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Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene (PCE)

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SUMMARY

When used in the risk assessment process, the output from physiologically based pharmacokinetic (PBPK) models has usually been considered as an exact estimate of dose, ignoring uncertainties in the parameter values used in the model and their impact on model predictions. We have collected experimental data on the variability of key parameters in a PBPK model for tetrachloroethylene (PCE) and have used Monte Carlo analysis to estimate the resulting variability in the model predictions. Blood/air and tissue/blood partition coefficients and the interanimal variability of these data were determined for tetrachloroethylene (PCE). The mean values and variability for these and other published model parameters were incorporated into a PBPK model for PCE and a Monte Carlo analysis (n = 600) was performed to determine the effect on model predicted dose surrogates for a PCE risk assessment. For a typical dose surrogate, area under the blood time curve for metabolite in the liver (AUCLM), the coefficient of variation was 25% and the mean value for AUCLM was within a factor of two of the maximum and minimum values generated in the 600 simulations. These calculations demonstrate that parameter uncertainty is not a significant potential source of variability in the use of PBPK models in risk assessment. However, we did not in this study consider uncertainties as to metabolic pathways, mechanism of carcinogenicity, or appropriateness of dose surrogates.

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INTRODUCTION

Physiologically based pharmacokinetic (PBPK) models have become useful tools for deriving internal dose estimates from exposure to chemicals by integration of information on the administered dose, the physiological structure of the mammalian species, and the physiochemical properties of the specific chemicals [1–3]. While these PBPK models provide a simplified description of a much more complex physiological system, the mammalian body, they still result in 30 to 40 parameters in the model structure. Fortunately, for most chemicals, only 25 to 30% of these parameters have significant enough impact on the model prediction to warrant consideration. Because these most critical parameters are chemical specific, they have to be determined for each chemical. In order for these models to find their application in determining excess risk from exposure to a chemical, the variability of the model parameters used in describing the pharmacokinetic data should be incorporated into the overall simulation of the pharmacokinetic data.

Previous studies have been conducted to address these issues in PBPK model parameter variability. Bois et al. [4] reported a risk assessment for tetrachloroethylene (PCE) exposure they conducte to consider the precision and sensitivity of pharmacokinetic models for mice, rats, and humans. They studied the precision of their risk estimate by treating PBPK model parameters as random variables and determined the range of risk estimates when parameter uncertainities were considered via Monte Carlo simulations. The results of the Monte Carlo simulations were analyzed to determine the pharmacokinetic model sensitivity to its parameters. The kinetic parameters defining the metabolic rate were the most important for the cases studied.

Farrar et al. [5] performed an uncertainty analysis on the input parameters for PBPK models and the resulting uncertainty in the output in a risk assessment of PCE. Using data from the literature as the basis for setting the preferred PBPK model parameter values, they developed probability distributions to express uncertainity in the model parameters. Each PBPK model parameter value had an accompanying uncertainty factor which ensured that values for any model parameter were within 95% of the parameter mean. By using Monte Carlo simulation with the Latin-hypercube procedure they were able to derive risk estimates for human PCE exposure at 50 ppm, the current Occupational Safety and Health Administration (OSHA) standard, based on three different dose surrogates.

In this study, the interanimal variability was determined for the blood/air and tissue/air partition coefficients of PCE. These data were then used with the published variation of body organ volumes and flow rates in a PBPK model for PCE and trichloroacetic acid (TCA) to determine the extent this parameter variation would have on model parameter output. These calculations demonstrate that the uncertainty associated with these particular parameters is within the range of other physiological parameters and most likely not a significant potential source of variability in the use of PBPK models in risk assessement. This study demonstrates the need for focusing on uncertainties as to metabolic pathways, mechanisms of carcinogenicity, appro-

priateness of dose surrogates, and interspecies differences in sensitivity to carcinogenesis.

MATERIALS AND METHODS

Determination of tissue and blood partition coefficients

A variation of the vial equilibrium method of Gargas et al. [6] was used to determine PCE blood/air or tissue/air partition coefficients in individual animals. Fresh tissues were homogenized and then ≈100 mg of muscle, liver, or kidney was smeared on the wall of a 25-ml liquid scintillation vial. Because of the high affinity of fat for PCE, only 50 mg of adipose tissue was used. Each vial was then injected with PCE from a standard bag and incubated for 3 h at 37°C, except fat, which was incubated for 4 h. For blood, 250 μl was placed in the scintillation vial with 250 μL of chemical and was incubated at 37°C with vortexing for 3 h. A headspace sample (1 ml) was injected onto a gas chromatograph (GC) equipped with a flame ionization detector (FID).

To validate the smear method, the vial equilibration method was used [6]. Rat tissues were used because they provided enough material to run both methods on the same sample. All incubation conditions were the same as the smear method except that the samples were prepared differently. The tissues were removed after the animals were euthanized, they were then homogenized in 0.09% physiological saline (1:3, w/v), and 2 ml of the mixture was pipetted into the 25-ml scintillation vials. Each vial was then injected with PCE from a standard bag and incubated for 3 h at 37°C, except fat, which was incubated for 4 h. The blood/air and tissue/air partitions were then calculated according to Gargas et al. [6].

Determination of in vivo metabolism by gas uptake

Individual mice were exposed to PCE vapor using a 0.7-l glass and aluminum closed nonrecirculating inhalation exposure chamber. Starting concentrations of PCE in the chamber were 200, 1000 and 3500 ppm. The decrease in PCE chamber concentration with time was monitored by hand sampling and injection on a GC every 5 min for the first hour, every 15 min until 4 h and every 30 min for the remainder of the exposure. To determine metabolic constants from these data, the decrease in chamber concentration with time for each starting concentration was simulated with the PBPK model and the model parameters describing metabolism of PCE (V_{max} (mg/h), K_m mg/l) and KFC (h^{-1}) were varied to achieve the best model prediction of the data at all concentrations.

Acute oral gavage of PCE in corn oil

Ten male B6C3F1 mice (30 to 35 g) per dose level received an acute oral gavage of PCE in corn oil (≈200 μl) at the two doses 0.536 and 1.072 g/kg/day, as used in the National Cancer Institute (NCI) [7] bioassay and five at a third dose (0.1 mg/kg) which would allow validation of the model at lower doses. Repeated blood samples

were taken at 1, 2, 3, 5, 7, 24, 26, 28 and 48 h after dosing to determine the kinetics of PCE and the major metabolite of PCE metabolism, trichloroacetic acid (TCA).

Blood sample preparation and analysis

Repeated blood samples were taken from the lateral tail vein of B6C3F1 mice using a 25-gauge needle attached to a hematocrit tube. Blood samples were extracted by vortexing for 1 h with 1 ml of hexane. One microliter of the hexane extract was injected onto a GC equipped with an electron capture detector (ECD). TCA blood samples were collected in 1 ml of cyclohexane. One hundred microliters of methanolic HCl derivatizing agent was then added to the blood samples and they were heated at 100°C for 30 min. The vials were then vortexed for 1 h and centrifuged. One microliter of the cyclohexane extract was injected onto the GC.

Statistical analysis

A one factorial analysis of variance with appropriate test of assumptions was done to determine the tissue/air partitions variability among animals, with the factor being animal (mice). To compare the methods (smear and saline) of obtaining tissue/air partitions, data taken from 2–3-month-old rats was analyzed using a two sample independent *t*-test with appropriate tests of assumptions.

A Monte Carlo simulation of 500 oral exposures to PCE was conducted for mice at 536 and 1072 mg PCE/kg body weight. The human oral exposure was 1 ppb. The dose surrogates area under the curve for blood (AUCB), area under the curve for liver (AUCL), and area under the curve for liver metabolite (AUCLM) for the above exposures were compared against various simulated independent variables. The comparison was done using Pearson's Correlational Analysis.

RESULTS

Blood and tissue partition coefficients

The mean and standard deviation for mouse and human PCE blood/air and tissue/air partition coefficients are shown in Table I. There was a significant difference ($P \leq 0.05$) between the mouse versus human blood/air partition coefficients, with the mouse value being almost twice that of the human value. The tissue/air partitions for the kidney, muscle, liver, and fat could not be statistically tested, because the human tissue samples were from one human subject, but the human values for the later three tissues were greater than for mouse, while the fat/air partition for mouse was greater than the human value.

The quotient calculated tissue/blood partitions were much larger for human tissues than for the mouse tissues because of the significantly lower human blood/air partition coefficient. This could not be statistically tested because the human tissue/air partitions were obtained from one human cadaver.

TABLE I

BLOOD/AIR, TISSUE/AIR, AND TISSUE/BLOOD PCE PARTITION COEFFICIENTS FOR MICE AND HUMANS

Mouse partition coefficients[a]				
Blood/air	fat/air	kidney/air	muscle/air	liver/air
21.47 ± 3.6	1510.8 ± 147	79.14 ± 12.32	79.14 ± 15.63	48.8 ± 5.6
	fat/blood	kidney/blood	muscle/blood	liver/blood
	70.37 ± 7.33	2.34 ± 0.26	3.69 ± 0.45	2.34 ± 2.53
Human partition coefficients				
Blood/air	fat/air	kidney/air	muscle/air	liver/air
11.58 ± 2.28	1450	58.64	70.45	61.10
	fat/blood	kidney/blood	muscle/blood	liver/blood
	125.2	5.06	6.11	5.28

[a] One standard deviation. $n = 7$ for mice, $n = 9$ for human blood/air values.

Saline dilution vs. smear partition coefficients

The determination of tissue/air partition coefficients by the two different methods produced partitions that were not statistically different for all four tissues examined. There was a nonsignificant difference in the fat/air partition coefficient, even though the saline method produced a lower partition than the smear method (Table II). The kidney/air and muscle/air partitions as determined by the two methods were almost identical, whereas the liver/air partition by the saline dilution method was about 23% higher than the smear method.

Variation in partition coefficients in B6C3F1 mice

The mean values for blood/air and tissue/blood partition coefficients for PCE in mice all had coefficients of variation of less than 20% (Table I). The liver/blood coefficent was the highest (17.5), whereas the fat/blood was the lowest (9.7%). The measured blood/air partition coefficient (21.5) was representative of the range of values for PCE in mice reviewed in the literature [8] by Hattis et al.

Determination of in vivo metabolism by gas uptake

The rate of in vivo metabolism of PCE determined by gas uptake was 0.2 mg/h/kg for the V_{max}, 2.0 mg/l for the K_m, with a first order metabolism rate of 2.0 $h^{-1}kg^{-1}$. Figure 1A depicts the PBPK analysis of the gas uptake curves without including a first-order rate of metabolism. By setting the V_{max} and K_m to 1.0 mg/l/h and 0.45 mg/l, the computer optimized values for these data. It was not possible to predict the decrease in chamber concentration in the two higher exposure groups. Adding a first-order metabolic rate constant of 2.0 h l^{-1} (Fig. 1B) decreased the initial rate of

TABLE II

COMPARISON OF PARTITION COEFFICIENT METHODS IN RATS

Partition	Smear vs. saline technique		
		smear	saline
Fat/air	n[a]	5	5
	mean	1437.200	1236.670
	SD	193.720	238.782
	CV	0.135	0.193
Kidney/air	n	5	5
	mean	51.260	51.660
	SD	27.196	13.240
	CV	0.531	0.256
Liver/air	n	5	5
	mean	50.200	61.960
	SD	24.260	27.310
	CV	0.483	0.441
Muscle/air	n	5	5
	mean	21.619	18.070
	SD	8.168	8.077
	CV	0.378	0.447

[a] n = number of animals, SD = standard deviation, CV = coefficient of variation.

decrease for the chamber concentration so that the simulation of 3500 ppm exposure provided a very good fit of the data.

PCE and TCA kinetics after acute oral gavage of PCE in corn oil

Blood kinetics of PCE with the model simulations of the data for three dose levels are shown in Figure 2A. The model provided excellent predictions of PCE blood levels for the first 10 h of the two highest oral doses, but underpredicted the presence of PCE still found in the blood of experimental animals past 24 h of dosing. The peak blood concentration of the 100 mg/kg dose group was underpredicted, but the data points from ≈5 to 30 h were predicted by the model simulation.

Blood kinetics of TCA with the model simulations of the data for the three PCE dose levels are shown in Figure 2B. The model provided an excellent prediction of the TCA blood time-course for the entire period of measurement of TCA blood levels. The peak concentration of TCA in blood in the lowest dose group again was underpredicted, as would be expected because there was an underprediction of the substrate for this metabolite. The model did provide a very good estimate of the TCA blood concentrations from ≈5 to 30 h after dosing, as it did with the PCE kinetic data.

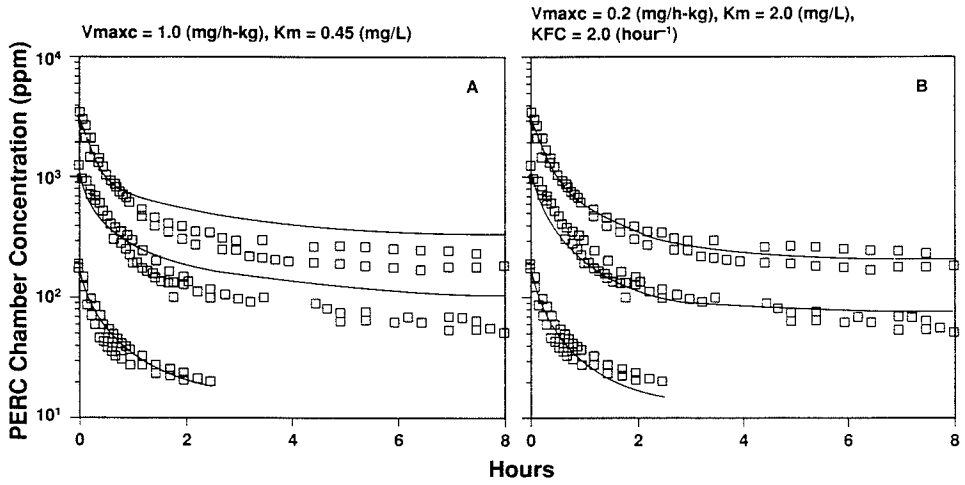

Fig. 1. Gas-uptake studies utilized to estimate the kinetic constants for saturable metabolism in mice. Data are ppm of tetrachloroethylene in the chamber atmosphere as a function of time. Experimental data are shown as symbols, whereas the computer simulations are presented as solid lines. (A) Simulation of the data with only saturable metabolism; (B) simulation of the data with saturable and first-order rates of metabolism.

Simulation of human exposure data

The ability of the model to predict human exposures to PCE was tested by analyzing some human data sets available in the literature. Stewart et al. [9] exposed human subjects to 101 ppm PCE for 6 h and then collected PCE in the expired breath. Figure 3A shows model simulations with the data of the human exposures from immediately following exposure out to ≈120 h after the end of the inhalation exposure. The model

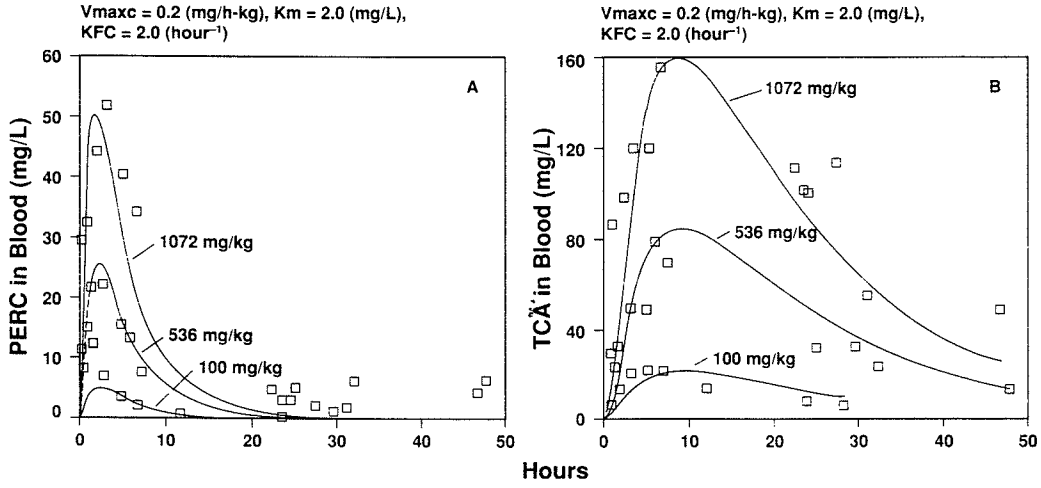

Fig. 2. Corn oil oral gavage studies of tetrachloroethylene at 0.1, 0.536, and 1.072 mg/kg doses of PCE. Experimental data are shown as symbols, whereas the computer simulations are presented as solid lines. (A) Simulation of PCE blood concentrations; (B) simulation of TCA blood concentrations. Other symbols and abbreviations as in Fig. 1.

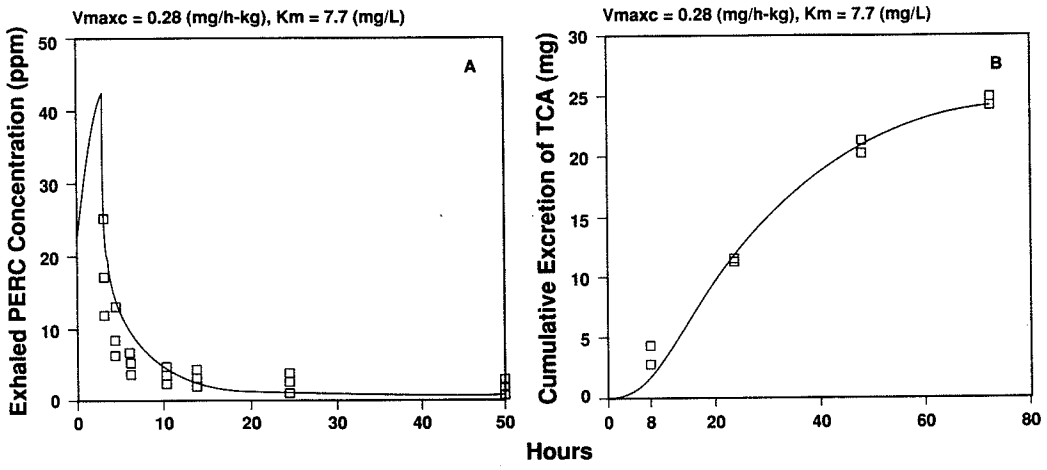

Fig. 3. Simulation (solid line) of human inhalation exposure data (symbols) from Stewart et al. [9]. (A) Concentration of PCE in exhaled breath of human subjects exposed to 101 ppm PCE for 6 h; (B) cumulative amount of TCA excreted in urine by human subjects exposed to 150 ppm PCE for 8 h. Other symbols and abbreviations as in Fig. 1.

provided a good overall prediction of the general shape of the expired breath time-course and was actually within the mean and standard deviation of most time points. The ability of the model to predict excretion of the primary metabolite of PCE, TCA, was examined (Fig. 3B) by simulating urinary excretion data for human subjects exposed to a concentration of PCE of 150 ppm for 8 h [9]. The model parameter responsible for controlling urinary excretion was fit to the available data to help set the human metabolism parameters for PCE.

Fernandez et al. [10] exposed humans to 100 ppm for varying lengths of time (1, 2, 4, and 8 h) and measured the exhaled concentration of PCE during and after exposure (Fig. 4). There was a very good agreement between the simulation and data for the 1-, 2-, and 4-h inhalation and postinhalation exhaled breath concentrations. The simulation of the 8-h inhalation exposure provided a good prediction of the data, but the simulation and data of the postexposure exhaled breath concentration did not agree with the simulation overpredicting the amount of exhaled PCE.

Correlation of model parameters with output variables

The correlation of nine model parameters with three representative dose surrogates for oral exposure of mice to PCE in corn oil at 536 and 1072 mg/kg and humans exposed at a steady state to 1 ppb PCE are shown in Tables III–V. For each model parameter and dose surrogate, the larger the correlation value, and the greater the effect of the model parameter on the dose surrogate calculated by the model. For the 536 mg/kg dose, the most significant negative correlation was the effect of pulmonary ventilation (QPC) on area under the curve for PCE in blood. QPC also had a significant negative correlation with concentration of PCE in the liver and area under the curve for metabolite in liver (AUCLM). The strongest positive correlation due to an

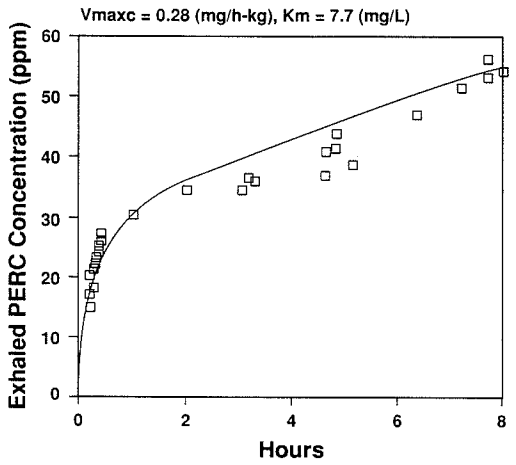

Fig. 4. Simulation (solid line) of exhaled PCE in human breath during and after inhalation exposure (data-symbols) to 100 ppm of PCE for 1, 2, 4, or 8 h. Fernandez et al. [10].

input model parameter occurred with the blood/air partition coefficient (PB). The same trend of negative correlation of QPC with AUCB was true for both the 1072 mg/kg dose and the 1 ppb human simulation.

TABLE III

CORRELATIONAL TABLE OF MODEL PARAMETERS AND OUTPUT FOR A GAVAGE DOSE OF 536 mg/kg IN MICE EXPOSED TO PCE

	AUCB	AUCL	AUCLM
QPC	−0.75[a]	−0.55[a]	−0.43[a]
QCC	0.07	−0.08[b]	−0.48[a]
BW	0.17[a]	0.14[a]	0.04
PL	−0.07	0.59[a]	−0.09[b]
PB	0.57[a]	0.44[a]	0.38[a]
VLC	−0.05	−0.04	0.37[a]
VSC	0.04	0.02	−0.12[a]
AUCB		0.74[a]	0.57[a]
AUCL			0.46[a]

[a] The row variable is highly correlated with column variable ($P < 0.01$).

[b] The row variable is correlated with column variable ($0.01 < P < 0.05$).

QPC, pulmonary ventilation (l/h/kg); QCC, cardiac output (l/h/kg); BW, body weight (kg); PL, liver/air partition coefficient; PB, blood/air partition coefficient; VLC, volume of the liver as % body weight; VSC, volume of the slowly perfused tissue as % body weight; AUCB, area under the curve for blood; AUCL, area under the curve for liver.

TABLE IV

CORRELATIONAL TABLE OF MODEL PARAMETERS AND OUTPUT FOR A GAVAGE DOSE OF 1072 mg/kg IN MICE EXPOSED TO PCE

	AUCB	AUCL	AUCLM
QPC	−0.78[a]	−0.62[a]	−0.50[a]
QCC	0.05	−0.07	−0.50[a]
BW	0.10[a]	0.06	−0.02
PL	−0.03	0.61[a]	−0.05
PB	0.60[a]	0.45[a]	0.39[a]
VLC	−0.06	−0.05	0.36[a]
VSC	0.01	0.03	−0.14
AUCB		0.74[a]	0.58[a]
AUCL			0.49[a]

[a] The row variable is highly correlated with the column variable ($P < 0.01$).

QPC, pulmonary ventilation (1/h/kg); QCC, cardiac output (1/h/kg); BW, body weight (kg); PL, liver/air partition coefficient; PB, blood/air partition coefficient; VLC, volume of the liver as % body weight; VSC, volume of the slowly perfused tissue as % body weight; AUCB, area under the curve for blood; AUCL, area under the curve for liver.

TABLE V

CORRELATIONAL TABLE OF MODEL PARAMETERS AND OUTPUT FOR HUMANS CONSUMING DRINKING WATER AT A CONCENTRATION OF 1 ppb OF PCE

	AUCB	AUCL	AUCLM
QPC	−0.72[a]	−0.62[a]	−0.44[a]
QCC	−0.06	−0.50[a]	−0.57[a]
BW	−0.15[a]	−0.15[a]	−0.43[a]
PB	0.69[a]	0.57[a]	0.45[a]
VKC	−0.09[b]	−0.07	−0.31[a]
VSC	0.078[b]	0.06	0.13[a]
AUCB		0.88[a]	0.71[a]
AUCL			0.89[a]

[a] The row variable is highly correlated with the column variable ($P < 0.01$).

[b] The row variable is correlated with the column variable ($0.01 < P < 0.05$).

QPC, pulmonary ventilation (1/h/kg); QCC, cardiac output (1/h/kg); BW, body weight (kg); PL, liver/air partition coefficient; PB, blood/air partition coefficient; VLC, volume of the liver as % body weight; VSC, volume of the slowly perfused tissue as % body weight; AUCB, area under the curve for blood; AUCL, area under the curve for liver.

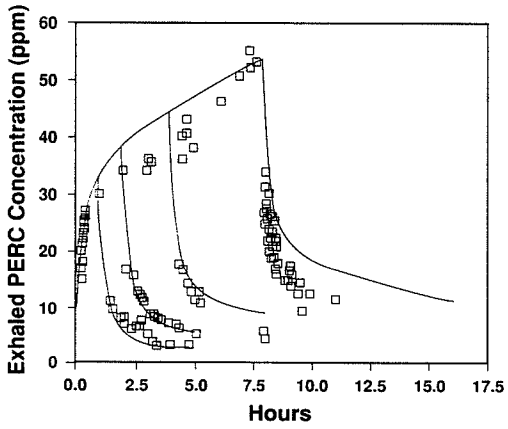

Fig. 5. Simulation (solid line) of exhaled PERC in human breath during inhalation exposure (dat-symbol) to ppm or PERC (Fernandez et al., 1976).

DISCUSSION

The blood/air partition coefficients measured for mice in our laboratory were approximately twice the human values. The mean mouse blood/air partition coefficient we measured (21.5) was in the middle of the range of values reported in the literature for mice (16.9 to 24.4), as reviewed by Hattis et al. [8]. If the range of blood/air partitions was calculated as the mean ± one standard deviation of the mean, then this range would encompass the range of values reported in the literature. It should be noted also that the published range includes other strains of mice in addition to those used in this study and also includes values determined for female mice.

As with the mouse blood/air partition coefficients, the human blood/air mean value we have determined experimentally (11.56) is very representative of the reported range of human values of 10.3 to 14.0 [8]. Again, if the mean ± one standard deviation of the mean were calculated, then this range would encompass the range of values reported in the literature. The reasons for this significant species difference are not known. Lam et al. [11] have proposed that the rat versus human species differences in affinity of some organic solvents for red blood cells versus plasma is a function of the greater affinity of rat versus human hemoglobin for hydorphobic solvents. Of greater importance than knowing the actual mechanism for the blood/air partition coefficient difference is that for modeling purposes. The rodent model can be scaled to human physiological values and we can then use the actual human blood/air partition values collected in the laboratory.

The in vivo metabolism of PCE as determined by gas uptake in this study required the addition of a first-order metabolic rate constant to provide an adequate fit of the data. It is known that PCE forms a conjugate with glutathione at a rate that appears first order, so that adding a first-order rate constant to improve prediction of gas-uptake data is not mechanistically unreasonable. The difficulty arises when the first-order pathway is used to generate TCA and not glutathione. This has not been a

proposed pathway for the generation of TCA from PCE metabolism, yet the oral pharmacokinetic data indicates the necessity of continued production of TCA at oral doses as great as 1072 mg/kg/day. If no first-order rate of PCE metabolism to TCA is incorporated into the metabolic analysis of the kinetic data, there is saturation of the Michaelis-Menten kinetics near the 0.536 mg/kg oral dose of PCE and not enough TCA is produced at the higher oral doses to reach the levels of TCA measured in the actual experimental studies. The urinary excretion data of Buben and O'Flaherty [12] support the necessity of continued TCA production at higher dose levels. Their studies showed that mice dosed with PCE continued to produce metabolites without complete saturation of metabolic pathways up to $\approx$ 2.0 g/kg, almost twice the concentration used in the bioassay and in this kinetic study.

One of the technical objectives of this research project was to develop a method to determine interanimal and intraanimal variability of tissue/air and blood/air partition coefficients. The determination of interanimal variability of each partition coefficient was necessary for setting the error bounds on the mean of each respective partition coefficient. The interanimal variability was necessary to allow a Monte Carlo simulation of the distribution of all partition coefficients. Because of the small tissue volumes of mice relative to rats, the species in which most partition coefficients has been determined, a technique was developed to measure partition coefficients in small tissue and blood volumes. This technique involved homogenizing the fresh tissue and smearing an appropriate volume on the inner wall of a tared vial. For tissues that do not distribute uniformly after homogenization in water and/or the amount of tissue per animal is small, it is possible to determine a partition coefficent.

Simulation of PCE and TCA after oral dosing at three different levels of PCE shows the ability of the PBPK model to not only predict concentration of the parent compound, PCE, but also to provide a very good description of the TCA kinetics. A validated PBPK model makes it possible to consider different dose surrogates to correlate with the cancer bioassay data for mice exposed by oral exposure. This will produce a risk assessment based more strongly on the biology and physiology of the species used in the cancer bioassay, while at the same time providing a rational means of incorporating human physiological and biochemical parameters into a mathematical framework for extrapolation to low-dose human exposures.

The usefulness of PBPK models in the risk assessment process can be strongly dependent on the variation of the basic model parameters. In this study, we have determined that the variation of blood/air and tissue/blood partition coefficients, key parameters in the simulation of pharmacokinetic data, is well within the variation of normal physiological variability. In this particular study of PCE, the analysis of the correlation of model parameters has shown that the pharmacokinetics of PCE is predominately controlled by the two main parameters which control PCE entry into the body by inhalation, the blood/air partition coefficient, PB, and pulmonary ventilation, QPC. The rate of exhalation of a compound is dependent upon the affinity of the compound for the blood, the PB, and the amount of air exchanged with the blood flowing to the lungs, the QPC.

A preliminary calculation of the risk to humans consuming 2 l of water per day contaminated with 1 μg/l of PCE is compared to two other previously calculated risk estimates (Table VI). The risk calculation presented in this paper (Table VI) is based on the animal kinetic/PBPK studies described and the PBPK simulation of human exposure data used to estimate human metabolism of PCE. The US Environmental Protection Agency (EPA) [13] risk estimate is based on the urinary excretion of TCA from the study of Buben and O'Flaherty [12], so as to utilize the available human urinary excretion of TCA. The Chen and Blancato [14] risk estimate is based on a PBPK model, which was used to analyze the data of Pegg et al. [15] and Schumann et al. [16] The EPA [13] risk estimate is 10-times higher than that calculated by Chen and Blancato [14] (Table VI) and approximately 30-times higher than the estimate of risk determined in this study. The major differences between the EPA estimate and that of Chen and Blancato [14], or the present risk estimates is in the use of a PBPK model to obtain data-based estimates of total parent absorption, which is less than 100%. In the classical EPA method, 100% of the parent chemical is assumed to be absorbed, while for PCE, experimental human inhalation exposures have shown that at higher concentrations, most of what is inhaled is exhaled.

The major differences between the Chen and Blancato risk estimate versus our analysis is the model parameter values and the sex of the animals used for the cancer dose–response relationship. Chen and Blancato [14] based their risk estimate on the data from female mice, whereas our analysis used male mice for the kinetic data collection and the male mouse NCI [7] cancer dose–response data. Also, Chen and Blancato [14] fit their PBPK model metabolism to predict the amount of PCE metabolites excreted in the urine. In the present study, we based our estimate of metabolism on disappearance of the PCE in gas-uptake studies, and by predicting the blood time-course of PCE and TCA.

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TABLE VI

COMPARISON OF PCE RISK AT A UNIT DOSE 1 μg/l IN WATER

EPA (classical – total metabolism)	1.5×10^{-6}
Chen and Blancato (PBPK – total metabolism)	1.5×10^{-7}
Gearhart et al. (PBPK – total metabolism)	4.9×10^{-8}

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