Running Title: Impact of Scaling Factor Variability

The impact of variation in scaling factors on the estimation of internal dose metrics: a case study using bromodichloromethane (BDCM)

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Abstract

Many physiologically based pharmacokinetic (PBPK) models include values for metabolic rate parameters extrapolated from *in vitro* metabolism studies using scaling factors such as mg of microsomal protein per gram of liver (MPPGL) and liver mass (FVL). Variation in scaling factor values impacts metabolic rate parameter estimates (Vmax) and hence estimates of internal dose used in dose response analysis. The impacts of adult human variation in MPPGL and FVL on estimates of internal dose were assessed using a human PBPK model for BDCM for several internal dose metrics for two exposure scenarios (single 0.25 liter drink of water or 10 minute shower) under plausible (5 μg/L) and high level (20 μg/L) water concentrations. For both concentrations, all internal dose metrics were changed less than 5% for the showering scenario (combined inhalation and dermal exposure). In contrast, a 27-fold variation in area under the curve for BDCM in venous blood was observed at both oral exposure concentrations, whereas total amount of BDCM metabolized in liver was relatively unchanged. This analysis demonstrates that variability in the scaling factors used for *in vitro* to *in vivo* extrapolation (IVIVE) for metabolic rate parameters can have a significant impact on estimates of internal dose under environmentally relevant exposure scenarios. This indicates the need to evaluate both uncertainty and variability for scaling factors used for IVIVE.

Key Words: *in vitro* to *in vivo* extrapolation (IVIVE), scaling factors, variation

Abbreviations: AML – amount metabolized in liver, AUC – area under curve, AUCv – AUC in venous blood, BDCM – bromodichloromethane, Calv – concentration in alveolar air, CV – venous blood concentration, CYP – cytochrome P450, FVL – fractional volume (mass) liver, IVIVE – in vitro to in vivo extrapolation, LSA – local sensitivity analysis, MPPGL – microsomal protein per gram of liver, MSP – microsomal protein, PBPK – physiologically based pharmacokinetic, SC – sensitivity coefficient

Introduction

Physiologically based pharmacokinetic (PBPK) models have demonstrated utility in refining dose response and extrapolation functions in human health risk assessment. Uncertainty in dose response assessments is reduced when tissue dose, rather than external dose is used as the expression of "dose", and when species extrapolation is based on concentration of toxicant at the biological target site/tissue (Lipscomb and Poet, 2008). The reliability of PBPK models is directly related to the accuracy of the parameter values used as model inputs. In particular, chemical-specific parameters describing metabolic clearance in the liver can have a major impact on measures of internal dose that are often used in risk assessment, such as predicted time course and area under the curve (AUC) for parent chemical or metabolites in blood in addition to steady state and maximum blood concentration (USEPA, 2006), as well as biomarkers of exposure such as chemical or metabolite concentrations in urine or exhaled breath.

The uncertainty associated with interspecies extrapolation of metabolic rate parameters is eliminated for human PBPK models where values for metabolic rate parameters have been experimentally-determined *in vitro* using human hepatic subcellular fractions (microsomes, cytosol) or hepatocytes. However, an additional source of uncertainty is added in that *in vitro* to *in vivo* extrapolation (IVIVE) of parameters (e.g., Vmax, K_M) is necessary for their incorporation into PBPK models. IVIVE for Vmax is accomplished using scaling factors such as mg microsomal protein per gram of liver (MPPGL) or the number of hepatocytes per gram of liver (hepatocellularity) depending upon the experimental system used, and as well as liver mass (Lipscomb & Poet, 2008).

Although most PBPK models treat parameter values as point estimates, in reality they are associated with variability within the human population. In the context of IVIVE for this

investigation, we focus on BDCM, which is metabolized by several members of the cytochrome P450 enzyme family (Zhao and Allis, 2002). The cytochromes P450 (CYP) are membrane-bound enzymes, typically isolated from the whole cell in the subcellular fraction called "microsomal protein" (MSP). Metabolic rate constants derived *in vitro* are often expressed in terms of the amount of chemical metabolized per minute per mg MSP, i.e. MPPGL. Both MPPGL and the mass of the liver per kg body mass (FVL) vary among the human population and both are required parameters in the extrapolation of *in vitro* derived metabolic rate constants to units of expression typically used in PBPK models (e.g., mg/hr-kg body mass) (Barter et al, 2007, 2008; Lipscomb and Poet, 2008).

The concentration of the various CYP forms in microsomal protein (pmol CYP form/mg MSP) also varies among the human population. Some previous efforts have aimed at quantifying this variability (e.g., Shimada et al., 1994; Snawder and Lipscomb, 2000) and incorporating it into PBPK modeling efforts (e.g., Lipscomb et al., 2003a, 2003b). However, mostly, the resulting efforts have combined the variability of the concentration of enzymes in MSP and the variability of MSP per gram liver, making it impossible to ascertain the separate impact of variability of MSP content (the impact of variability in liver mass has rarely been determined). The present work uses a "fixed" (implied) concentration of CYP per mg MSP, and investigates the influence of measures of variability in MPPGL and FVL, which are directly applicable to any of the membrane-bound enzyme families, on estimates of internal dose and common biomarkers of exposure using a human BDCM model under environmentally realistic exposure scenarios. Monte Carlo analysis was used to evaluate the propagation of variability (in MPPGL and FVL) through the model to give an estimate of the variability in several relevant measures of internal dose and biomarkers of exposure (USEPA, 1997).

Methods

A recently published PBPK model for BDCM in the human was re-parameterized (MPPGL, FVL) and used to quantify the impact of variability in MPPGL and liver mass on various BDCM dose metrics. The model structure, assumptions and parameterization have been previously described in detail (Kenyon et al., 2015) and are presented in summary in the Appendix. The model and subsequent analyses were implemented in acslXtreme 3.0.2.1 (The AEgis Technologies Group; Huntsville, AL). Model outputs (responses) evaluated included AUC for BDCM in venous blood (AUCv), amount of BDCM metabolized in liver (AML), venous blood BDCM concentration (CV), and exhaled breath concentration (Calv); both CV and Calv have also been used as biomarkers of exposure for BDCM in environmental exposure studies. Two typical household exposure scenarios were simulated assuming a water concentration of 5 or 20 µg BDCM per liter - a ten minute shower (combined dermal and inhalation exposure) and a single ¼ liter drink (oral) of water; length of the simulation in both cases was 2 hrs. These water concentrations were selected on the basis of national survey data to approximate median and upper 90th percentiles for BDCM in finished drinking water derived from surface waters (USEPA 2005a).

I*n vitro* microsomal metabolism parameters, V_{max} and K_M for the human hepatic metabolism of BDCM were 1.74±0.094 nmoles/min-mg MSP and 2.61±0.55 µM, respectively. The data are expressed as mean ± standard error and the human microsomes were from a commercially prepared pooled source derived from 200 individuals (Kenyon et al., 2015). The scaling calculations, using the equation below, were incorporated directly into the model code to treat MPPGL and liver mass (FVL) as variables in the subsequent Monte Carlo analysis. LR is the rate of BDCM metabolism in liver in units of µg/hr-kg BW (Lipscomb and Poet, 2008).

LR = Vmax (mass/time-mg protein) x MPPGL (mg MSP/g liver) x FVL (g liver /kg BW) (1)

The *in vitro* K_M was divided by the liver:blood partition coefficient to express the concentration in venous blood at equilibrium with liver, rather than in liver itself; this assumes that the concentration in the *in vitro* suspension adequately represents the concentration in liver resulting in the half-maximal rate of metabolism, and adjusts the *in vitro* K_M to reflect the concentration of BDCM in blood at equilibrium with liver (USEPA, 2005b; Kenyon, 2012).

Monte Carlo analysis was used to evaluate impacts of variability of MPPGL and FVL on model outputs (AUCv, AML, CV, Calv) under two different exposure scenarios. This enabled determination of statistical dependencies between scaling factors used as simulation inputs (FVL, MPPGL) and model responses or outputs under specific distributional assumptions. Model responses selected for evaluation were those that were either likely to be measured in water use studies (CV, Calv) as well as toxicologically relevant measures of internal dose (AUCv, AML). Estimation of the variance in model responses was achieved by randomly sampling the parameters of interest (MPPGL, FVL) from defined distributions and running the model for a larger number of iterations (10,000). Distributional characteristics are provided in Table 1 and were based on data from Lipscomb et al. (2003a, b) and Young et al. (2009). FVL and MPPGL are assumed to vary independently. These data were selected because the complete original data sets were available enabling calculation of all needed distributional descriptors (Table 1).

Local sensitivity analysis (LSA) was used to evaluate both the time-dependent and dose-dependent sensitivity of model outputs evaluated (CV, Calv, AUCv, AML) to the parameters varied (MPPGL, FVL). Additional parameters were also included in the LSA based on having high influence in previous analyses (Kenyon et al., 2015). For the drinking scenario, these included fractional blood flow to liver (FQL), liver:blood partition coefficient, fractional blood

flow and mass of the GI tract, alveolar ventilation, cardiac output, and oral absorption coefficient. For the showering scenario, these included alveolar ventilation rate, cardiac output, blood:air partition coefficient, skin permeation coefficient, and blood flow to skin. 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 (Schlosser, 1994; Clewell et al. 1994).

Results and Discussion

Results for the oral route exposure at 5 and 20 μg/L are shown in Table 2 and Figures 1 and 2. In the case of AUC for BDCM in venous blood there was an approximately 27-fold difference between the minimum and maximum values at both water concentrations, whereas little difference was observed between the minimum and maximum values for amount of BDCM metabolized in liver (Table 2). Large differences (~16-fold) between minimum and maximum values were also observed for both peak venous blood BDCM concentration (Fig. 1) and peak exhaled breath BDCM concentration (Fig. 2) at both water concentrations. Results for the showering exposure (inhalation, plus dermal routes) at 5 and 20 μg/L are shown in Table 2 and Figures 3 and 4. All dose metrics evaluated were changed by less than 10% at both water concentrations for showering (combined dermal and inhalation) exposure.

In the case of oral exposure, variation in hepatic scaling factors had clear impact on model outputs that are related to parent chemical (AUCv, CV, Calv), but not on the dose metric for hepatic metabolism (AML). The most likely explanation for this latter observation is that at typically low environmental exposure concentrations, hepatic biotransformation is not saturated and variability in the parameters FVL and MPPGL are impacting hepatic Vmax which is not an

influential parameter under these exposure conditions (Kenyon et al., 2015); put another way, delivery of parent chemical to liver in blood is the rate limiting step in hepatic biotransformation under these conditions. The lack of impact of variation in hepatic scaling factors on internal dose metrics following showering may be attributable to the physiology of inhalation and dermal absorption, in that compounds absorbed into the systemic circulation are not subjected to the potential for first-pass metabolism in liver or intestine, as are compounds consumed orally (Lehman-McKeeman, 2013).

Results of the sensitivity analysis for MPPGL and FVL are summarized in Table 3 for all dose metrics and both exposure scenarios. Since no dose-dependent differences in results were observed at the two different water concentrations simulated, only the results for 5 μg/L are shown; this is an expected result since hepatic CYP metabolism is in the linear range (i.e., not saturated) at either exposure level. The final value of the sensitivity coefficient (at the end of the 2 hour simulation) is reported since this value was generally consistent over the time course of the simulation. Both MPPGL and FVL were highly influential for oral exposure for dose metrics related to parent chemical, whereas all parameters related to liver metabolism were ranked as low influence for total amount of BDCM metabolized. This latter finding also supports the conclusion that metabolism being linear (not saturated) at low exposure concentrations explains the lack of impact of variability is scaling factors for total hepatic BDCM metabolized (AML). For showering exposures, parameters directly related to metabolic scaling were non-influential for all dose metrics evaluated, which is consistent with dose metrics being relatively unchanged by variation in scaling factors. Additional sensitivity analysis results (data not shown) confirmed earlier results in terms of the same parameters being highly influential or in some cases non-influential for specific dose metrics and exposure scenarios (Kenyon et al., 2015).

This analysis demonstrates that variability in scaling factors used for IVIVE for hepatic biotransformation can have a clear impact on internal dose metrics that are both metric- and route-dependent at environmentally realistic exposure levels. These results also indicate the importance of evaluating both uncertainty and variability for scaling factors used for IVIVE. In the context of total exposure, our results are likely generalizable to many low level environmental contaminants of concern in drinking water or food because for many of these pollutants human exposures are sufficiently low that overall clearance is most heavily influenced by hepatic clearance following oral exposure and in addition, multiple routes of exposure are a common concern.

However, the overall importance of consideration of the impact of measured variability in scaling factors related to hepatic metabolism is most appropriately considered both in the context of human exposure to environmental contaminants and concerns related to risk assessment. Measures of central tendency for scaling factors for hepatic *in vitro* metabolism such as MPPGL and hepatocellularity (cells per gram of liver) have been characterized because of their importance for interpretation of *in vitro* drug metabolism data in the pharmaceutical industry where oral exposure is a major concern. For example, following an extensive review and meta-analysis of the literature Barter et al (2007) recommended weighted geometric means of 32 mg/g (29-34, 95% CI) and 99 x 106 cells/g (74-131, 95% CI) for human MPPGL and hepatocellularity, respectively. These investigators noted that while inter-individual variation was significant for both scaling factors, differences were not attributable to covariates such as gender, cigarette smoking or alcohol consumption. In the pharmaceutical industry the oral route of exposure is particularly relevant, evaluation of the potential for hepatic first pass metabolism is critical and doses utilized render metabolic saturation a possibility (Houston, 1994). For

environmental contaminants, additional concerns related to metabolism in both target and non-target tissues can be critical, and overall there is a paucity of data needed to scale *in vitro* metabolism data to the *in vivo* situation for extrahepatic metabolism, as well as characterization of the associated variability.

Another important concern is whether age-related differences in scaling factors, including those for hepatic metabolism, are of sufficient magnitude that both they and their variability need to be characterized to appropriately scale *in vitro* metabolism data for various aged populations (Barter et al., 2007, 2008). Specifically, in a follow-up to their original investigation (Barter et al., 2007), Barter et al. (2008) confirmed an inverse relationship between both MPPGL and hepatocellularity and age that had been noted in the first study, although further study is needed to fully characterize this variability. While characterizing the variability in these and other scaling factors is essential, evaluation of the actual impact of this variability is most accurately assessed in the context of a PBPK model. In summary, IVIVE for biotransformation pathways requires tissue-specific estimates of both central tendency (mean, GM) and variability (SD, GSD) to provide the most scientifically robust basis for extrapolation.

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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Table 1. Parameter distribution descriptors used in the Monte Carlo analysis.

Distribution Descriptors	Parameter	
	FVL	MPPGL
Shape	Normal	Lognormal
Mean (GM)	0.0244	52.9
SD (GSD)	0.0109	1.476
Lower Limit	0.0136	27.9
Upper Limit	0.0415	100

Arithmetic mean and standard deviation (SD) used for normal distribution; geometric mean (GM) and geometric standard deviation (GSD) used for lognormal distribution.

Figures for FVL are recalculated from Young et al., 2009; figures for MPPGL were reported in Lipscomb et al. (2003a). Lower and upper limits are 5th and 95th percentiles.

Table 2. Statistical characteristics for internal dose measures area under the curve (AUCv) for BDCM in venous blood (μg-hr/L) and total amount of BDCM metabolized in liver (AML, μg) for specific inhalation and oral exposures.

Exposure Scenario[1], BDCM water concentration	Dose Metric	Mean	S.D.	Minimum	Maximum
Drink (1/4 liter)	AUCv	2.12×10^{-04}	1.07×10^{-04}	3.62×10^{-05}	9.63×10^{-04}
5 μg/L	AML	1.24	4.17×10^{-03}	1.21	1.25
Drink (1/4 liter)	AUCv	8.46×10^{-04}	4.27×10^{-04}	1.45×10^{-04}	3.85×10^{-03}
20 μg/L	AML	4.97	1.67×10^{-02}	4.85	4.99
Shower (10 min)	AUCv	1.34×10^{-02}	1.04×10^{-04}	1.33×10^{-02}	1.42×10^{-02}
5 μg/L	AML	1.29	4.94×10^{-03}	1.25	1.30
Shower (10 min)	AUCv	5.38×10^{-02}	4.18×10^{-04}	5.31×10^{-02}	5.68×10^{-02}
20 μg/L	AML	5.15	1.98×10^{-02}	5.01	5.18

[1]Length of the model simulation was 2 hours for both scenarios and the model was parameterized as described in Tables 1 and 2 of Kenyon et al. (2015) and summarized in Appendix Table 1. AUCv and AML, which are both cumulative dose metrics, are values at end of 2 hour simulation.

Table 3. Sensitivity analysis results for drinking and showering exposures to 5 μg/L BDCM in water

Model Response (Output)	Parameter	Drink ¼ L 5 μg/L BDCM in water		Shower 10 min 5 μg/L BDCM in water	
		Sensitivity Coefficient	Ranking	Sensitivity Coefficient	Ranking
BDCM in Venous Blood (CV)	MPPGL	-1.008	high	-0.024	low
	FVL	-1.044	high	-0.080	low
Exhaled Breath BDCM Concentration (Calv)	MPPGL	-1.008	high	-0.024	low
	FVL	-1.044	high	-0.080	low
AUC for BDCM in Venous Blood (AUCv)	MPPGL	-0.995	high	-0.011	low
	FVL	-0.993	high	-0.009	low
Amount BDCM metabolized in Liver (AML)	MPPGL	0.004	low	0.005	low
	FVL	0.005	low	0.007	low

Figure Legends

Figure 1. Plots for venous blood BDCM (ng/L) as mean ± 3 SD based on Monte Carlo simulations utilizing the distributional characteristics for MPPGL and FVL shown in Table 1 for an oral exposure to water as a single ¼ liter drink containing 5 (A) or 20 (B) µg/L BDCM.

Figure 2. Plots for exhaled breath BDCM (µg/m^3) as mean ± 3 SD based on Monte Carlo simulations utilizing the distributional characteristics for MPPGL and FVL shown in Table 1 for an oral exposure to water as a single ¼ liter drink containing 5 (A) or 20 (B) µg/L BDCM.

Figure 3. Plots for venous blood BDCM (ng/L) as mean ± 3 SD based on Monte Carlo simulations utilizing the distributional characteristics for MPPGL and FVL shown in Table 1 for a 10 minute showering exposure to water containing 5 (A) or 20 (B) µg/L BDCM.

Figure 4. Plots for exhaled breath BDCM (µg/m^3) as mean ± 3 SD based on Monte Carlo simulations utilizing the distributional characteristics for MPPGL and FVL shown in Table 1 for a 10 minute showering exposure to water containing 5 (A) or 20 (B) µg/L BDCM.

Appendix – Summary Description of Human BDCM Model

Table 1. Physiological parameters for the human BDCM model

Parameter, units	Value	Footnote
Height, cm	160 - 190	[1]
Body Weight (BW), kg	65-91	[1]
Alveolar ventilation Rate (QP), L/h-m^2	212.4	[2]
Alveolar Deadspace, unitless	0.238	
QPC to Cardiac Output (CO) Ratio, RQPCO unitless	0.8	[3]
Fractional Blood Flows, unitless		[4, 5]
Richly Perfused Tissue Group	0.75	[5,6]
Liver	0.09	
Gastrointestinal Tract	0.16	
Kidney	0.15	
Poorly Perfused Tissue Group	0.25	[6]
Fat	0.05	
Blood Flow to Skin, L/min-m^2	0.58	[6]
Compartment Volume, unitless		[3]
Blood fraction of BW	0.079	[7]
Blood as arterial	0.25	
Blood as venous	0.75	
Richly perfused fraction of BW	0.20	[8]
Poorly perfused fraction of BW	0.80	
GI tract fraction of BW	0.0165	
Liver fraction of BW	0.026	
Fat fraction of BW	0.07 – 0.20	[1]
Kidney fraction of BW	0.004	
Volume GI tract lumen, L	2.1	[3]
Skin thickness, mm	2.0	[9]

[1]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 fat fraction volume data were also available from Leavens et al. (2007) estimated based on skin fold thickness; average value used for general simulations was 0.11.

[2]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).

[3]Cardiac Output, QC = QP/RQPCO.

[4]Physiological parameters from (Brown et al. 1997) unless otherwise specified.

[5]Fractional blood flows to individual tissues are scaled to cardiac output.

[6]Richly (QRP) and poorly perfused (QPP) tissues calculated by difference subtracting out blood flows from liver, kidney and gut for QRP and subtracting out fat and skin volumes for QPP. Blood flow to skin is scaled on the basis of body surface area.

[7]Volume of blood compartment is scaled to BW and volume of arterial and venous compartments are scaled to total blood volume.

[8]Tissue volumes to tissues are scaled to BW. Volume of skin is calculated as skin thickness multiplied by surface area.

[9]Skin thickness is average value for thickness of dermis and epidermis (Laurent et al. 2007).

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

Parameter, units	Value			Footnote
	Male	Female	Average	
Partition coefficients, unitless				
Blood:Air	17.33	14.61	15.97	[1]
Liver:Blood	1.77	2.09	1.93	[2]
Gut:Blood	1.77	2.09	1.93	[3]
Kidney:Blood	1.90	2.25	2.08	[2]
Fat:Blood	30.35	36.00	33.2	[2]
Skin:Blood	2.68	3.18	2.91	[4]
RPTG:Blood	1.77	2.09	1.93	[3]
PPTG:Blood	0.72	0.85	0.78	[2]
Skin diffusion coefficient, cm/h	0.18			[5]
Skin:water partition coefficient	5.6			[5]
Oral absorption coefficient, h^{-1}	8.3			[6]
Vmax CYP Liver, µg/h-kg $BW^{0.75}$	4.13×10^4			[1]
KM CYP Liver, µg/L	221			[1]
Kf GST Liver, 1/h-kg $BW^{0.75}$	0.0079			[7]

[1]Experimentally determined.

[2]Calculated by dividing rat tissue:air partition coefficient (Lilly et al. 1997) by human blood:air partition coefficient.

[3] Gut:air and rapidly perfused tissue:air partition coefficients were assumed to be the same as liver:air.

[4] Skin:air partition coefficient (Haddad et al. 2006) used with human blood air partition coefficient to calculate skin:blood partition coefficient.

[5] 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).

[6]Estimated on basis of Tmax from oral time course data of Leavens et al. (2007).

[7]Estimated from in vitro clearance of BDCM from pooled human liver cytosol (Ross and Pegram 2003).