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RESEARCH ARTICLE

Species and sex-dependent toxicokinetics of 1-bromopropane: the role of hepatic cytochrome P450 oxidation and glutathione (GSH)

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Abstract

1. The objectives of the current studies were to evaluate the factors influencing the toxicokinetics of 1-bromopropane (1-BP) in rodents after intravenous (IV) and inhalation exposure.
2. F-344 rats were administered 1-BP via IV bolus injection at 5 and 20 mg/kg and blood concentration determined versus time. F-344 rats and B6C3F1 mice were also exposed to starting inhalation concentrations 70, 240, 800 and 2700 ppm 1-BP in a closed gas uptake system and chamber 1-BP levels were monitored for 6 h. Plasma bromide concentrations were determined to estimate total metabolized dose. Rats were pretreated with chemical inhibitors of cytochrome P450 and glutathione (GSH) synthesis, prior to exposure to 1-BP at 800 ppm within inhalation chambers.
3. Systemic clearance of 1-BP in rat was rapid and decreased with increasing dose. As inhalation chamber concentration of 1-BP increased, the terminal elimination rates decreased. Half-life of 1-BP in rats following inhibition of P450 (9.6 h) or depletion of GSH (4.1 h) increased relative to controls (2.0 h) at 800 ppm. The percentage of 1-BP metabolized decreased with increasing inhalation exposure. Hepatic levels of GSH were significantly lowered regardless of the exposure level in both rats and mice. Chamber concentration–time curves were fit to a two compartment model which was used to estimate metabolic rate constants.
4. These data suggest that in rat, 1-BP clearance is saturable and that elimination is highly dependent on both P450 and GSH-dependent metabolism. This investigation in rodents may provide an understanding of interspecies differences in toxicokinetics and eventually aid translation of animal studies to human risk assessment.

Keywords

1-Bromopropane, glutathione, P4502E1, toxicokinetics

History

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Introduction

1-Bromopropane (1-BP) is a halogenated alkane, introduced into the workplace as an ozone depleting alternative (ODA) solvent after the discovery of the reproductive and hematopoietic toxicities of 2-brompropane (2-BP) in workers (Ichihara et al., 1996, 1997; Kim et al., 1996; Yu et al., 1999, 2001). Since its approval as an ODA from the US Environmental Protection Agency (EPA) (USEPA, 2007), its usage is estimated to be around 20 million pounds/year, and is thus categorized as a high-production volume chemical for electronic parts cleaning and dry cleaning as well as in the synthesis of pharmaceuticals and pesticides (Eisenberg & Ramsey, 2010). Its significant usage in the industry may result in widespread human exposure in the workplace (Anderson et al., 2010; NIEHS, 1999; NTP, 2013). Since the first report of the neurotoxicity of 1-BP in animals (Yu et al., 1998), a dozen human cases of 1-BP associated neurotoxicity have been reported among workers exposed to 1-BP (Ichihara et al., 2002, 2004a,b,c; Majersik et al., 2007; Raymond & Ford, 2007; Samukawa et al., 2012; Smith et al., 2011). Most recently, the National Toxicology Program (NTP) reported a dose-dependent effect of carcinogenesis in both rats and mice (Morgan et al., 2011; NTP, 2013). The potential for human exposure to 1-BP and the reports of adverse effects associated with occupational exposure to high levels of 1-BP have increased the need to understand the mechanism of these adverse effects in animal models as a means of understanding human risk in workers.

The acute toxicity of 1-BP is relatively low by inhalation exposure: 4 h LC50, 7000 ppm in rats and LD50 exceeding 2 g/kg in rats and mice by other routes of exposure. In the first reported animal study of 1-BP, exposure to 1000 ppm of 1-BP for 6 weeks in male rats (Yu et al., 1998, 2001), all 9 rats in the 1-BP treatment group began to walk with a paddle-like gait, their hindlimbs dragging along the ground with the plantar surface of the hind paw upwards. The motor nerve conduction velocity became considerably slower than the control after four weeks' exposure and histological examination showed the degeneration of the peripheral nerves

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(Ichihara et al., 2000c; Yu et al., 1998, 2001). Male rats exposed to 1-BP at 800 ppm or greater exhibit a decrease in prostate and seminal vesicle weights, epididymal motile sperm rate, abnormal sperm morphology and a concentration and time-dependent decrease in the number of spermatogonia (Ichihara et al., 2000c; Wang et al., 2003).

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 inhalation study between F344 and Wistar rats, neurotoxicity characterized by distal latency was more significant in F344 than in Wistar rats along with lower gene expression of glutathione-S-transferase (GST) Yc2 subunit and NAD(P)H dehydrogenase, quinone 1. Furthermore, mice were reported to be more susceptible than rats to 1-BP regarding hepatotoxicity and reproductive toxicity (Liu et al., 2009). Hepatotoxicity and male reproductive toxicity were compared among the three 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. Hepatic CYP2E1 levels, GST activity, reduced GSH status and NAD(P)H:quinone oxidoreductase and heme oxygenase-1 mRNA levels were measured. Histopathological evaluation of liver damage showed significantly larger area of necrosis and more degenerative lobules in the order of BALB/cA > C57BL/6J > DBA/2J. BALB/cA mice showed a higher CYP2E1 protein level and lower total GSH content and GST activity in the liver than DBA/2J strain. These results indicate that BALB/cA mice are the most susceptible to hepatotoxicity of 1-BP among the three strains tested, and that CYP2E1, GSH level/GST activity may contribute to the susceptibility to 1-BP hepatotoxicity. Exposure to atmospheres of $\geq$50 ppm of 1-BP also decreased sperm count and sperm motility and increased sperms with abnormal heads in all three strains mice in a dose-dependent manner, suggesting mice are far more susceptible than rats. Furthermore, genetically modified mice deficient in the oxidative regulatory pathway Nrf2 also demonstrated increased hepatotoxicity (Liu et al., 2009, 2010). In a recent long-term animal study from the NTP (Morgan et al., 2011; NTP, 2013), inhalation exposure of male and female F344/N rats to 1-BP resulted in significantly increased incidences of adenomas of the large intestine and skin neoplasms. 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.

In previous studies, we demonstrated differences in the metabolism of 1-BP between mice and rats (Garner et al., 2006, 2007). In contrast to a previous report by Jones & Walsh (1979), we have shown that P450-mediated oxidation is a major pathway of 1-BP elimination following inhalation and intravenous (IV) exposure in rats and mice (Garner et al., 2006). In both species, the principal P450-mediated urinary metabolite is 1-bromo-2-hydroxypropanol (2OH-BP), which is also conjugated with GSH or glucuronic acid or further metabolized to highly reactive metabolites such as bromoacetone (Garner et al., 2006). In rats, as 1-BP dose increased the percentage of the dose exhaled as unchanged parent increased while the percentage of the dose exhaled as CO_2 or excreted in the urine decreased. In mice, the mercapturic acid conjugate of 2OH-BP was the predominant metabolite in urine regardless of dose. In mice, metabolism and disposition were relatively insensitive to dose, suggesting that the mouse possesses a higher capacity for P450-mediated elimination of 1-BP than rat. These previous results suggested that species or strain differences in the metabolic activity of P450 enzymes and GST pathways could lead to a significantly different toxicokinetics of 1-BP, which could contribute to difference in susceptibility to 1-BP toxicity. The objectives of the current studies were to further examine the toxicokinetics of 1-BP in rats and mice after IV and inhalation exposure. The comparative investigation on the species and sex differences in the toxicokinetics of 1-BP provides critical information in understanding of differential susceptibility of toxicological effects and may help to understand the mechanisms of neurotoxicity, reproductive toxicity and carcinogenesis observed in rat and mice.

Materials and methods

A portion of these methods described below was summarized in RTI (2005).

Chemicals

Neat 1-BP (99% pure) was purchased from Aldrich Chemical Company (Milwaukee, WI). The identity and purity of these test articles was confirmed by nuclear magnetic resonance and gas chromatography/mass spectrometry (GC/MS). Alkamuls was procured from Sanofi-Aventis (Bridgewater, NJ). All other reagents were obtained from Sigma-Aldrich (St. Louis, MO).

Animals

Male and female Fischer 344 rats and B6C3F1 mice were obtained from Charles River, Inc. (Portage, MI). Certified Purina Rodent Chow (5002) and tap water were provided *ad libitum*. Temperature was maintained at 64–79 °F and relative humidity at 30–70%. Light/darkness was set to cycle at 12-h intervals. Animal care and experiment protocols were approved by the Institutional Animal Care and Use Committee (IACUC), and the animal care laws and Guidelines on experimental animals were strictly followed throughout the experiments.

Toxicokinetic studies of 1-BP following IV administration to rats

Rats ($n = 5$), implanted with indwelling exterior jugular cannulae, were dosed (1.0 mL/kg) by IV bolus injection of 5 and 20 mg/kg 1-BP formulated in isotonic saline/alkamuls (95:5, v:v) into a lateral tail vein. Blood samples (250 µL) were collected from each rat via jugular cannulae immediately prior to dosing and then after dosing at 0.033, 0.083, 0.167, 0.33, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 and 4.0 h. The blood was placed in silylated 2-mL vials, which were immediately sealed with a silicon-Teflon® crimp top (DuPont, Wilmington, DE). After each sample collection, the blood volume was replaced with an equal volume of plasma

(prepared fresh from donor animals). Collected samples were then stored at −20 °C until analyzed by headspace GC as described below. Blood and urine were collected at 0- and 7-h time points for determination of bromide ion concentrations. At 24 h post-dose, animals were humanely euthanized by CO_2 asphyxiation, the livers excised, hepatic microsomes prepared (Omura & Sato, 1964). The microsomal rate of *p*-nitrophenol hydroxylation, a marker for CYP2E1 activity, was determined as previously reported (Mathews et al., 1996).

To determine blood concentration of 1-BP, the samples in 2 mL crimp top vials were thawed at room temperature and then heated for 1 h at 38 °C in a dry bath incubator (Fischer Scientific, Pittsburg, PA). Using a gas tight syringe, 300 µL of headspace gas from each sample was then injected onto a Hewlett Packard 6890 GC (Palo Alto, CA) with a 6 m × 0.32 mm GS-GASPRO column (J&W Scientific, Torrance, CA) and an electron capture detector (ECD). The oven temperature was held at 90 °C for 2 min, ramped up to 150 °C over 2 min (30 °C/min), and then maintained at 150 °C for another 2 min; the ECD temperature was maintained at 250 °C with a N_2 makeup flow rate of 60 mL/min and an anode gas N_2 flow rate of 6 mL/min. The blood 1-BP concentrations were calculated based on a standard curve developed from blood samples spiked with 1-BP and analyzed in an analogous manner. The linear range of this system was 0.01 to 10 µg/mL.

Gas uptake inhalation of 1-BP in male and female rats and mice

The gas uptake chamber was constructed based on Gargas et al. (1986) and as detailed in Garner et al. (2007). Initial gas uptake system experiments were performed to validate operation of the system and to estimate chamber volume and compound loss over time. A standard curve was produced by making 1-mL injections of 1-BP-generated atmospheric standards. Chamber volume was calculated by dividing the amount of 1-BP introduced to the chamber by the resultant initial 1-BP concentration. Scrubbing material was then placed in the internal trap to determine if there was any adsorption or chemical loss in its presence. Approximately 110 g of lithium hydroxide and 4 g of citric acid were added to their respective traps, followed by the introduction of a known amount of 1-BP. Carcasses of rats and mice were placed in the chamber containing 800 ppm 1-BP to determine the rate of compound adsorption to the skin and fur over time.

Gas uptake experiments were performed in a closed exposure system at starting inhalation concentrations of 70, 240, 800 and 2700 ppm 1-BP in both male and female rats and mice ($n = 4$ per experimental condition). Concentration of 1-BP within the chamber was monitored with a Hewlett Packard 5890 GC (Palo Alto, CA) with a flame ionization detector (GC/FID). The GC/FID was outfitted with a Phenomenex stainless steel GC column (0.2% Carbowax 1500 m, 80/100; Carbopack C, 6′). The GC oven was maintained under isothermal conditions at a temperature of 150 °C. FID was maintained at 300 °C with an airflow rate of 375 mL/min, and a hydrogen gas flow rate of 35 mL/min. Helium carrier gas flow was maintained at 25 mL/min. Prior to initiation of gas uptake experiments, approximately 110 g of lithium hydroxide and 4 g of citric acid were added to their respective traps. A single rat or four mice were then added to the chamber, and after an acclimation period of 30 min, 1-BP was introduced into the gas uptake chamber to achieve the desired generated atmosphere of 1-BP. Chamber atmosphere (1 mL) was sampled every 4 min for 6 h and analyzed by GC/FID. During the course of each experiment, chamber temperature, relative humidity and oxygen levels were monitored by the WorkBench data acquisition software. At the termination of the exposure (6 h), animals were humanely sacrificed, their livers excised for determination of GSH levels and blood was collected for plasma bromide analysis as described below.

Determination of the effect of GSH depletion or inhibition of cytochrome P450 on the gas uptake kinetics of 1-BP

Because GSH and cytochrome P450s are known to be involved in the elimination of 1-BP via our previous study (Garner et al., 2006), the gas uptake kinetics of 1-BP were evaluated in experiments using D,L-buthionine (S, R)-sulfoximine (BSO), an inhibitor of GSH synthesis (Manning & Franklin, 1990); and 1-aminobenzotriazole (ABT), a known inhibitor of cytochrome P450s (Balani et al., 2002). Prior to exposure to 1-BP at 800 ppm within a gas uptake chamber, two rats were pretreated orally for three days with 1000 mg/kg BSO. An additional rat was administered ip 50 mg/kg ABT at 2 h prior to a single 800 ppm exposure of 1-BP. Gas uptake studies with pretreated animals were not subject to further replication due to mortality/moribundity issues during the first experiment with the pretreatment. Animals pretreated with BSO died ∼5 h after initiation of 1-BP exposure.

Determination of hepatic-reduced glutathione in rats and mice

Hepatic levels of reduced GSH were measured after derivatization with *o*-phthalaldehyde using the method of Akerboom & Sies (1981). Hepatic S9 was prepared from rat liver by homogenizing liver in ice-cold 0.1 M potassium phosphate (pH 8.0) and then centrifuging at 9000*g* for 20 min. Assay mixtures contained 950 µL of assay buffer (0.1 M potassium phosphate, pH 8.0 and 0.005 M EDTA), 10 µL of 9000 *g* S9, and 50 µL of *o*-phthalaldehyde. Fluorescence intensity was measured at an excitation wavelength of 350 nm and an emission wavelength of 420 nm. A standard curve was prepared using authentic standards of reduced GSH.

Bromide ion analysis in rat plasma and urine

Aliquots of rat plasma and urine samples collected from kinetic studies were analyzed for bromide ion content by ion chromatography (IC) using conductivity detection. The ion chromatography system consisted of a water pump system fitted with a Dionex IonPac AS11 (Sunnyvale, CA) (25 cm × 4 mm ID) and a Dionex Ion Pac AG11 guard column with a Dionex conductivity detector (3 µs). A linear ternary gradient system was employed with a flow rate of 2 mL/min. Mobile phase Eluent #1 consisted of 100% water, Eluent #2 was 5 mM NaOH, and Eluent #3 was 100 mM NaOH. Following injection of sample, mobile phase was ramped linearly from 90/10 (#1/#2) to 100% (#2) in 6 min and held for

4 min; changed to 65/35 (#2/#3) in 0.5 min; then reversed to 90:10 (#1/#2) in 0.5 min. Approximately 200 µL of each plasma sample was transferred into a polypropylene tube, diluted with 2 mL of deionized water, mixed, and then extracted by passing dilutions through a Dionex On-Guard® RP-SPE cartridge (Sunnyvale, CA). The first 1 mL of dilution was allowed to pass uncollected to waste and the remaining volume was collected in an autosampler vial for analysis. Bromide ion concentrations were calculated from a standard curve prepared using sodium bromide standards prepared in blank urine or plasma.

Toxicokinetic modeling of 1-BP concentrations

Mean blood 1-BP concentration–time data were analyzed by model-independent methods using WinNonlin 5.01 (Pharsight, Cary, NC). A biexponential function was used to generate pharmacokinetic parameters for each set of data (weighting: $1/y^2$). Toxicokinetic parameters calculated included areas under the concentration–time curves from time zero to infinity ($AUC_{0-\infty}$), systemic clearances (CLs), volumes of distribution at steady state (V_{ss}), mean residence time (MRT) and terminal elimination half-lives ($t_{1/2}$). Parameters were reported as the estimated value ± SE of estimate.

The chamber 1-BP concentration–time curves were evaluated using WinNonlin (Pharsight Corporation; Apex, Cary, NC) pharmacokinetic software to estimate the terminal elimination rate constant (λ_z) and half-lives of elimination ($t_{1/2}$). Concentration–time data were fitted against a two compartment gas uptake model based upon the methods of Filser et al. (1995) as shown in Figure 1 and then metabolism constants were estimated from this model. GSH conjugation and oxidative metabolism have been shown to play a major role in the elimination of 1-BP (Garner et al., 2007). Therefore, both saturable oxidative metabolism and first-order elimination were incorporated in the two compartment gas uptake model as shown in Figure 1. This model also incorporated loss of compound from the chamber due to hair adsorption. Data were fitted to the model and equilibrium and metabolism constants were estimated.

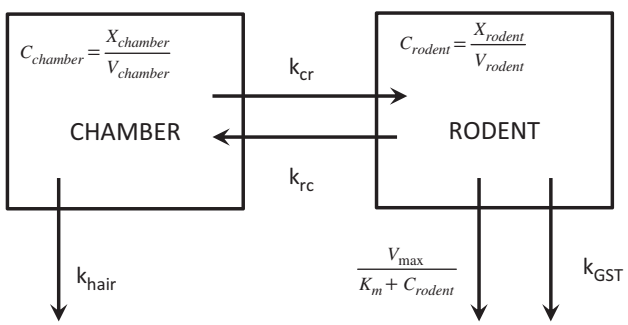


Figure 1. Schematic of a two compartmental model of 1-BP gas uptake in rodents. This model is based on Filser et al. (1995). Glutathione conjugation and oxidative metabolism pathways were incorporated in the model. Both saturable oxidative metabolism (V_{max}) and first-order elimination (K_{GST}) were incorporated. This model also incorporated loss of compound from the chamber due to hair adsorption (Khair). Data were fitted to the model and equilibrium and metabolism constants were estimated. Vch, Chamber volume (L) (4.3 L); Vrodent, rodent volume (L); Krc, rodent to chamber rate constant (h^{-1}); Kcr, chamber to rodent rate constant (h^{-1}); Khair, hair adsorption rate constant (h^{-1}) (0.0441 h^{-1}), K_{GST}, conjugation constant (h^{-1}); V_{max}, metabolic maximal velocity (mg/h/kg); K_m, Michaelis–Menten constant (mg/L).

Statistics

All the data presented are the mean ± SE. Statistical significance was examined where necessary using Student's *t*-test. A *p* value of ≤0.05 denoted the presence of a statistically significant difference. Statistical analysis was conducted with JMP software (SAS, Cary, NC).

Results

IV pharmacokinetics in rats

Mean blood 1-BP concentration–time curves following 5 and 20 mg/kg iv bolus doses of 1-BP in male Fischer 344 rats ($n = 5$) are displayed in Figure 2(A). After *iv* bolus administration of 1-BP to male Fischer 344 rats, maximum 1-BP concentrations in blood were achieved immediately and declined rapidly in a biphasic manner. Pharmacokinetic parameters are listed in Table 1. Systemic clearance at 5 and 20 mg/kg (177 ± 22 and 125 ± 12 mL/min/kg, respectively) was less than cardiac output (296 mL/min/kg) but much larger than hepatic (55 mL/min/kg) and renal (36.8 mL/min/kg) blood flows, either singly or combined (Davies & Morris, 1993). The estimated systemic clearance decreased as dose increased from 5 to 20 mg/kg ($p < 0.05$). The estimated V_{ss} values for the 5 and 20 mg/kg doses (3.1 ± 0.7 and

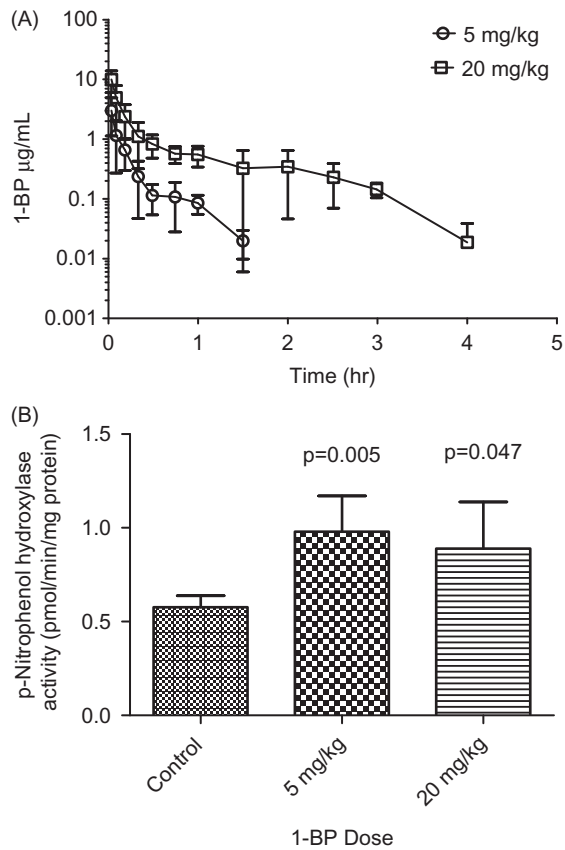


Figure 2. Concentration of 1-BP in blood (A) and activity of p-NPH in hepatic microsomes (B) following single bolus IV administration of 1-BP to rats via lateral tail vein to male F-344 rats. Data points represent mean of individual blood data from $n = 5$ rats. Error bars represent standard deviation of mean.

Table 1. 1-BP pharmacokinetic parameters[a] in male F-344 rats derived after IV bolus administration of 1-BP.

		5 mg/kg		20 mg/kg	
Parameter	Units	Mean	SE[b]	Mean	SE
$AUC_{0-\infty}$	μg · h/mL	0.47	0.06	2.68	0.26
$t_{1/2}$	h	0.39	0.08	0.85	0.09
CL_{sys}	mL/min/kg	177	22	125	12
MRT	h	0.29	0.05	0.87	0.10
V_{ss}	mL	3.1	0.7	6.5	1.1

Abbreviations of estimated and secondary parameters: $AUC_{0-\infty}$, Area under the concentration–time curve from time zero to infinity; $t_{1/2}$, apparent terminal half-life; Cl, systemic clearance; MRT, mean residence time; V_{ss}, volume of the distribution at steady state.
[a]Pharmacokinetic parameters calculated from mean 1-BP blood concentrations ($n = 5$ rats).
[b]Standard error of estimated parameter mean.

Table 2. Plasma bromide concentration following intravenous administration of 1-BP to male rats.

	Plasma bromide		
Dose (mg/kg)	0 h (μg/mL)	7 h (μg/mL)	% of Dose in body[a]
5	ND	3.8 ± 1.1	28.4 ± 7.6
20	ND	12.9 ± 3.7	36.6 ± 9.5

ND, Not detected. The estimated limit of detection (LOD) is ~259 ng/mL for bromide.
[a]Estimated using $V_{d,Br}$ (0.34 L/kg, Lilly et al., 1997).

6.5 ± 1.1 L/kg, respectively) exceeded total body water (0.67 L/kg) (Davies & Morris, 1993). The average elimination half-life ($t_{1/2}$) was 0.39 ± 0.08 and 0.85 ± 0.09 h for the respective doses.

Bromide is liberated during metabolism of 1-BP (Jones & Walsh, 1979). Therefore, free bromide was quantitated in plasma samples collected at 7 h post-dosing to determine the extent of the bromide liberating metabolic pathways in the clearance of 1-BP. Bromide was not detected in pre-dose samples nor in urine after dosing (data not shown). Mean plasma bromide concentrations 7 h following the 20 mg/kg dose (12.9 ± 3.7 μg/ml) were approximately 3.4-fold higher than those obtained from the 5-mg/kg dose group (3.8 ± 1.1 μg/ml) (Table 2). This was similar to the approximate 2.6-fold difference between the actual 1-BP doses administered. To estimate the proportion that bromide liberating metabolic pathways contribute to the clearance of 1-BP, estimates of total dehalogenated dose were made based on levels of free bromide in plasma samples collected at 7 h post 1-BP dosing. In the rat, bromide is eliminated extremely slowly ($t_{1/2}$ ~200 h) (Pavelka et al., 2000) and distributes primarily into extracellular water (V_d 0.34 L/kg) (Lilly et al., 1997). Thus, the total amount of bromide in the body can be estimated from plasma concentration by the following calculation:

$$Br^-_{total} = V_d \times C_{plasma}. \quad (1)$$

Total bromide in the body was estimated to account for ca. 28% and 37% of the administered doses, respectively, at 6 h following IV administration of 5 and 20 mg/kg 1-BP.

We have demonstrated that in mice 1-BP is eliminated in part by cytochrome P4502E1 (Garner et al., 2007). To explore the potential for 1-BP to modulate levels of this enzyme, hepatic activity of p-nitrophenol hydroxylase (pNPH) was measured in livers following IV administration of 1-BP at 5 and 20 mg/kg. Activity of pNPH in liver microsomes was increased approximately 1.5-fold by 24 h following IV administration of 1-BP at 5 and 20 mg/kg ($p \leq 0.05$ relative to controls) (Figure 2B).

Estimation of loss rates in gas uptake chamber

The chamber volume was estimated to be 4.3 ± 0.25 L. Plots of chamber 1-BP concentrations in an empty chamber, and one that contained rat and mouse carcasses were generated, respectively. There was an approximate 1.5 ± 1.5% loss of 1-BP from the empty gas uptake chamber atmosphere over a 6-h period. However, chamber atmosphere 1-BP loss in the presence of rat and mouse carcasses was significant. Loss of 1-BP to hair was similar between rat and mouse with estimated adsorption rate constants of 0.0415 and 0.0444 h^{-1}, respectively.

Estimation of *in vivo* metabolic parameters for 1-BP in male and female rats by gas uptake inhalation

Mean concentrations of 1-BP within inhalation chambers are plotted versus time for each exposure level in male (Figure 3A) and female rats (Figure 3B). Generally, 1-BP was rapidly removed from the chamber by the rats at all concentrations. At a given starting concentration, male rats tended to eliminate 1-BP from the chamber more rapidly than females. The mean first-order rate constants (λ_z) and half-lives ($t_{1/2}$) of the terminal elimination data for male and female rats are listed for each dose group in Table 3. With both males and female rats, there was a gradual decrease in the terminal slope of the curve (λ_z) with increasing initial chamber concentration and therefore a concomitant increase in half-life ($t_{1/2}$). With the increase in doses, the increase in half time in the female rats is much more pronounced than in the male rats.

Plasma bromide concentrations are summarized in Table 4. With the increase in inhalation concentrations, the concentration of bromide in plasma increased in both male and female rats. At a given inhalation level, the concentrations of plasma bromide were higher in the female rats than male rats. The total amount of absorbed dose metabolized by dehalogenation was estimated by conversion of plasma Br^- concentrations to total body Br^- (Equation (1)) in the rats and dividing those molar equivalents by the total moles of 1-BP eliminated from the chamber. In the male rats, about 73% of inhaled dose was metabolized with a corresponding release of bromide. With increasing inhalation concentrations, the percentage metabolized decreased significantly, from 73% to about 42%. In the female rats, about 80% of the inhaled 1-BP was metabolized via bromide release at low inhalation concentration. With the increase in inhaled concentration of 1-BP, the percentage metabolized via bromide release decreased, to about 30% of total inhaled 1-BP (significantly lower than in the male rats). These values suggest that as 1-BP exposures increase, significant amounts of 1-BP are absorbed but not eliminated via de-halogenation (Figure 4). As exposure increased to 800 ppm, 1-BP moles

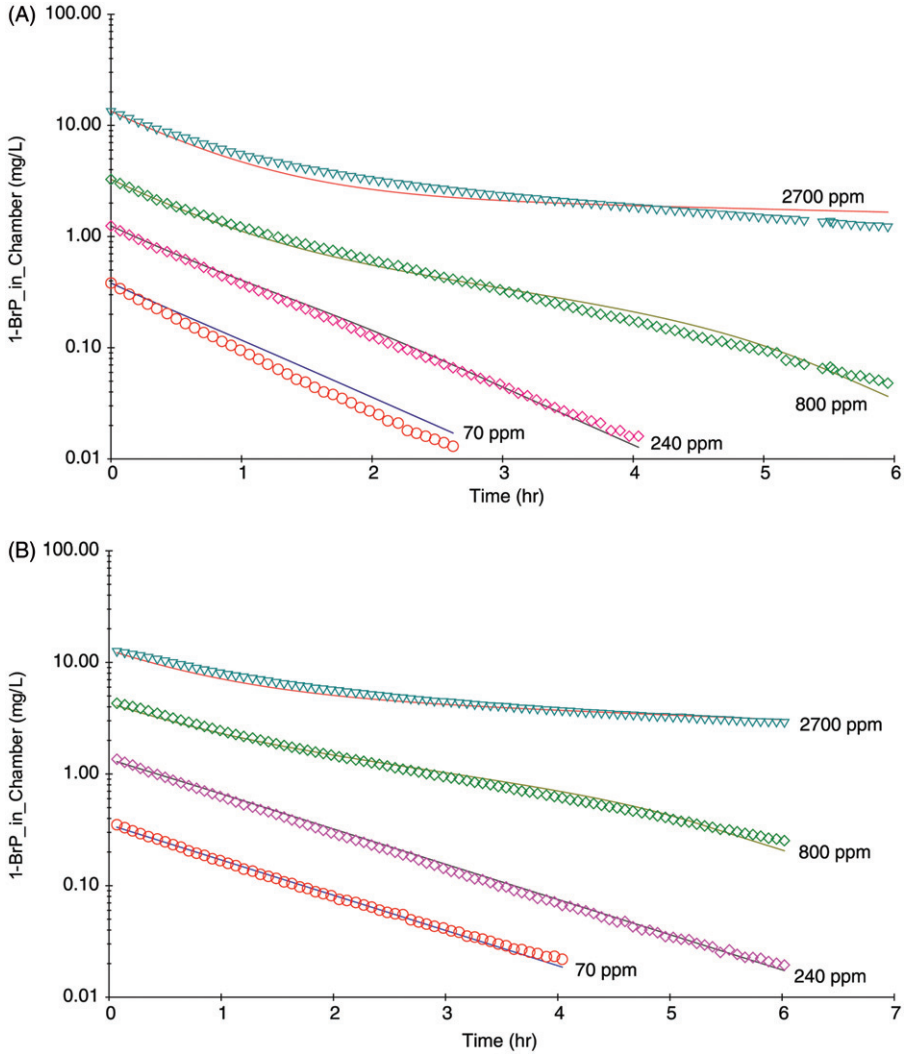


Figure 3. Mean chamber 1-BP concentrations during gas uptake experiments with male (A) and female (B) Fischer 344 rats. Chamber 1-BP concentrations were fit to a two compartment model based on the work of Filser et al. (1995) to estimate kinetic parameters. Model estimates are presented as solid lines.

Table 3. Estimated first-order terminal rate constants in male and female rats following 1-BP exposures in gas uptake studies.

Dose group (ppm)	Actual dose[a] (ppm)	Number of rats	Animal weight[a] (kg)	Moles of 1-BP per kg[a,b] (moles/kg)	λ_z[c] (1/h)	$t_{1/2}$[d] (h)
Male						
70	103 ± 20	4	0.361 ± 0.014	43 ± 8	1.30 ± 0.130	0.5 ± 0.06
240	274 ± 71	4	0.325 ± 0.017	122 ± 30	1.17 ± 0.060	0.6 ± 0.03
800	848 ± 62	4	0.342 ± 0.012	377 ± 30	0.67 ± 0.102	1.1 ± 0.22
2700	3167 ± 139	4	0.357 ± 0.012	1358 ± 51	0.29 ± 0.014	2.4 ± 0.11
Female						
70	108 ± 18	4	0.171 ± 0.017	105 ± 29	0.67 ± 0.109	1.0 ± 0.14
240	275 ± 15	4	0.192 ± 0.012	235 ± 17	0.73 ± 0.018	1.0 ± 0.03
800	880 ± 20	4	0.192 ± 0.011	743 ± 50	0.40 ± 0.106	2.0 ± 0.71
2700	3096 ± 15	4	0.184 ± 0.005	2650 ± 149	0.12 ± 0.012	6.1 ± 0.70
800 w/1-ABT[e]	884	1	0.179	799	0.07	9.6
800 w/BSO[f]	830	1	0.182	737	0.17	4.1

[a]Values expressed as mean ± standard deviation.
[b]Calculated from initial weight of 1-BP introduced to the gas uptake system.
[c]First-order rate constant associated with the terminal portion of chamber concentration versus time plot (log-linear).
[d]Terminal half-life associated with the terminal portion of the chamber concentration versus time plot (log-linear).
[e]Female rat was pretreated with an ip dose of 50 mg/kg of 1-aminobenzotrizole (1-ABT) 4 h prior to uptake experiment.
[f]Female rat was pretreated orally with BSO for 3 days, 1000 mg/kg/day prior to uptake experiment.

removed from the chamber began to diverge from Br^- equivalents within the animal in both male and female rats. Plasma Br^- equivalents in females trended lower than was observed in males but lacked statistical significance ($p = 0.07$).

GSH conjugation and oxidative metabolism have been shown to play a major role in the elimination of 1-BP (Garner et al., 2007). Therefore, both saturable oxidative metabolism and first-order elimination were incorporated in the two compartment gas uptake model as shown in Figure 1, which is

Table 4. Concentration of bromide in plasma of male and female rats following 1-BP exposure in gas uptake studies.

Intended dose[a] (ppm)	Actual dose (ppm)	N	Bromide plasma concentration (μg/g)	% 1-BP metabolized[b] 6.5 h
Males				
2700	3152 ± 144	4	151.5 ± 30.5	42 ± 7
800	821 ± 54	4	70.8 ± 10.5	71 ± 15
240	254 ± 72	4	26.5 ± 2.8	82 ± 3
70	101 ± 25	4	13.2 ± 7.8	73 ± 19
Females				
2700	3041 ± 167	4	205 ± 56	29 ± 7
800	879 ± 20	5	130 ± 42	69 ± 22
240	276 ± 15	5	49.2 ± 5.2	83 ± 9
70	108 ± 18	4	20.0 ± 3.7	83 ± 24

[a]Target initial chamber 1-BP concentration expressed in ppm.
[b]The percentage of the dose was calculated using the following formula:
$\%\text{BrP metabolized} = \frac{X_{Br^-}}{X_{1-BrP}} \times 100\%$,
where X_{Br^-} is the millimoles of bromide in the body and X_{1-BrP} is the millimoles of 1−BrP removed from chamber during exposure.

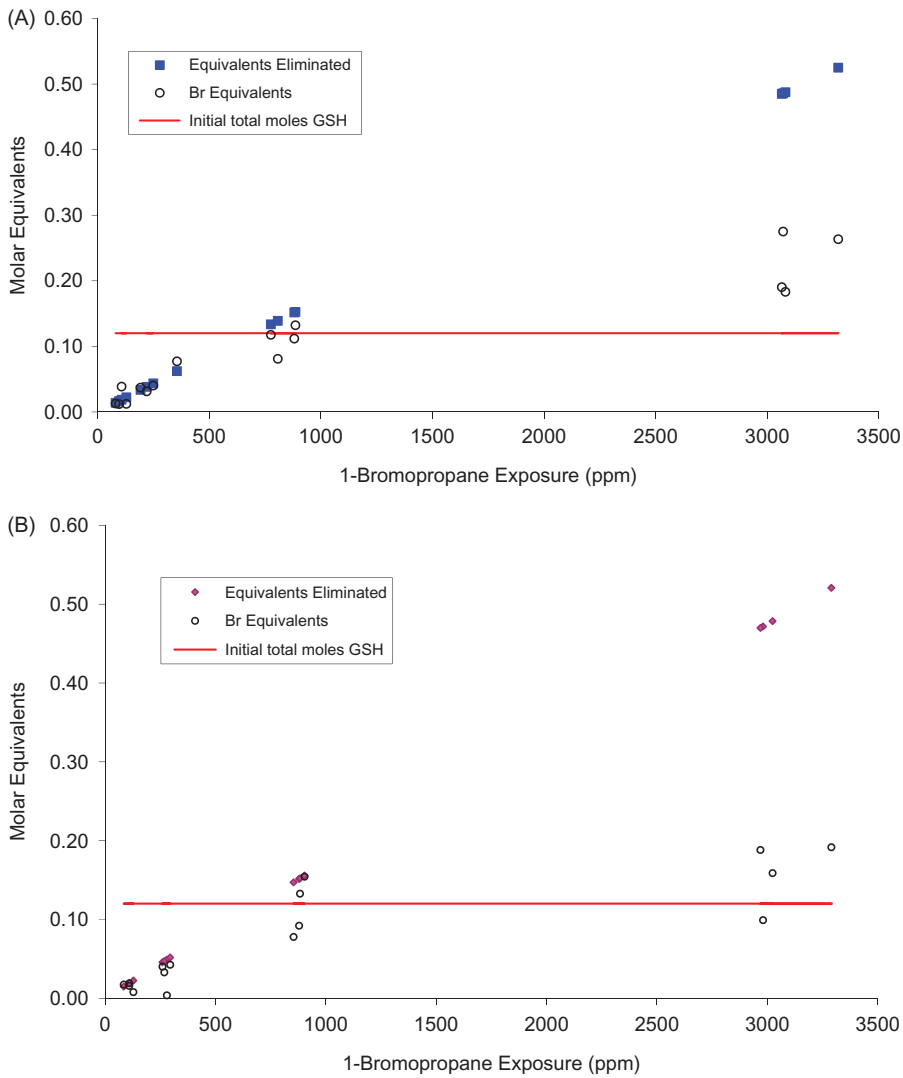


Figure 4. 1-BP equivalents eliminated from chamber and de-halogenated following inhalation exposure to male (A) and female (B) rats.

modified from Filser et al. (1995). This model also incorporated loss of compound from the chamber due to hair adsorption. Data were fitted to the model and equilibrium and metabolism constants were estimated (Table 5). Curve fit was adequate as visualized in Figure 3. Estimated oxidative metabolism rate constants (V_{max} and K_{GST}) for male rats were approximately twice those of female rats (Table 5). Furthermore, the K_{m1} for oxidative metabolism in male was significantly larger than that estimated in females, indicating that oxidative metabolism of 1-BP by females may be saturated at lower concentrations than that in males.

The impact of P450 inhibitor and GSH depletor on the toxicokinetics of 1-BP

A pilot experiment designed to explore the mechanism of 1-BP elimination following inhalation exposure was conducted. In this experiment, a female rat was pre-dosed with 1-ABT (a potent inhibitor of P450) prior to an exposure to 800 ppm 1-BP. A second female rat was pretreated with BSO (a depletor of hepatic GSH) prior to a similar 800 ppm 1-BP exposure. Concentrations of 1-BP within the chamber following these treatments are shown in Figure 5. Untreated rats absorbed and cleared 1-BP from the chamber rapidly with a terminal $t_{1/2}$ of approximately 1 h (Table 3). Pretreatment of the rat with ABT, known potent inhibitor of P450s, causes a dramatic change in 1-BP elimination relative to the control animals, with a change in terminal half-life from 2.0 to 9.6 h. Pretreatment with BSO, known to lower levels of reduced GSH, caused an increase in half-life from 2.0 to 4.0 h. Notably, the animal pretreated with BSO died ~5 h after initiation of exposure. Gas uptake studies with pretreated animals were not subject to further replication due to mortality/morbidity issues.

Table 5. Estimated metabolic rate constants in male and female rats following 1-BP exposures in gas uptake studies.

		Male rats[a]		Female rats	
Parameter	Units	Mean	SE	Mean	SE
V_{max}	(mg/h/kg)	0.227	0.009	0.143	0.001
K_m	(mg/L)	0.215	0.077	0.009	0.003
k_{GST}	(h-1)	0.00011	0.00636	0.00005	0.00002
k_{CR}	(h-1)	5.292	0.130	3.086	0.000
k_{RC}	(h-1)	0.089	0.007	0.074	0.001

[a]Values expressed as estimated mean ± standard error of mean.

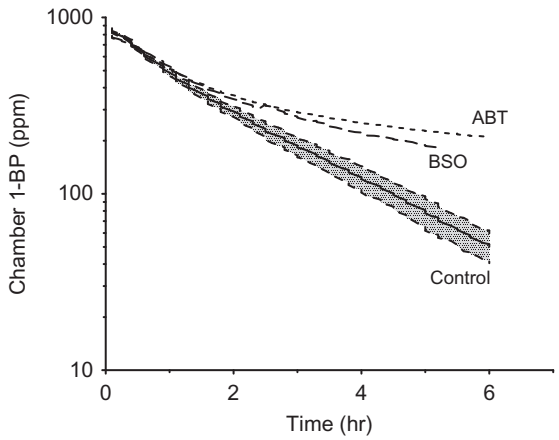


Figure 5. Chamber 1-BP concentrations during gas uptake experiments in female Fischer 344 rats pretreated with 1-aminobenzotrizole (ABT) or BSO. Rat was pretreated with an ip dose of 50 mg/kg of ABT 4 h prior to inhalation. Rat was pretreated orally with (BSO) for three days, 1000 mg/kg/day prior to inhalation.

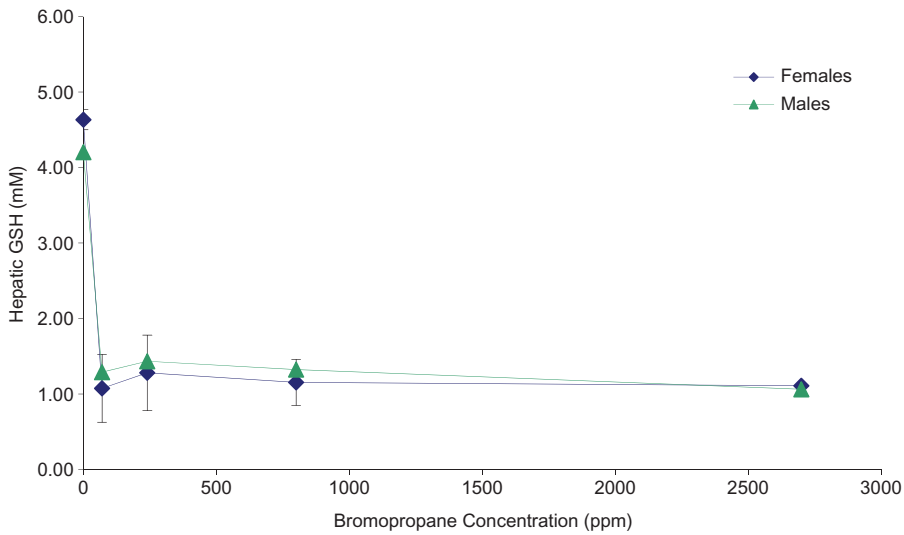
Dose-dependent changes of the GSH level in male and female rats

Hepatic levels of reduced GSH in rats 6 h following inhalation exposure to 1-BP are shown in Figure 6. GSH concentrations were lowered ca. 80% relative to control regardless of 1-BP chamber concentration.

Estimation of *in vivo* metabolic parameters for 1-BP in male and female mice by gas uptake inhalation

The mean chamber 1-BP atmosphere concentration–time data from mouse inhalation studies are plotted for each exposure in Figure 7. Generally, 1-BP was rapidly removed from the chamber. At starting concentrations of 800 ppm or above males tended to eliminate 1-BP from the chamber more rapidly than females. The mean first-order rate constants (λ_z) and half-lives ($t_{1/2}$) of the terminal elimination curves for male and female rats are listed for each dose group in Table 6. With both males and females, there was a gradual decrease in the terminal slope of the curve and therefore a concomitant increase in half-life. Metabolism data from mouse studies showed that unlike rat, direct conjugation of parent 1-BP with GSH is insignificant at doses up to 100 mg/kg (Garner et al., 2006). Furthermore, experiments with CYP2E1 knockout mice demonstrated that CYP2E1-mediated metabolism made the predominant contribution to elimination of 1-BP in mice (Garner et al., 2007). Therefore, only saturable oxidative metabolism was incorporated in the two compartment mouse in the gas uptake inhalation system. In fact, incorporation of

Figure 6. Hepatic levels of reduced glutathione in rats 6 h following inhalation exposure to 1-BP.



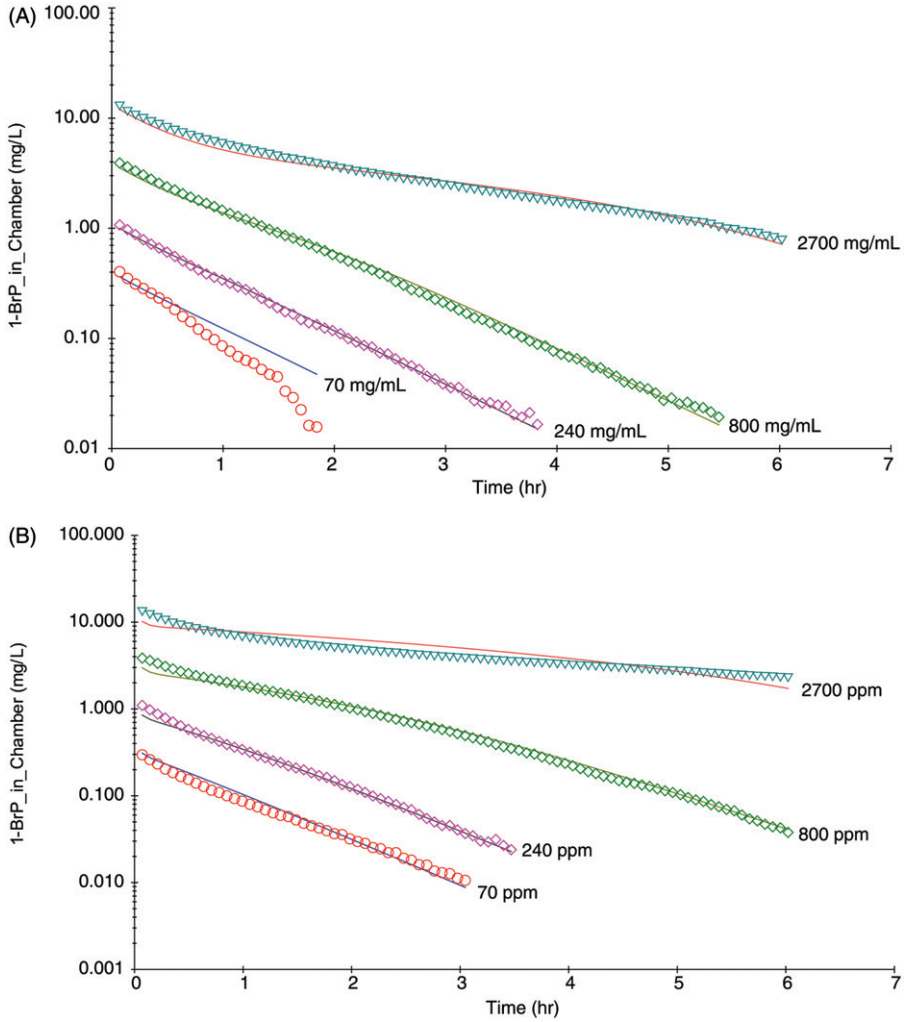


Figure 7. Mean chamber 1-BP concentrations during gas uptake experiments with male (A) and female (B) mice. Chamber 1-BP concentrations were fit to a two compartment model based on the work of Filser et al. (1995) to estimate kinetic parameters. Model estimates are presented as solid lines.

Table 6. Estimated first-order terminal rate constants in male and female mice following 1-BP exposures in gas uptake studies.

Dose group (ppm)	Actual dose[a] (ppm)	Number of mouse units[b]	Animal weight[c] (kg)	λ_z[d] (1/h)	$t_{1/2}$[e] (h)
Males					
70	122	2	0.111	1.02	0.68
240	263	2	0.115	1.08	0.64
800	858	2	0.124	0.77	0.9
2700	2859	2	0.136	0.18	3.8
Females					
70	100	2	0.0880	1.81	0.38
240	257	2	0.0925	1.06	0.66
800	796	2	0.1011	0.99	0.7
2700	2900	2	0.1030	0.36	1.91

[a]Calculated from initial weight of 1-BP introduced to the gas uptake system.
[b]Four mice per unit.
[c]Total mouse weight within exposure chamber.
[d]First-order rate constant associated with the terminal portion of chamber concentration versus time plot (log-linear).
[e]Terminal half-life associated with the terminal portion of the chamber concentration versus time plot (log-linear).

Table 7. Estimated metabolic rate constants in male and female mice following 1-BP exposures in gas uptake studies.

		Male mice[a]		Female mice	
Parameter	Units	Mean	SE	Mean	SE
V_{max}	(mg/h/kg)	0.329	0.009	0.234	0.006
K_m	(mg/L)	5.222	0.388	5.897	0.236
k_{CR}	(h-1)	0.10	0.01	1.23	0.29
k_{RC}	(h-1)	0.329	0.009	0.234	0.006

[a]Values expressed as estimated mean ± standard error of mean.

GST first-order elimination resulted in poor fits or models that would not converge (data not shown). This model also incorporated loss of compound from the chamber due to hair adsorption. Data were fitted to the model and equilibrium and metabolism constants were estimated (Table 7). Curve fit was adequate (Figure 7). Estimated metabolic constants (V_{max}) for males were slightly larger than that of females, with a male/female ratio that was similar to that in rat. The value of V_{max} was larger in mice regardless of gender than the corresponding rat value. Unlike in rat, K_m in male and female mice was similar. Simulation of systemic 1-BP as shown in Figure 8 reflects that higher doses are required for saturation in mice relative to rat (Figure 8).

Discussion

There is a growing body of toxicological data regarding the systemic- and organ-specific effects of 1-BP in animals and also in humans (Ichihara et al., 2000a,b,c, 2012; Liu et al., 2010; Mohideen et al., 2013; Morgan et al.,

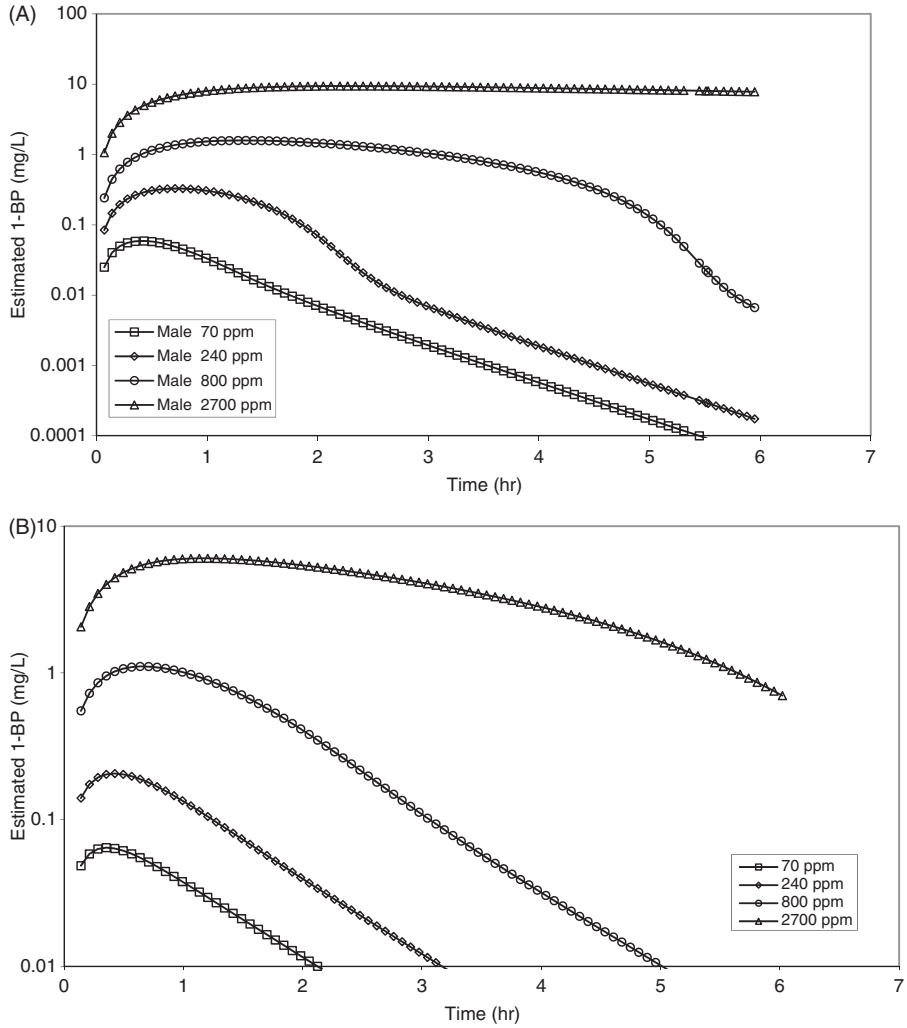


Figure 8. Estimated whole body levels of 1-BP in rat (A) and mouse (B) based the two compartmental model during simulated inhalation exposure to 1-BP.

2011; NTP, 2013; Yu et al., 1998, 2001). However, there are only a limited number of reports on factors contributing to the understanding of the toxicokinetics difference among species, which is potentially linked to the susceptibility of 1-BP toxicity. Our current study was designed to examine the species and sex-dependent toxicokinetics as well as the effect of hepatic cytochrome P450 oxidation and GSH conjugation pathways.

As demonstrated in our IV bolus administration of 1-BP to male Fischer 344 rats, systemic clearance was rapid. The estimated V_{ss} values for 1-BP greatly exceeded total body water and indicated extensive distribution of 1-BP into tissues. Systemic clearance was greater than hepatic and renal blood flows, either singly or combined, suggesting that the majority of 1-BP is cleared from the body by routes other than hepatic metabolism and urinary excretion. This is consistent with data from disposition studies which demonstrated that exhalation of unchanged 1-BP in breath is a major route of 1-BP elimination from the body (Garner et al., 2006). Our previously published disposition data showed that residual equivalents in tissue were relatively minor 48 h post-dose, suggesting that although initial distribution may be extensive, tissue accumulation is insignificant (Garner et al., 2006). Given the rapid clearance and high V_{ss} of 1-BP, the average elimination half-life was short (0.8–0.14 h). Thus, approximately 99% of 1-BP was eliminated from the body by 3 h post-dose. Bromine is released from 1-BP by either oxidative metabolism or via conjugation with GSH (Garner et al., 2006). The plasma bromide levels after IV administration to rats suggested that approximately 30% of administered 1-BP was subject to metabolism by either route. This is in agreement with previously published data where 1-BP was excreted rapidly as parent compound in expired air, as CO_2, and as several metabolites in urine following iv and inhalation administration (Garner et al., 2006). The similarity of metabolic profiles of urine following *iv* and inhalation exposure suggests that qualitatively metabolic fate is independent of route of exposure in rat but is dose dependent.

In rat inhalation studies, in both male and females, there was a gradual decrease in the terminal slope of the curve and therefore a concomitant increase in half-life, suggesting that one or more routes of elimination was saturated as chamber concentration increased (Figure 3). At a given starting concentration, males tended to eliminate 1-BP from the chamber more rapidly than females. This phenomenon was reflected in the estimated metabolic constants (V_{max} and K_{GST}), which for males were approximately twice those of females (Table 5). Furthermore, the estimated K_m for oxidative metabolism in females was significantly lower than that estimated in males, indicating that oxidative metabolism of females may be saturated at lower concentrations than that of males. This is in agreement with 1-BP

metabolism studies wherein we have shown that as IV doses in rats increase there was saturation of oxidative metabolism, resulting in a significant shift from oxidative metabolism to pathways of GSH conjugation and expiration of unchanged 1-BP (Garner et al., 2006).

Measurements of the systemic concentration of bromide, a surrogate for dehalogenation metabolism, suggested that in rat at inhalation exposures less than 800 ppm nearly all of the chamber 1-BP was absorbed and subsequently metabolized, releasing molar equivalents of bromide ion. However at concentrations higher than 800 ppm, significant amounts of 1-BP are systemically absorbed but not eliminated via de-halogenation. After a bolus IV dose approximately 34% of administered dose is converted to bromide. The IV dose is a ''single pass'' whereas the whole body chamber allows for rebreathing of exhaled, un-metabolized 1-BP. Thus, the combined data suggest that at higher initial concentrations in inhalation chamber there may be un-metabolized 1-BP remaining within the body at the end of the exposure period. This further supports the concept of saturation of elimination pathways at higher exposures. As exposure increased to 800 ppm, 1-BP moles removed from the chamber began to diverge from moles of Br^- (de-halogenated equivalents) within the animal (Figure 4), suggesting that below 800 ppm, nearly all of the absorbed dose was metabolized. In rats, GSH conjugation has been shown to play a major role in the elimination of 1-BP in these studies, hepatic levels of reduced GSH in rats 6 h following inhalation exposure to 1-BP were lowered by ca. 80% relative to control regardless of 1-BP chamber concentration. Significantly, Br^- molar equivalents within both male and female rats approached a limit that corresponded with the calculated upper range of rat hepatic GSH molar levels. This suggests that following high exposures to 1-BP, where GSH has been depleted and oxidative metabolism has also been saturated, then significant amounts of un-metabolized 1-BP may remain within the systemic circulation for extended periods. Output from the two-compartment model (Figure 4) agrees with this hypothesis.

Plots of the chamber 1-BP concentration–time data in mice showed that only at the highest concentrations studied there is saturation of clearance in mice during inhalation exposure (Figure 7). With both males and females at 800 ppm and higher, there was a decrease in the terminal slope of the curve and therefore a concomitant increase in half-life, suggesting that elimination was saturated as chamber concentration increased beyond 800 ppm. At chamber concentrations less than 800 ppm, male and female mice eliminated 1-BP at approximately similar rates (Table 6). At concentrations of 800 ppm or higher was there a divergence in elimination rates, with male mice eliminating 1-BP from the chamber more slowly than females. This phenomenon was reflected in the estimated metabolic V_{max}, which for males were 1.5 times that of females, and the K_m which were approximately equal (Table 7). This suggests that only at the highest exposures, where oxidative metabolism has been saturated, it is expected that 1-BP exposures should be significantly elevated systemically for extended periods during the exposure.

The majority of the work preceding these studies have suggested that conjugation of 1-BP with GSH is the predominant metabolic pathway (Barnsley et al., 1966). This report and prior reports from this lab (Garner et al., 2006, 2007) have shown that P450-mediated oxidative metabolism plays a major role in the systemic clearance of 1-BP. Pretreatment of a rat with 1-ABT, a potent inhibitor of cytochrome P450 caused a dramatic change in 1-BP elimination rate relative to that observed in the rats, with a five-fold increase in terminal half-life from 2.0 to 9.6 h. Pretreatment with BSO, an inhibitor of GSH synthesis, doubled the half-life from 2.0 to 4.0 h. Results of these studies suggest that both CYP450 and GSH are critical to the toxicokinetics of inhaled 1-BP in rat. In our current studies, we observed major changes in toxicokinetic parameters determined from gas uptake experiments after pretreatment to either inhibit cytochromes P450 or lower GSH systemically. Though only a single animal was used, these data suggest that the toxicokinetics of 1-BP in rat are profoundly affected by previously observed changes in route of metabolism induced with the inhibitors 1-ABT and BSO. In our earlier metabolism work, we showed that in rat, oxidative metabolites were the major form excreted in urine. Only as dose increased to 100 mg/kg iv did the proportion of urinary metabolites shift to significantly favor formation of N-acetyl-S-propylcysteine, derived from direct GSH conjugation with parent compound. Additionally, following inhibition of P450-mediated metabolism with ABT, rat urine metabolites were reduced from 10 to a single major metabolite, N-acetyl-S-propylcysteine which accounted for >90% of total radioactivity.

Plots of the chamber 1-BP concentration–time data demonstrated that only at the highest concentrations studied is there saturation of the routes of clearance in mice. Interestingly, in ^{14}C studies the disposition and urinary metabolite profile in mice after IV administration was unchanged with increasing dose (Garner et al., 2007). Though mice are known to have a higher capacity for GSH conjugation of halogentated solvents and reactive molecules than rats (Gargas et al., 2008) it is noteworthy that with 1-BP there is significantly more flux via oxidative metabolism pathways relative to direct conjugation in mice (Garner et al., 2007). This suggests a high oxidative metabolic capacity in mice relative to that of rats (Figure 8). Further metabolism data revealed an important distinction between mice and rats. Whereas rats produced both directly GSH-conjugated parent and also oxidative metabolites, mice only produced a single oxidative metabolite, 2-hydroxybromopropane which was then conjugated with GSH. Over half of the urinary metabolites present in rat urine after inhalation are derived from this initial P450-derived metabolite. This metabolite is then conjugated with GSH or glucuronic acid or further metabolized to reactive metabolites such as bromoacetone or α-bromohydrin. Indeed, inhibition of cytochrome P450 with potent suicide inhibitor 1-ABT completely eliminated all 1-bromo-2-propanol-derived metabolites in rat. However, CYP2E1 knockout mice still produced this metabolite, implying that other P450s also contribute to 2 hydroxylation of 1-BP (Garner et al., 2007). Results from our current study, as well as previously reported, suggest strongly that oxidation of 1-BP mediated via cytochrome P450 is a significant if not the predominant metabolic pathway at low body burdens of 1-BP. Kim et al. (1999) examined the inducibility of hepatic cytochrome P450 isoenzymes in microsomes of

Sprague-Dawley rats following 1-BP inhalation exposure for eight weeks (Kim et al., 1999). 1-BP treatment resulted in significantly increased p-NPH activity. Western blot analyses from 1-BP-treated rats showed increase in CYP2E1 levels. The authors concluded that CYP2E1 isoenzyme is possibly responsible for 1-BP metabolism but did not demonstrate this directly. The present work has demonstrated that a single exposure to 1-BP is sufficient to induce hepatic CYP2E1, the major enzyme in 1-BP elimination (Garner et al., 2007). There are no data, currently, to determine the impact of repeated low level exposure to 1-BP on either systemic pharmacokinetics or metabolism.

Thus, our current data suggest 1-BP toxicokinetics and disposition may be sensitive to environmental and/or genetic factors which may have impact on P450 or GST expression or activity. This is in agreement with data we have previously shown in which gas uptake kinetics of 1-BP by CYP2E1−/− mice were significantly different from that of wild-type mice (Garner et al., 2007).

Following IV or inhalation 1-BP exposure in rat and mouse the predominant pathway of 1-BP oxidation is hydroxylation to form 1-bromo-2-propanol (bromoisopropanol) (Garner et al., 2006). The metabolism of 1-BP in rodents may be dose-dependent and was found to be different in mice and rats. In rats, as 1-BP dose increased the percentage of the dose exhaled as unchanged parent increased while the percentage of the dose exhaled as CO_2 or excreted in the urine decreased. After administration of 1-BP to rats pretreated with 1-ABT, a potent, general cytochrome P450 inhibitor, the urinary metabolite profile changed from mercapturates of several P450-mediated metabolites to a single mercapturic acid metabolite derived from direct conjugation of parent compound with GSH (presumably via GST). In mice, the mercapturic acid conjugate of 2OHBP was the predominant metabolite in urine regardless of dose. That is, in rats but not mice, the proportion of 1-BP metabolized via P450 oxidation relative to pathways dependent on direct GSH conjugation is inversely proportional to dose. In mice, metabolism and disposition were relatively insensitive to dose, suggesting that the mouse possesses a higher capacity for P450-mediated elimination of 1-BP than rat.

Overall, these studies described here demonstrated that 1-BP is cleared rapidly from the circulation in rats following IV administration and that clearance is dependent on dose. Hepatic CYP2E1 activity is induced after a single administration of 1-BP. During inhalation studies 1-BP was rapidly absorbed from the chamber atmosphere by both rats and mice. Both species and sex-related differences in 1-BP were observed and dose dependency in clearance was noted. Hepatic levels of reduced GSH were lowered relative to control regardless of initial atmosphere concentration of 1-BP. Changes in CYP and GSH status resulted in profound changes in uptake and elimination of atmospheric 1-BP. These data suggest that the systemic exposure of 1-BP in rats and mice may be sensitive to factors which modulate oxidative and GSH-mediated metabolic pathways. Species or strain differences in the metabolic activity of P450 enzymes and GST pathways could lead to a significantly different toxicokinetics of 1-BP, which could contribute to difference in susceptibility to 1-BP toxicity.

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Declaration of interest

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