *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge Cambridgeshire; New York.

RTI Internation. (2005). Final study report of bromopropane Studies of chemical disposition in mammals, National Institute of Environmental Health Sciences, pp. 1–516.

Stelljes, M. E., and Wood, R. R. (2004). Development of an occupational exposure limit for n-propylbromide using benchmark dose methods. *Regulat. Toxicol. Pharmacol.* **40**, 136–150.

Valentine, H., Amarnath, K., Amarnath, V., Li, W., Ding, X., Valentine, W. M., and Ichihara, G. (2007). Globin s-propyl cysteine and urinary *N*-acetyl-*S*-propylcysteine as internal biomarkers of 1-bromopropane exposure. *Toxicol. Sci.* **98**, 427–435.

Wang, H., Ichihara, G., Ito, H., Kato, K., Kitoh, J., Yamada, T., Yu, X., Tsuboi, S., Moriyama, Y., Sakatani, R., *et al.* (2002). Biochemical changes in the central nervous system of rats exposed to 1-bromopropane for seven days. *Toxicol. Sci.* **67**, 114–120.

Wang, H., Ichihara, G., Ito, H., Kato, K., Kitoh, J., Yamada, T., Yu, X., Tsuboi, S., Moriyama, Y., and Takeuchi, Y. (2003). Dose-dependent biochemical changes in rat central nervous system after 12-week exposure to 1-bromopropane. *Neurotoxicology* **24**, 199–206.

Yamada, T., Ichihara, G., Wang, H., Yu, X., Maeda, K., Tsukamura, H., Kamijima, M., Nakajima, T., and Takeuchi, Y. (2003). Exposure to 1-bromopropane causes ovarian dysfunction in rats. *Toxicol. Sci.* **71**, 96–03.

Yoon, M., Nong, A., Clewell, H. J., Taylor, M. D., Dorman, D. C., and Andersen, M. E. (2009). Evaluating placental transfer and tissue concentrations of manganese in the pregnant rat and fetuses after inhalation exposures with a PBPK model. *Toxicol. Sci.* **112**, 44–58.

Yu, X., Ichihara, G., Kitoh, J., Xie, Z., Shibata, E., Kamijima, M., Asaeda, N., Hisanaga, N., and Takeuchi, Y. (1999a). Effect of inhalation exposure to 2-bromopropane on the nervous system in rats. *Toxicology* **135**, 87–93.

Yu, X., Ichihara, G., Kitoh, J., Xie, Z., Shibata, E., Kamijima, M., and Takeuchi, Y. (2001a). Neurotoxicity of 2-bromopropane and 1-bromopropane, alternative solvents for chlorofluorocarbons. *Environ. Res.* **85**, 48–52.

Yu, X., Ichihara, G., Kitoh, J., Xie, Z. L., Shibata, E., Kamijima, M., Asaeda, N., and Takeuchi, Y. (1998a). Preliminary report on the neurotoxicity of 1-bromopropane, an alternative solvent for chlorofluorocarbons. *J. Occupat. Health* **40**, 234–235.

Yu, X., Kamijima, M., Ichihara, G., Li, W., Kitoh, J., Xie, Z., Shibata, E., Hisanaga, N., and Takeuchi, Y. (1999b). 2-Bromopropane causes ovarian dysfunction by damaging primordial follicles and their oocytes in female rats. *Toxicol. Appl. Pharmacol.* **159**, 185–193.

Yu, X., Kubota, H., Wang, R., Saegusa, J., Ogawa, Y., Ichihara, G., Takeuchi, Y., and Hisanaga, N. (2001b). Involvement of Bcl-2 family genes and Fas signaling system in primary and secondary male germ cell apoptosis induced by 2-bromopropane in rat. *Toxicol. Appl. Pharmacol.* **174**, 35–48.

Yu, X. Z., Johanson, G., Ichihara, G., Shibata, E., Kamijima, M., Ono, Y., and Takeuchi, Y. (1998b). Physiologically based pharmacokinetic modeling of metabolic interactions between n-hexane and toluene in humans. *J. Occupat. Health* **40**, 293–301.

Yu, X. Z., Kamijima, M., Ichihara, G., Li, W. X., Kitoh, J., Xie, Z. L., Shibata, E., Hisanaga, N., and Takeuchi, Y. (1999c). 2-Bromopropane causes ovarian dysfunction by damaging primordial follicles and their oocytes in female rats. *Toxicol. Appl. Pharmacol.* **159**, 185–193.