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Evaluation of Physiologically Based Pharmacokinetic Models in Risk Assessment: An Example with Perchloroethylene

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One of the more problematic aspects of the application of physiologically based pharmacokinetic (PBPK) models in risk assessment is the question of whether the model has been adequately validated to provide confidence in the dose metrics calculated with it. A number of PBPK models have been developed for perchloroethylene (PCE), differing primarily in the parameters estimated for metabolism. All of the models provide reasonably accurate simulations of selected kinetic data for PCE in mice and humans and could thus be considered to be "validated" to some extent. However, quantitative estimates of PCE cancer risk are critically dependent on the prediction of the rate of metabolism at low environmental exposures. Recent data on the urinary excretion of trichloroacetic acid (TCA), the major metabolite of PCE, for human subjects exposed to lower concentrations than those used in previous studies,[1] make it possible to compare the high- to low-dose extrapolation capability of the various published human models. The model of Gearhart et al.,[2] which is the only model to include a description of TCA kinetics, provided the closest predictions of the urinary excretion observed in these low-concentration exposures. Other models overestimated metabolite excretion in this study by 5- to 15-fold. A systematic discrepancy between model predictions and experimental data for the time course of the urinary excretion of TCA suggested a contribution from TCA formed by metabolism of PCE in the kidney and excreted directly into the urine. A modification of the model of Gearhart et al.[2] to include metabolism of PCE to TCA in the kidney at 10% of the capacity of the liver, with direct excretion of the TCA formed in the kidney into the urine, markedly improved agreement with the experimental time-course data, without altering predictions of liver metabolism. This case study with PCE demonstrates the danger of relying on parent chemical kinetic data to validate a model that will be used for the prediction of metabolism.

Keywords PBPK Modeling, Perchloroethylene, Risk Assessment

INTRODUCTION

Pharmacokinetic and mechanistic information provides valuable insight into processes of primary interest in toxicology and risk assessment: receptor-specific target tissue doses and responses. Accordingly, the most recent guidelines, final or draft, for cancer and noncancer risk assessment[3,4] have emphasized the importance of considering what is known about the pharmacokinetics and mode of action of a chemical to support quantitative dosimetry in the estimation of acceptable levels of chemical exposure. These changes in the guidelines facilitate the application of more sophisticated techniques for the incorporation of pharmacokinetic and mechanistic data into the dosimetric calculations performed in a risk assessment.[5] Physiologically based pharmacokinetic (PBPK) models are particularly attractive for this purpose because they can be used to quantitatively describe the metabolism and disposition of a chemical in both experimental animals and humans, resulting in more accurate dosimetry estimates.[6,7]

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As the development of PBPK models has flourished, there have been increasing efforts to incorporate these models into the risk assessment process.[8] The first use of a PBPK model in an agency risk assessment was in 1987, when the U.S. Environmental Protection Agency (EPA) updated its inhalation risk assessment for methylene chloride using the PBPK model of Andersen et al.[9] The model used in this case was relatively simple, with five tissue compartments and two metabolic pathways, but uncertainty regarding model structure and parameterization was nevertheless a major source of controversy.[10] Since this first consideration of a PBPK model in risk assessment, more complex models have been proposed for use, driven by the need to quantitatively describe relevant physiological processes.[8] For example, upper respiratory tract dosimetry models have now been developed[11,12] that divide the nasal cavity into multiple compartments to describe the exposure of the tissue areas most vulnerable to chemical insult. In general, model structure should be as complex or as simple as necessary to describe the pertinent in vivo processes; however, it is critical that these model structures rely on sufficient data in their development to validate their use in the context of the risk assessment.[13] Evaluation of the suitability of a particular PBPK model for use in a risk assessment can be a difficult undertaking, particularly when the model is being used to predict a dose metric that is not directly measurable, such as the average tissue concentration of a reactive intermediate produced during metabolism. In such cases, where it is necessary to validate the model based on predictions of quantities other than the preferred dose metric, additional care is needed to assure that the validation data are adequately informative regarding the performance of the model for the intended use.[14] Sensitivity and uncertainty analysis are useful techniques for performing this evaluation.[15,16]

Often when a risk assessment is to be conducted, more than one PBPK model is available for the chemical of interest. This was the case for the methylene chloride risk assessment,[10] and it is currently an issue for several chemicals for which regulatory toxicity criteria are in the process of being developed, including trichloroethylene (TCE)[17–20] and perchloroethylene (PCE).[2,21–30] The alternative models will typically differ in the data used for their development and validation, and may have structural differences reflecting both the desired level of detail and the nature of the intended application. In addition, the alternative models may address different routes of exposure or simulate kinetics in different species or strains. Whenever multiple models are available, questions will naturally arise as to which model provides the most accurate estimates of the dose metrics to be used in the risk assessment. Differences in model structure and parameterization can have a significant impact on the estimation of dose metrics, and hence on the estimation of acceptable levels of chemical exposure.[31,32] In these cases, it is critical that all of the available quantitative data on the pharmacokinetics of a given chemical, and not just the data used for the development of a specific model, be considered during comparative model evaluation.

PCE provides a particularly suitable example of the issues associated with comparative model evaluation. A number of applications of PBPK modeling in a risk assessment for PCE have been described.[2,21,24,33,34] Several analyses have also been performed to evaluate the uncertainty and/or variability in PBPK models of PCE and the implications of this uncertainty/variability for a PCE risk assessment.[23,24,29,31,32,35] This issue has been a major concern in ongoing risk assessment efforts for PCE at both the U.S. Environmental Protection Agency (EPA)[36] and the California EPA.[34] In particular, variability in the prediction of human dosimetry was a critical issue in the recent derivation of a public health goal (PHG) for PCE[34] by the California EPA Office of Environmental Health Hazard Assessment (OEHHA). PHGs are intended to be based on risk assessments conducted using the most current principles, practices, and methods.[37] In the case of PCE, OEHHA determined that the level of variability associated with predictions of the desired human dose metric (fraction metabolized) justified the use of upper bound estimates from a published population variability analysis.[23]

The purpose of this review is to conduct a comparison of the various published PBPK models for PCE, and to identify critical issues to be considered when applying these models in a risk assessment. After a brief summary of the most pertinent information regarding the carcinogenicity and pharmacokinetics of PCE, the available PBPK models will be described. The importance of the data with which the models are validated will be demonstrated by comparing the predictions of the various models with recently published data on human exposures at relatively low concentrations. This evaluation of the PBPK models for PCE provides an example of how it is possible to deal with the problem of PBPK model structure and parameter selection without resorting to upper bound approaches, such as those employed in the derivation of the PHG for PCE.

Carcinogenicity of PCE

Epidemiological studies provide conflicting evidence regarding the carcinogenic potential of PCE in humans following inhalation or oral exposure.[38–47] In general, these studies either did not indicate statistically significant increases in cancer incidence or were largely confounded by coexposure to other chemicals.[48–51] A recent comprehensive review concluded that "the current epidemiological evidence does not support a conclusion that occupational exposure to PCE is a risk factor for cancer of any specific site."[51] Because the results of epidemiological studies are inconclusive, estimates of the human carcinogenic potential of PCE have relied mainly on chronic studies conducted in rodents.[33,34,52]

Only two chronic bioassays addressing the potential carcinogenicity of PCE have been conducted that are usable for risk assessment purposes: an oral gavage study[53] and an inhalation study.[54] The published U.S. EPA[33,52] risk assessments for PCE,

as well as the PHG for PCE,[34] are based on the tumorigenicity observed in these studies.

In the chronic oral study,[53] groups of male and female Osborne–Mendel rats or $B6C3F_1$ mice were administered two dose levels of PCE in corn oil by gavage 5 days per week for 78 weeks, followed by 32 (rats) or 12 (mice) weeks of observation. Accounting for several dose adjustments made during the study, time-weighted average doses were 471 and 941 mg/kg/day in male rats, 474 and 949 mg/kg/day in female rats, 536 and 1072 mg/kg/day in male mice, and 386 and 772 mg/kg/day in female mice. Statistically significant increases in the incidence of hepatocellular carcinomas were observed in treated mice of both sexes, compared to control incidences (Table 1). Survival was significantly lower in treated mice than in controls, suggesting that the optimum dose may have been exceeded. Significant early mortality was also observed in rats, mainly due to PCE-related toxic nephropathy. Because of the reduced survival, the rat study was not considered adequate for evaluation of the carcinogenicity of PCE.[53] This study has also been questioned because the PCE administered was stabilized with epichlorohydrin, a strong alkylating agent that is itself an animal carcinogen.

In the inhalation study,[54] both $B6C3F_1$ mice and Fischer 344 rats were exposed to PCE, 6 hours/day, 5 days/week, for 103 weeks. Mice were exposed to concentration of 0, 100, or 200 ppm PCE, while rats were exposed to concentrations of 0, 200, or 400 ppm. In mice of both sexes, there were statistically significantly increased incidences of hepatocellular adenomas and carcinomas (combined) in treated animals compared to corresponding controls (Table 1). In rats, the only endpoint that was statistically significantly increased compared to concurrent controls was mononuclear cell leukemia in both males and females. The National Toxicology Program (NTP)[54] considered these tumors as contributing to the evidence of carcinogenicity of PCE in rats; however, the U.S. EPA Science Advisory Board concluded that these results did not provide a scientific basis to associate mononuclear cell leukemia in rats with inhalation exposure to PCE.[55] This type of leukemia, which occurs spontaneously in Fischer 344 rats, does not occur in humans; therefore, its relevance to human health is uncertain.[56] A nonsignificant

TABLE 1
Tumor incidence in chronic bioassays with perchloroethylene

		Doses and incidence			
Study	Sex/species	Dose (mg/kg/day)	Fatal tumors	Incidental tumors	Number of animals at risk
NCI, 1977—oral[53]	Male mouse		Hepatocellular carcinoma		
		0	0	2	20
		536	19	13	50
		1072	17	10	50
	Female mouse		Hepatocellular carcinoma		
		0	0	0	20
		386	8	11	50
		772	13	6	50
NTP, 1986—inhalation[54]	Male mouse		Hepatocellular adenoma or carcinoma		
		0[a]	1	16	49
		100[a]	17	14	49
		200[a]	14	27	50
	Female mouse		Hepatocellular adenoma or carcinoma		
		0[a]	2	2	48
		100[a]	5	12	50
		200[a]	24	14	50
	Male rat		Mononuclear-cell leukemia		
		0[a]	19	9	50
		200[a]	26	11	50
		400[a]	28	9	50
	Female rat		Mononuclear-cell leukemia		
		0[a]	8	9	50
		200[a]	20	10	50
		400[a]	19	10	50

increase in renal tubular-cell adenomas and carcinomas observed in male rats only was considered to be treatment related.[54] In contrast, no tumors were observed in groups of 96 male and 96 female Sprague-Dawley rats exposed to PCE concentrations of 300 or 600 ppm, 6 hours/day, 5 days/week, for 52 weeks, and observed for the rest of their lives.[57] The reason for this apparent discrepancy is unknown, but may be related to differences in rat strain and/or exposure duration.

In 1985, the U.S. EPA classified PCE in weight-of-evidence group "C—Possible Human Carcinogen," and calculated risk estimates based on the incidence of hepatocellular carcinoma in mice in the oral gavage study,[53] using the then-current default cross-species scaling based on body surface area.[52] An inhalation unit risk of 4.8×10^{-7} $(\mu g/m^3)^{-1}$ was obtained using estimates of metabolized dose (based on total urinary excretion of metabolites) from pharmacokinetic studies in mice[58] and humans.[59] A drinking-water unit risk of 1.5×10^{-6} $(\mu g/L)^{-1}$ was also derived assuming 100% metabolism of ingested PCE. In 1986, the agency revised its PCE inhalation risk estimate[33] based on the results of the inhalation bioassay.[54] The risk estimates were again calculated on the basis of metabolized dose using the same approach as the previous assessment. The resulting inhalation unit risk, based on the incidence of liver adenoma/carcinoma, was 5.8×10^{-7} $(\mu g/m^3)^{-1}$, almost identical to the value derived from the oral study. Similar unit risks were also obtained for the other endpoint evaluated: leukemia in male and female rats.

Metabolism and Mode of Action of PCE

PCE is a volatile lipophilic compound that is readily absorbed following inhalation, oral, or dermal exposure in both animals and humans, and is distributed primarily to the fat.[49] Based on urinary excretion of metabolites, the metabolism of PCE is expected to be limited. For example, after an oral dose of 800 mg/kg in rats, excretion of metabolites in the urine accounted for only 2% of the dose.[60] Metabolism of PCE in humans is also very limited, based on metabolites measured in the urine.[61,62] The metabolic pathway of PCE appears to be qualitatively similar in mice, rats, and humans (Figure 1). However, the significant quantitative differences in the rate and extent of metabolism among the species are thought to be responsible for corresponding species differences in target organ doses, and hence responses.[1,49,63–68] Formation of TCA via cytochrome P-450 (CYP) oxidation is the principal route of metabolism in all three species, representing approximately 60% of total metabolism in both rats and mice,[69] and essentially all of the urinary excretion observed after PCE exposure.[60,70] Excretion of TCA in urine therefore provides a useful measure of PCE metabolism.

Two alternative (but not mutually exclusive) modes of action have been frequently discussed to explain the liver carcinogenicity of PCE in mice: (1) cytotoxicity associated with reactive intermediates produced during the oxidative metabolism of PCE,[33,52] and (2) metabolism to TCA, a peroxisome proliferator.[64] CYP oxidation of PCE results in formation of PCE oxide

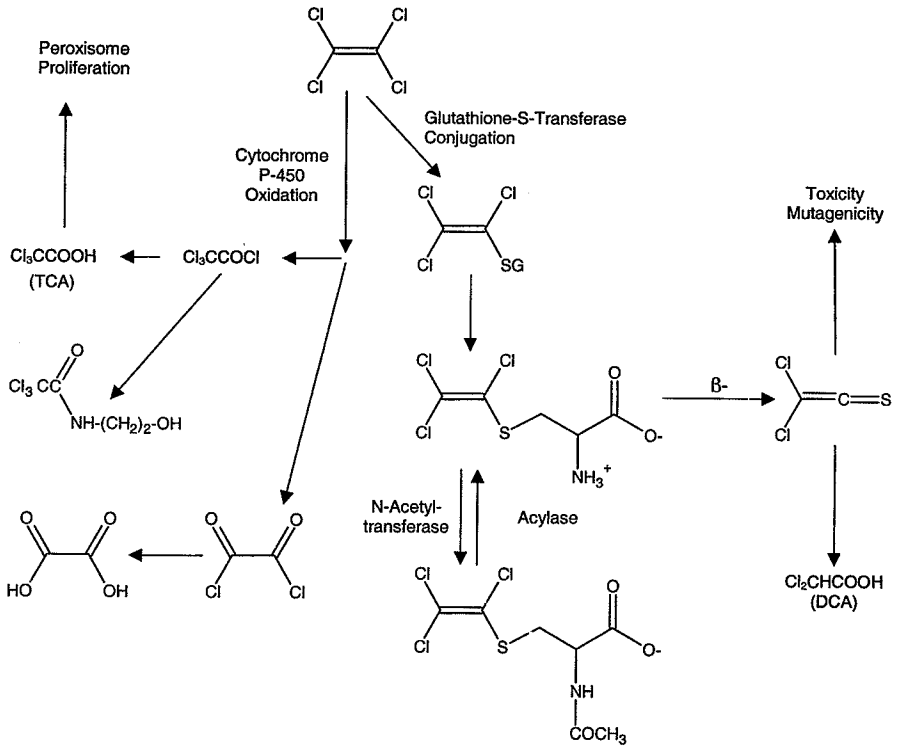

FIG. 1. Metabolism of perchloroethylene (adapted from Vamvakas et al., 1993[60].)

and trichloroacetyl chloride (Figure 1); both can react with macromolecules.[67,71,72] Oxidative metabolism of PCE is quantitatively greater in mouse than rat hepatocytes,[64] and greater in rat than in human liver.[1,32] Trichloroacetylated protein residues have been detected in the livers of mice[73] and rats,[67,71,72] indicating reaction of some trichloroacetyl chloride with proteins. Adduct formation in human liver has not been directly examined. Since trichloroacetylated proteins were much lower in blood from human volunteers exposed to 10 and 40 ppm PCE than from similarly exposed rats, humans should also have a correspondingly lower level of hepatic binding.[67] While the toxicological significance of these protein adducts is unclear, oxidative damage to lipids and DNA provides a more direct indication of potential for cellular toxicity. PCE-induced oxidative stress has not been examined in mice; however, vitamin E in mice receiving very high doses of PCE (3000 mg PCE/kg orally for 15 days) partially protected the mice from liver toxicity.[74,75] Rats administered single doses of PCE (100, 500, or 1000 mg/kg) showed no evidence of either lipid peroxidation or oxidative DNA damage.[76]

Of some interest, a recent study comparing indices of oxidative stress in female dry cleaners exposed to time-weighted average concentrations below 5 ppm versus laundry workers matched by age, race, and smoking status reported significantly decreased levels of leukocyte 8-hydroxydeoxyguanosine (an extensively used biomarker of exposure to oxygen-based radicals) in the former group.[77] Several experimental observations offer potential mechanistic explanations for reduced levels of the marker in these workers. First, pretreatment of rats with clofibrate (a peroxisome proliferating hypolipidemic drug) or TCA markedly mitigated the lipoperoxidative response following acute challenge with TCA.[78] Since this effect was linked to both markers of peroxisome proliferation and increased expression and activity of CYP4A, the observation indicates that it is mediated by peroxisome proliferator-activated receptor α (PPARα). The authors suggested that the observed reduction of the peroxidative response in pretreated animals was due to the shift in the expression of CYP isoforms from those that participate in lipid peroxidation to those that do not.[78] Human hepatocytes contain lower levels of PPARα than those of rodents and do not undergo peroxisome proliferation. However, the increases in CYP4A in response to peroxisome proliferators, including TCA, have been reported in cultured human cells.[79,80] TCA produced by PCE metabolism in humans might exert a similar PPARα-mediated protective effect. PPARα ligands inhibit inflammatory processes in many tissues, acting via a variety of molecular mechanisms.[81–83] Although no studies have demonstrated any anti-inflammatory efficacy of TCA, it may share this property with other peroxisome proliferators.

Although some of the kidney toxicity of high doses of PCE observed in male rats is attributable to $\alpha 2\mu$-globulin accumulation,[84] kidney tumorigenicity has been proposed to be associated with formation of the glutathione conjugate in the liver followed by conversion to the cysteine conjugate in the kidney, where it is activated by β-lyase to form a highly reactive metabolite, dichlorothioketene.[67,69,84–86] This pathway appears to be significantly less active in humans than in rats, suggesting that humans may be less susceptible to PCE-induced nephrotoxicity.[1,66,68]

PBPK Modeling of PCE

With few exceptions, the PBPK models for PCE share the simple four-compartment structure (liver, fat, rapidly perfused tissues, and slowly perfused tissues) and steady-state description of lung equilibration developed by Ramsey and Andersen[87] for styrene. Only one of the published models[2] provides a description of the kinetics of TCA, the major metabolite of PCE. None of the models provide a description of the glutathione conjugation metabolic pathway that has been implicated in the kidney lesions produced by PCE. Therefore, it is not possible to estimate dose metrics for kidney toxicity as has been done in the case of TCE.[88] For the most part, the differences between the models reflect the different data used by the authors in their development. To simplify comparison, animal and human modeling studies are described separately, followed by a discussion of studies focusing on the uncertainty and variability in the models.

Animal Modeling Studies

Several pharmacokinetic modeling studies have been performed to characterize the kinetics and metabolism of PCE in the mouse[2,21,22,29] and, in some cases, the rat.[21,22,29] Two of these models have been applied in the estimation of internal dose metrics for use in risk assessment.[2,21] Development of PBPK models of PCE has also been conducted in the rat and dog by Dallas et al.[25–28] These rat and dog models, which were developed to describe blood and exhaled air concentrations of PCE, would likely be useful to provide parent chemical dosimetry estimates for toxicity studies conducted in these species. However, since the cancer risk assessment for PCE is based on tumors in the mouse, these models of PCE kinetics in the rat and dog have not been considered in the present analysis.

Chen and Blancato[21] developed a PBPK description of the pharmacokinetics of PCE as part of a U.S. EPA[33] risk assessment. Their model structure was based on the styrene model of Ramsey and Andersen.[87] Physiological parameters were taken from a compilation of values used in previously published PBPK models,[89] and partition coefficients were obtained from the Air Force toxicology laboratory at Wright-Patterson Air Force Base.[90] Metabolism parameter values in rat and mouse were estimated by fitting the model to published data on total metabolism following inhalation and oral exposures to radiolabeled PCE,[70,91] assuming a single oxidative CYP pathway that followed Michaelis–Menten kinetics. No attempt was made to compare predictions of the model with other kinetic data. Using this model, Chen and Blancato[21] estimated daily production of total metabolites in male and female mice following exposure to

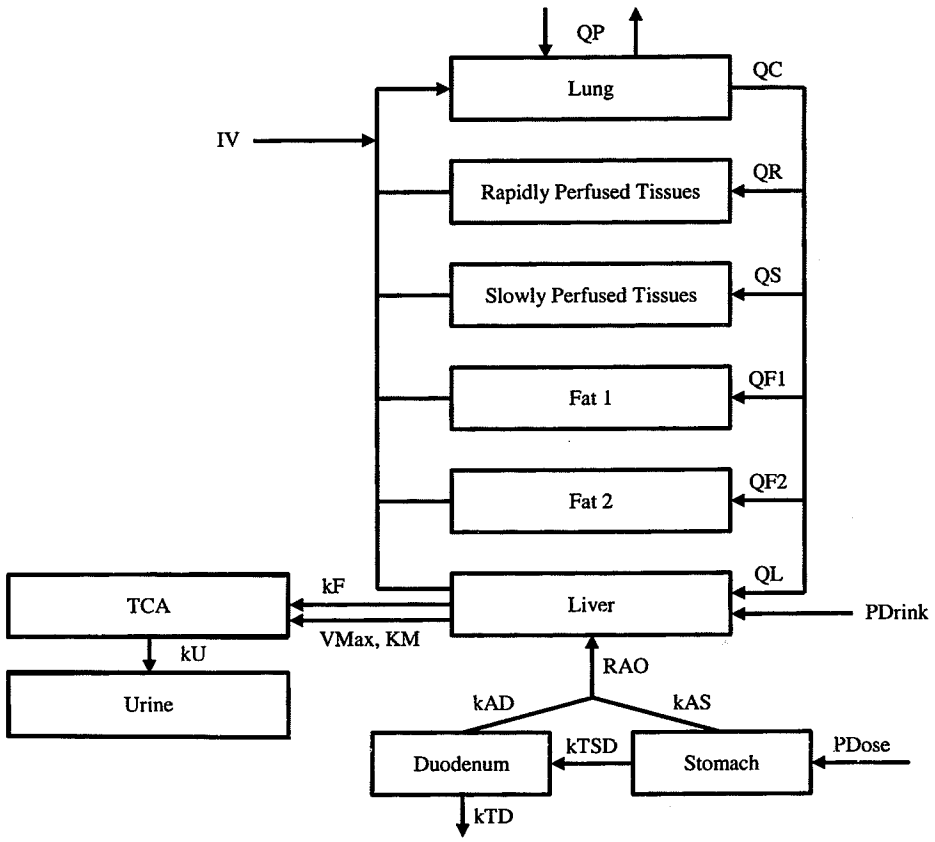

FIG. 2. Structure of perchloroethylene PBPK model of Gearhart et al. (1993).[2] *Note*: A description of the parameters is provided in Table 2. For some parameters, a "C" has been added to the abbreviation to indicate a "scaled" value.

PCE either by gavage or inhalation under the bioassay exposure conditions.[53,54]

Ward et al.[22] developed a similar PBPK model for PCE in the mouse and rat. Metabolism parameter values were estimated by fitting the model to experimental data sets on total metabolism and total metabolite excretion. In addition to the disposition data of Pegg et al.[91] and Schumann et al.[70] relied on by Chen and Blancato,[21] data on total urinary excretion of metabolites[58] were used, assuming that urinary excretion accounted for 65% of metabolism. Again, no attempt was made to compare the model with other kinetic data. In contrast to the model of Chen and Blancato,[21] which included only a saturable Michaelis–Menten description of the metabolism of PCE, the Ward et al.[22] model included both a saturable and a linear component in the equation for metabolism. They assumed that the saturable pathway represented oxidative CYP metabolism and that the linear pathway represented conjugation with glutathione; however, no experimental data were presented to support this assumption.

Gearhart et al.[2] conducted targeted experimental studies to support the development of a more comprehensive mouse PBPK model for PCE. Unfortunately, the publication does not provide a complete documentation of the model structure and parameters; however, it was possible to obtain a copy of the actual model code from the principal developer (J. M. Gearhart, personal communication). As a check on the model code, it was possible to reproduce each of the figures in Gearhart et al.[2] A diagram of the Gearhart et al.[2] model is shown in Figure 2, and the parameters are provided in Table 2. The Gearhart et al.[2] model differed from the earlier models in that it included two fat compartments with different perfusion ratios. The value of including multiple fat compartments has since been demonstrated for a related compound, TCE.[92] Another refinement was the use of a two-compartment description of oral uptake (Figure 2); a similar description has also been used successfully for TCE.[18] More significantly, the Gearhart et al.[2] model also described the kinetics of the principal metabolite, TCA, using a single-compartment model with first-order urinary excretion, and assuming that the amount of TCA produced represents 60% of the total amount of PCE metabolized.[69] The kinetic parameter values for TCA were taken from a description of the same metabolite in a model of TCE.[17] To determine the metabolic parameters for the mouse model, Gearhart et al.[2] conducted closed-chamber PCE clearance studies at concentrations of 200, 1000, and 3500 ppm PCE. In addition, concentrations of PCE and TCA were measured in the blood of mice following gavage administration of doses comparable to those used in the NCI[53] study. These oral data were

TABLE 2
Parameters for perchloroethylene PBPK model of Gearhart et al. (1993)[2]

Name	Definition	Mouse	Human
BW	Body weight (kg)	0.035	70.0
QCC	Cardiac output (L/h/kg$^{0.75}$)	18.0	13.0[a]
QPC	Alveolar ventilation rate (L/h/kg$^{0.75}$)	30.0	18.0[a]
Blood flows (fraction of cardiac output)			
QFC1	Fat tissue 1	0.04	0.03
QFC2	Fat tissue 2	0.03	0.02
QLC	Liver	0.16	0.23
QRC	Rapidly perfused tissues	0.59	0.70
QSC	Slowly perfused tissues	0.41	0.30
Tissue volumes (fraction of body weight)			
VDC	TCA volume of distribution	0.24	0.1
VFC1	Fat tissue 1	0.05	0.16
VFC2	Fat tissue 2	0.02	0.05
VLC	Liver	0.057	0.026
VRC	Rapidly perfused tissues	0.21	0.11
VSC	Slowly perfused tissues	0.64	0.65
Partition coefficients			
PB	Blood:air	20.0	11.58
PF	Fat:blood	75.0	125.2
PL	Liver:blood	2.4	5.28
PR	Rapidly perfused tissue:blood	2.2	5.06
PS	Slowly perfused tissue:blood	3.3	6.11
Metabolism parameters			
VMaxC	Maximum rate of metabolism (mg/hr/kg$^{0.75}$)	0.2	0.28
KM	Michaelis-Menten affinity constant (mg/L)	2.0	7.7
kFC	Pseudo-first order metabolism (kg$^{0.25}$/hr)	2.0	0.0
FTCA	Fraction of PCE metabolized to TCA in liver	0.6	0.6
Oral absorption parameters (/h)			
kAS	Absorption from stomach	0.0	—[b]
kAD	Absorption from duodenum	0.3	—
kTSD	Transfer from stomach to duodenum	2.0	—
kTD	Excretion from duodenum	0.0	—
Urinary clearance parameter (kg$^{0.25}$/h)			
kUC	TCA urinary clearance	0.035	0.12[c]

[a] Values used for simulation of experimental subjects. Values used for human dose metric calculations were 16.5 for QCC and 24.0 for QPC.

[b] Human oral exposure from drinking water described by continuous zero-order intake.

[c] Original value used in Gearhart et al. (1993)[2] model. Revised human model and dose metric calculations used a value of 0.023, based on value used for TCA in a PBPK model of trichloroethylene (Clewell et al., 2000).[18] Revised model also assumed metabolism of PCE in the kidney with a VMaxC of 0.028.

used to validate the kinetic parameters for PCE and TCA. The description of metabolism in the model was similar to that used by Ward et al.,[22] including both saturable and linear metabolism components. However, both components were assumed to represent oxidative CYP metabolism, producing TCA. This assumption was necessary to provide a consistent description of both the closed-chamber PCE clearance data and the data on TCA concentrations following oral gavage with PCE. The parameter values for this model are shown in Table 2.

Reitz et al.[29] conducted 6-h inhalation studies with PCE in the mouse and rat. Metabolism parameter values were then estimated by fitting a PBPK model to the postexposure time course for the exhalation of PCE, assuming Michaelis–Menten kinetics. The resulting estimates for both the capacity (V_{max}, mg/h) and affinity (K_m, mg/L) of PCE metabolism were roughly a factor of 2 higher than the values estimated by Chen and Blancato[21] from disposition data, but the estimated clearance (V_{max}/K_m) was similar. The model was then used with the estimated parameter values to predict closed-chamber gas uptake studies. The metabolism parameter values estimated for the rat provided model predictions that were in good agreement with the closed-chamber data. However, in order to obtain agreement with the closed-chamber data in the mouse it was necessary to double the K_m estimated from the postexposure exhalation data.

Human Models

A number of studies have been performed to develop PBPK models of PCE in the human,[2,21,22,29,30] and in two cases[2,21] the resulting models have been applied in the estimation of internal dose metrics for use in risk assessment.

Chen and Blancato[21] developed a PBPK description of the pharmacokinetics of PCE in the human as part of a U.S. EPA[33] risk assessment. This model used the same structure as their rodent model. Physiological parameter values were taken from a compilation of values used in previously published PBPK models,[33] and partition coefficients were obtained from the Air Force toxicology laboratory at Wright-Patterson Air Force Base.[90] Metabolism parameter values in the human were estimated from fitting of published data on total urinary excretion of the metabolite TCA for 72 h following inhalation exposures to PCE,[93,94] assuming that the excretion of TCA over the 72 h postexposure represented 30% of total metabolism. This estimate, which is lower than would be expected from disposition data in animals, was based on the fit of the model to data on exhaled air concentrations of PCE postexposure.[93] No attempt was made to compare predictions of the model with other published kinetic data.

Ward et al.[22] also developed a model for PCE in the human. In contrast to their description of the rat and mouse, which included both saturable and linear metabolism, the human model only included a saturable oxidative pathway. Metabolism parameter values were estimated by fitting the model to data on the urinary excretion of TCA from workers exposed to PCE in the course of their work.[95] The predictions of the model were then compared

with published data on exhaled air concentrations for controlled inhalation exposures ranging from 72 to 198 ppm.[93,94,96] The authors concluded that the human metabolic parameters could be predicted by assuming equal K_m across species and scaling the rat V_{max} by body weight raised to the three-quarters power, and that similar scaling of V_{max} from the mouse overestimated human metabolism. However, the human metabolic parameter values estimated in this study must be viewed with caution because they were estimated from data for individuals exposed under uncontrolled conditions in the workplace, with exposure concentration estimates based on the average of measurements taken at various sites in different workshops.

Gearhart et al.[2] developed a human model of PCE that, like the mouse model on which it was based, includes two fat compartments in the parent chemical description, and also describes the production, distribution, and excretion of the principal metabolite, TCA. The parameters for the metabolism of PCE in the human were estimated by fitting the model to data on the time course of urinary excretion of TCA following inhalation exposure to PCE,[93] assuming that TCA represents 60% of the total metabolism of PCE in the human, as it does in the mouse and rat.[69] As in the case of the Ward et al.[22] model, only a single saturable pathway was described in the human. The predictions of the model were then compared with published data on exhaled air concentrations for controlled inhalation exposures.[93,96]

Reitz et al.[29] developed a human version of their PBPK model for PCE by estimating in vivo metabolic parameter values in humans based on a parallelogram approach using in vivo data in mice and rats plus in vitro data in mice, rats, and humans. A sensitivity analysis of the PBPK model revealed that the most significant uncertainties were in the techniques used to estimate rates of PCE metabolism in humans.

Loizou[30] developed a PBPK model for PCE in the human to investigate workplace exposures. Using the PCE metabolism and partitioning parameters from Gearhart et al.,[2] he was able to successfully simulate experimental data on exhaled PCE concentrations following both inhalation[93] and dermal[97] exposures, as well as data on the time course of blood concentrations and urinary excretion of TCA following inhalation of PCE.[1,94] The model was then used to analyze occupational exposure data from dry-cleaning operations.

Uncertainty Analyses

Farrar et al.[35] reparameterized the U.S. EPA[33] model using distributions of parameters rather than single values, and conducted a Monte Carlo analysis to evaluate the uncertainty in risk estimates resulting from the uncertainty in the parameters. They found that an upper-bound estimate (the 97.5th percentile) of the human dose metric for amount of PCE metabolized per volume of liver was approximately fourfold higher than the median estimate.

Bois et al.[24] conducted a similar analysis using the same model structure evaluated by Farrar et al.[35] As in Farrar et al.,[35] instead of estimating single values for each parameter in the model, probability distributions were specified. Estimates of metabolic parameter values in the rodent were obtained by fitting multiple disposition studies,[58,69,70,98] assuming saturable Michaelis–Menten metabolism. Estimates of metabolized doses in the mouse and rat were similar to those estimated by Chen and Blancato.[21] In the case of the human, Bois et al.[24] relied on urinary excretion data from occupational studies of Japanese workers[61,95] to estimate maximum likelihood estimates of the metabolism parameter values. The fraction of total metabolite excreted in the urine was assumed to be 65%, based on data from animals.[69,91,98,99] Bois et al.,[24] then used the model to estimate metabolized dose of PCE in rats and mice, following inhalation exposure in the NTP[54] study, and in humans exposed to 1 ng/L in air. As in Farrar et al.,[35] parameter values were described by probability distributions rather than fixed values, with a Monte Carlo approach used to generate a distribution of estimates. The model was coupled with a multistage model to evaluate the precision of the resulting risk assessment. In looking at the variability in the model parameters, they assessed risks that ranged from 0.04 per million (5th percentile) to 6.8 per million (95th percentile), with a median risk estimate of 1.6 per million, very similar to the results of Farrar et al.[35]

Hattis and coworkers[31,32] compared parameters and results from a number of published and unpublished PBPK models for PCE in the human, including the models of Chen and Blancato,[21] Ward et al.,[22] and Farrar et al.[35] All of the models compared were variations on same basic framework described by Chen and Blancato[21]; that is, they all represented models of parent chemical absorption, distribution, and clearance, with no description of metabolite kinetics. The model of Gearhart et al.,[2] which includes a description of the kinetics of the principal metabolite, TCA, was not available at the time this comparison was conducted. Some models did, however, include a nonspecific compartment for total unexcreted metabolites, to facilitate modeling of data on total urinary metabolite excretion. The most important differences noted between the various models were in their descriptions of the metabolism of PCE, particularly in the use of linear or saturable metabolism, or both. In the first study, Hattis et al.[32] evaluated the variability in predictions of total metabolism in animals exposed at the lowest concentrations used in the NTP bioassays and in humans exposed at 1 ppm. While predictions of the various models were in relatively good agreement in the mouse (within a factor of 3), the authors found substantial (60-fold) differences in model predictions of metabolism in the human, which they concluded were primarily related to the choice of data used for estimating the metabolic parameter values. In the second study,[31] predictions of the various human models were compared with data on fractional absorption of inhaled PCE, as well as measured concentrations of PCE in alveolar air and venous blood. Overall, the model predictions showed a systematic departure from the observed alveolar air and blood levels that the authors suggested might be resolved with more sophisticated description of the fat compartment.

Gearhart et al.[2] performed a Monte Carlo analysis with their mouse and human models, following the approach of Farrar et al.,[35] and incorporating estimates of the uncertainty in the parameters from statistical analyses of repeated measurements conducted as part of the experimental effort. They found that, in general, the upper and lower bound estimates for model-predicted dose metrics were within a factor of 2 of the median estimates. These results are consistent with the conclusions of Farrar et al.,[35] who found a somewhat greater variability for risk estimates, because the observed variation in the risk estimates reflects the variation in both the animal and human dose metrics.

Bois et al.[23] applied the Markov-chain Monte Carlo (MCMC) technique to evaluate the population variability of the metabolism of PCE in the human, using the same model structure as in their earlier study.[24] In this hierarchical Bayesian approach, prior estimates of the distributions for each of the parameters, as well as for the uncertainty in the distributional parameters (mean and variance) themselves, are used as input to the MCMC algorithm along with experimental data sets that are considered informative regarding the population distribution of the parameters. The algorithm then combines the information from the priors and the data in a Bayesian framework to obtain posterior estimates of the population distributions of the parameters. In the case of the analysis conducted by Bois et al.,[23] only one experimental data set was used in the MCMC: measured concentrations of PCE in blood and exhaled air for 1 week following exposures to 72 or 144 ppm PCE in an inhalation chamber for 4 h.[94]

The resulting estimates of fractional metabolism obtained by Bois et al.[23] were strongly dependent on exposure concentration. For exposure at 50 ppm, the predicted 95% confidence interval for fractional metabolism ranged from 0.52 to 4.1%, while for exposure at 1 ppb the 95% confidence interval ranged from 15 to 58%. The authors concluded that the model predictions for fractional metabolism at the higher concentration were consistent with the data in Monster et al.[94] Indeed, the average excretion of TCA in these studies was approximately 6 and 11 mg at 72 and 144 ppm, respectively, while the corresponding average net uptake of PCE reported by Monster et al. (1979) was 455 and 945 mg; therefore, excretion of TCA accounted for around 1% of the net uptake of PCE at both exposure levels. Bois et al.[23] attributed the difference in fractional metabolism between high and low exposure concentrations to saturation occurring between 1 and 10 ppm.

EVALUATION OF ALTERNATIVE PBPK MODELS FOR USE IN A PCE RISK ASSESSMENT

The appropriate evaluation of a PBPK model is strongly dependent on its intended application. The criteria for a model intended to codify quantitative hypotheses and support experimental design and inference in conjunction with mechanistic studies are likely to be quite different from those for a model intended to perform the dosimetric extrapolations needed in a risk assessment. In this review, the evaluation of the various PBPK models is conducted from the viewpoint of their potential use in a cancer risk assessment for PCE, and does not consider their value as a tool for conducting research on the metabolism and mechanism of toxicity of PCE.

Results of Parameter Sensitivity Analyses

Given the relatively large number of parameters in a PBPK model, even the simple models that have been developed for PCE, a parameter-by-parameter comparison would be impractical. Fortunately, only a few of the parameters typically have a significant impact on the dose metric predictions of the models.[15] Based on sensitivity analyses conducted with several of the PBPK models for PCE,[2,24,29] it is possible to restrict consideration to the key parameters for the prediction of metabolized dose in the animal bioassays and in human steady-state exposure conditions.

Based on the observed correlation between the model parameters and the predicted rate of metabolism during continuous human exposures, Bois et al.[24] found that the most important parameters were those for metabolism, partitioning, and, to a lesser extent, the ventilation rate, while those for tissue volumes and blood flows were relatively unimportant. Using the same correlational approach for estimating sensitivity, Gearhart et al.[2] obtained similar results for both continuous human ingestion and mouse gavage. Reitz et al.[29] determined the analytical sensitivity of predictions of metabolism to each of the model input parameters for inhalation exposure of mice and humans. Their results were consistent with the correlation analyses, in that the most critical parameters for the prediction of amount metabolized were found to be the capacity and affinity of metabolism, the blood:air partition coefficient, and, at low concentrations in the human, the ventilation rate. Of these critical parameters, those for metabolism are clearly the most uncertain. The blood:air partition coefficients for PCE are well characterized in both mice and humans,[2] and ventilation rates, while varying across individuals and work loads, are also reasonably well characterized.[100,101] Therefore, it is concluded that evaluation of the alternative models should focus on their metabolism parameters.

Comparison of Mouse Models

Based on the evaluation performed by Hattis et al.,[31,32] it is clear that the greatest differences in the predictions of the PBPK models for PCE are in the case of the human rather than the mouse. Hattis et al.[32] found only a threefold variation in the model predictions for metabolism in the mouse, while similar predictions in the human varied by 60-fold. Nevertheless, there are a few points worth considering with regard to the modeling of metabolism in the mouse. In particular, any comparison of the metabolism predictions of the PBPK models for mice is complicated by the fact that some of the models use a single saturable term, while others use both saturable and linear terms. Moreover, the assignments of the pathways in the two-pathway

models differ. Ward et al.[22] assumed that the saturable pathway represented oxidative CYP metabolism while the linear pathway represented conjugation with glutathione, whereas Gearhart et al.[2] assumed that both pathways represented oxidative CYP metabolism. In both cases only a single saturable pathway was used for the human. A similar use of multiple components in the description of CYP oxidation in the mouse and rat, but not the human, was also required in the PBPK model for another haloethene, vinyl chloride.[14] In that case, two saturable components were included, one representing high-affinity, low-capacity metabolism by CYP 2E1, and the other representing lower affinity, higher capacity metabolism, presumed to be due to other isozymes of the CYP family. The observation that multiple CYP isozymes contribute to the metabolism of PCE in the mouse and rat[102,103] provides support for this description. Therefore, it is likely that the combination of saturable and linear components in the model of Gearhart et al.[2] represents an empirical description of PCE metabolism by multiple CYP isozymes with widely different affinities and capacities. This interpretation may also apply to the metabolism description in the model of Ward et al.[22]

From the viewpoint of using the models in a risk assessment, the main interest is the ability of the model to estimate amount metabolized in the PCE bioassays. Therefore, an approach similar to that used by Hattis et al.[32] for comparing the various models would seem to be the most reasonable. That is, the predictions of the models for the dose metric of interest for the risk assessment, the amount metabolized in this case, can be compared for an exposure similar to those used in the bioassays. The exposure chosen for the present analysis is shown in Figure 3, which compares the measured blood concentrations of TCA produced by a 6-h inhalation exposure of mice to PCE at 400 ppm[64] with the predicted TCA blood concentrations obtained using the PBPK model of Gearhart et al.[2] This data set, which reproduces the highest exposure in the inhalation bioassay,[54] was not used in the development of any of the models being compared. The estimates of total metabolism obtained with several different models (Table 3) are within a factor of 2, consistent with the results obtained by Hattis et al.[32] This agreement is not surprising since most of the mouse models have been calibrated against data on metabolite formation and/or excretion that are highly dependent on the extent of metabolism.

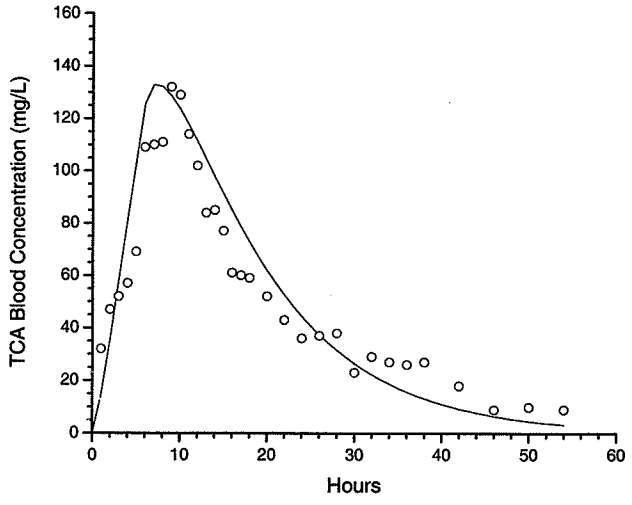

FIG. 3. Predicted (curve) and experimental (symbols) blood concentration of TCA for a 6-h inhalation exposure of mice to perchloroethylene at 400 ppm (Odum et al., 1988),[64] using the PBPK model of Gearhart et al. (1993).[2]

TABLE 3
Total metabolism following 6-h inhalation exposure of mice to perchloroethylene at 400 ppm, predicted with different PBPK models

Model	Total metabolism
Chen and Blancato, 1987[21]	2.09
Ward et al., 1988[22]	2.43
Ward et al., 1988[22a]	1.66
Bois et al., 1990[24]	2.06
Gearhart et al., 1993[2]	2.28
Reitz et al., 1996[29]	3.09

[a]Predicted metabolism by saturable pathway only.

Comparison of Human Models

The much greater variability in metabolism predictions observed by Hattis et al.[32] in the human reflects the greater variety of data used to calibrate the human models. Also, few human data are available that provide information concerning the rate of metabolism. There are no studies of radiolabel disposition in the human, and the interpretation of urinary excretion data is complicated by the longer half-life of metabolites in humans versus rodents.[1,104] Therefore, most of the human models have relied to some extent on data for the in vitro or in vivo kinetics of the parent chemical to infer the rate of metabolism. For example, in the human model of Bois et al.,[23] the metabolic parameters are based on blood and exhaled air concentrations of PCE after the end of an inhalation exposure.[94]

The use of postexposure parent chemical concentration data for metabolism parameter estimation is problematic because the predictions of PBPK models for volatile chemicals like PCE during the postexposure period in human inhalation studies provide only an indirect indication of the amount metabolized, particularly for compounds with relatively low blood:air partition coefficients.[14] Moreover, the human postexposure kinetics of volatiles are typically dependent on a number of uncertain parameters apart from those for metabolism. For example, in the case of methylene chloride inhalation in the human,[15] some of the largest sensitivities for predictions of blood concentrations during the postexposure period are to the parameters for the volume, blood flow, and partitioning of the fat, and for the blood flow to the liver. In the case of vinyl chloride,[14] varying the

metabolic clearance 10-fold had little impact on the fit of the PBPK model to data on parent chemical concentrations during and after a constant concentration inhalation exposure to 2.5 ppm vinyl chloride for 30 min. However, data from inhalation exposures to similar concentrations of the same compound using a closed rebreathing chamber provided a much better estimate of metabolic clearance. These studies demonstrate that highly uncertain parameters are likely to have more influence than the metabolic parameter values on the postexposure PCE concentrations predicted by the model for human inhalation exposures.

Another important determinant of postexposure parent chemical kinetics for volatiles such as PCE is the subject's activity level, due to its effects on both ventilation rate and the associated redistribution of blood flows.[105] Therefore, even less confidence can be placed in metabolic parameter values estimated from studies conducted in the workplace, such as those used to parameterize the human models of Ward et al.[22] and Bois et al.[24]

Subsequent to the publication of the human PBPK models for PCE described earlier, a new experimental study was reported in which human subjects were exposed to lower concentrations of PCE than in previous studies (10, 20, or 40 ppm for 6 h), and the urinary excretion of TCA was measured for 72 h postexposure.[1] Blood concentrations of TCA were also reported at two time points following the 10- and 40-ppm exposures. Since this study was not used in the development of any of the published models, and since it was conducted at lower concentrations than previous studies, it provides an opportunity to validate the metabolism predictions of the various models. In order to make use of this time-dependent metabolite formation and excretion data to infer total metabolism, the PBPK model of Gearhart et al.,[2] which includes a description of TCA formation and excretion, was exercised for the conditions of the exposure. The predictions of the Gearhart et al.[2] model, using the original parameter values (Table 2), are compared with the experimental data in Figure 4. With no adjustment of the parameter values, the predicted blood concentrations of TCA are in good agreement with the experimental data, while the model overpredicts the rate of excretion by roughly a factor of 2.

Although the purpose of this review was not to develop yet another PBPK model for PCE, there appeared to be a systematic discrepancy between the predictions of the Gearhart et al.[2] model and the experimental data for the time course of urinary excretion of TCA.[1] Specifically, the model was only able to simulate the rapid early excretion of TCA at the expense of overestimating excretion at longer times (Figure 4). Reducing the urinary excretion rate for TCA from the original value of 0.12 to the value of 0.023 used in a PBPK model for trichloroethylene[18] resulted in better agreement with the urinary excretion of TCA

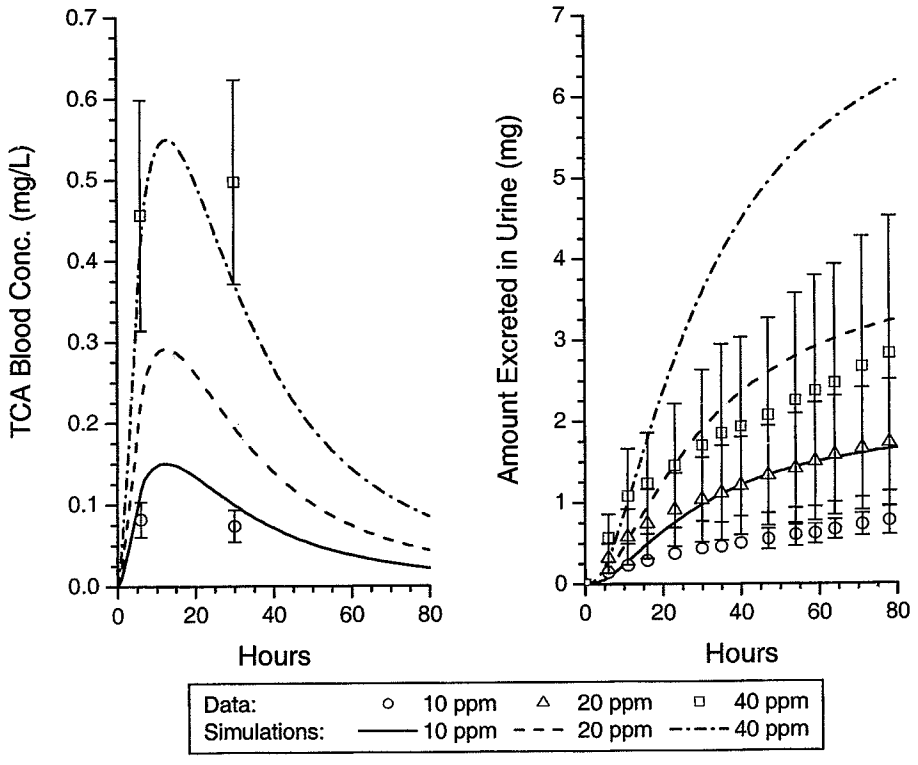

FIG. 4. Predicted (curves) and experimental (symbols) blood concentrations (left) and urinary excretion (right) of TCA for 6-h inhalation exposures of human subjects to PCE at 10, 20, and 40 ppm (Volkel et al., 1998),[1] using the original PBPK model of Gearhart et al. (1993).[2] Data are plotted as means ± one standard deviation. Error bars do not appear where the standard deviation was smaller than the symbol used to plot the mean.

at the end of the measurement period, but underestimated the initial rate of excretion (not shown). The nature of these discrepancies suggested the need to include metabolism of PCE to TCA in the kidney, with direct excretion of the TCA formed in the kidney into the urine (rather than into the systemic volume of distribution). Accordingly, the model of Gearhart et al.[2] was modified to incorporate these elements, and the relative capacity of PCE metabolism in the kidney was estimated by fitting the data of Volkel et al.[1] Better agreement with the experimental time course for TCA excretion was obtained when the metabolic capacity of the kidney was assumed to be 10% of the capacity of the liver, and the value of V_{maxc} in the liver was reduced from 0.28 to 0.24 (Figure 5). The pharmacokinetic parameters for TCA in the modified model were taken from a published PBPK model for trichloroethylene.[18] While the agreement of the modified model with the data on TCA excretion does not necessarily imply that the kidney contributes to the metabolic clearance of PCE, such a possibility is supported by data indicating that several CYP isoforms, including 2E1, 2A6, and 3A4, contribute to the metabolism of anesthetics in human kidney.[106]

In order to evaluate the predictions of the other human PBPK models, the selected PCE model was coupled to a submodel of TCA pharmacokinetics from a PBPK model for trichloroethylene.[18] The results of this approach are illustrated in Figure 6, where the PCE parent chemical model is from Ward et al.[22] A comparison of the predictions across the human models is presented in Figure 7. All of the human models overpredict the urinary excretion of TCA in the Volkel et al.[1] study, ranging from a factor of 2 for the model of Gearhart et al. to a factor of more than ten for the model of Reitz et al.[29] More importantly, two of the models[22,23] demonstrate a concentration dependence (reflected in the slope of the lines connecting the predictions) that differs significantly from that of the experimental observations. These two models have in common the use of lower values for the metabolism affinity parameter (K_m less than 1 mg/L) as compared to the models that more closely predict the observed concentration dependence (K_m greater than 1 mg/L).

The predictions of the human models for the fraction of PCE metabolized for continuous inhalation at 1 ppb or steady-state ingestion of 2 L/day of drinking water at 1 μg/L are presented in Table 4. The relationships of the model predictions, which

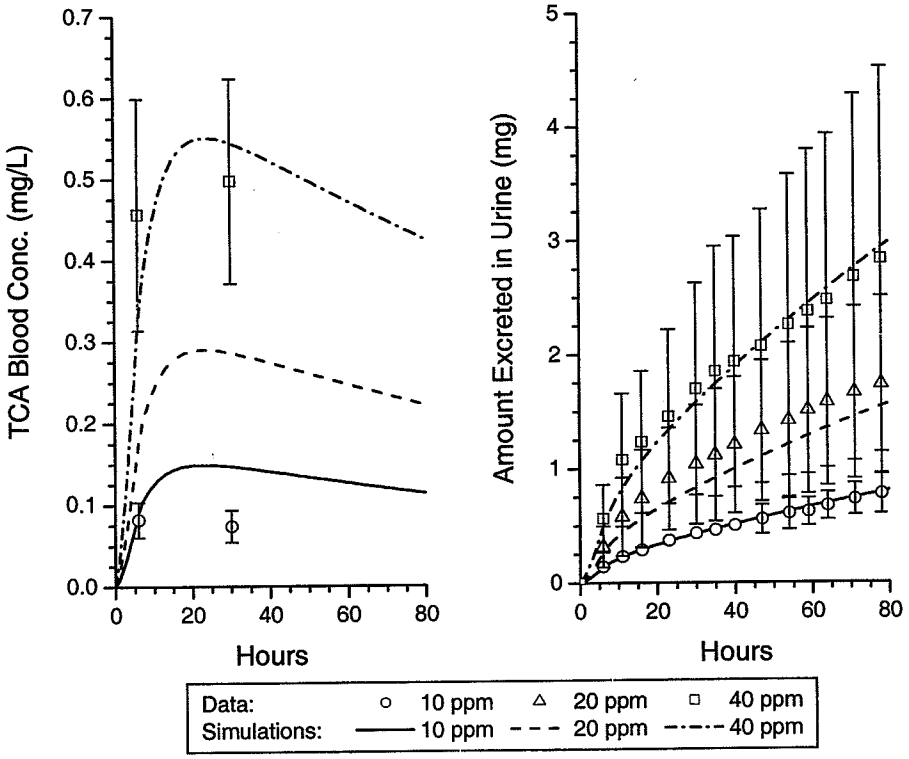

FIG. 5. Predicted (curves) and experimental (symbols) blood concentrations (left) and urinary excretion (right) of TCA for 6-h inhalation exposures of human subjects to perchloroethylene at 10, 20, and 40 ppm (Volkel et al., 1998),[1] using a modification of the PBPK model of Gearhart et al. (1993) that included metabolism of PCE to TCA in the kidney, with direct excretion of the TCA formed in the kidney into the urine. Published pharmacokinetic parameters for TCA (Clewell et al., 2000)[18] were used. To obtain the simulations shown in this figure, values of 0.24 and 0.024 were used for V_{max} in the liver and kidney, respectively. Data are plotted as means ± one standard deviation. Error bars do not appear where the standard deviation was smaller than the symbol used to plot the mean.

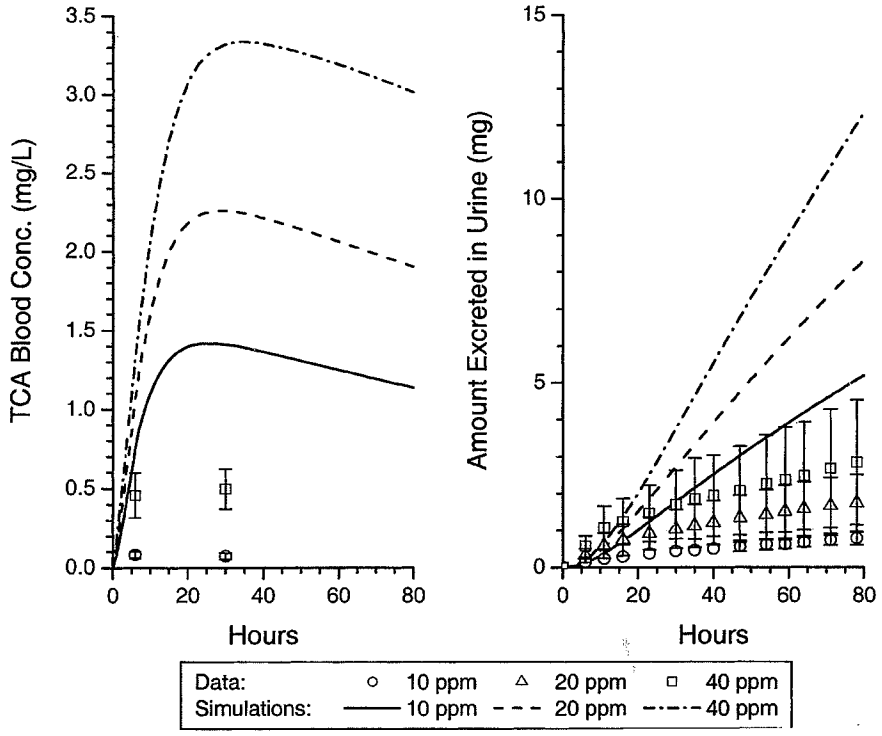

FIG. 6. Predicted (curves) and experimental (symbols) blood concentrations (left) and urinary excretion (right) of TCA for 6-h inhalation exposures of human subjects to perchloroethylene at 10, 20, and 40 ppm (Volkel et al., 1998),[1] using the PCE parameter values from the PBPK model of Ward et al. (1988),[22] together with a published pharmacokinetic submodel for TCA (Clewell et al., 2000).[18] Data are plotted as means ± one standard deviation. Error bars do not appear where the standard deviation was smaller than the symbol used to plot the mean.

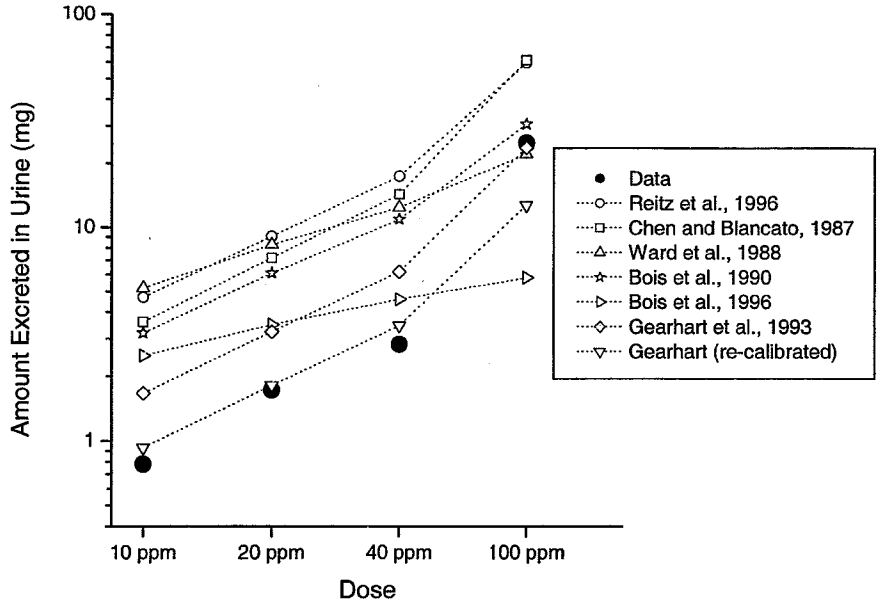

FIG. 7. Predicted (dotted lines and open symbols) and experimental (solid symbols) total urinary excretion of TCA at 72 h postexposure, for inhalation exposures of human subjects to PCE at 10, 20, and 40 ppm for 6 h (Volkel et al., 1998)[1] and at 100 ppm for 8 h (Fernandez et al., 1976).[93] Predictions were obtained using the PCE parameter values from the indicated PBPK model, together with a published pharmacokinetic submodel for TCA (Clewell et al., 2000).[18] The recalibrated Gearhart et al. (1993) model includes metabolism in the kidney, as described in the text.

TABLE 4
Fraction metabolized during continuous human exposure to perchloroethylene at 1 ppb in air or 1 μg/L in water, predicted with different PBPK models

	Fraction metabolized	
Model	Inhalation	Oral
Bois et al., 1996[23]	0.36	0.54
Ward et al., 1988[22]	0.114	0.265
Reitz et al., 1996[29]	0.077	0.179
Bois et al., 1990[24]	0.069	0.142
Chen and Blancato, 1987[21]	0.053	0.123
Gearhart et al., 1993[2]	0.011	0.026

span more than an order of magnitude, are similar to those in Figure 7, except for the two models with the shallower concentration dependence of metabolism,[22,23] which predict relatively higher fractional metabolism at these very low exposures. Based on the performance of the various models in predicting the data of Volkel et al.,[1] it can be concluded that metabolism estimates obtained with the model of Gearhart et al.[2] would provide the most reliable dose metrics for a PCE risk assessment.

EVALUATION OF RISK ASSESSMENT IMPLICATIONS

As discussed earlier, cancer risk assessments for PCE have generally been based on the liver tumors observed in the mouse bioassays,[53,54] using metabolized dose as the metric. The rationale for the use of metabolized dose as the measure of internal exposure is that the liver carcinogenicity of PCE is associated with the production of reactive moieties during the oxidative metabolism of PCE.[33,52] In order to compare metabolized doses across species it is necessary to define the basis for equivalence. In the published U.S. EPA[33,52] risk assessments, the basis of equivalence for lifetime cancer risk was assumed to be the lifetime average daily amount of PCE metabolized per unit body surface area (actually calculated as body weight raised to the $\frac{2}{3}$ power). That is, the amount metabolized in the human assumed to be equivalent to an amount metabolized in the mouse was obtained by multiplying the mouse value (in mg) by the ratio of the human and mouse body weights (in kg) raised to the $\frac{2}{3}$ power. This scaling approach is referred to as body surface area scaling, and for many years it was the default method of cross-species scaling for cancer risk assessment used by the U.S. EPA. The justification for its use was uncertainty regarding potential differences in sensitivity between rodents and humans.[107] In the OEHHA[34] PHG derivation, body surface area scaling (to the $\frac{3}{4}$ power) is also applied to the amount metabolized per unit body weight estimated by PBPK modeling. However, the recent U.S. EPA[108] cancer guidelines indicate that when pharmacokinetic tissue dosimetry is used in a risk assessment, no body surface area scaling should be performed. This change in policy reflects the conclusions of an interagency working group analysis of cross-species scaling.[109] The appropriate pharmacokinetic dose metric for a lesion produced by a short-lived reactive product of metabolism is the daily amount metabolized divided by the volume of the tissue in which it is produced,[9] in this case the liver.

Another proposed mode of action for the liver carcinogenicity of PCE is the production of the stable metabolite, TCA.[64] In fact, TCA, a peroxisome proliferating compound that has been shown to be hepatocarcinogenic in mice, is considered a leading candidate as the basis for the mouse liver carcinogenicity of TCE.[110] An appropriate dose metric for liver carcinogenicity under this mode of action would be the integral over time of the concentration of TCA (and/or DCA) in the liver, or as a surrogate, in the blood.[110] This integral is referred to as the area under the curve (AUC), and if divided by the time period over which it is calculated yields the average concentration over that period.

Having defined the alternative dose metrics, it is necessary to select the PBPK model that should be used to estimate them. The present evaluation of the published PBPK models for PCE is consistent with the results of earlier comparisons indicating that the predictions of metabolism in the mouse obtained with different models are in reasonable agreement, but the predictions of metabolism in the human vary substantially. Therefore, it is important that the estimates of metabolized dose used in a risk assessment for PCE be obtained with a model that has been adequately validated for that purpose. Based on the comparative evaluation of the predictive capability of the human models for the data of Volkel et al.[1] the model of Gearhart et al.[2] appears to provide the most reliable dosimetry in the human. In addition, the predictions of the Gearhart et al.[2] model for the kinetics of TCA have been validated in both the mouse and the human (Figures 3 through 5). The revision of the Gearhart et al.[2] model to include metabolism in the kidney, described earlier, did not significantly alter predictions of liver metabolism, but did affect predictions of AUC for TCA. In the case of TCA AUC, the use of the revised model produced a higher (more conservative) result. Therefore, the revised model was used for the risk assessment calculations performed in this study.

The dose metrics calculated with the model of Gearhart et al.,[2] as revised to include kidney metabolism, are provided in Table 5. The parameter values used for these calculations are listed in Table 2. In order to reflect liver exposure to TCA, the model-predicted human dose metrics for AUC TCA in the blood would need to be adjusted for the differences in plasma binding between mice and humans. Data on the binding of TCA in the plasma of rats and humans[111] suggest that TCA in plasma is bound to a much greater extent in the human (~80%) than in the rat (~50%). More recent data[104] confirm these results and show that the fraction bound in the mouse is even lower (~20%). Assuming binding of TCA in liver cells is similar across species, it can be estimated that the liver to plasma TCA concentration ratio in the human would be about 25% of the ratio in the mouse. Moreover, the observed dose response for the binding is such

TABLE 5
Perchloroethylene dose metrics (lifetime average daily dose)[a]

Study	Species	Concentration or dose	BW	Mg metabolized/kg liver	Mg metabolized/kg body weight	TCA AUC (mg-h/L)
NTP[54]	Male mouse	100 ppm	0.037	286	16.3	502
		200 ppm		536	30.6	944
NTP[54]	Female mouse	100 ppm	0.032	296	16.9	501
		200 ppm		555	31.6	941
	Human	1 ppm	70	0.88	0.0228	4.18[c]
		1 μg/m^3		1.30e − 4	3.36e − 6	6.15e − 4[c]
		1 mg/kg/day		1.00	0.026	4.80[c]
		1 mg/L[b]		0.029	0.00075	0.138[c]

[a]Dose metrics estimated using Gearhart et al. (1993)[2] model, as revised to include kidney metabolism. Model parameter values are listed in Table 2.

[b]Assuming 70 kg individual consuming 2 L/day drinking water.

[c]Human dose metric for TCA in blood obtained from model has been divided by 4 to adjust for differences in mouse and human plasma binding of TCA (Clewell and Andersen, 2004).[110]

that at lower human exposures the binding would be greater than 80%, while at higher mouse exposures the binding would be lower than 20%. Thus using the average daily AUC of TCA in the plasma as the dose metric would overestimate the liver exposure to TCA in the human by at least fourfold, and possibly more. A pragmatic resolution of this problem is to simply adjust the model-predicted human dose metrics for AUC TCA by the ratio of the mouse and human free TCA fractions in plasma; that is, the human dose metrics should be divided by a factor of 4.[110]

Lifetime risk estimates obtained with the three different dose metrics are presented in Table 6. These estimates were based on the incidence of hepatocellular adenoma/carcinoma in the mouse in the PCE inhalation bioassay[54] using a time-to-tumor model to consider animal survival (TOX_RISK, V3.5, ICF Consulting, Inc., Fairfax, VA). The inhalation bioassay results were used for both inhalation and oral risk calculations to avoid the potential confounding effects of the corn oil vehicle on risk estimates derived from oil gavage studies.[14] This approach has also been recommended for TCE.[110] In the absence of definitive information on the mode of action, the conservative default approach in the new U.S. EPA[108] cancer guidelines, linear low-dose extrapolation, was used. In this approach, a 95% lower bound on the dose associated with a 10% increase in tumor incidence is calculated, and the risk R at dose D is estimated from the LED_{10} by the formula $R = (0.1)D/LED_{10}$. In this case, both the LED_{10} and D represent metabolized doses, so the PBPK model must be used to calculate the human metabolized dose, D, associated with a given environmental exposure.

Risk estimates are provided in Table 6 using all three dose metrics; however, the risk estimates based on the different metrics should not be considered equally likely. There is not actually any biological justification for the use of amount metabolized per unit body weight as a dose metric. As discussed by

TABLE 6
Lifetime risk estimates for continuous exposure to perchloroethylene

	Mg metabolized/kg liver			Mg metabolized/kg body weight			TCA AUC		
Dose metric	Male	Female	Geometric mean	Male	Female	Geometric mean	Male	Female	Geometric mean
LED_{10}	25.8	45.2	34.1[a]	1.5	2.6	2.0	45.4	76.3	58.9
Unit risks									
per ppm	3.4e − 3	2.0e − 3	2.6e − 3[a]	1.6e − 3	8.8e − 4	1.2e − 3	9.2e − 3	5.5e − 3	7.1e − 3
per μg/m^3	5.0e − 7	2.9e − 7	3.8e − e7[a]	2.3e − 7	1.3e − 7	1.7e − 7	1.4e − 6	8.1e − 7	1.0e − 6
per mg/kg-day	3.9e − 3	2.2e − 3	2.9e − 3[a]	1.8e − 3	1.0e − 3	1.3e − 3	1.1e − 2	6.3e − 3	8.1e − 3
per μg/L[b]	1.1e − 7	6.4e − 8	8.5e − 8[a]	5.1e8	2.9e8	3.8e8	3.0e7	1.8e − 7	2.3e − 7

[a]Preferred dose metric (mg metabolized in liver/kg liver).

[b]Assuming 70-kg individual consuming 2 L/day drinking water.

Andersen et al.,[9] the use of amount metabolized in the liver per unit liver weight provides a reasonable surrogate for the steady-state concentration of a reactive intermediate produced during metabolism, assuming constant stoichiometry and sufficiently high reactivity to rule out transport away from the target tissue before reaction. No similar rationale can be made for dividing the amount metabolized in a particular tissue (e.g., the liver) by the body weight. On the other hand, although the AUC for TCA has been suggested as a dose metric for the liver carcinogenicity of TCE,[110] there does not appear to be equal justification in the case of PCE. The liver toxicity of PCE is greater than that of TCE for the same level of metabolite generation.[58] If the liver carcinogenicity of both PCE and TCE were due to the effects of TCA, one would expect similar potency estimates (LED_{10}s) for these two chemicals using the AUC TCA dose metric. In fact, for TCE-induced mouse liver tumors in the inhalation bioassays, the mean of the LED_{10}s for AUC TCA is 926 mg-h/L,[110] as opposed to the LED_{10} values of 45.4 and 76.3 mg-h/L obtained for male and female mice, respectively, for the PCE inhalation bioassay (Table 6). Similarly, the average daily AUC for TCA at its lowest carcinogenic dose in the mouse (1 g/L TCA in drinking water) is on the order of 1600 mg-h/L.[110] Thus, the production of TCA in the PCE bioassays does not appear to be adequate, in itself, to account for the observed tumorigenicity, leaving the generation of reactive metabolites, or a combination of processes that includes the generation of reactive metabolites, as the most likely mode of action.

Using the preferred dose metric, amount metabolized in the liver per unit liver weight, the mean inhalation unit risk predicted using the PBPK model is 3.8×10^{-7} $(\mu g/m^3)^{-1}$, which is very similar to the inhalation unit risk of 5.8×10^{-7} $(\mu g/m^3)^{-1}$ estimated by the U.S. EPA[33] in its last published risk assessment for PCE. The U.S. EPA[33] inhalation unit risk was also based on the mouse liver tumors in the NTP[54] bioassay, but was obtained using estimates of metabolized dose (based on total urinary excretion of metabolites) from pharmacokinetic studies in mice[58] and humans,[59] and assuming equivalence across species on a milligrams metabolized per body surface area per day basis. The mean drinking water unit risk predicted using the Gearhart et al.[2] PBPK model is 8.5×10^{-8} $(\mu g/L)^{-1}$, which is roughly an order of magnitude lower than the unit risk of 1.5×10^{-6} $(\mu g/L)^{-1}$ estimated by the U.S. EPA[52] in its last published oral risk assessment for PCE. This U.S. EPA[52] unit risk was based on the mouse liver tumors in the NCI[53] bioassay, with equivalence across species defined on a milligrams ingested per body surface area per day basis, assuming 100% metabolism of ingested dose. Given the similar predictions of the alternative PBPK models in the mouse, coupled with the tendency of several of the human models to overestimate metabolism for low human exposures by roughly 5- to 15-fold (Figure 7), risk estimates obtained with models other than that of Gearhart et al.[2] could be expected to produce risk estimates as much as an order of magnitude higher than those in Table 6.

It is also of interest to evaluate the impact of variation in the PBPK model estimates of fractional metabolism in the human on the PHG for PCE derived by OEHHA.[34] In its risk assessment, OEHHA[34] converted estimates of PCE intake via inhalation, oral, and dermal exposure routes, expressed as drinking water volume equivalents, to metabolized doses using upper bound estimates (95% upper confidence limits) of the fraction of PCE metabolized from the PBPK uncertainty analysis of Bois et al.[23] These upper bound estimates were 58% and 79% for the inhalation and oral routes, respectively.[34] However, as discussed earlier, it appears that the model of Bois et al.[23] greatly overestimates fractional metabolism in humans at the low exposures of interest for risk assessment (Figure 7, Table 4). Therefore, the upper bound estimates of fractional metabolism obtained with this model must be considered highly suspect.

Using the Gearhart et al.[2] model, the estimates of the fraction of PCE metabolized in the liver following inhalation and oral exposure are 1.1%, and 2.6%, respectively (Table 4), about 50- and 30-fold lower than the upper bound estimates used by OEHHA (2001). Using intakes derived using these estimates of fractional metabolism (Table 7) with the mean of the male and female potency estimates from Table 6 for the metric selected by OEHHA, milligrams metabolized per kilogram body weight ($0.1/LED_{10} = 0.049$), the PHG estimated with the Gearhart et al.

TABLE 7
Estimated exposure to perchloroethylene in drinking water for an average California resident

	Avg. dose (mg/kg/day)	Exposure: vol. equiv. (L/day)	Fraction metabolized (UCL)	Metabolized vol. equiv. (L/day)	Percent of total dose
Inhalation	1.27×10^{-6}	3.54	0.011	0.039	38.7
Ingestion					
Water	7.16×10^{-7}	2.00	0.026	0.052	
Produce, meat, etc.	2.48×10^{-8}	0.07	0.026	0.002	
Total ingestion	7.41×10^{-7}	2.07	0.026	0.054	53.6
Dermal uptake	2.51×10^{-7}	0.70	0.011	0.0077	7.7
Dose sum	2.26×10^{-6}	6.31		0.1007	100

(1993) model is 14 μg/L:

$$C = \frac{1 \times 10^{-6} \times 70\ \text{kg}}{(0.049 \times 0.054\ \text{L/d}) + (0.049 \times 0.0467\ \text{Leq/day})} = 0.014\ \text{mg/L} = 14\ \text{ppb}$$

This PHG estimate is about 240-fold higher than the OEHHA[34] proposal of 0.056 μg/L. Of this difference, about a factor of 7 is due to the inappropriate use of body surface area scaling in the derivation of potency estimates by OEHHA,[34] and the rest is primarily due to the different estimates of fractional metabolism in the human.

CONCLUSIONS

PCE provides a useful case study to highlight some of the issues associated with the comparative evaluations of PBPK models for use in a risk assessment. A number of structurally similar PBPK models have been developed for PCE, differing primarily in the parameter values estimated for metabolism, although one of the models[2] also includes a description of the kinetics of the principal metabolite, TCA. All of the models provide reasonably accurate simulations of some of the pharmacokinetic data available for PCE in mice or humans, and could therefore be considered, to some extent, to be validated. However, while similar predictions of metabolism are obtained with the alternative models in the mouse, predictions of metabolism in the human with different models vary considerably. This species difference in the variation of the PBPK model estimates of metabolism seems to stem from the different kinds of data used to identify the metabolism parameter values in mice and humans. All of the mouse models made use of data that were highly informative regarding metabolism, including radiolabel disposition, metabolite excretion, or closed-chamber clearance studies. Many of the human models, on the other hand, relied on parent chemical kinetic data that do not directly reflect metabolism.

The use of parent chemical kinetic data to infer metabolic parameter values or validate model estimates of metabolism in the human can be highly misleading, because it is often the case that other uncertain parameters can strongly influence model predictions of parent chemical kinetic behavior. Therefore, demonstrating the agreement of a human model with data on the kinetics of the parent chemical may give a false sense of validation if the use of the model in the risk assessment is to predict the rate of metabolism. To avoid this problem it is crucial that time-dependent sensitivity analysis be conducted with the model under the conditions of the exposure to assure that the metabolic parameter values are identifiable from the available data.[15]

The use of in vitro studies to identify human metabolic parameter values is limited by the difficulty of obtaining reliable estimates of the in vivo affinity, making it necessary to adjust the in vitro affinities measured in human tissues on the basis of the relationship between in vitro and in vivo estimates in the rodent.[29] A more direct approach would be to use estimates of the maximum velocity of metabolism from in vitro studies together with estimates of intrinsic clearance (V_{max}/K_m) from in vivo rebreathing studies.

In the case of the evaluation of the PCE human models, recent data[1] on the urinary excretion of TCA, the major metabolite of PCE, at lower exposure concentrations (10 to 40 ppm) than had been reported previously, made it possible to compare the high- to low-dose extrapolation capability of the various published models. We found that the model of Gearhart et al.[2] which was the only model to include a description of TCA kinetics, gave the closest predictions of the urinary excretion observed in the low-concentration exposures (within a factor of 2). Other models overestimated metabolite excretion in this study by 5- to 15-fold. Clearly, there are several advantages associated with the addition of a description of metabolite kinetics to a parent chemical model, even when the risk assessment is based on the rate of parent chemical metabolism rather than tissue exposure to the metabolite. Including a description of a major metabolite allows data on the time course of metabolite kinetics and excretion to be more readily used for metabolism parameter estimation or validation, and reduces the uncertainty associated with the tendency in human studies to collect urine for too short a time to ensure that all of the metabolite has been excreted.

Based on the systematic nature of the discrepancy between the predictions of the model of Gearhart et al.[2] and the data on the time course of the urinary excretion of TCA in the Volkel et al.[1] study, we suspected that TCA generated from the metabolism of PCE in the kidney was being excreted directly into the urine. Adding a quantitative description of this hypothesis to the model greatly improved the ability of the model to simulate the data. Although the success of the description does not in itself demonstrate the validity of the hypothesis, several CYP isoforms, including 2E1, 2A6, and 3A4, have been shown to contribute to the metabolism of compounds similar to PCE in human kidney.[106]

The wide discrepancies between the predictions of the human PCE models resulted in large part from the fact that the metabolic parameter values in each model were estimated on the basis of only a small subset of the available kinetic data—in some cases only a single study. Undoubtedly, if all of the data used in the development of previously published models were included in the development of subsequent models, the predictions of the later models would inevitably come closer together. Ideally, a PBPK model should be compared with all relevant data regarding the parameters to which the dose metric predictions are sensitive, rather than focusing only on one or two studies. This presupposes the use of sensitivity and uncertainty analysis to identify the parameters of concern (those that have the most influence on the dose metric estimate and are the least certain), as well as of time-dependent sensitivity analysis[15] to select the experimental data that are most informative for those parameters.

Cancer risk estimates for PCE based on liver tumors in mice in the NTP study were estimated with the Gearhart et al.[2] model, using lifetime average daily amount metabolized per volume liver as the dose metric. The resulting inhalation risk estimate for lifetime exposure to PCE at 1 μg/m^3 was 0.4 per million. The

corresponding risk for oral exposure at 1 mg/kg/day was 3 per thousand. Risk estimates using other published PCE PBPK models would be higher by as much as an order of magnitude, due to the tendency of the models to overestimate human metabolism.

Finally, it is important to note that PBPK analyses alone provide limited insight into species differences in another response element critical for human health risk assessment: pharmacodynamics. In seeming contrast with the results of high-dose rodent studies, available epidemiological studies do not support a conclusion that PCE is carcinogenic in humans. Unfortunately, neither the mouse nor the rat appears to be an appropriate animal model for prediction of potential human carcinogenic responses to PCE, due both to differences in metabolic capabilities and to less well understood differences in cellular responses.[49] Thus, further studies to elucidate the mechanism(s) of action of PCE in different species will be of great value in improving the quality of human health risk assessments for this important compound.

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