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Development and application of a human PBPK model for bromodichloromethane to investigate the impacts of multi-route exposure

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ABSTRACT: As a result of its presence in water as a volatile disinfection byproduct, bromodichloromethane (BDCM), which is mutagenic, poses a potential health risk from exposure via oral, dermal and inhalation routes. We developed a refined human physiologically based pharmacokinetic (PBPK) model for BDCM (including new chemical-specific human parameters) to evaluate the impact of BDCM exposure during showering and bathing on important measures of internal dose compared with oral exposure. The refined model adequately predicted data from the published literature for oral, dermal and bathing/showering exposures. A liter equivalency approach (L-eq) was used to estimate BDCM concentration in a liter of water consumed by the oral route that would be required to produce the same internal dose of BDCM resulting from a 20-min bath or a 10-min shower in water containing 10 μg l^{-1} BDCM. The oral liter equivalent concentrations for the bathing scenario were 605, 803 and 5 μg l^{-1} BDCM for maximum venous blood concentration (Cmax), the area under the curve (AUCv) and the amount metabolized in the liver per hour (MBDCM), respectively. For a 10-min showering exposure, the oral L-eq concentrations were 282, 312 and 2.1 μg l^{-1} for Cmax, AUC and MBDCM, respectively. These results demonstrate large contributions of dermal and inhalation exposure routes to the internal dose of parent chemical reaching the systemic circulation, which could be transformed to mutagenic metabolites in extrahepatic target tissues. Thus, consideration of the contribution of multiple routes of exposure when evaluating risks from water-borne BDCM is needed, and this refined human model will facilitate improved assessment of internal doses from real-world exposures. Published 2015. This article has been contributed to by US Government employees and their work is in the public domain in the USA.

Keywords: bromodichloromethane; multi-route exposure; PBPK model

Introduction

The brominated trihalomethane (THM), bromodichloromethane (BDCM) and other disinfection byproducts (DBPs) occur in finished drinking water as a result of natural components in source waters (organic water, bromine and iodine) reacting with disinfectants (chlorine and chloramine). Beyond oral intake of water, human exposure to DBPs via multiple routes (dermal, inhalation) is common during various household water use activities including bathing/showering, cooking, hand washing, and dish and clothes washing (Lynberg *et al.,* 2001; Nuckols *et al.,* 2005).

In evaluating the relationship between household exposures and the concentration of BDCM in the blood, it has been demonstrated that much higher levels of BDCM can be attained in blood after activities that involve dermal and inhalation exposure (showering and bathing) compared with oral exposure (Backer *et al.,* 2000; Leavens *et al.,* 2007). For example, Backer *et al.* (2000) compared post-exposure blood levels of THMs after subjects drank 1 liter of water in a 10-min period or took a 10-min bath or shower with water containing approximately the same levels of THMs. At 10 min post-exposure, the median levels of BDCM in venous blood were 3.8, 17, and 19.4 pg ml^{-1} (ppt) for drinking, bathing and showering exposures, respectively. In another study with human volunteers, Leavens *et al.* (2007) examined the relationship between oral (single 0.25-l drink, mean dose 146 ng kg^{-1}) and dermal (forearm immersion for 1 h, estimated mean dose 155 ng kg^{-1}) exposure to BDCM in water (~36 μg l–1) and BDCM pharmacokinetics. Peak venous blood concentrations of BDCM ranged from 0.4 to 4.1 ng l^{-1} (ppt) after oral exposure and 39 to 170 ng l^{-1} (ppt) for dermal exposure with the mean area under the curve (AUC) of 149 ($AUC_{0\rightarrow\infty}$) and 11, 800 ng min l^{-1} ($AUC_{0\rightarrow1440}$) for oral and dermal exposures, respectively. These studies demonstrate that activities involving dermal and inhalation exposure result in much higher blood concentrations and hence the greater overall distribution of BDCM to the systemic circulation compared with oral exposure. This difference is almost certainly a result of extensive first-pass intestinal and hepatic metabolism that occurs after oral exposure.

The distribution of THMs in drinking water varies depending upon geographic location, source water, season and disinfection method. In national surveys, BDCM is typically the second most prevalent THM (after chloroform) and most abundant brominated THM (BrTHM). In general, BrTHM concentrations are higher in drinking water when the raw water is from surface water, rather than groundwater sources (USEPA, 2005). In one national survey conducted by the U.S. EPA under the Information Collection Rule

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(ICR in 1997–1998), the mean, median and 90th percentile concentrations for BDCM in distribution system samples originating from surface water were 8.6, 10.2 and 20.3 ppb, respectively (USEPA, 2005). Interestingly, another potential source of exposure to THMs that may carry an increased risk of adverse health effects is swimming in disinfected pool water (Zwiener *et al.,* 2007; Font-Ribera *et al.,* 2010; Kogevinas *et al.,* 2010). It is particularly noteworthy that the disinfection method used (Lee *et al.,* 2009) and the amount of bromide in source water (Krasner *et al.,* 1989) strongly influences the formation of BrTHMs.

BDCM is carcinogenic in animal models with oral gavage exposure resulting in kidney carcinomas in both rats and mice, as well as large intestine carcinomas in rats (USEPA, 2005). Epidemiological studies report an increased risk of both bladder and colon cancer for individuals exposed to DBPs (Villanueva *et al.,* 2014), with the most compelling evidence being for bladder cancer (Cantor *et al.,* 2010). With regard to potential mechanisms for these effects, BrTHMs, including BDCM, have been found to be mutagenic via metabolic activation by the enzyme, glutathione-*S*-transferase theta-1 (GSTT1) in bacterial (Salmonella) systems (DeMarini *et al.,* 1997; Pegram *et al.,* 1997;). In addition, Ross and Pegram (2003, 2004) have demonstrated that GSTT1 is functional in the urinary tract of humans, rats and mice, and that the GSTT1-mediated metabolism of BDCM produces reactive intermediates that covalently bind DNA, suggesting that BDCM could be a mutagenic carcinogen. Moreover, Cantor *et al.* (2010) found that people with the GSTT1 +/+ and GSTT1 +/- genotypes were at a significantly greater risk of developing bladder cancer than GSTT1 null subjects when exposed to tap water with total THM levels $> 49\ \mu g\ l^{-1}$ [odds ratio (OR) = 2.2, 95% confidence interval (CI) = 1.1–4.3], a finding that implicates the BrTHMs in the etiology of DBP-associated bladder cancer because brominated THMs, but not chloroform, are metabolized to mutagenic intermediates by GSTT1.

Two competing and toxicologically significant biotransformation pathways for BDCM have been identified in multiple tissues – an oxidative pathway (mainly deactivation) leading to CO_2 mediated by cytochrome P450 (primarily CYP2E1) and an activation pathway mediated by GST (Lilly *et al.,* 1997a; Allis *et al.,* 2002; Ross and Pegram, 2003, 2004). The CYP pathways have been associated with CO_2/CO production (Stevens and Anders, 1981) and free radical production which may lead to high-dose acute toxicity (Tomasi *et al.,* 1985). The GSTT1-mediated formation of reactive BDCM-GSH conjugates is the only known mutagenic pathway of BDCM metabolism (Ross and Pegram, 2003, 2004). The mutagenic metabolites are very unstable and highly reactive, and their effect, therefore, occurs only within the cells where they are generated (Landi *et al.,* 1999; Ross and Pegram, 2003, 2004). In other words, the unstable and reactive mutagenic metabolites of BrTHMs would not survive transport out of liver cells to be systemically distributed to extrahepatic target cells.

Ross and Pegram (2004) evaluated the relative efficiency of the CYP and GSTT1 pathways in rat non-target (liver) and target (kidney, large intestine) tissues for carcinogenic effects based on comparison of intrinsic clearance (Vmax/Km). CYP-catalyzed oxidation of BDCM was 16-fold more efficient in the liver compared with the kidney and large intestine; whereas, the efficiency of the hepatic GST pathway was only 1.7- and 3.5-fold greater than in the kidney and large intestine, respectively. On the basis of these data, Ross and Pegram (2004) hypothesized that relatively greater amounts of GSH pathway reactive intermediates could be formed in extrahepatic target tissues in the rat relative to the liver and that target tissue biotransformation might account for the carcinogenic response observed in these tissues. Greater flux through the GSTT1 pathway may be even more significant in the human urinary tract, because little to no CYP2E1 has been found in the human kidney (Amet *et al.,* 1997; Cummings *et al.,* 2000). Given that dermal and inhalation exposures result in higher levels of BDCM in the blood compared with the oral route, and thus a greater potential for extrahepatic biotransformation of parent chemical, the issue of multi-route exposure is of toxicological concern. This concern also extends to other potential extrahepatic effects of BDCM and brominated THMs including developmental toxicity (Danileviciute *et al.,* 2012; Rivera-Nunez and Wright, 2013).

The objective of this work was to develop a refined human physiologically based pharmacokinetic (PBPK) model for BDCM, and to utilize that model to evaluate the impact of multi-route exposure on various internal dose metrics for BDCM under environmentally relevant exposure conditions. PBPK models for BDCM have been published previously for both rodents (Da Silva *et al.,* 1999; Lilly *et al.,* 1997a, 1998) and humans (Haddad *et al.,* 2006; Tan *et al.,* 2007). Earlier multi-route human PBPK models for BDCM, in which BDCM was one of multiple THMs or volatiles in the model, were developed to interpret biomonitoring data (Tan *et al.,* 2007) and indoor exposure assessment (Haddad *et al.,* 2006). Our focus is to evaluate the toxicological implications of multi-route exposure with particular emphasis on how various routes of exposure contribute to internal dose metrics that could be relevant to cancers associated with exposure to DBPs in epidemiologic studies (Cantor *et al.,* 1987, 1998, 2010). Enhancements in the current model include using new experimentally determined chemical-specific human parameters and human data from diverse sources, unavailable at the time earlier models were published, for model evaluation. In addition, both local (LSA) and global (GSA) sensitivity analysis were used to determine which model inputs (parameters) were most influential for specific model responses (e.g. toxicologically relevant dose metrics or experimental measurements for which data are or may become available).

Methods

Model structure, assumptions and implementation

The model structure is shown in Fig. 1. Tissue groups/compartments were included based on consideration of route of exposure [lungs, skin and gastrointestinal (GI) tract], metabolism (liver), storage (fat), as well as body mass balance and future expansion of the model (kidney, poorly and richly perfused tissue groups). The individual compartments are connected by the systemic circulation. The model has distinct arterial and venous blood compartments, and tissues are described as homogeneous well-mixed compartments. Metabolism is described by two simultaneous metabolic pathways in the liver – oxidative metabolism via microsomal cytochrome P450s (Michaelis-Menten) and a first order GST pathway (Ross and Pegram, 2004). Oral absorption and skin absorption are described as first-order processes. The skin surface area is estimated on the basis of height and body weight (Gehan and George, 1970). Alveolar ventilation was determined from the minute volume, scaled to the skin surface area with correction for dead space (Altman and Dittmer, 1971). Key differential equations for the model are provided in the Appendix. The model was implemented in AcslX 3.0.2.1 (The AEgis Technologies Group, Huntsville, AL, USA).

Model parameterization. Final physiological and chemical-specific parameters are presented in Tables 1 and 2, respectively. An initial model parameterization for the chemical-specific parameters was utilized as the basis for preliminary local sensitivity analysis (using methods described in sensitivity analysis section) to

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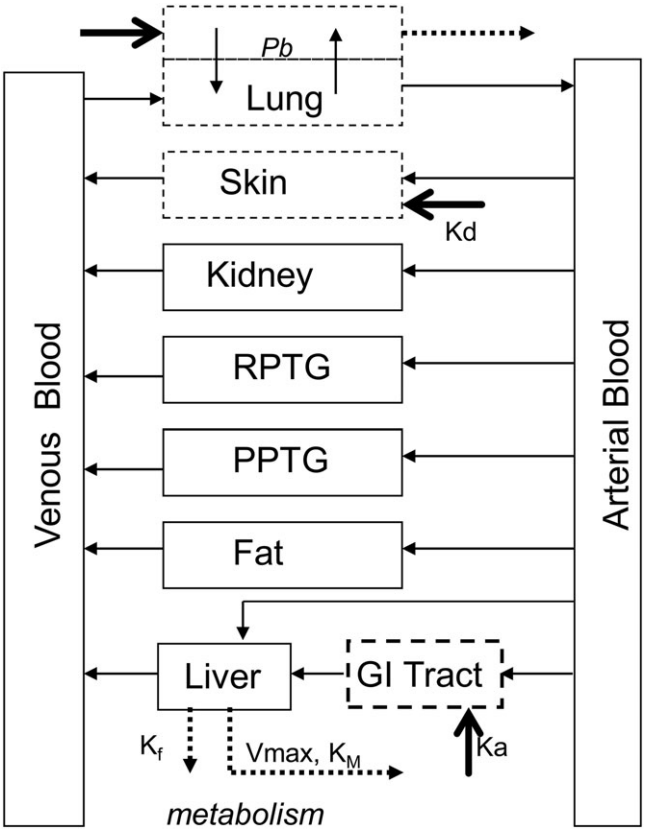

Figure 1. Schematic diagram of the human bromodichloromethane (BDCM) model. Thick black arrows indicate routes of entry to specific tissue compartments (dotted lines). Thin solid lines and arrows indicate blood flow for organs connected by systemic circulation.

help guide decisions on experimental data needs. Because metabolism parameters for the CYP pathway (V1CBDCM, KM1BDCM) and the oral absorption coefficient (KABDCM) were highly influential parameters for all model responses of interest for oral exposure (i.e. venous blood BDCM, AUC for venous blood concentration and the amount of BDCM metabolized in the liver per hour), independent estimation of these parameters using the most appropriate data was a high priority. In addition, because the blood: air partition coefficient (PBBDCM) was highly influential for both venous blood and exhaled breath BDCM for showering exposure, and is utilized to calculate other tissue: blood partition coefficients, determining this parameter experimentally was also considered important.

The time to achieve a maximum blood concentration (Tmax) has high sensitivity to the oral absorption coefficient for BDCM (KABDCM) (Kenyon *et al.,* 2009). Thus, KABDCM was varied to obtain the range of reported Tmax values (5–30 min) reported in Leavens *et al.* (2007) and the mid-point of the range was used as the central tendency estimate in the model (Table 2). This kinetic description assumes that oral bioavailability from water is complete (100%) and that oral absorption of BDCM follows first-order kinetics.

Experimental blood: air partition coefficient determination

Gender-specific blood: air partition coefficients (PBBDCM) were determined using the vial equilibration technique (Gargas *et al.,* 1989) modified as described previously (El-Masri *et al.,* 2009). Commercially purchased human whole blood (Research Blood Components, LLC, Brighton, MA, USA) from fasted-status individual donors was used ($n = 6$ per gender). Gender-specific differences in PBBDCM were evaluated for statistical significance using a *t*-test.

Experimental microsomal metabolism determination

Metabolism parameters were determined with commercially purchased pooled (200 individuals) human microsomes (XTreme 200 pool; XenoTech, Kansas City, MO, USA) to provide an estimate of population central tendency. Stock solutions of BDCM (20–80 mM) were prepared in 5% Alkamuls EL-620 (Rhone-Poulenc, Cranbury, NJ, USA) in crimp-sealed 10-ml silanized vials (Supelco, Bellefonte, PA, USA) and placed in a sonicating water bath with gentle agitation and heat (32°C) to solubilize the BDCM. Aliquots of the stock solution were added to 100 mM sodium phosphate buffer, pH 7.4, in sealed 10-ml vials to produce BDCM working solutions (10-ml volume to minimize headspace). Incubations with human microsomes were conducted in the sealed silanized vials with a final 2-ml volume containing the following: 100 mM Na_2HPO_4, pH 7.4; 3.1 mM $MgCl_2$; 11.9 mM glucose-6-phosphate; 1.25 mM NADP, 2 units of glucose-6-phosphate dehydrogenase (prepared in 5 mM sodium citrate, pH 7.4); and 0.2–0.3 mg of microsomal protein. After the vial was sealed, an appropriate aliquot of the BDCM working solution was added with a gas-tight syringe, and the mixture was preheated at 37°C with vortex agitation for 5 min. Headspace concentrations of control vials remained constant at 5, 12 and 19 min, thus demonstrating that headspace: liquid equilibrium of BDCM was attained by 5 min. Metabolism was initiated by the addition of NADP in 100 mM Na_2HPO_4 buffer, and the vials were incubated at 37°C for 28 min with continued vortex agitation. Control incubations did not contain NADP.

Headspace samples (20–50 μl), taken from the vials after 5-min pre-incubation and at 7-min intervals thereafter, were manually injected into an Agilent 7890A gas chromatograph (Wilmington, DE, USA) equipped with a Restek RTX-Volatiles 0.53 mm ID, 2-μm film, 60-m column (Bellefonte, PA, USA) and micro-electron capture detector (μECD). The gas chromatography (GC) analysis was isothermal (160°C oven temperature) with the following parameter settings: front inlet temperature = 240°C; split ratio = 10; split flow = 55 ml min^{-1}; total inlet flow = 63.5 ml min^{-1}; column carrier (He) flow (constant) = 5.5 ml min^{-1}; μECD temperature = 300°C; and make-up (N_2) flow = 60 ml min^{-1}. The retention time of BDCM was 3.1 min and the baseline with no additional peaks was attained 6 min after injection. BDCM peak areas for known concentrations were used to generate a standard curve ($R^2 = 0.992$), and known quantities of BDCM were injected daily prior to all experiments.

Metabolism experiments were performed in triplicate at each nominal starting BDCM concentration in the incubation mixture (1.25, 2.5, 5, 10, 20 and 40 μM). The concentration of BDCM did not decrease in the control vials, and, therefore, measurable metabolism did not occur in the absence of NADP. BDCM headspace: incubation mix partition coefficients were calculated for each control sample and used to calculate the initial rate (0–7 min, 0 time is after 5-min pre-incubation and the addition of NADP) of BDCM metabolism for each microsomal metabolism experiment. V_{max} and K_m values were calculated using the SigmaPlot Enzyme Module 1.3 (Systat Software, Inc., San Jose, CA, USA).

Incorporating biotransformation pathways in the PBPK model

Two competing and toxicologically significant metabolic pathways for BDCM were incorporated in the model – an oxidative pathway mediated by cytochrome P450 (CYP) and an activation pathway

Table 1. Physiological parameters for the human bromodichloromethane (BDCM) model

Parameter, units	Symbol	Value	Footnote
Height, cm	Height	160 – 190	a
Body Weight, kg	BW	65-91	a
Alveolar ventilation Rate, L/h-m^2	QPC	212.4	b
Alveolar Deadspace, unitless	Deadspace	0.238	
QPC to Cardiac Output Ratio, unitless	RQPCO	0.8	c
Fractional Blood Flows, unitless			d,e
Richly Perfused Tissue Group	FQRP	0.75	e,f
Liver	FQL	0.09	
Gastrointestinal Tract	FQG	0.16	
Kidney	FQK	0.15	
Poorly Perfused Tissue Group	FQPP	0.25	f
Fat	FQF	0.05	
Blood Flow to Skin, L/min-m^2	QSKSA	0.58	f
Compartment Volume, unitless			d
Blood fraction of BW	FVBD	0.079	g
Blood as arterial	FVART	0.25	
Blood as venous	FVVEN	0.75	
Richly perfused fraction of BW	FVRP	0.20	h
Poorly perfused fraction of BW	FVPP	0.80	
GI tract fraction of BW	FVGI	0.0165	
Liver fraction of BW	FVL	0.026	
Fat fraction of BW	FVF	0.07 – 0.20	a
Kidney fraction of BW	FVK	0.004	
Volume GI tract lumen, L	VLUM	2.1	c
Skin thickness, mm	LSK	2.0	i

[a]Height and BW are experiment specific (Leavens *et al.,* 2007). Average height and BW used for general simulations were 178 cm and 74 kg, respectively (based on the subject average) in Leavens *et al.* (2007). Individual subject-specific FVF data were also available from Leavens *et al.* (2007) estimated based on skin fold thickness. Average FVF used for general simulations was 0.11.
[b]Minute ventilation rate was scaled to skin surface area (SA) in m^2, QP = QPC* SA * (1-Deadspace). SA was estimated on the basis of height and weight as SA = 0.0239*(Height$^{0.417}$)*(BW $^{0.517}$) (USEPA, 2011).
[c]Cardiac Output, QC = QP/RQPCO.
[d]Physiological parameters from (Brown *et al.,* 1997) unless otherwise specified.
[e]Fractional blood flows to individual tissues are scaled to cardiac output (QC), i.e. QL = FQL *QC, QG = FQG *QC, QK = FQK * QC, and QF = FQF * QC.
[f]Richly (QRP) and poorly perfused (QPP) tissues calculated respectively, as follows: QRP = (FQRP*QC)-QL-QK-QG and QPP = (FQPP*QC)-QF-QSK. Blood flow to skin (QSK) is calculated as QSK = QSKSA * SA * 60 min/hr.
[g]Volume of blood compartment is scaled to BW and volume of arterial (Vart) and venous (Vven) compartments are calculated respectively, as VART = FVART*VBD and VVEN = FVVEN*VBD.
[h]Tissue volumes to tissues are scaled to BW with richly (VRP) and poorly perfused (VPP) tissue volumes calculated respectively, as follows: VRP = FVRP*BW-VL-VGI-VBD-VK and VPP = FVPP*BW-VF-VSK. Volume of skin (VSK) is calculated as VSK = LSK*SASK.
[i]LSK is average value for thickness of dermis and epidermis (Laurent *et al.,* 2007).

mediated by GST enzymes (Lilly *et al.,* 1997b; Allis *et al.,* 2002; Ross and Pegram, 2003, 2004). Based on *in vitro* data, kinetics for the CYP pathway and the GST pathway in the liver were described as saturable (Michaelis–Menten) and first order, respectively (Lilly *et al.,* 1997a; Allis *et al.,* 2002; Ross and Pegram, 2004). Metabolism parameters (V1CBDCM, KM1BDCM) for the CYP pathway were determined based on the microsomal metabolism studies described in the previous section. The clearance parameter for the GST pathway (VFCBDCM) was derived from *in vitro* cytosolic clearance of BDCM estimated using pooled liver human cytosol (Ross and Pegram, 2003).

The *in vitro* metabolism rate for the CYP pathway (V1CBDCM) and clearance for the GST pathway (VFCBDCM) were scaled to the whole liver (assuming a liver fraction of BW as 0.026) and subsequently scaled to BW$^{0.75}$ in the PBPK model (Kenyon, 2012; Lipscomb and Poet, 2008). *In vitro* biotransformation rate data expressed as mass per time-mg microsomal (MMPGL) or cytosolic (MCPGL) protein per gram of liver were scaled to a rate for whole liver (LR) *in vivo* based on the following equation:

$$\mathrm{LR} = \mathrm{Vmax}(\mathrm{mass/time\ mg\ \ protein}) \times \mathrm{MMPGL}(\mathrm{mg/g\ liver}) \times \mathrm{LW}(\mathrm{g/kg\ BW}) \quad (1)$$

In vitro hepatic clearance (LC$_i$) in units of volume/time-mg microsomal or cytosolic protein was similarly scaled to the whole liver using the equation:

$$\mathrm{LC} = \mathrm{LC_i}(\mathrm{volume/time\ mg\ \ protein}) \times \mathrm{MCPGL}(\mathrm{mg/g\ \ liver}) \times \mathrm{LW}(\mathrm{g/kg\ \ BW})$$

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Table 2. Chemical-specific parameters in the human bromodichloromethane (BDCM) model

Parameter Symbol	Symbol	Value			Footnote
		Male	Female	Average	
Partition coefficients, unitless					
Blood:Air	PBBDCM	17.33	14.61	15.97	a
Liver:Blood	PLBDCM	1.77	2.09	1.93	b
Gut:Blood	PGBDCM	1.77	2.09	1.93	c,b
Kidney:Blood	PKBDCM	1.90	2.25	2.08	b
Fat:Blood	PFBDCM	30.35	36.00	33.2	b
Skin:Blood	PSKBDCM	2.68	3.18	2.91	d,b
RPTG:Blood	PRPBDCM	1.77	2.09	1.93	c
PPTG:Blood	PPPBDCM	0.72	0.85	0.78	b
Skin diffusion coefficient, cm/h	KBDCM	0.18			e
Skin:water partition coefficient	PWSBDCM	5.6			e
Oral absorption coefficient, h^{-1}	KABDCM	8.3			f
Vmax CYP Liver, μg/h-kg $BW^{0.75}$	V1CBDCM	4.13×10^4			a
KM CYP Liver, μg/L	KM1BDCM	221			a
Kf GST Liver, 1/h-kg $BW^{0.75}$	VFCBDCM	0.0079			g

[a]Experimentally determined in this laboratory. See Methods for details.
[b]Calculated by dividing rat tissue:air partition coefficient (Lilly *et al.,* 1997a) by human blood:air partition coefficient.
[c]Gut:air and rapidly perfused tissue:air partition coefficients were assumed to be the same as liver:air.
[d]Skin:air partition coefficient (Haddad *et al.,* 2006) used with human blood air partition coefficient to calculate skin:blood partition coefficient.
[e]Skin diffusion coefficient determined with method using aqueous solution across human skin (Xu *et al.,* 2002). Skin:water partition coefficient calculated on basis of water:air partition coefficient (Batterman *et al.,* 2002) divided by skin:air partition coefficient (Haddad *et al.,* 2006).
[f]Estimated on basis of Tmax from oral time course data of Leavens *et al.* (2007). See text for details.
[g]Estimated from in vitro clearance of BDCM from pooled human liver cytosol (Ross and Pegram, 2003).

Scaling factors used for MMPGL and MCPGL were 32 mg microsomal protein per g liver and 80 mg cytosolic protein per g liver, respectively (Barter *et al.,* 2007; Cubitt *et al.,* 2009). The *in vitro* K_M (KM1BDCM) was divided by the liver: blood partition coefficient in order to express the concentration in venous blood at equilibrium with the liver, rather than in the liver itself; this assumes that the concentration in the *in vitro* suspension adequately represents the concentration in the liver resulting in the half-maximal rate of metabolism and adjusts the *in vitro* KM to reflect the concentration of BDCM in blood at an equilibrium with the liver (USEPA *et al.,* 2005).

Model evaluation. The published literature was searched to identify studies that had sufficient information to allow simulation of exposure and comparison to pharmacokinetic data in humans. Data from these studies with various water use scenarios were used to evaluate the ability of the already parameterized model to predict the BDCM blood concentration (Backer *et al.,* 2000, 2008; Lynberg *et al.,* 2001; Nuckols *et al.,* 2005; Leavens *et al.,* 2007; Silva *et al.,* 2013). These studies are summarized in Table 3. Subject- and study-specific input parameters were used when available. If subject-specific data were not available, average values from the *Exposure Factors Handbook* (USEPA, 2011) were used that corresponded to available demographic information for the age range or gender-specific information provided in the studies from which the data was drawn.

For showering scenarios, if air concentration data were unavailable, they were estimated based on water concentration and scenario-specific information such as water flow rate, temperature and air exchange rates using a showering model (Tan *et al.,* 2006). Study-specific showering parameters were used when available or default values were based on Tan *et al.* (2006). Parameters (from Tan *et al.,* 2006 or 2007 in parentheses) in the showering model were the number of air changes per hour (air exchange 15), the volume of the shower in liters (VOLSHWR 2400), water temperature in°C (temp 37), water flow rate in liters per hour (qw, 454.2, 50th percentile) and mass transfer coefficient of BDCM in liters per hr (KOLA, 504). For bathing scenarios, if air concentration data were not provided in the original publication, they were estimated based on published data for the observed relationship between water and air concentration for BDCM under controlled bathing conditions (Kerger *et al.,* 2000). Pre-exposure BDCM blood levels reported in these environmental exposure studies were incorporated into the PBPK model as background initial concentration in the blood. Given the assumptions sometimes necessary to simulate water use scenarios and the aggregate reporting of data in household water use studies, if model predictions fell within twofold or close to the reported range of BDCM blood concentrations, then model predictive ability can be considered adequate.

For the Leavens *et al.* (2007) study, individual subject (body weight, height and fat volume) and time-course data for BDCM in venous blood were available after precisely controlled and quantified oral and dermal exposures. In such a situation, the model's overall ability to predict the experimental data can be effectively visualized for an entire group of subjects by plotting the model-predicted values on the *x*-axis and the observed values on the *y*-axis. If the PBPK model exactly predicted the observed data,

Table 3. Study description and sources for bromodichloromethane (BDCM) physiologically based pharmacokinetic (PBPK) model evaluation data

Reference	Exposure Description	BDCM in water (μg/l)	BDCM in air (μg/m^3)	Subjects	Data Description
Leavens *et al.*, 2007[a]	Dermal exposure of arm and forearm, 1 hr	60.4 34-134	n/a	N = 8-10 adults, 9 male, 1 female	Whole blood BDCM time course during and following exposure for individual subjects
	Oral exposure single 0.25 L drink	43.0 24.1-76.7	n/a	N = 10 adults, 9 male, 1 female	Whole blood BDCM time course during and following exposure for individual subjects
Backer *et al.*, 2000[b]	Showering, 10 min, 7.2 L/min	6.27 ± 0.686	43.3 (11.3)	N = 10, adult male and female	Whole blood BDCM, pre-exposure and 10 and 30 min post exposure
	Bathing, 10 min	6.22 ± 0.713	3.67	N = 11, adult male and female	
	Drinking, 1 L in 10 min	5.52 ± 0.204	n/a	N = 10, adult male and female	Whole blood BDCM, pre-exposure 10 and 60 min post exposure
Lynberg *et al.*, 2001[c]	Showering (GA)	13.5 (12.4 - 16)	93.3 (24.3)	N = 25, adult females	Whole blood BDCM, pre-showering and post showering (~30 min apart)
	Showering (TX	12.2 (8.8 - 12.8)	84.3 (22.0)	N = 25, adult females	
Nuckols *et al.*, 2005[d]	Showering (NC)	32	54 (57.6) [221]	N = 4 adults, 3 male and 1 female	Whole blood BDCM, 5 min pre-exposure and post-exposure
	Bath (NC)	25	12 (13.6)		
	Showering (TX)	12	23 (19.8) [76.0]	N = 3 adults, 1 male and 2 female	
	Bath (TX)	9	7 (5.3)		
Backer *et al.*, 2008[e]; Silva *et al.*, 2013	Showering, 10 min, 40°C, 5.6 – 6.7 L/min	21 (18 – 24)	70.9 (35.8) [138]	N = 99 adults, males and females 18-44 yrs old	Whole blood BDCM pre-exposure and 10 and 30 min post showering

[a]BDCM in water is study mean and range. Individual water concentration and subject-specific data (weight, height, and percent body fat) were available and used for simulations.
[b]BDCM in water is mean ± SD. For showering, air concentration estimated using showering model; Kerger *et al.* (2000) UEC-based estimated in (). Bathing scenario air concentration simulated using Kerger *et al.* (2000) UEC-based estimate. Blood BDCM concentration provided as median and 10th, 25th, 75th and 90th percentiles (from figure in paper).
[c]BDCM in water is median and interquartile range (IQR). Data not available on length of shower or shower flow rate. Air concentration estimated using showering model with Kerger *et al.* (2000) UEC-based estimated in (). Blood BDCM concentration provided as median and IQR.
[d]BDCM in water is median. Duration in shower or bath was 10 minutes. Median air concentrations measured during showering and bathing in this study were used in simulations. Air concentrations estimated by Kerger *et al.* (2000) UEC method are in () and shower-model based estimates are in [square brackets]. Blood BDCM concentration provided as median and range.
[e]BDCM in water is median and IQR. The median air concentration and IQR were 75.0 and 59.1 – 86.2 μg/m^3, respectively based on a 10-minute integrated shower stall air sample (Backer *et al.*, 2008. Air concentrations estimated by Kerger *et al.* (2000) UEC method are in () and shower-model based estimates are in [square brackets]. Blood BDCM data provided as geometric mean and 95% confidence interval in da Silva *et al.* (2013).

such a 'scatter plot' would result in a so-called unity line, a straight line with a 0.0 intercept and slope of 1.0 (Kenyon *et al.*, 2008).

Model application. Two water use activities – drinking a liter of water over a 10-min period or showering for 10 min – were simulated for water containing the same concentration of BDCM (10 or 30 ppb) to compare different measures of internal dose. The PBPK model was also used together with a liter-equivalency (L-eq) type methodology (Krishnan and Carrier, 2008), to estimate the equivalent drinking water concentration in μg l^{-1} necessary to achieve the same internal dose metrics for a 10-min showering or 20-min bathing exposure to 10 ppb (μg l^{-1} BDCM) in water. For purposes of this analysis, airborne concentrations of BDCM were calculated on the basis of the unit exposure concentration (UEC, i.e. μg m^{-3} per μg l^{-1}) relationships for BDCM reported by Kerger *et al.* (2000) in their field studies of residential showering and bathing. The purpose of this type of analysis is to evaluate the contribution from showering and bathing (inhalation plus dermal) exposures compared with oral exposure for specific internal dose measures of toxicological relevance. The selected exposures represented typical daily activities, e.g. an individual showering or bathing event compared to oral consumption of a liter of water. Dose

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metrics evaluated included the maximum concentration of BDCM (Cmax) and AUC for BDCM in venous blood, as well as total amount of BDCM metabolized in the liver per hour (MBDCM). In addition, the separate contribution of dermal or inhalation exposure alone during showering or bathing was evaluated for the L-eq analysis.

Sensitivity analysis comparison

GSA was performed in AcslXtreme 3.0.2.1 using the Morris method (Morris, 1991) to provide a coarse ranking of importance of all model parameters. To implement the Morris GSA method in AcslXtreme, it is necessary to set ranges for input parameters that are allowed to vary under the assumption of a uniform distribution. For physiological parameters, partition coefficients, and the dermal absorption coefficient, ranges were set as ± one standard deviation (SD) from the average value used in the model (Tables 1 and 2) assuming a coefficient of variation of 30%. This assumption has been used in reverse dosimetry applications of PBPK models (e.g. Tan *et al.,* 2007). V_{max} and K_m for the CYP pathway were bounded at four- and two-fold variation surrounding the point estimates, respectively, based on data from Leavens *et al.* (2007) and Zhao and Allis (2002), respectively. In the case of the oral absorption coefficient (KABDCM), upper and lower bounds were set to correspond to the values obtained when T_{max} was set to 5 or 30 min which is the range reported in Leavens *et al.* (2007). The Morris method as implemented in AcslXtreme assumes a uniform distribution which is appropriate for a screening level analysis. Algorithmic settings used in the analysis were 100, 25 and 1000, for p, jump, and Ns, respectively. P is the number of values in discretized parameter range (divides up parameter range into p-1 ranges or hypercubes); jump is the step size in computing effects (effectively computing a number of local sensitivities); and Ns is the number of samples (AEgisTechnologies, 2010). These algorithmic settings were selected to optimize analysis performance, i.e. multiple test runs were done until no changes were seen in the overall ranking.

As LSA has historically been more commonly applied to PBPK models (Schlosser, 1994), LSA was performed to compare screening level GSA results. LSA utilized the central difference method with sensitivity coefficients (SC) normalized to both parameter and response, and parameters were categorized as having low ($SC < 0.2$), medium ($0.2 < SC < 0.5$) or high sensitivity ($SC > 0.5$) to the response being evaluated based on the highest absolute value of the SC (Clewell *et al.,* 1994).

Responses evaluated included the BDCM venous blood concentration (CvBDCM), concentration in exhaled breath (Calv), area under the curve (AUCv) for BDCM in blood and total BDCM metabolized in the liver per hour (MBDCM); the first two responses were selected because they are measured in some human exposure studies and the second two are toxicologically relevant internal dose metrics. Simulation conditions for sensitivity analysis were selected to approximate a typical environmental exposure level of 10 $\mu g\,l^{-1}$ (ppb) BDCM in water for both oral (single 0.25-l drink) and showering (10 min) exposure scenarios.

Results

Model parameterization – experimental data

Human blood: air partition coefficients were 17.33 ± 0.68 and 14.61 ± 0.76 for males and females, respectively, and the difference was statistically significant ($P < 0.001$) between genders. Values are expressed as the mean ± SD. For the purposes of model simulations, gender-specific values were used when a study-subject gender was specified; otherwise average values were used (Table 2).

In vitro metabolism parameters, V_{max} and K_m for the human microsomal metabolism of BDCM were 1.74 ± 0.094 nmoles min^{-1} mg microsomal protein (17.14 $\mu g\,h^{-1}$-mg MSP^{-1}) and 2.61 ± 0.55 μM (221 $\mu g\,l^{-1}$), respectively (Fig. 2). Data are expressed as the mean ± standard error. The human microsomes were from a pooled source derived from 200 individuals, and these values should, therefore, provide a reasonable surrogate measure of population central tendency. These values were scaled for use in the PBPK model as described in methods and shown in Table 2.

Model evaluation. The model was parameterized prior to testing its ability to predict the evaluation data which are described in detail in Table 3. The Leavens *et al.* (2007) study is unique in that subject-specific (height, BW and FVF) and exposure scenario-specific (water concentration data and time course measurements of venous blood concentration) were available for individual subjects for controlled exposures. Examples of subject-specific model predictions compared with data are shown in Fig. 3A and B for dermal and oral data, respectively. To concisely display data compared with model predictions for all study subjects using a single set of reference parameters (Tables 1 and 2), the predicted versus observed blood concentrations are plotted in Fig. 4A and B for dermal and oral data, respectively. The line shown in these graphs is the 'unity line' (slope = 1, intercept = 0) along which all data points would fall if there were absolute agreement between observed data and model predictions. For the dermal data (Fig. 4A), overall ~70% of predictions were within threefold of the observed data points with roughly equal scatter above and below the unity line. The model tended to consistently under-predict the data at time points beyond 3 h and for some subjects the predicted peak concentration was lower compared with the data. For the oral data (Fig. 4B), overall 66% of predictions were within threefold of the observed data with the model tending to over-predict the data at earlier time points. In the Leavens *et al.* (2007) study, the

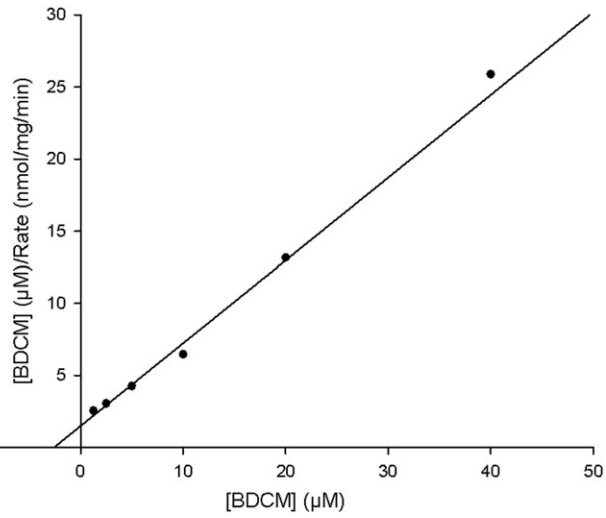
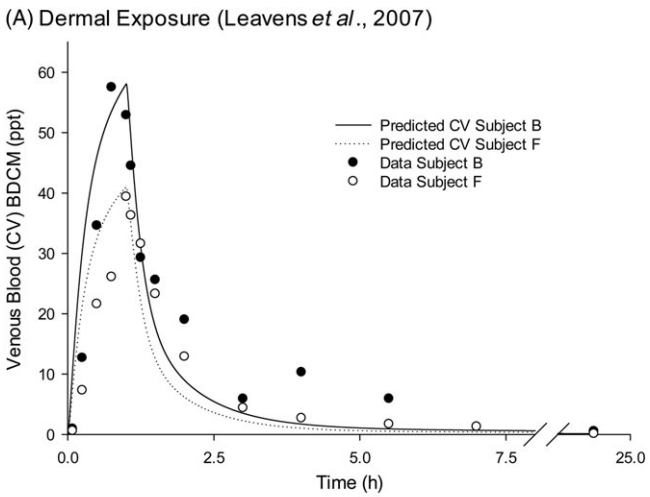


Figure 2. Hanes–Woolf kinetics plot of *in vitro* bromodichloromethane (BDCM) metabolism data over a range of substrate concentrations. A Michaelis–Menten kinetics analysis of these data yielded a Vmax of 1.74 nmoles min^{-1} mg MSP^{-1} and a Km of 2.61 μM. Each data point is the mean initial velocity from two to three replicate metabolism experiments using a pool of human microsomes derived from 200 individuals.



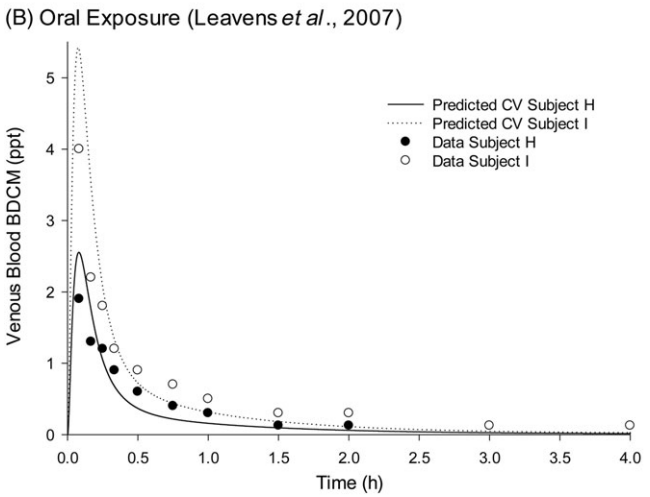
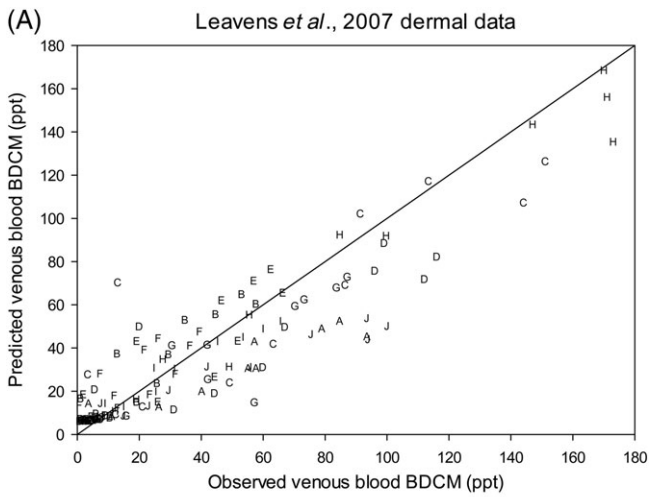


Figure 3. (A) Model predictions (lines) compared with data (symbols) for venous blood concentration (ppt) data from Leavens *et al.* (2007) for selected subjects with an arm immersed in water containing bromodichloromethane (BDCM) for 1 h. (B) Model predictions (lines) compared with data (symbols) for venous blood concentration (ppt) data from Leavens *et al.* (2007) for selected subjects ingesting BDCM in water as a single 0.25-l drink.



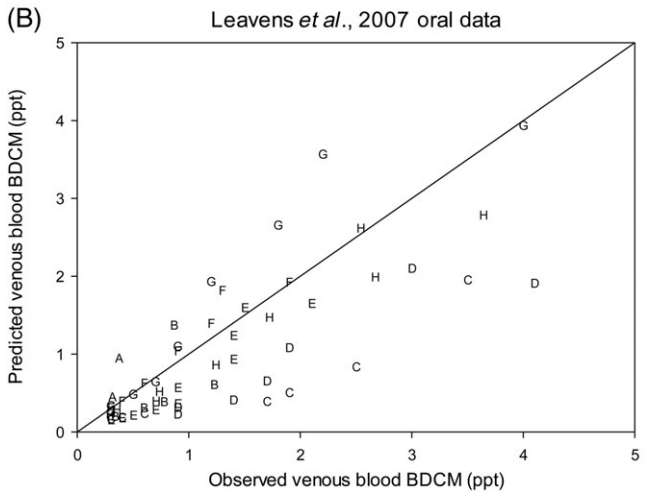


Figure 4. (A) Model predicted versus observed venous blood concentration (ppt) data from Leavens *et al.* (2007) for subjects with an arm immersed in water containing bromodichloromethane (BDCM) for 1 h. Letters correspond to individual subject data ($n = 10$) at different time points. The solid line is the 'unity line' along which all points would align if there was absolute agreement between model-predicted and experimentally observed venous blood concentrations. Points above the line represent instances where the model is over-predicting the data and points below the line represent instances where the model is under-predicting the data. (B) Model predicted versus observed venous blood concentration (ppt) data from Leavens *et al.* (2007) for subjects ingesting BDCM in water as a single 0.25-l drink. Letters correspond to individual subject data ($n = 8$) at different time points. The solid line is the 'unity line' along which all points would align if there was absolute agreement between model-predicted and experimentally observed venous blood concentrations. Points above the line represent instances where the model is over-predicting the data and points below the line represent instances where the model is under-predicting the data.

antecubital vein was being sampled and the model predictions are for the central venous compartment; some studies have reported that blood concentrations of pharmaceuticals in peripheral veins may differ markedly from both the central venous and arterial blood concentrations (Chiou, 1989a, b). In general, given that these model simulations were performed using a single set of reference parameters (Tables 1 and 2) that cannot take into account variation in highly influential parameters (e.g. V1CBDCM and KM1BDCM), the overall fits are adequate.

Additional model evaluation data available in the published literature involves water use activities such as bathing and showering. When the model predictions (using single point central tendency parameters, Tables 1 and 2) are compared with data and data ranges for these studies (Table 4), generally the model predictions fall either very close to or within the range of the data (Backer *et al.,* 2000, 2008; Lynberg *et al.,* 2001) or are at least within a factor of two or three-fold (Nuckols *et al.,* 2005). In the case of the Nuckols *et al.* (2005) data, the model consistently over-predicted the data, which may be a consequence of there being a degree of uncertainty in interpreting the actual sampling time post-showering or bathing based on the study description. Simulating water use scenarios typically involves both uncertainty and variability that are not well captured by a single reference set of model parameters, e.g. (i) individual subject data that would be desirable (e.g. height and body weight) are often unavailable, which necessitates use of population averages; (ii) for showering and bathing scenarios, air concentration data were sometimes not available and had to be estimated using a shower model with varying

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Table 4. Model evaluation: predictions compared with data (venous blood BDCM) for environmental BDCM exposures[a]

Study	Activity	Data (ppt)	Model Point Prediction (ppt)
Backer *et al.,* 2000[b]	Showering	19.4 (15-26)	30.0
		10.3 (8-14)	11.2
	Bathing	17 (7.5–28)	31.9
		9.9 (4-16)	11.6
	Drinking	3.8 (2.5-7)	1.8
		2.8 (2-5)	0.74
Lynberg *et al.,* 2001	Showering (GA)	38 IQR: 26-69	65.6
	Showering (TX)	43 IQR: 31-60	59.8
Nuckols *et al.,* 2005 (NC)[c]	Showering	93 (64-95)	145
	Bathing	41 (40-43)	175
Nuckols *et al.,* 2005 (TX)[c]	Showering	28 (26-31)	52.6
	Bathing	36 (26-65)	69.9
Backer *et al.,* 2008; Silva *et al.,* 2013[d]	Showering	69 IQR: 54-88	81.9
		32.6 (31.6-33.7)	29.2

[a]Studies are described in greater detail in Table 3. Tap water and air measurements from individual studies were used where reported. Showering lasted ~10 min in most studies with post exposure blood samples collected at 10 and 30 or 60 min post exposure. Air concentrations not reported in individual studies were estimated using a showering model (Tan *et al.,* 2007) or based on an equation from Kerger *et al.* (2000) for bathing. Data are median BDCM in venous blood. IQR is interquartile range.
[b]Data are 10 and 30 min post exposure for showering and bathing exposures and 10 and 60 min post exposure for oral studies. Values for range are 10th to 90th percentiles of data.
[c]Values in parentheses are ranges.
[d]Median and IQR provided in Backer *et al.* (2008) for 10 min post shower. Values for 30 min post shower are geometric mean values with 95% confidence interval in parentheses from Silva *et al.* (2013).

availability of necessary and influential input parameters (e.g. air exchange and shower volume); and (iii) the impact of variability in the air and water concentration as well as showering/bathing parameters are not captured.

Model application. Differences in three measures of internal dose are compared for three common water use activities (drinking, bathing and showering) for two BDCM water concentrations in Fig. 5. At both common (5 ppb) and realistically higher (10 ppb) exposure levels, much more parent chemical reaches the systemic circulation with combined inhalation and dermal exposure (showering) compared with oral exposure (Cmax, AUCv), whereas the opposite is true for the hepatic metabolism of BDCM. Using the L-eq methodology, to produce by oral exposure on a liter equivalent basis, the same internal doses that are predicted to result from a 20-min bathing exposure to water containing 10 $\mu g\,l^{-1}$ BDCM would require the drinking water to contain 605, 803 and 5 $\mu g\,l^{-1}$ BDCM for Cmax, AUCv, and MBDCM, respectively. For a 10-min showering exposure to BDCM, the oral equivalent water concentrations would be 282, 312 and 2.1 $\mu g\,l^{-1}$ for Cmax, AUC and MBDCM, respectively. Fig. 6 indicates the contribution of inhalation exposure and dermal exposure routes for both bathing and showering. Inhalation contributes far less to internal dose compared with dermal for both bathing and showering. The contribution from inhalation is in the range of 5–7% for bathing and 18–25% for showering; this difference reflects the relatively greater

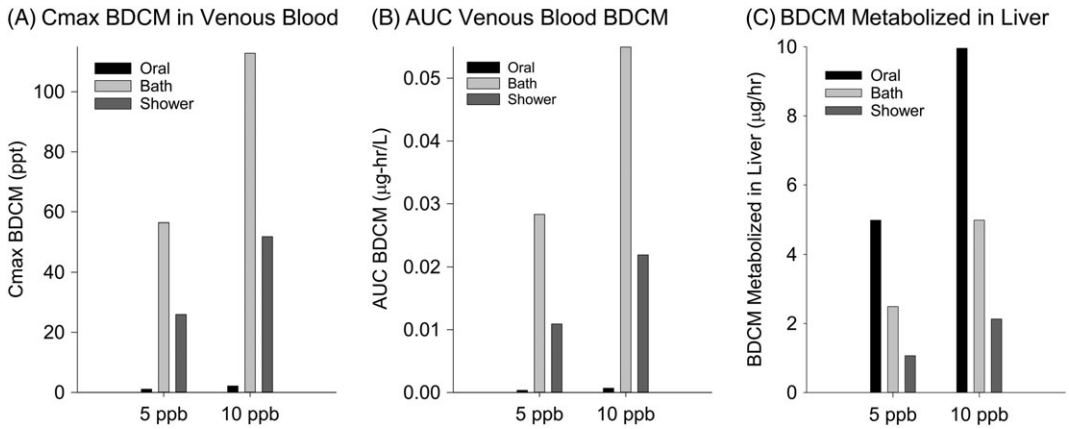

Figure 5. Internal dose metric comparison for water use activities drinking, bathing and showering using water containing 5 or 10 ppb ($\mu g\,l^{-1}$) bromodichloromethane (BDCM). Drinking event assumes 1 l of water consumed over a 10-min period, showering and bathing events assume 10- and 20-min exposure (dermal + inhalation), respectively, with a total simulation time of 1 h for all activities. BDCM air concentrations for bathing and showering scenarios estimated based on Kerger *et al.* (2000) UEC relationships.

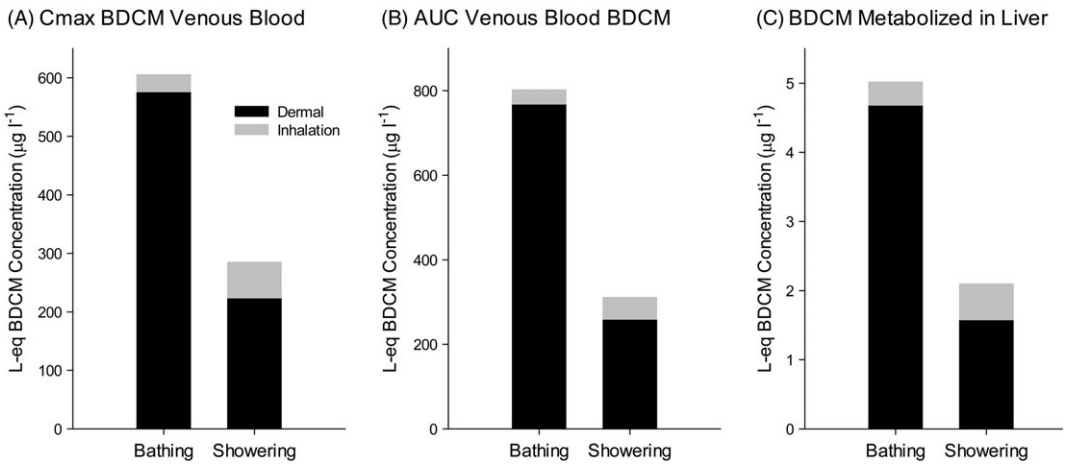

Figure 6. Liter Equivalency Analysis – ingested water concentration (assuming 1 l of water consumed) required to produce the same value for the dose metrics, maximum concentration of bromodichloromethane (BDCM) in venous blood (A), area under the curve for BDCM in venous blood (B), and amount of BDCM metabolized in liver per hour (C), resulting from a 20-min bathing or 10-min showering event with 10 ppb ($\mu g\,l^{-1}$) BDCM in water. The individual contributions of inhalation and dermal routes of exposure are represented as stacked bars.

volatilization (and hence air transfer) that occurs during showering compared with bathing (Kerger *et al.,* 2000).

Local and global sensitivity analysis comparison

Results for LSA are shown in Tables 5 and 6 for oral exposure and showering exposure to 10 $\mu g\,l^{-1}$ BDCM in water, respectively. Parameters are ranked on the basis of the maximum value of the SC achieved over the course of the simulation and further categorized as high, medium, or low influence based on criteria proposed by Clewell *et al.* (1994), i.e. high – $SC > 0.5$, medium – $0.2 > SC < 0.5$ and low – $SC < 0.2$. For oral exposure (Table 5), there was considerable overlap in parameters ranked as a high influence for all of the responses evaluated; these included parameters describing alveolar ventilation (QPC) and QPC to cardiac output ratio (RQPCO) and gut-related parameters for blood flow (FQG), volume (FVGI) partitioning (PGBDCM) and absorption (KABDCM). For oral exposure, the parameters for CYP metabolism (V1CBDCM and KM1BDCM) and liver partitioning (PLBDCM) were also a high influence for the concentration of BDCM in venous blood and exhaled breath, as well as AUC for BDCM in venous blood.

In the case of showering exposure for LSA, the parameters having a high influence for venous blood and exhaled breath BDCM concentration (Table 6), were the blood: air partition coefficient (PBBDCM), QPC to cardiac output ratio (RQPCO), alveolar ventilation (QPC), parameters related to skin for blood flow (QSKSA), thickness (LSK), partitioning (PSKBDCM), absorption (KBDCM) and fractional skin surface area exposed (FSASK), as well as shower stall volume and blood flow to the GI tract (FQG). For BDCMAUC in venous blood under the showering scenario (Table 6), the parameters ranked as high influence were the QPC to cardiac output ratio (RQPCO), fractional skin surface area exposed (FSKSA) and skin absorption (KBDCM). In the case of the amount of BDCM metabolized in the liver (MBDCM), the high influence parameters were alveolar ventilation (QPC), cardiac output (RQPCO), shower volume (VOLSHWR), fractional blood flow to the gut (FQG) and fractional skin surface area exposed (FSASK).

Quantitative results for GSA using the Morris screening method are illustrated graphically in Figs. 7 and 8 for oral and showering exposure to 10 $\mu g\,l^{-1}$ BDCM in water, respectively. In these figures, the mean SC for each parameter (averaged over the time period of the simulation) is plotted on the *x*-axis and the corresponding SD is plotted on the *y*-axis as a means to display the overall results of screening level GSA (McNally *et al.,* 2011). The advantage of this presentation format is that it provides a better quantitative sense of how parameters compare to each other. For oral exposure, the parameters that were clearly influential for both BDCM concentration in venous blood and AUC were those governing absorption (KABDCM), liver partitioning and metabolism via the CYP pathway (PLBDCM, V1CBDCM and KM1BDCM). These same parameters, as well as the blood: air partition coefficient were influential for exhaled breath BDCM concentration, and oral absorption (KABDM) stood out from other parameters as influential for the amount of BDCM, metabolized in the liver (MBDCM). In the case of showering exposure, a number of parameters were highly influential for the various responses. For BDCM concentration in venous blood and AUC, there was considerable overlap in the influential parameters, i.e. FSASK, RQPCO, KBDCM, QPC, QSKSA, FQG and VOLSHWR. The same parameters were also influential for exhaled breath BDCM with the addition of blood: air partition coefficient (PBBDCM) as most influential. For amount of BDCM metabolized in liver (MBDCM), the influential parameters were fractional skin surface area exposed (FSASK), dermal absorption coefficient (KBDCM), alveolar ventilation (QPC), fractional blood flow to the gut (FQG) and showering parameters [VOLSHWR, air exchange and mass transfer coefficient (KOLA)]. In general, parameters ranked in the top one-third based on the Morris GSA (highlighted in darkest gray in the tables) were also ranked as high influence based on the LSA. Conversely, parameters ranked in the bottom one-third based on the Morris GSA, were also ranked as a low influence in the LSA.

Discussion

Multi-route human PBPK models have been published in which BDCM was one of multiple THMs or volatiles in the model. These models were developed to interpret biomonitoring data (Tan *et al.,* 2007) and for indoor exposure assessment (Haddad *et al.,* 2006), whereas the emphasis of our modeling effort is on the contribution of various routes of exposure to internal dose metrics that could be relevant to adverse outcomes associated with exposure to DBPs in epidemiologic studies (Cantor *et al.,* 1987, 1998, 2010). The scientific and biological plausibility of this model was

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Table 5. Local sensitivity analysis parameter ranking – oral exposure, single 0.25-l drink of water containing 10 ppb bromodichloromethane (BDCM)[a]

Response: CvBDCM		Response: Calv		Response: AUCv		Response: MBDCM	
Parameter	Rank	Parameter	Rank	Parameter	Rank	Parameter	Rank
FQG	1.39	FQG	1.39	RQPCO	-1.46	KABDCM	0.93
RQPCO	-1.30	RQPCO	-1.35	QPC	1.46	PGBDCM	-0.80
QPC	1.30	QPC	1.30	FQG	1.46	FQG	0.79
V1CBDCM	-1.00	V1CBDCM	-1.00	PLBDCM	-1.00	FVGI	-0.79
KM1BDCM	1.00	KM1BDCM	1.00	V1CBDCM	-1.00	QPC	0.79
PLBDCM	-0.99	PLBDCM	-0.99	KM1BDCM	1.00	RQPCO	-0.79
KABDCM	0.92	PBBDCM	0.95	KABDCM	0.94	Deadspace	-0.25
PGBDCM	-0.77	KABDCM	0.92	PGBDCM	-0.84	V1CBDCM	0.05
FVGI	-0.75	PGBDCM	-0.77	FVGI	-0.83	KM1BDCM	-0.05
FVK	-0.54	FVGI	-0.75	FVBD	-0.60	FVL	0.04
FVBD	-0.48	FVBD	-0.48	FVK	-0.48	PLBDCM	0.01
Deadspace	-0.41	Deadspace	-0.41	Deadspace	-0.46	FQL	>0.01
PPPBDCM	-0.37	PPPBDCM	-0.37	FQL	0.35	PPPBDCM	>0.01
FQL	0.35	FQL	0.35	PPPBDCM	-0.14	PBBDCM	>0.01
FQF	-0.33	FQF	-0.33	PRPBDCM	-0.14	FQF	>0.01
PRPBDCM	0.23	PRPBDCM	0.23	FQF	-0.10	PRPBDCM	>0.01
PBBDCM	0.19	FVL	-0.11	PBBDCM	0.07	FVBD	>0.01
FVL	-0.11	FQK	0.07	FVL	0.07	FVF	>0.01
FQK	0.07	FVF	0.05	FQK	0.06	FQK	>0.01
FVF	0.05	QSKSA	-0.03	PKBDCM	-0.03	FVK	>0.01
QSKSA	-0.03	PFBDCM	-0.03	FVF	0.02	PKBDCM	>0.01
PFBDCM	-0.03	PKBDCM	-0.03	QSKSA	>0.01	QSKSA	>0.01
PKBDCM	-0.03	FVK	-0.03	PSKBDCM	>0.01	PFBDCM	>0.01
FSASK	-0.02	FSASK	-0.02	LSK	>0.01	PSKBDCM	>0.01
LSK	0.02	LSK	0.02	PFBDCM	>0.01	LSK	>0.01
PSKBDCM	0.01	PSKBDCM	0.01	FSASK	>0.01	FSASK	>0.01
VFCBDCM	>0.01	VFCBDCM	>0.01	VFCBDCM	>0.01	VFCBDCM	>0.01
VLUM	>0.01	VLUM	>0.01	VLUM	>0.01	VLUM	>0.01

[a]Parameters are ranked and shaded (dark to light) as high, medium or low influence based on criteria proposed by Clewell *et al.* (1994), i.e. high – SC > 0.5, medium – 0.2 < SC < 0.5, and low SC < 0.2. The highest absolute value of the SC achieved over the course of the simulation was used to rank the parameters.

improved and strengthened by including more chemical-specific parameters that were determined experimentally using human tissues and data. These included skin permeation, oral absorption and metabolism parameters, as well as male and female human blood: air partition coefficients. The use of human metabolism data in this model eliminates the uncertainties related to species differences present in previous models that relied upon rodent metabolism parameters. Obtaining gender-specific partition coefficients reduced uncertainty in these parameters for individual male and female subjects from the Leavens *et al.* (2007) study. In addition, human data from diverse sources unavailable at the time earlier models were published were used for model evaluation, and global sensitivity analysis was applied to the model.

The measured human blood: air partition coefficients in this study (males: 17.33 ± 0.68; females: 14.61 ± 0.76) reflect interindividual variation because data from different subjects were used. Our measurements are about 40% lower than those (26.6 ± 1.4) reported in another study (Batterman *et al.,* 2002) that also used the vial equilibration method. However, owing to limited details (Batterman *et al.,* 2002), it is unclear whether the reported SD represents experimental variation in measurement versus interindividual variation. In addition, the fasted versus fed status of the subjects providing the blood is not known for the Batterman *et al.* (2002) study, whereas the subjects providing the blood in our study had blood samples taken in the morning prior to eating which could result in lower lipid levels in the blood. For 1,3-butadiene, it has been demonstrated that the blood: air partition coefficient can be increased 20–40% in humans with borderline high triglyceride levels after ingesting a standardized milkshake (Lin *et al.,* 2002). Interestingly, Lilly *et al.* (1997a) reported a blood: air partition coefficient of 31.4 in male F344 rats, and rat blood: air partition coefficients of di- and tri-haloalkanes were 2.1 ± 0.4-fold higher than those of humans (Gargas *et al.,* 1989). The rat: human ratio using the present data is 1.8 for males and 2.1 for females.

The scaled *in vitro* Vmax and Km were derived experimentally from a human hepatic microsomal pool of 200 subjects and thus can be considered a surrogate measure of population central tendency for metabolism via the CYP pathway. Given that venous blood BDCM concentration (CvBDCM) is sensitive to changes in the rate of BDCM metabolism via the CYP pathway (Table 5), the Leavens *et al.* (2007) oral data could also reasonably be used to estimate the CYP Vmax (V1CBDCM). If one performs this exercise, i.e. optimizing V1CBDCM using individual subject oral data while holding all other parameters constant, the result is 1.76×10^4 μg h^{-1} kg BW

Table 6. Local sensitivity analysis parameter ranking – showering exposure, 10 min in water containing 10 ppb bromodichloromethane (BDCM)[a]

Response: CvBDCM		Response: Calv		Response: AUCv		Response: MBDCM	
Parameter	Rank	Parameter	Rank	Parameter	Rank	Parameter	Rank
RQPCO	1.00	RQPCO	0.95	RQPCO	0.79	QPC	1.27
QSKSA	-0.84	PBBDCM	0.95	FSASK	0.75	VOLSHWR	0.88
QPC	-0.82	QSKSA	-0.84	KBDCM	0.74	FQL	0.74
FSASK	0.78	VOLSHWR	-0.83	QSKSA	0.73	FQG	0.66
FQG	-0.74	QPC	-0.82	PSKBDCM	-0.72	FSASK	0.55
KBDCM	0.70	FSASK	0.78	LSK	-0.72	KBDCM	0.51
PSKBDCM	-0.67	FQG	-0.74	FVBD	-0.52	RQPCO	-0.51
LSK	-0.67	KBDCM	0.70	QPC	-0.50	KOLA	0.46
FVK	-0.49	LSK	0.62	FQG	-0.45	QW	0.41
VOLSHWR	-0.45	PSKBDCM	0.60	VOLSHWR	-0.43	Deadspace	-0.40
FQL	-0.42	Airexchange	-0.45	FQL	-0.25	Airexchange	-0.36
FVBD	-0.41	KOLA	0.44	PRPBDCM	-0.23	FVGI	-0.29
FQF	-0.40	FQL	-0.42	KOLA	0.23	QSKSA	0.29
PPPBDCM	-0.32	FQF	-0.40	FQK	0.22	PGBDCM	-0.27
Airexchange	-0.27	QW	0.39	Airexchange	-0.22	PSKBDCM	-0.26
Deadspace	0.26	PPPBDCM	-0.32	QW	0.21	LSK	-0.26
PRPBDCM	0.25	Deadspace	0.26	Deadspace	0.16	PRPBDCM	-0.14
KOLA	0.24	PRPBDCM	0.25	FVL	0.12	PBBDCM	0.12
FQK	0.24	FVBD	-0.25	PKBDCM	-0.11	FVBD	0.10
QW	0.21	FVL	-0.13	PPPBDCM	-0.10	PPPBDCM	-0.08
PBBDCM	0.19	FQK	-0.07	FQF	-0.10	FQF	-0.08
FVL	0.13	FVGI	-0.06	PBBDCM	0.09	FVL	0.07
PKBDCM	-0.12	PFBDCM	-0.04	FVK	-0.06	FQK	0.06
FVGI	-0.06	TEMP	0.03	FVGI	0.06	V1CBDCM	0.06
PFBDCM	-0.04	PKBDCM	-0.03	TEMP	0.02	KM1BDCM	-0.06
TEMP	0.02	FVK	-0.02	FVF	>0.01	PKBDCM	-0.03
FVF	0.02	FVF	0.02	PLBDCM	>0.01	TEMP	0.03
V1CBDCM	-0.01	V1CBDCM	-0.01	V1CBDCM	>0.01	FVK	-0.02
KM1BDCM	0.01	KM1BDCM	0.01	KM1BDCM	>0.01	PLBDCM	0.01
PLBDCM	-0.01	PLBDCM	-0.01	PFBDCM	>0.01	FVF	>0.01
PWSBDCM	>0.01	PWSBDCM	>0.01	PWSBDCM	>0.01	PFBDCM	>0.01
PGBDCM	>0.01	PGBDCM	>0.01	PGBDCM	>0.01	PWSBDCM	>0.01
VFCBDCM	>0.01	VFCBDCM	>0.01	VFCBDCM	>0.01	VFCBDCM	>0.01

[a]Parameters are ranked and shaded (dark to light) as high, medium or low influence based on criteria proposed by Clewell *et al.* (1994), i.e. high – SC > 0.5, medium – 0.2 < SC < 0.5, and low SC < 0.2. The highest absolute value of the SC achieved over the course of the simulation was used to rank the parameters.

with a range of 6.73×10^3 to 3.11×10^4. These data are mutually supportive because the estimate for V1CBDCM derived from the human hepatic microsomal pool data (4.13×10^4 μg h^{-1} kg BW) is close to V1CBDCM estimates based on the Leavens *et al.* (2007) oral individual subject data. Lilly *et al.* (1997a) estimated a BDCM Vmax in rats of 1.28×10^4 μg h^{-1} kg based on *in vivo* plasma bromide levels after constant concentration inhalation exposures, which is within ~ threefold of the estimate based on *in vivo*-scaled *in vitro* human hepatic microsomal data.

This BDCM model also incorporates hepatic first order metabolism via the GST pathway based on data from pooled human hepatic cytosol. Although the hepatic GST pathway itself does not contribute significantly to overall metabolic clearance, mechanistic data and epidemiology findings suggest that tissue-specific metabolism via the GST pathway could significantly contribute to BDCM-induced genotoxicity in target organs. Future studies in our laboratory will address pharmacokinetic and pharmacodynamic linkages in human target tissues of greatest concern and how they compare relative to the liver.

The ability of the BDCM human model to predict data from the literature using a single set of reference parameters was surprisingly good given uncertainties common to data from human water use studies. These uncertainties are related to both exposure and physiology and also highlight the issues inherent in utilizing a single set of parameters to evaluate model predictive ability. On the exposure side, uncertainties in air concentration are relatively greater compared with the water concentration. The reasons are twofold: (i) in some cases air concentration was not measured and must, therefore, be estimated or modeled; and (ii) when the air concentration was measured it was sometimes unclear from the original reports whether or not the samples were taken in the subject's breathing zone, which would be optimal, versus an area sample.

To better understand the issues related to air concentration it is instructive to compare measured versus modeled or estimated air

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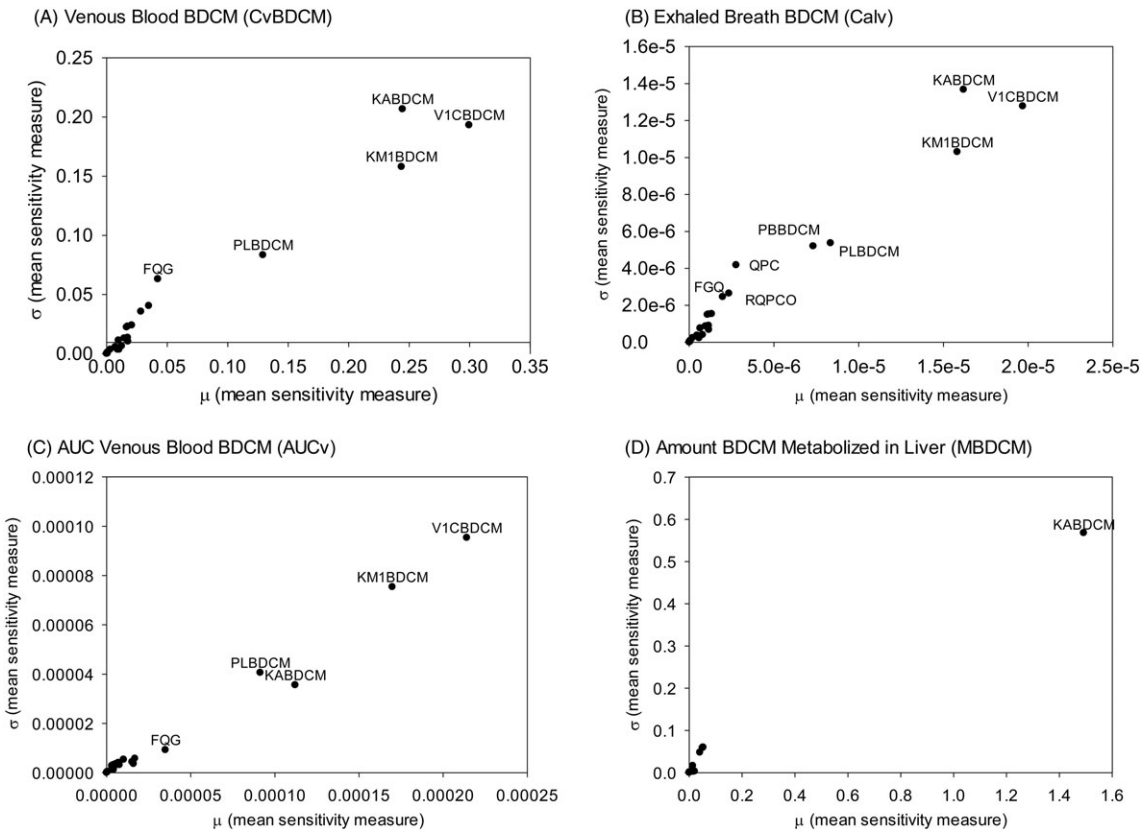

Figure 7. Morris screening level global sensitivity analysis for an oral exposure scenario of a single 0.25-l ingestion of water containing 10 ppb bromodichloromethane (BDCM) for (A) BDCM in venous blood (CvBDCM), (B) BDCM in exhaled breath (Calv), (C) area under the curve for BDCM in blood (AUCv) and (D) total amount of BDCM metabolized per hour (MBDCM).

concentrations for cases in which data are available. For bathing, one can use the UEC approach based on Kerger *et al.* (2000) in comparison to cases where air concentration was measured for bathing scenarios. Doing so reveals that estimated air concentrations are remarkably close to measured air concentrations for bathing scenarios (see Table 3, Nuckols *et al.,* 2005, bathing entries) which increases confidence in the use of these estimates in cases where air concentration data are unavailable (e.g. Backer *et al.,* 2000). In contrast, for showering scenarios, if one compares measured data versus the UEC approach or the shower model-estimated approach, the differences are greater. For example, in the case of the Nuckols *et al.* (2005) data (Table 3), the measured, UEC-approach and shower model estimates for air concentration are 54, 58 and 221 $\mu g\, m^{-3}$, respectively for the North Carolina site, and 23, 20 and 76 $\mu g\, m^{-3}$, respectively for the Texas site. For the data of Backer *et al.* (2008), the differences are even more evident in that the measured, UEC-approach and shower model estimates for air concentration are 70.9, 35.8 and 138 $\mu g\, m–^{3}$, respectively. For the Nuckols *et al.* (2005) data it was not possible to determine the location of air sampling, whereas for the Backer *et al.* (2008) study an integrated 10-min air sample was collected while study participants were showering. The UEC relationships published by Kerger *et al.* (2000) were developed based on air sampling that occurred in the showering or bathing area that would have approximated the human breathing zone. Another source of uncertainty is the use of aggregated exposure and subject data available in published reports, because the correlation between individual measurements of water, air and blood concentrations is lost. Many physiological and chemical-specific parameters that are influential (based on sensitivity analysis) are also often not readily measurable, and variability in these parameters is not captured by model predictions where a single set of reference parameters is used.

The dose metric comparisons and the L-eq analysis presented here to provide perspective on the relative contributions of both different activities and different routes of exposure to various measures of internal dose. The L-eq concentrations for Cmax and AUCv for BDCM in blood are strikingly higher (by approximately 100-fold) compared to the amount of BDCM metabolized in the liver for showering and bathing exposures. Physiologically this difference is driven by first-pass metabolism in the liver and GI mucosa that limits the amount of BDCM reaching the systemic circulation after oral exposure as reflected by internal dose metrics related to hepatic metabolism. In contrast, showering and bathing activities (inhalation and dermal exposure) result in a much greater initial availability of BDCM into the systemic circulation and hence greater distribution to other organs. If BDCM metabolism in extra-hepatic target tissues is necessary for BDCM-induced carcinogenicity, then the magnitude of the contribution of inhalation and dermal exposure to the BDCM internal dose is a significant toxicological issue that needs to be addressed in the context of total exposure.

A striking feature described here for the first time and highlighted by the L-eq analysis is that dermal exposure contributes more heavily to internal BDCM dose during bathing and showering compared with inhalation irrespective of the dose metric (Fig. 6). This prediction is consistent with high levels of BDCM in venous blood observed during and after dermal only exposure in the Leavens *et al.* (2007) study. Evaluation of the contribution to

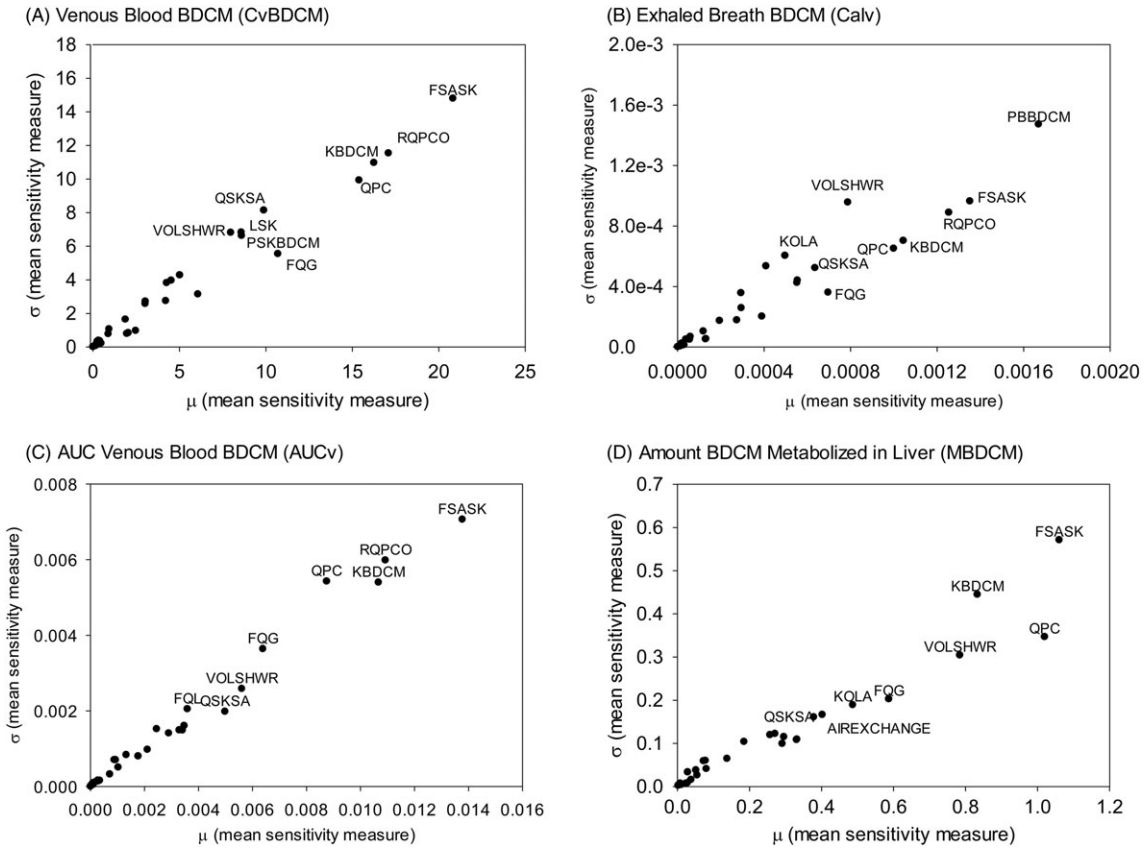

Figure 8. Morris screening level global sensitivity analysis for showering exposure for 10 min in water containing 10 μg l^{-1} bromodichloromethane (BDCM) for (A) BDCM in venous blood (CvBDCM), (B) BDCM in exhaled breath (Calv), (C) area under the curve for BDCM in blood (AUCv) and (D) the total amount of BDCM metabolized per hour (MBDCM).

the internal dose of dermal or inhalation routes of exposure during showering or bathing has been limited (Jo *et al.,* 1990; Xu and Weisel, 2005a, b). In one study directly evaluating the comparative contribution of dermal and inhalation routes of exposure, chloroform was measured in exhaled breath during inhalation-only showering compared with normal showering. In this study, Jo *et al.* (1990) estimated approximately equal contributions of inhalation and dermal routes of exposure to the chloroform internal dose. This difference between chloroform and BDCM is reasonable given that chloroform is more volatile and less permeable to skin than BDCM. The large contribution of dermal exposure to internal dose predicted during showering or bathing also raises the issue of the internal exposure to BDCM, as well as other THMs, during other common activities that result in dermal exposure for longer periods than typical showering and bathing activities such as swimming in disinfected pool water and hot tub use (Zwiener *et al.,* 2007).

Comparison of the results of local and global sensitivity analysis as applied to the BDCM model was undertaken to determine if both methods would identify the same parameters as important or influential. Sensitivity analysis (SA) is commonly used during PBPK model development and evaluation to determine which parameters (inputs) are most influential or important for specific model responses (e.g. toxicologically relevant dose metrics or experimental measurements) under defined exposure and time course conditions (Schlosser, 1994). Historically, LSA has been commonly used in SA of PBPK models (Barton *et al.,* 2007). More recently, GSA has been recommended as a means to better characterize the impact of uncertainty and variability in model parameters on model responses, although GSA can be more computationally intensive and requires more information regarding parameter ranges (Barton *et al.,* 2007; Loizou *et al.,* 2008). A variety of GSA methods exist that differ in their assumptions, ease of implementation and computational intensity. We chose to use the Morris method, which is characterized as a screening method to allow ease of comparison with LSA results, particularly given that the Morris method is considered global because it is determined by computing averages of local measures over the input parameter range (under the assumption of a uniform distribution) resulting in two sensitivity measures for each parameter, μ and δ (McNally *et al.,* 2011). In general, results of the LSA and GSA rankings were similar in that common sets of parameters were identified in both the top 1/3 and bottom 1/3 for any given response (CvBDCM, Calv, AUCv and MBDCM) and exposure (oral or shower) combination. The most likely explanation is that the scenarios used for sensitivity analysis reflect realistic human environmental exposures over which the modeled physiological processes are principally linear; the limitations cited for LSA are that it is generalizable only for linear models and does not identify interactions between parameters, whereas GSA evaluates sensitivity over the entire parameter range (defined by the modeler) and is robust for identifying parameter interactions in the presence of non-linear processes (McNally *et al.,* 2011; Saltelli *et al.,* 2005).

Results of sensitivity analysis in the later stages of model development confirmed the results of preliminary analyses in highlighting the importance (high influence) of chemical-specific parameters for both commonly measured responses in human

studies (venous blood and exhaled breath BDCM concentration) and important internal dose metrics (AUC for BDCM in venous blood, amount of BDCM metabolized in liver per hour). In particular, parameters for metabolism via the CYP oxidative pathway are strongly influential for all model responses evaluated via the oral route. Additionally, the importance of model parameters for skin absorption (from *in vitro* studies using human skin) for dermal plus inhalation route model responses and an oral absorption parameter (estimated on the basis of human *in vivo* data for drinking water exposures) are also confirmed by sensitivity analysis.

Although BDCM is a good prototype chemical for brominated THMs owing to its occurrence and relatively larger database, environmental exposures to multiple DBPs are the exposure reality. One concern in regards to BDCM exposure is that there is potential for competitive metabolic interactions with other THMs metabolized by CYPs, especially CYP 2E1 (Da Silva *et al.,* 1999, 2000). Such interactions have the potential to alter both observed blood concentration-time curves and exposure of target organs to BDCM. With typical environmental exposures to THMs, hepatic metabolic saturation is unlikely as metabolism is limited by the rate of blood flow to the liver. Other multiple DBP PBPK model analyzes have also concluded that metabolism is likely to be blood flow-limited at environmental exposure levels (Tan *et al.,* 2007). However, the effects of competitive metabolic interactions, both between and within pathways, in the target tissues of greatest concern have not been evaluated in humans and are an important consideration for population-based risk, especially given the association of increased bladder cancer risk with specific polymorphisms in GST enzymes (Cantor *et al.,* 2010) and reports of low CYP2E1 activity in the urinary tract (Amet *et al.,* 1997). The present lack of these data is due, in part, to difficulties involved in obtaining relevant human tissues and cells for the research, but future investigations in this laboratory will focus on this issue.

In summary, this refined BDCM model provides an improved tool for human multi-route analysis and internal dose estimation for this prevalent drinking water contaminant and a scientifically robust core structure for future addition of key pharmacodynamic linkages. Our model analysis indicates a large contribution of inhalation and especially dermal exposure (e.g. from showering) to the internal dose of parent BDCM reaching the systemic circulation and thus available for extra-hepatic (target tissue) metabolism. Based on the hypothesis that metabolism in target tissues is important for cancer development, non-oral exposures could contribute significantly to the amount of BDCM available for metabolism in these sites and hence the potential for adverse effects. Overall, this analysis indicates the importance of considering the contribution of multiple routes of exposure to BDCM and similarly metabolized chemicals to provide a complete evaluation of potential risk of adverse health outcomes.

Disclaimer

The research described in this manuscript has been reviewed by the National Health and Environmental Effects Research Laboratory, U. S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

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Conflict of interest

The Authors did not report any conflict of interest.

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Appendix: Key model rate equations

The BDCM model has eight compartments with 23 rate equations. The distribution to the tissues from the systemic circulation was assumed to be blood-flow limited, and the tissue compartments were assumed to be well stirred.

For non-metabolizing tissues (richly-, poorly-perfused, fat and kidney), the rate of change of BDCM in the tissue (*Ai*) is described as

$$\frac{dxAi}{dt} = Qi*(Ca - Cvi) \quad (1)$$

where *Qi* is tissue blood flow, *Ca* is arterial concentration and *Cvi* is the tissue venous blood concentration of BDCM leaving the tissue.

Gut $$\frac{dAg}{dt} = Qg*(Ca - Cvg) + RoBDCM \quad (2)$$

Gut absorption $$RoBDCM = k_a*Clu*Vlu \quad (3)$$

where ka is the oral absorption constant, Clu is the lumen concentration and Vlu is the lumen volume.

Liver $$\frac{dAl}{dt} = Ql*Ca + Qg*Cvg - (Ql + Qg)*Cvl - RmBDCM \quad (4)$$

Total Liver metabolism $$RmBDCM = R1BDCM + R2BDCM \quad (5)$$

CYP pathway $$R1BDCM = (V1BDCM*Cl)/(Km1BDCM + Cl) \quad (6)$$

GST pathway $$R2BDCM = V2BDCM*Cl \quad (7)$$

Skin $$\frac{dAsk}{dt} = Qsk*(Ca - Cvsk) + RdBDCM \quad (8)$$

$$\text{Dermal Absorption RdBDCM} = Kd*SAsk*10*\left(cwater_{in} - \frac{Csk}{PwsBDCM}\right) \quad (9)$$

where Kd is the dermal permeability constant (cm/hr), $cwater_{in}$ and 10 is for conversion to L/h.

Arterial blood $$\frac{dCa}{dt} = (Qc*Cv + Qp*inh_{conc})/\left(\frac{Qp}{PbBDCM} + Qc\right) \quad (10)$$

Venous blood:

$$\frac{dAv}{dt} = Qrp*Cvrp + Qpp*Cvpp + Ql*Cvl + Qf*Cvf + Qk*Cvk + Qsk*Cvsk - Qc*Cv \quad (11)$$

$$Cv = Av/Vven$$

Equations for describing air and water concentrations based on the shower model from Tan *et al.* (2006):

Water concentration exiting the shower:

$$cwater_{out} = cwater_{in} \times exp^{-\left(\frac{kolA}{qw}\right)} + \frac{inh_{conc}}{H} \times \left(1 - exp^{-\left(\frac{kolA}{qw}\right)}\right)$$

where cw_{in} is the BDCM concentration in water entering the shower, kolA is the mass transfer rate constant ($l\,h^{-1}$) for BDCM from water to air, qw is the shower water inflow rate ($l\,h^{-1}$), inh_{conc} is the air concentration, and H is the Henry's law constant for equilibrium of BDCM between air and water concentrations.

The air concentration of BDCM (inh_{conc}) is determined based on the amount of BDCM transferred from the shower water, the amount of BDCM exchanged via air flow between the shower and bathroom, and the amount exchanged via respiration from the showering subject. The rate of accumulation of BDCM in the air is calculated as:

$$\frac{dA_{air}}{dt} = Qw \times (cwater_{in} - cwater_{out}) - QA \times (inh_{conc} - C_{room}) + Rinh_{shower}$$

Where

$$inh_{conc} = \frac{A_{air}}{volshwr}$$

And the rate of exchange due to respiration is:

$$Rinh_{shower} = Qp \times \left(\frac{Ca}{PbBDCM} - inh_{conc}\right)$$

The airflow from the room to the shower (QA) is the product of the number of airexchanges per hour (Airexchange) and the volume of the shower (VOLSHWR). The concentration in the bathroom (C_{room}) was assumed to be zero.