AAMRL-TR-90-072
Volume III

DEVELOPMENT AND VALIDATION OF METHODS FOR APPLYING PHARMACOKINETIC DATA IN RISK ASSESSMENT

VOLUME III OF VII: TETRACHLOROETHYLENE

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December 1990

Final report for the period May 1987 through September 1990

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AAMRL-TR-90-072, Volume III

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

JAMES N. McDOUGAL, Maj, USAF, BSC
Deputy Director, Toxic Hazards Division
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	DECEMBER 1990	Final Report - May 1987 - September 1990

4. TITLE AND SUBTITLE
DEVELOPMENT AND VALIDATION OF METHODS FOR APPLYING PHARMACOKINETIC DATA IN RISK ASSESSMENT
VOLUME III OF VII VOLUMES: TETRACHLOROETHYLENE

5. FUNDING NUMBERS
PE 62202F
PR 6302
TA 630213
WU 63021303
Accession #333560

6. AUTHOR(S)

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Clement International Corporation
K.S. Crump Division
1201 Gaines Street
Ruston, LA 71270

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AAMRL, Toxic Hazards Division
HSD, AFSC
Wright-Patterson AFB, OH 45433-6573

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
AAMRL-TR-90-072

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Perchloroethylene (PCE) is a chemical of relevance to the Air Force Installation Restoration Program (IRP) efforts. Pharmacokinetics of PCE and available PCE PBPK were reviewed and a preliminary risk assessment was prepared. The physiologically based pharmacokinetic (PBPK) model developed for PCE was used to examine the behavior of PCE in mouse and human systems. Uncertainty analysis was applied to the PBPK models used in the risk assessment study.

14. SUBJECT TERMS
tetrachloroethylene; PCE; pharmacokinetic; models; risk

15. NUMBER OF PAGES
98

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

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FOREWORD

This report has been prepared by Clement International Corporation, K.S. Crump Division, for the Department of the Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Paterson Air Force Base in response to a request to investigate the incorporation of pharmacokinetic modeling into quantitative risk assessment. This report contains the results of this multiyear effort and reflects the changes in direction and priorities as this project has evolved. The Project Director was Dr. Kenny Crump and the Principal Investigator for this project was Mr. Bruce Allen; other investigators who provided technical support and internal peer review were Drs. Crump and Annette Shipp. Mr. Allen was assisted in the pharmacokinetic modeling and analyses primarily by Mr. Christopher Rambin and by Ms. Robinan Gentry. The sensitivity analyses were conducted by Mr. David Farrar, Dr. Crump, Dr. Richard Howe, and Mr. Allen. The software was developed by Ms. Cynthia Van Landingham, Mr. William Fuller, Mr. Eric Brooks, Dr. Howe, and Mr. Allen. The authors wish to acknowledge the support provided by Dr. Jeffery Fisher and Lt. Col. Harvey Clewell, who are at the Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Paterson Air Force Base, and Drs. Melvin Andersen and Michael Gargas, formerly with the Harry G. Armstrong Aerospace Medical Research Laboratory and now with CIIT.

PREFACE

This volume of the final report presents the work that has been completed for tetrachloroethylene (PERC, perchloroethylene). This volume is divided into two parts. Part 1 presents a detailed review of the pharmacokinetic data for PERC. Two physiologically based pharmacokinetic (PBPK) models of PERC are examined in detail. The models are used in preliminary risk assessments for PERC.

Part 2 presents the PBPK modeling work that has been completed by Clement personnel. That modeling extends the models discussed in Part 1. In addition, a revised risk assessment is presented, one based on liver tumors in the mouse and using dose surrogates estimable from the extended models. A discussion of the uncertainties presented in the Introduction (Volume I) is also included, with emphasis on how some of the uncertainties have been treated in the revised risk assessment.

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VOLUME III

PART 1 OF 2 PARTS

REVIEW OF TETRACHLOROETHYLENE PHARMACOKINETICS AND PBPK MODELING; PRELIMINARY RISK ASSESSMENT

A. INTRODUCTION

Tetrachloroethylene (PERC) is a commercially important chemical used as a solvent. Unfortunately, PERC is also a frequent contaminant of drinking water supplies. The pharmacokinetic literature available for PERC is reviewed in Appendix III-1-A. This part of the document examines preliminary physiologically based pharmacokinetic (PBPK) models that have been proposed for PERC and the effect of incorporating pharmacokinetic considerations into the risk assessment process. The PERC example illustrates the general approach that can be adopted for the incorporation of pharmacokinetics and pharmacokinetic modeling in risk assessment.

The models considered in this part of the document (and which are the subject of the sensitivity/uncertainty analysis discussed in Volume VI) are variants of the PBPK model proposed by Ramsey and Andersen (1984) for styrene. Reitz and Nolan (1986) and Hattis et al. (1986) have described PBPK models that have at least four compartments including the liver, richly perfused tissues, poorly perfused tissues, and the fat group. In the model of Hattis et al., when applied to humans, another compartment, the muscle group, is also included. The dynamics of absorption of PERC by inhalation are derived from simple algebraic equations describing the arterial and venous concentrations in terms of the alveolar ventilation, the blood flow rate through the lung, and the blood/air partition ratio. Metabolism is assumed to occur only in the liver and follows saturable, Michaelis-Menten kinetics. Appendix III-1-B contains the systems of equations that define the two models. These two models will be compared in this section with respect to the estimation of the input parameters and with respect to their use in risk assessment.

These specific models of PERC pharmacokinetics are probably inadequate to completely explain the carcinogenic effect of PERC. Expansion of the existing models to include considerations of trichloroacetic acid formation is discussed in Part 2 of this volume. Nevertheless, the models of Reitz and Nolan and Hattis et al. can be used to demonstrate the potential and problems of using PBPK modeling in risk assessment. Thus, estimates of delivered dose, and ultimately of risks, for the two models will be compared and contrasted. The discussion of both models illustrates the differences that can be attributed to choices made in the process of PBPK modeling. The differences noted are examples of the uncertainties discussed in the Introduction to this report (Volume 1).

B. PARAMETER ESTIMATION FOR PBPK MODELS

1. Partition Coefficients and Physiological Parameters

Hattis et al. (1986) employed a regression technique to obtain partition coefficient estimates for PERC in human tissues. Using data on partitioning for 24 chemicals, Hattis et al. estimated regression formulas that expressed tissue/gas coefficients in terms of the oil/gas and water/gas partition coefficients. For each tissue, a multiple regression analysis was employed using a model equation of the form

$$(1) \qquad R_{tissue/gas} = b_0 + b_1 R_{oil/gas} + b_2 R_{water/gas},$$

where $R_{x/y}$ represents the partition coefficient of the chemical between the components x and y. The resulting coefficients, b_0, b_1, and b_2, together with

literature values of the partition coefficients of PERC in oil and water were used to estimate the human tissue/gas partition coefficients. However, a similar analysis performed on rat data compared less favorably with the available experimental data. In the case of rats then, experimental data formed the basis of the partition coefficient estimates. Mice were assumed to have the same partition coefficient parameter values as rats, that is, the same tissue/blood partition coefficients.

In contrast, Reitz and Nolan (1986) employed partition coefficients for rats and mice that were obtained from Wright-Patterson Air Force Base (WPAFB) laboratories. Richly perfused tissues were assumed to behave as the liver does with respect to partitioning. Direct human partition coefficient estimates were available only for blood/air. The partition coefficients for other tissues, compared to air, were set equal to the average of the corresponding rat and mouse values. Human tissue/blood coefficients were calculated by dividing the averaged values by the measured human blood/air coefficient.

Hattis et al. (1986) set rat and mouse tissue volumes and flow rates to reference values taken from Arms and Travis (1987). Hattis et al. (1986) assumed different ventilation and cardiac output rates (and, therefore, different compartment perfusion rates) for periods of sleeping and waking in humans. Those rates, as well as the human tissue volumes, were consensus values derived from various sources. The active rates corresponded to "shoeworker" (light work) activity levels, while the sleeping rates were substantially smaller.

In contrast, Reitz and Nolan (1986) fixed ventilation and cardiac output rates for humans for all times. Reitz and Nolan did not discuss the sources for the physiological parameter values used in their rodent or human models.

2. Metabolic Pathways and Rate Constants

Both PERC models assumed that the liver was the sole site of metabolism. Moreover, a single, saturable pathway mediated by the MFO enzyme system was the only proposed mechanism of PERC transformation. The metabolic parameters used by Reitz and Nolan for rats and mice were based on the data of Pegg et al. (1979) and Schumann et al. (1980). When the PBPK model with the previously estimated rat physiological and partition coefficient parameters was run and compared to the data from Pegg et al., the optimal values for rat Vmax and Km were obtained. The value of Km was assumed to be the same for rats and mice. The data of Schumann et al. then allowed estimation of the mouse Vmax, again by optimization. The same procedure was followed for estimation of the human Vmax: the human Km was set to the value estimated for rats, human physiological and partition coefficient parameters were input into the model, and the model results were compared to data from Monster (1979) to obtain the best estimate of Vmax.

The study by Ohtsuji et al. (1983) was the basis of the metabolic parameter estimation done by Hattis et al. for humans. The Vmax and Km values that reproduced the metabolic rate determined by Ohtsuji et al. (1983) were estimated, after adjusting for metabolites that did not show up in the urine (an adjustment based on animal data). The data of Pegg et al. (1979) and Schumann et al. (1980) were used to estimate Vmax and Km for rats and mice, respectively.

Tables III-1-1 and III-1-2 display the values of the parameters used in the models. The parameter values in conjunction with the equations found in Appendix III-1-B completely define these models. Note that some of the parameters (ventilation rate, cardiac output, perfusion rates, tissue volumes, and metabolic rate constants) were scaled according to body weight. The output variables estimated by the PBPK models can thus be based specifically on the body weights of the animals used in carcinogenicity bioassays.

C. RISK ESTIMATION USING PBPK MODELING

The estimation of human cancer risks posed by PERC exposure followed the procedure outlined in the Introduction to this report (Volume I).

For each of the carcinogenicity bioassays testing PERC (NCI, 1977; NTP, 1986), the carcinogenic responses whose rates were significantly elevated in a dosed group over those in the control group (Fisher's exact test at the 0.05 level of significance) were chosen for use in the estimation of human risk. Those responses included hepatocellular tumors in male and female mice (NCI, 1977; NTP, 1986), mononuclear cell leukemia in male and female rats (NTP, 1986), and tubular cell adenomas or carcinomas in male rats (NTP, 1986).

Each tumor response was associated with certain dose surrogates (measures of delivered dose) that were thought to be potentially relevant to the production of that type of tumor.

As a general measure of dose throughout the body, and as substitute for site-specific concentrations for cancers such as leukemia that do not correspond to any of the compartments in the PBPK models, area under the PERC arterial blood concentration curve was used as a surrogate dose for all

tumors. Moreover, area under the PERC concentration curve in the target organ was also used. For the mouse hepatocellular tumors, liver was the target organ. For the rat kidney tumors, the richly perfused tissue compartment was assumed to be the target; kidneys were included as one component of that compartment.

In addition, virtual concentration of reactive metabolite (represented by integrated amount of PERC metabolized divided by liver volume) was examined as a dose surrogate for use with the mouse liver tumors. Because metabolism was assumed to occur only in the liver, and because a reactive metabolite would not leave the site of its formation, this dose surrogate was not used in conjunction with any of the other observed tumor responses. Neither of the PBPK models included parameters necessary to describe the distribution of any metabolite outside the liver. Part 2 of this volume addresses this issue.

The relevant dose surrogate values were estimated for each dose group in a bioassay (Tables III-1-3 to III-1-6). The estimation used the PBPK models discussed above, the administered dose level (inhalation concentration or gavage dose), and dose-group-specific body weights. The dose surrogate values and the associated response rates comprised the input for the multistage model (Crump, 1979). Both maximum likelihood and upper bound estimates of risk were derived. If more than one surrogate dose was relevant to a particular tumor, then that response was analyzed by the multistage model using each of the surrogate doses as input dose values. A version of the program GLOBAL82 (Howe and Crump, 1982) was used to implement the multistage model. For the risk calculations, extra risk, defined by

$$(2) \qquad (P(d) - P(0))/(1 - P(0)),$$

was used, where P(d) is the probability of observing a tumor during a lifetime when exposed to dose d. The number of stages in the multistage model was set to one (a one-hit model) and to the maximum number estimable (i.e., one less than the number of dose groups). Consequently, two sets of risk estimates were produced for each response and dose surrogate combination.

Human risks corresponding to concentrations of PERC in drinking water ranging from 0.1 ppb to 10 ppm were estimated. It was assumed that an average individual drinks 2 liters of water each day.

In order for the human risk corresponding to a drinking water concentration to be calculated by the multistage model, the concentration was converted to the same units as were used for the input doses associated with each animal dose group. [The input, dose-group doses were the surrogate doses relevant to the tumor in question.] Thus, the drinking water concentrations were used as input into the PBPK models with the appropriate human parameters to yield estimates of the surrogate doses corresponding to those used in the input to the multistage models (Table III-1-7). The estimated human dose surrogate values were then used in the multistage model and risks were calculated.

For comparison, risks were also estimated without consideration of pharmacokinetics. In that case, the doses used as input to the multistage model were the administered doses converted to mg/kg/day. The drinking water concentrations for which risk estimates were desired were also converted to mg/kg/day equivalents, assuming a 70 kg human drinks 2 liters of water per

day. The assumption underlying risk estimates derived in this manner is that rodents and humans are equally susceptible to PERC-induced cancer when exposed to the same amount of PERC expressed in terms of mg/kg/day.

Inhalation exposures (NTP, 1986) were converted to mg/kg/day by assuming breathing rates used in the PBPK models. The two models (Reitz and Nolan, 1986; Hattis et al., 1986) differed with respect to breathing rate values. Thus, risk estimates obtained using the various dose terms (mg/kg/day or a surrogate dose measure) were compared only when breathing rates were equal. (This entailed two conversions of inhalation doses to mg/kg/day, one using the Reitz and Nolan rodent breathing rates and one using the Hattis et al. rodent breathing rates.)

The risk estimates derived using the applied doses or using dose surrogates are shown in Table III-1-8 (for the Reitz and Nolan (1986) model) and Table III-1-9 (for the Hattis et al. (1986) model). The response rates from the bioassays (as well as the mg/kg/day doses obtained by using the model estimates of breathing rates) are shown in Table III-1-10.

If one focuses on the upper bound risk estimates, as EPA does for example, then the risk associated with 1 ppm in drinking water ranged from 2.07×10^{-5} to 1.53×10^{-4} when using the experimentally administered doses to estimate risks. The ranges corresponding to the PBPK-derived dose surrogates were very similar for the two PERC PBPK models. When based on the dose calculations of Reitz and Nolan (Table III-1-8) the ranges were 5.0×10^{-5} to 3.08×10^{-3}, 2.20×10^{-5} to 8.72×10^{-5}, and 3.17×10^{-5} to 9.53×10^{-4} for the three dose surrogates evaluated, PERC in the target, MFO metabolite in the target, and PERC in arterial blood, respectively. For the dose surrogate values calculated by the Hattis et al. model the corresponding ranges were 1.58×10^{-5}

to 1.84×10^{-3}, 3.31×10^{-5} to 8.76×10^{-5}, and 2.86×10^{-5} to 8.21×10^{-4}, respectively (Table III-1-9).

D. DISCUSSION

Some nonlinearities attributable to saturation of the MFO metabolic pathway are apparent for the dose levels to which the rodents were exposed . The doses administered to rodents either by inhalation or gavage were high enough that clear evidence of metabolic saturation was seen. Therefore, when the metabolite was the basis for the surrogate dose, the PBPK models estimated lower surrogate dose values than would have been expected if linear metabolism had occurred. On the other hand, the values of the surrogates based on parent concentrations were higher at high doses than would be the case if metabolism were nonsaturable. For humans, metabolism was linear for most of the relatively low drinking water concentrations of interest (Table III-1-7). Apparently, these concentrations were low enough that the amount consumed in 2 liters of water per day only begins to lead to nonlinearities at the top of the range, i.e., at around 10 ppm.

The two PERC PBPK models predicted roughly the same values for corresponding surrogate doses for all species. Differences in parameter values produced slight differences among estimates in rodents, with the largest difference noted for mice. In mice, when exposed by inhalation, the Hattis et al. model predicted metabolite concentrations two to three times smaller than predicted by the Reitz and Nolan model (Table III-1-3). When applied to gavage dosing (Table III-1-5), the metabolite concentrations were more similar, but the liver PERC concentrations differed by two- to three-fold

between the two models. Slightly more than a three-fold difference was noted for concentrations of PERC in human richly perfused tissue (Table III-1-7).

The differences the risk estimates obtained using the different surrogates, and in particular as compared to the approach using administered doses, are important considerations from a regulatory point of view. That is to say, if the incorporation of pharmacokinetics into risk assessment procedures is deemed to be appropriate, then regulatory actions may depend on the risks derived using the pharmacokinetically adjusted surrogate doses. Even if formal pharmacokinetic modeling is not considered in regulatory decision making, the risk estimates based on such surrogate doses inform the estimation of the uncertainty associated with whatever risk estimates are used.

For almost every data set taken from PERC bioassays, risks based on the parent concentration either in the target organ or in the arterial blood were higher than those based on the administered doses (Tables III-1-8 and III-1-9). The exceptions were for male and female rat mononuclear cell leukemia (for both the Reitz and Nolan model and the Hattis et al. model) and for the male rat kidney tumors when the parent-in-target surrogate values are estimated by the Hattis et al. model. The risk estimates based on the Reitz and Nolan model differed from the corresponding estimates derived in the traditional manner more than the estimates based on the Hattis et al. model did. The largest difference was noted when the PERC liver concentration surrogate (estimated by Reitz and Nolan) was used in conjunction with the hepatocellular carcinoma response in the female mice in the NTP study; in that case the risk estimates differed by more than two orders of magnitude. Risk estimates based on the arterial blood concentration surrogate were more

similar to those derived in the traditional manner than were the estimates based on the parent-in-target surrogate. [Note the difference due solely to the estimation of respiratory rate. The risk estimates derived using the experimentally applied doses in mice tested by NTP (Tables III-1-8 and III-1-9) differed only because the respiratory rate assumed for mice by Reitz and Nolan differed from that assumed by Hattis et al. This was reflected in the differences in estimated applied doses (Table III-1-10).]

The use of the metabolite dose surrogate reduced risk estimates, although the difference was small in some cases (e.g., when based on the hepatocellular carcinoma response among male mice tested by NCI, Tables III-1-8 and III-1-9). At least part of this effect can be explained by the differences in the metabolic parameters for mice and humans (note that the only species with a liver cancer response, and thus the only species for which the metabolite-based surrogate has been calculated, was the mouse). The difference in metabolic parameter values entails that at low doses more PERC is metabolized by mice than by humans.

For risk estimation purposes, the metabolic differences can be expressed in terms of the difference between the experimental doses (in particular the lowest experimental dose) and the dose for which a risk estimate is desired. The greater the difference (e.g., the larger the ratio between the lowest experimental dose and the dose for which a risk estimate is desired), the smaller the desired risk estimate tends to be.

Consider virtual concentration of metabolite (estimated by the Reitz and Nolan model) used as the dose surrogate to estimate risks from the NTP results related to hepatocellular carcinomas in male mice (Table III-1-8). The upper bound risk estimate using that surrogate was 4.02 times smaller than the

estimate obtained using administered dose. The ratio of dose values corresponding to the lowest experimental dose (1.47E+3, Table III-1-3) and to the 1 ppm drinking water concentration for which risk estimates were desired (5.20E-2), Table III-1-7) was 2.8E+4. This was 4.26 times larger than the ratio obtained using the corresponding mg/kg/day administered doses. Therefore, the risk estimate was smaller when using the virtual concentration of metabolite dose surrogate, by a factor almost the same as the relationship between the ratios.

The example given above is a slight simplification because the risk estimates do not depend solely on the lowest experimental dose. In fact, the difference between 4.26, the factor by which the ratios discussed above differ, and 4.02, the factor by which the risk estimates differ, was due to the nonlinear transformation of the experimentally applied doses to get the metabolite-based dose surrogate values. The nonlinearity was associated with the saturation of metabolism that was becoming apparent at the higher dose levels. If no saturation had been evident, the risk estimates would have differed to the same extent as the ratios between lowest experimental dose and the dose for which risk estimates were desired.

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Table III-1-1

Parameters Used in PERC PBPK Model Runs; All Species in Reitz and Nolan Model, Mice and Rats in Hattis *et al.* Model

	Reitz and Nolan			Hattis *et al.*	
	Mouse	Rat	Human	Mouse	Rat
Alveolar Ventilation (ℓ/hr) (QP)	$28*BW^{.74}$	$15*BW^{.74}$	$15*BW^{.74}$	$12.5*BW^{.74}$	$21*BW^{.74}$
Cardiac Output (ℓ/hr) (QC)	$28*BW^{.74}$	$15*BW^{.74}$	$15*BW^{.74}$	$15*BW^{.74}$	$15*BW^{.74}$
Blood Flow Rates (ℓ/hr): QC*					
Liver (QL)	.25	.25	.24	.25	.25
Fat (QF)	.05	.05	.05	.09	.09
Richly Perfused (QR)	.51	.51	.52	.51	.51
Slowly Perfused (QS) (Muscle (QM))[a]	.19	.19	.19	.15	.15
Tissue Volumes (ℓ): BW*					
Liver (VL)	.04	.04	.0314	.06	.04
Fat (VF)	.05	.07	.231	.10	.07
Richly Perfused (VR)	.05	.05	.0371	.05	.05
Slowly Perfused (VS) (Muscle (VM))[a]	.78	.75	.621	.70	.75
Partition Coefficients					
Blood/Air (PB)	16.9	18.8	10.3	18.9	18.9
Liver/Blood (PL)	3.01	3.74	5.88	3.72	3.72
Fat/Blood (PF)	48.3	87.1	119.1	109	109
Richly Perfused/ Blood (PR)	3.01	3.74	5.88	3.72	3.72
Slowly Perfused/Blood (PS) (Muscle/ (PM))[a]	2.59	1.06	3.1	1.06	1.06
Metabolic Constants					
V_{max} (mg/hr)	$8.34*BW^{.74}$	$1.27*BW^{.74}$	$0.346*BW^{.74}$	$1.49*BW^{.70}$	$2.26*BW^{.70}$
K_m (mg/ℓ)	4.5637	4.5637	4.5637	2.49	25.21

[a]In the Reitz and Nolan model, a slowly perfused tissue compartment is included. Hattis *et al.* label the fourth compartment the muscle group.

Table III-1-2

Parameters Used in PERC PBPK Model
Developed by Hattis *et al.* for Humans

Parameter	Sleeping	Active
Alveolar Ventilation (QP) (ℓ/hr)	288	682.8
Cardiac Output (QC) (ℓ/hr)	348	503.64
	Blood Flow Rates (ℓ/hr)	
Liver (QL)	84.0	73.44
Fat (QF)	15.0	35.4
Richly Perfused (QR)	177.0	232.2
Slowly Perfused (QS)	6.0	6.0
Muscle (QM)	66.0	156.6
	Tissue Volumes (ℓ)	
Liver (VL)	2.476	2.476
Fat (VF)	15.024	15.024
Richly Perfused (VR)	6.037	6.037
Slowly Perfused (VS)	12.5	12.5
Muscle (VM)	34.756	34.756
	Partition Coefficients	
Blood/Air (PB)	13.71	13.71
Liver/Blood (PL)	4.73	4.73
Fat/Blood (PF)	104.2	104.2
Richly Perfused/Blood (PR)	2.05	2.05
Slowly Perfused/Blood (PS)	8.0	8.0
Muscle/Blood (PM)	3.56	3.56
	Metabolic Constants	
V_{max} (mg/hr)	9.08	9.08
K_m (mg/ℓ)	21.11	21.11

Table III-1-3

Estimates of Mouse Average Daily Values of Surrogate Doses in the Liver, Arterial Blood, and Richly Perfused Tissue Resulting from Inhalation Exposure to Perchloroethylene (PERC)

Experimental Dose (ppm)[c]	REITZ AND NOLAN[a]			HATTIS[b]		
	Liver		Arterial	Liver		Arterial
	MFO-Metabolite[d]	PERC[e]	Blood-PERC[e]	MFO-Metabolite[d]	PERC[e]	Blood-PERC[e]
MALE						
100	1.47E+3	9.23E+1	3.42E+1	6.09E+2	1.15E+2	3.45E+1
200	2.06E+3	2.17E+2	7.72E+1	8.32E+2	2.86E+2	8.18E+1
FEMALE						
100	1.53E+3	9.25E+1	3.43E+1	6.35E+2	1.16E+2	3.48E+1
200	2.15E+3	2.18E+2	7.74E+1	8.69E+2	2.88E+2	8.22E+1

[a]Derived from the physiologically based pharmacokinetic model of Reitz and Nolan (1986).
[b]Derived from the physiologically based pharmacokinetic model of Hattis et al. (1986).
[c]Experimental doses utilized in the NTP study (1986), 6 hours/day, 5 days/week.
[d]Average amount of metabolite (mg) formed by way of the MFO pathway, per day per liter of tissue; units in mg/ℓ/day.
[e]Average daily area under the concentration * time curve; units in mg * hr/ℓ/day.

Table III-1-4

Estimates of Rat Average Daily Values of Surrogate Doses
in the Liver, Arterial Blood, and Richly Perfused Tissue
Resulting from Inhalation Exposure to Perchloroethylene (PERC)

Concentration in Air (ppm)[c]	REITZ AND NOLAN[a]				HATTIS[b]			
	Liver		Arterial	Richly	Liver		Arterial	Richly
	MFO-Metabolite[d]	PERC[e]	Blood-PERC[e]	Perfused-PERC[e]	MFO-Metabolite[d]	PERC[e]	Blood-PERC[e]	Perfused-PERC[e]
MALE								
200	3.07E+2	3.53E+2	9.70E+1	3.63E+2	4.57E+2	3.47E+2	9.72E+1	3.61E+2
400	4.26E+2	7.40E+2	2.02E+2	7.54E+2	6.56E+2	7.29E+2	2.02E+2	7.50E+2
FEMALE								
200	3.44E+2	3.54E+2	9.72E+1	---[f]	5.20E+2	3.48E+2	9.74E+1	---[f]
400	4.80E+2	7.42E+2	2.02E+2	---	7.51E+2	7.32E+2	2.02E+2	---

[a]Derived from the physiologically based pharmacokinetic model of Reitz and Nolan (1986).
[b]Derived from the physiologically based pharmacokinetic model of Hattis et al. (1986).
[c]Experimental doses utilized in the NTP study (1986), 6 hours/day, 5 days/week.
[d]Average amount of metabolite (mg) formed by way of the MFO pathway, per day per liter of tissue; units in mg/ℓ/day.
[e]Average daily area under the concentration * time curve; units in mg * hr/ℓ/day.
[f]This surrogate dose was not calculated for female rats; the values are expected to be very similar to those calculated for male rats.

Table III-1-5

Estimates of Mouse Average Daily Values of Surrogate Doses in the Liver and Arterial Blood Resulting From Gavage Exposure to Perchloroethylene (PERC)

Sex	Experimental Time Weighted Average Dose (mg/kg/day)[c]	REITZ AND NOLAN[a]			HATTIS[b]		
		Liver		Arterial Blood	Liver		Arterial Blood
		MFO-Metabolite[d]	PERC[e]	PERC[e]	MFO-Metabolite[d]	PERC[e]	PERC[e]
Male	536	1.52E+3	2.91E+2	7.81E+1	1.00E+3	7.46E+2	1.70E+2
	1072	1.92E+3	6.11E+2	1.64E+2	1.18E+3	1.57E+3	3.59E+2
Female	386	1.35E+3	1.93E+2	5.18E+1	9.06E+2	4.90E+2	1.12E+2
	772	1.74E+3	4.19E+2	1.13E+2	1.12E+3	1.07E+3	2.45E+2

[a]Derived from the physiologically based pharmacokinetics model of Reitz and Nolan (1986).
[b]Derived from the physiologically based pharmacokinetics model of Hattis et al. (1986).
[c]Experimental doses utilized in the NCI (1977) study.
[d]Average amount of metabolite (mg) formed by way of the MFO pathway, per day, per liter of tissue; units in mg/ℓ/day.
[e]Average daily area under the concentration * time curve; units in mg * hr/ℓ/day.

Table III-1-6

Estimates of Rat Average Daily Values of Surrogate Doses
in the Liver and Arterial Blood Resulting from
Gavage Exposure to Perchloroethylene (PERC)

Sex	Experimental Time Weighted Average Dose (mg/kg/day)[c]	REITZ AND NOLAN[a] Liver MFO-Metabolite[d]	REITZ AND NOLAN[a] Liver PERC[e]	REITZ AND NOLAN[a] Arterial Blood PERC[e]	HATTIS[b] Liver MFO-Metabolite[d]	HATTIS[b] Liver PERC[e]	HATTIS[b] Arterial Blood PERC[e]
Male	471	4.57E+2	1.61E+3	3.55E+2	6.04E+2	1.19E+3	2.46E+2
	941	5.70E+2	3.20E+3	7.04E+2	8.08E+2	2.35E+3	4.88E+2
Female	474	4.84E+2	1.41E+3	3.10E+2	6.34E+2	1.01E+3	2.10E+2
	949	6.08E+2	2.85E+3	6.28E+2	8.54E+2	2.06E+3	4.26E+2

[a]Derived from the physiologically based pharmacokinetics model of Reitz and Nolan (1986).
[b]Derived from the physiologically based pharmacokinetics model of Hattis et al. (1986).
[c]Experimental doses utilized in the NCI (1977) study.
[d]Average amount of metabolite (mg) formed by way of the MFO pathway, per day, per liter of tissue; units in mg/ℓ/day.
[e]Average daily area under the concentration * time curve; units in mg * hr/ℓ/day.

Table III-1-7

Estimates of Human Average Daily Values of Surrogate Doses
in the Liver and Arterial Blood Resulting from
Exposure to Perchloroethylene (PERC) in Drinking Water

Concentration in Drinking Water (ppm)	REITZ AND NOLAN[a]				HATTIS[b]			
	Liver		Arterial Blood-PERC[d]	Richly Perfused-PERC[d]	Liver		Arterial Blood-PERC[d]	Richly Perfused-PERC[d]
	MFO-Metabolite[c]	PERC[d]			MFO-Metabolite[c]	PERC[d]		
0.0001	5.20E-6	3.82E-5	4.26E-6	2.50E-5	5.15E-6	2.96E-5	3.83E-6	7.85E-6
0.00088[e]	4.58E-5	3.36E-4	3.74E-5	2.20E-4	4.53E-5	2.60E-4	3.37E-5	6.91E-5
0.01	5.20E-4	3.82E-3	4.25E-4	2.50E-3	5.18E-4	2.98E-3	3.87E-4	7.84E-4
0.1	5.20E-3	3.82E-2	4.25E-3	2.50E-2	5.14E-3	2.96E-2	3.83E-3	7.84E-3
1	5.20E-2	3.82E-1	4.25E-2	2.50E-1	5.14E-2	2.96E-1	3.83E-2	7.84E-2
10	5.17E-1	3.82E0	4.25E-1	2.50E0	5.12E-1	2.96E0	3.83E-1	7.84E-1

[a]Derived from the physiologically based pharmacokinetic model of Reitz and Nolan (1986).
[b]Derived from the physiologically based pharmacokinetic model of Hattis et al. (1986).
[c]Average amount of metabolite (mg) formed by way of the MFO pathway, per day per liter of tissue; units in mg/ℓ/day.
[d]Average daily area under the concentration * time curve; units in mg * hr/ℓ/day.
[e]The concentration in drinking water EPA has estimated to correspond to a one-in-a-million risk.

Table III-1-8

Extra Lifetime Human Cancer Risks Corresponding to Exposures of 1 ppm Perchloroethylene (PERC) in Drinking Water; Reitz and Nolan Surrogate Dose Estimates

Data Set	Experimentally Applied Dose[a] Extra Risk		Based on Surrogate Doses[b] PERC in Target[c] Extra Risk		MFO Metabolite at Target Extra Risk		PERC in Arterial Blood Extra Risk	
	MLE[d]	Upper	MLE	Upper	MLE	Upper	MLE	Upper
NTP male mice - hepatocellular carcinoma	7.24E-5[e] (7.24E-5)[f]	9.09E-5 (9.09E-5)	1.26E-3 (1.26E-3)	1.79E-3 (1.79E-3)	1.66E-5 (1.66E-5)	2.26E-5 (2.26E-5)	3.97E-4 (3.94E-4)	5.57E-4 (5.57E-4)
NTP female mice - hepatocellular carcinoma	6.96E-6 (7.60E-5)	6.72E-5 (9.62E-5)	7.39E-4 (1.95E-3)	2.18E-3 (2.50E-3)	6.08E-10 (2.15E-5)	1.18E-5 (2.73E-5)	1.81E-4 (6.02E-4)	6.38E-4 (7.68E-4)
NTP male mice - hepatocellular adenoma or carcinoma	1.32E-4 (1.32E-4)	1.63E-4 (1.63E-4)	2.23E-3 (2.23E-3)	3.08E-3 (3.08E-3)	7.69E-10 (2.66E-5)	3.44E-5 (3.62E-5)	6.90E-4 (6.90E-4)	9.53E-4 (9.52E-4)
NCI male mice - hepatocellular carcinoma	6.37E-5 (6.37E-5)	8.94E-5 (8.94E-5)	5.88E-4 (5.88E-4)	8.40E-4 (8.40E-4)	2.36E-5 (5.35E-5)	3.09E-5 (8.72E-5)	2.44E-4 (2.44E-4)	3.48E-4 (3.48E-4)
NCI female mice - hepatocellular carcinoma	6.23E-5 (6.23E-5)	8.07E-5 (8.07E-5)	6.31E-4 (6.31E-4)	8.17E-4 (8.17E-4)	1.70E-5 (1.70E-5)	2.20E-5 (2.20E-5)	2.61E-4 (2.60E-4)	3.38E-4 (3.38E-4)

Table III-1-8 (continued)

Extra Lifetime Human Cancer Risks Corresponding to Exposures of 1 ppm Perchloroethylene (PERC) in Drinking Water; Reitz and Nolan Surrogate Dose Estimates

Data Set	Experimentally Applied Dose[a] Extra Risk		Based on Surrogate Doses[b]							
			PERC in Target[c] Extra Risk		MFO Metabolite at Target Extra Risk		PERC in Arterial Blood Extra Risk			
	MLE[d]	Upper	MLE	Upper	MLE	Upper	MLE	Upper		
NTP male rat - mononuclear cell leukemia	2.43E-4 (2.43E-4)	2.98E-4 (2.98E-4)	--- (---)	--- (---)	--- (---)	--- (---	1.24E-4 (1.24E-4)	2.32E-4 (2.32E-4)		
NTP female rat - mononuclear cell leukemia	1.38E-4 (1.38E-4)	1.71E-4 (1.71E-4)	--- (---)	--- (---)	--- (---)	--- (---)	1.03E-4 (1.03E-4)	1.79E-4 (1.79E-4)		
NTP male rat - tubular cell adenoma or adeno-carcinoma (kidney)	1.30E-5 (1.30E-5)	2.29E-5 (2.29E-5)	2.24E-5 (2.24E-5)	5.00E-5 (5.00E-5)	--- (---)	--- (---)	1.42E-5 (1.42E-5)	3.17E-5 (3.17E-5)		

[a]Risk estimates based on the experimental doses administered to test animals, as reported in the NTP study or NCI study. Units of mg/kg body weight/day assumed to yield equivalent risks across species.

[b]Risk estimates based on surrogate doses derived from the physiologically based pharmacokinetic model of Reitz and Nolan (1986) and Table III-1-7.

[c]Target tissue was the liver compartment for hepatocellular carcinoma and adenoma, and the richly perfused tissue compartment for the kidney tubular cell adenoma or carcinoma.

[d]Maximum likelihood estimate and 95% upper confidence limit on extra lifetime cancer risks.

[e]Risks estimated by fitting the multistage model (degree equal to one less than the number of dose groups) to the data.

[f]In parentheses are the risks estimated by fitting a one-hit model to the data.

Table III-1-9

Extra Lifetime Human Cancer Risks Corresponding to Exposures of 1 ppm Perchloroethylene (PERC) in Drinking Water; Hattis et al. Surrogate Dose Estimates

Data Set	Experimentally Applied Dose[a]		Based on Surrogate Doses[b]					
			PERC in Target[c]		MFO Metabolite at Target		PERC in Arterial Blood	
	Extra Risk		Extra Risk		Extra Risk		Extra Risk	
	MLE[d]	Upper	MLE	Upper	MLE	Upper	MLE	Upper
NTP male mice - hepatocellular carcinoma	1.62E-4[e] (1.62E-4)[f]	2.04E-4 (2.04E-4)	7.40E-4 (7.40E-4)	1.06E-3 (1.06E-3)	4.02E-5 (4.02E-5)	5.48E-5 (5.48E-5)	3.35E-4 (3.35E-4)	4.77E-4 (4.77E-4)
NTP female mice - hepatocellular carcinoma	1.56E-5 (1.70E-4)	1.50E-4 (2.16E-4)	5.39E-4 (1.17E-3)	1.36E-3 (1.49E-3)	3.55E-9 (5.19E-5)	2.88E-5 (6.60E-5)	1.98E-4 (5.20E-4)	5.82E-4 (6.63E-4)
NTP male mice - hepatocellular adenoma or carcinoma	2.97E-4 (2.97E-4)	3.65E-4 (3.65E-4)	1.32E-3 (1.32E-3)	1.84E-3 (1.84E-3)	4.53E-9 (6.43E-5)	8.27E-5 (8.76E-5)	5.94E-4 (5.94E-4)	8.21E-4 (8.21E-4)
NCI male mice - hepatocellular carcinoma	6.37E-5 (6.37E-5)	8.94E-5 (8.94E-5)	1.77E-4 (1.77E-4)	2.53E-4 (2.53E-4)	3.88E-5 (3.88E-5)	5.05E-5 (5.05E-5)	1.00E-4 (1.00E-4)	1.43E-4 (1.43E-4)
NCI female mice - hepatocellular carcinoma	6.23E-5 (6.23E-5)	8.07E-5 (8.07E-5)	1.92E-4 (1.92E-4)	2.48E-4 (2.48E-4)	2.56E-5 (2.56E-5)	3.31E-5 (3.31E-5)	1.08E-4 (1.08E-4)	1.40E-4 (1.40E-4)

Table III-1-9 (continued)

Extra Lifetime Human Cancer Risks Corresponding to Exposures of 1 ppm Perchloroethylene (PERC) in Drinking Water; Hattis et al. Surrogate Dose Estimates

Data Set	Experimentally Applied Dose[a] Extra Risk MLE[d]	Experimentally Applied Dose[a] Extra Risk Upper	Based on Surrogate Doses[b] PERC in Target[c] Extra Risk MLE	PERC in Target[c] Extra Risk Upper	MFO Metabolite at Target Extra Risk MLE	MFO Metabolite at Target Extra Risk Upper	PERC in Arterial Blood Extra Risk MLE	PERC in Arterial Blood Extra Risk Upper
NTP male rat - mononuclear cell leukemia	1.74E-4 (1.74E-4)	2.13E-4 (2.13E-4)	--- (---)	--- (---)	--- (---)	--- (---)	1.12E-4 (1.12E-4)	2.09E-4 (2.09E-4)
NTP female rat - mononuclear cell leukemia	9.88E-5 (9.88E-5)	1.22E-4 (1.22E-4)	--- (---)	--- (---)	--- (---)	--- (---)	9.28E-5 (9.28E-5)	1.62E-4 (1.62E-4)
NTP male rat - tubular cell adenoma or adeno-carcinoma (kidney)	9.30E-6 (9.30E-6)	1.63E-5 (1.63E-5)	7.06E-6 (7.06E-6)	1.58E-5 (1.58E-5)	--- (---)	--- (---)	1.28E-5 (1.28E-5)	2.86E-5 (2.86E-5)

[a]Risk estimates based on the experimental doses administered to test animals, as reported in the NTP study or NCI study. Units of mg/kg body weight/day assumed to yield equivalent risks across species.

[b]Risk estimates based on surrogate doses derived from the physiologically based pharmacokinetic model of Hattis et al. (1986) and Table III-1-7.

[c]Target tissue was the liver compartment for hepatocellular carcinoma and adenoma, and the richly perfused tissue compartment for the kidney tubular cell adenoma or carcinoma.

[d]Maximum likelihood estimate and 95% upper confidence limit on extra lifetime cancer risks.

[e]Risks estimated by fitting the multistage model (degree equal to one less than the number of dose groups) to the data.

[f]In parentheses are the risks estimated by fitting a one-hit model to the data.

Table III-1-10
PERC Bioassay Data Used in Risk Calculations

Data Set	Experimental Doses (mg/kg/day) Standard Breathing Rates[a]	Reitz & Nolan Breathing Rates[b]	Hattis *et al.* Breathing Rates[c]	Response Rates
NTP male mice -	0	0	0	7/49
hepatocellular	282	192.60	85.98	25/49
carcinoma	565	381.22	170.19	26/50
NTP female mice -	0	0	0	1/45
hepatocellular	282	201.86	90.12	13/43
carcinoma	565	401.37	179.28	36/49
NTP male mice -	0	0	0	16/49
hepatocellular	282	192.60	85.98	31/49
adenoma or carcinoma	565	381.22	170.19	40/50
NCI male mice -	0	...[d]	...[d]	2/20
hepatocellular	215	---	---	32/49
carcinoma	309	---	---	27/48
NCI female mice -	0	---	---	0/20
hepatocellular	155	---	---	19/48
carcinoma	429	---	---	19/48
NTP male rat -	0	0	0	28/50
mononuclear cell	103	107.26	150.17	37/50
leukemia	206	214.53	300.34	37/50
NTP female rat -	0	0	0	18/50
mononuclear	103	122.41	171.37	30/50
cell leukemia	206	247.92	347.08	29/50
NTP male rat -	0	0	0	1/49
tubular cell adenoma	103	107.26	150.17	3/49
or adenocarcinoma	206	214.53	300.34	4/50
(kidney)				

[a]The standard breathing rates are 0.01104 m^3/8 hrs for mice and 0.048 m^3/8 hrs for rats.

[b]The rates assumed by Reitz and Nolan (1986) are $28 \times BW^{.74}$ ℓ/hr for mice and $15 \times BW^{.74}$ ℓ/hr for rats.

[c]The rates for the Hattis *et al.* (1986) model are scaled as $12.5 \times BW^{.74}$ ℓ/hr for mice and $21.BW^{.74}$ ℓ/hr for rats.

[d]Revised estimates not calculated; the NCI (1977) study administered doses by gavage so calculation of daily doses is not affected by breathing rates.

APPENDIX III-1-A

REVIEW OF THE LITERATURE

ON THE PHARMACOKINETICS OF TETRACHLOROETHYLENE

APPENDIX III-1-A

REVIEW OF THE LITERATURE ON THE PHARMACOKINETICS OF TETRACHLOROETHYLENE

The pharmacokinetics of tetrachloroethylene (PERC, perchloroethylene) have been reported for rats exposed by drinking water, gavage, and inhalation, and for mice exposed by gavage and inhalation. In general, the data indicate that disposition of PERC is a saturable, dose dependent process, and that PERC is primarily cleared, unchanged, by expiration, and by metabolism and urinary excretion.

Inhalation is the main route of human exposure, although PERC contamination of drinking water indicates that oral ingestion is possible. Pulmonary absorption into blood occurs via alveolar air (EPA, 1983). Several parameters influence the uptake of PERC including: inspired air concentration, pulmonary ventilation, duration of exposure, and the rates of diffusion into and solubility in blood and tissue. After tissue and body equilibrium are reached, uptake is then balanced by elimination and other routes, including metabolism (EPA, 1983).

Absorption and Tissue Distribution

Disposition of PERC following oral and inhalation exposure to Sprague-Dawley rats was investigated by Pegg et al. (1979). For the oral route, radiolabeled PERC in corn oil was administered by gavage at single doses of 1 or 500 mg/kg. Those exposed by inhalation received 10 or 600 ppm radiolabeled PERC for 6 hours. Extensive monitoring of blood concentrations was performed on all animals, which were maintained in metabolism cages and killed 72 hours after exposure. Analysis of rat tissues, including liver, kidney, fat, brain,

lung, heart and adrenal, indicated little distribution of radioactivity after oral exposure. At the low dose, the greater amounts of radioactivity were recovered from the liver, fat, and adrenal tissues (0.0008, 0.0006, and 0.0005 µmol eq/g of tissue, respectively). However, at the high dose the greater recoveries were reported in fat, kidney, and liver tissues (0.272, 0.137, and 0.097 µmol eq/g of tissue, respectively). Analysis of tissues of rats exposed at 10 ppm indicated that liver tissue retained the greatest amount of radioactivity, followed by kidney, fat and lung tissues (0.0047, 0.0018, 0.0018, 0.0012 µmol eq/g of tissue, respectively). At the high dose of 600 ppm, kidney, liver and fat tissues accounted for recoveries of 0.167, 0.096 and 0.082 µmol eq/g tissue, respectively.

Frantz and Watanabe (1983) administered radiolabeled PERC at 150 ppm (8.1 mg/kg) to male Sprague-Dawley rats in drinking water for 12 hours. Total radioactivity recovered as both parent and metabolites was approximately 14.1 µmole equivalents and varied with the drinking water volume consumed. A mean body burden of approximately 8.1 mg PERC/kg was found. Small levels of radioactivity were found in the liver and kidneys (11 and 10 nmol eq/g of tissue respectively), with smaller levels found in other tissues, including fat, lung, heart and adrenals (6, 6, 7, and 7 nmol eq/g of tissue, respectively).

Hake et al. (1976) exposed males and females to 25, 50, 100, or 150 ppm PERC by inhalation for 1, 3, 5.5, or 7.5 hours/day. The authors concluded that PERC was rapidly absorbed and excreted via the lungs. Amount absorbed at a given vapor concentration was reported to be related to the respiratory minute volume.

In contrast, Monster et al. (1979) found total body uptake to be influenced more by lean body mass than by respiratory minute volume or adipose tissue. Six male volunteers were exposed by inhalation for 4 hours to approximately 72 ppm and 144 ppm PERC during rest and to 142 ppm PERC at rest combined with a work load on a bicycle ergometer. Uptake/minute decreased during the exposure due to a decrease in retention. Uptake was about 25% higher during the first exposure hour at rest than during the last exposure hour.

Results of an experiment by Jakobson et al. (1982) indicated that PERC can be transported through the skin. PERC was applied to the skin of guinea pigs and blood uptake was measured. Blood concentrations increased rapidly within one hour, peaking at about 30 minutes after exposure began, then decreased slightly in spite of the fact that exposure was continued. At 30 minutes, PERC concentration was 1.1 µg/ml and decreased to 0.63 µg/ml after 6 hours post-exposure. Due to the lipophilic nature of PERC, the decrease was attributed to transport from the blood to adipose tissue.

A study using humans reported contrasting results. Stewart and Dodd (1964) exposed five individuals by immersing one thumb of each in a beaker of PERC. The authors concluded that there was virtually no way that toxic amounts of PERC could be absorbed through the skin during normal use or exposure.

In a study by Riihimaki and Pfaffli (1978), three individuals wearing full face piece respirators and dressed in thin cotton pajamas and socks were exposed to 600 ppm PERC for 3.5 hours. During each "midhour," each person exercised on a bicycle ergometer for 10 minutes. The conclusion was that

concentrations of PERC found in the workplace were not likely to result in significant amount of absorption through the skin.

A mathematical model was used by Guberan and Fernandez (1974) to calculate the uptake and distribution of PERC in the body. They predicted that fatty tissues would show the slowest rate of elimination due to the high lipid solubility of PERC. To develop the model, serial breath concentration decay data were obtained from 25 volunteers after exposure to 50 or 150 ppm PERC for up to 8 hours. The model predictions agreed with reported data. PERC is believed to accumulate in body tissues that have a high lipid content (Savolainen, 1981; Fernandez et al., 1976). Accumulation of PERC in adipose tissue increases linearly with length of exposure and will continue during repeated exposures until equilibrium is reached (Savolainen, 1981). After equilibrium, further exposure will not influence accumulation (Schumann et al., 1980).

Metabolism

Available information on the metabolism of PERC suggests that it is a rate-limited process that proceeds according to Michaelis-Menten kinetics (Filser and Bolt, 1979; Pegg et al., 1979; Schumann et al., 1980; EPA, 1986). The blood/air partition coefficient of 1o reflects the fact that PERC has a very low solubility in water, while the fat/blood partition coefficient of 90 suggests that this chemical is likely to partition in adipose tissues of the body (Monster, 1979). It is believed that PERC is metabolized by the mixed-function oxidase (MFO) system to epoxide intermediates (Buben and O'Flaherty, 1985). The epoxide may then be subject to hydration and, subsequently, formation of tetrachloroethylene glycol (Leibman and Ortiz, 1975). The

epoxide and glycol formed from PERC are believed to spontaneously rearrange to trichloroacetyl chloride, which is then rapidly hydrolyzed to trichloroacetic acid (TCA), the most commonly reported metabolite.

Moslen et al. (1977) provided evidence of metabolism of PERC via the MFO pathway. Rats that were pretreated with inducers of the hepatic MFO system showed a significant increase in trichlorinated urinary metabolites, including TCA excretion, after a single oral dose of PERC at 0.75 ml/kg.

Costa and Ivanetich (1980) reported that the cytochrome P-450 enzyme system may be the only enzyme system utilized in the conversion of PERC to free trichloroacetate and the trichloroacetyl portion covalently bound to cellular constituents. The epoxide formed is capable of binding to cellular macromolecules and is thought to be the toxic intermediate. There is some disagreement in the literature concerning metabolic endpoints. While trichloroacetic acid is believed and generally reported to be the primary metabolite, some researchers have reported other metabolites in varying amounts, including oxalic acid (Yllner, 1961), trichloroethanol (Ikeda and Ohtsuji, 1972), and ethylene glycol (Moslen et al., 1977). The study by Daniel (1963) which has been described in the following discussion, reported trichloroacetic acid and inorganic chloride as the only urinary metabolites detected.

The metabolism of PERC in mice exposed briefly by inhalation was studied by Yllner (1961). Mice were exposed for 2 hours to radiolabeled PERC, resulting in doses of approximately 1.3 mg/g body weight. Twenty percent of the radioactivity was recovered as metabolites in urine in the percentages indicated: trichloroacetic acid (52%), oxalic acid (11%), and dichloroacetic

acid (trace). Eighteen percent of the urinary metabolites were not extractable and were, therefore, not identified.

Buben and O'Flaherty (1985) reported that PERC, when administered by gavage to mice, was metabolized to trichloroacetic acid. In fact, in this experiment trichloroacetic acid was identified as the sole metabolite. Other experiments substantiate the fact that trichloroacetic acid has been identified as a primary urinary metabolite in rats and man (Ogata et al., 1971; Daniel 1963; Ikeda and Ohtsuji, 1972; Ikeda, 1977; Mitoma et al., 1985).

In contrast, the work of Pegg et al. (1979) reported the major metabolite to be oxalic acid. As stated previously, rats were exposed to PERC by gavage (1 or 500 mg/kg, single dose) and inhalation (10 or 600 ppm for 6 hours). Regardless of dose or route, the major metabolite was identified by gas chromatography-mass spectrometry as oxalic acid. The chromatography analysis exhibited a second, minor peak that was not identified, but neither the parent compound nor trichloroacetic acid was detected. Oxalic acid has been identified as a metabolite by other authors including Yllner (1961) and Dmitrieva (1967).

In a study which compared metabolism of PERC in humans, rats, and mice, Ikeda and Ohtsuji (1972) reported finding trichloroacetic acid and trichloroethanol in post-exposure urine of all three species. Rats and mice were exposed to equal concentrations of PERC vapor. The major metabolite in both species was found to be trichloroacetic acid, but, on a mg/kg body weight basis, mice metabolized almost four times more PERC to this endpoint than did rats. Trichloroethanol accounted for 38% of the total trichlorocompounds recovered from rat urine, while accounting for only 17% of the total recovered from urine of mice. The human subjects were workers, and therefore subject to

a wide range of exposures. In those workers exposed to relatively low doses of PERC (20 to 70 ppm), trichloroethanol and trichloroacetic acid were recovered in approximately equal amounts. In those exposed at higher concentrations (200 to 400 ppm), trichloroethanol/trichloroacetic acid ratio was approximately 2/3. Urinary excretion data suggests that humans have a limited capacity to metabolize PERC. Ohtsuji et al. (1983) offered further confirmation of limited metabolic capabilities of PERC in humans, which was reportedly about 2% (EPA, 1983). In this study, personal monitoring of exposure and comparison of urinary trichlorocompounds suggested that metabolic capacity becomes saturated at about 100 ppm (678 mg/m^3) in air.

Mitoma et al. (1985) examined urinary metabolite patterns and extent of metabolism in rats and mice given oral doses of 1000 mg/kg and 900 mg/kg, respectively, 5 days per week for 4 weeks followed by a single radiolabeled dose. The majority of the dose was excreted in expired air in both species, although the amount metabolized in rats and mice showed a four-fold difference, with rats metabolizing 5.1% and mice 22.2% of the dose. Trichloroacetic acid was the primary metabolite in both species. When actual amounts of metabolized PERC were examined, expressed in mmol/kg body weight, again mice metabolized two to four times more PERC than rats.

Schumann et al. (1980), as previously described, stated that their experiment was designed to enable comparison of data resulting from their work on mice and that of Pegg et al. (1979) on rats. Comparisons of the data indicated that the mouse metabolized 8.5 times more PERC per kilogram body weight than did the rat after inhalation of 10 ppm PERC, and 1.6 times more after ingestion of 500 mg/kg PERC.

In summary, the metabolism of PERC has been studied in mice, rats, and humans. These studies indicate that metabolism of PERC is rate-limited and proceeds according to Michaelis- Menten kinetics. The path of this metabolism, via the cytochrome P-450 mixed-function oxidase system, is believed to be similar in the species tested.

Elimination

In the Franz and Watanabe study (1983), in which rats were administered 150 ppm PERC for 12 hours in drinking water, 90% of the recovered radioactivity was found in expired air (88% from parent compound, 2% from carbon dioxide) and 7% in urine. Of the 88% of radioactivity from PERC recovered from expired air, 96% of that amount was recovered during the first 24 hours following the initiation of exposure, with a half-life for pulmonary elimination of 7.1 hours.

Recovery of radioactivity following oral exposure in rats indicated that PERC was primarily eliminated in the expired air as the parent compound, with recovery of 72% in the 1 mg/kg dose group and 90% in the 500 mg/kg dose group (Pegg et al., 1979). Expired carbon dioxide accounted for only 2.5% (low dose) and 0.5% (high dose) of recovered radioactivity. The half-life for pulmonary elimination was approximately 7 hours for either dose. Urinary excretion of nonvolatile metabolites accounted for 17% (low dose) and 5% (high dose) of the recovered radioactivity; while 6% (low dose) and 4% (high dose) were recovered from the feces, also in the form of nonvolatile metabolites. After oral exposure, peak concentrations in the blood were observed after 1 hour. Disappearance from whole blood followed first order kinetics with a half-life of 6 hours.

Daniel (1963) reports similar kinetics for oral exposure in Wistar rats. PERC, [^{36}Cl]-labeled, was administered by gavage in doses of 1.75 and 13 μCi. The study has certain deficiencies in reporting, including the fact that the vehicle, if any, was not identified and radioactivity in expired air from the high dose group was not reported. Within 48 hours following the 1.75 μCi dose, 98% of radioactivity was recovered in expired air. The remaining 2% was excreted in the urine over 18 days.

Exposure of rats to PERC by inhalation resulted in recovery of 68% to 88% of radioactivity in the form of parent compound in expired air in the 10 ppm and 600 ppm dose groups, respectively (Pegg et al., 1979). Radioactivity recovered as radiolabeled carbon dioxide in expired air accounted for 4% (low dose) and 0.7% (high dose). Pulmonary elimination of PERC exhibited a half-life of approximately 7 hours for either dose. After inhalation exposure was halted, blood concentrations declined according to first order kinetics, also exhibiting a half-life of 7 hours.

Comparison of these studies on rats shows that, regardless of route of exposure, the fate of PERC does not appreciably change. The study by Pegg et al., which tests at a high and low dose, indicated a potential shift to increased pulmonary elimination at high doses. The shift is attributed to saturable metabolism, i.e., when metabolic capacity is surpassed, a greater amount of the compound is eliminated unchanged by the lungs. The values reported for half-lives of pulmonary elimination and elimination from the blood, regardless of route of exposure, were consistently close to 7 hours.

The pharmacokinetics of PERC following inhalation and oral exposure to mice have been studied by Schumann et al. (1980). B6C3F1 male mice were exposed to either 10 ppm PERC for 6 hours or 500 mg/kg PERC by gavage. Two

other dosages were given, but were not tested for recovery. The test chemical was radiolabeled and that delivered by gavage was dissolved in corn oil. Recovery of radioactivity 72 hours after inhalation exposure at 10 ppm showed that 20% was recovered from expired air (12% parent compound, 8% carbon dioxide), while 63% was found in the urine. The finding that metabolism and urinary excretion was the major elimination pathway for this low dose inhalation exposure was in direct contrast to reported results from similar studies conducted on rats. After the single oral dose of 500 mg/kg, 84% of radioactivity was recovered from expired air, primarily as the parent compound, while only 10% was recovered from urine. The disposition following the oral route was in agreement with that reported in orally dosed rats (Pegg et al., 1979). In fact, Schumann et al. stated that their experiment was designed to enable comparison of data resulting from their work on mice and that of Pegg et al. on rats. Comparisons of the data indicated that the mouse metabolized 8.5 times more PERC per kilogram body weight than did the rat after inhalation of 10 ppm PERC, and 1.6 times more after ingestion of 500 mg/kg PERC.

Yllner (1961) exposed female mice to PERC vapor for 2 hours at a concentration which resulted in an approximate dose of 1.3 mg/g body weight. Absorption of the solvent was reported to be 70%, of which 90% was excreted within 4 days. Of the amount recovered, 70% was in expired air, 20% in urine, and less than 5% in feces. Metabolites identified were discussed in the previous section.

Stewart et al. (1961) investigated the uptake and decay of PERC in humans exposed by inhalation to 194 ppm for 83 to 187 minutes. Blood concentrations were monitored as well as the amount of test compound in

expired air and urine. Blood concentrations rose slowly during the exposure periods, but the compound was not present at the limit of detection 30 minutes after exposure was ended indicating rapid clearance from the blood. PERC in post-exposure expired air demonstrated prolonged exponential decay. Detectable amounts were still present in expired air 94 hours after exposure to 194 ppm for 83 minutes. Urine samples that had been obtained every 30 minutes during and after exposure had no detectable amounts of PERC.

Pulmonary elimination of PERC was extensively investigated in a study by Stewart et al. (1974). Several sets of experiments were performed, but one in which men were exposed to 100 ppm PERC for 5 days in the first week, and to 20 ppm in the second week was utilized by Hattis et al. in their work on pharmacokinetic modeling. Extensive data were reported on pre- and post-exposure levels of PERC in alveolar air, confirming the conclusion from earlier work that PERC is rapidly excreted, unchanged, via the lungs.

Additional studies of the pharmacokinetics of inhalation exposure to humans have been published (Monster et al., 1979; Monster, 1979; Monster and Houtkooper, 1979). These studies reported results of a series of experiments in which human volunteers were exposed for 4 hours to PERC in amounts of 72 ppm (at rest), 144 ppm (at rest), and 142 ppm (at rest combined with a work load). The same six subjects were utilized in each experiment, with 2 weeks allowed for recovery between exposures. Concentrations of PERC were measured in expired air and in blood and amounts of the primary metabolite, trichloroacetic acid, were measured in urine. Uptake of the test chemical in resting subjects was approximately linear at these doses; i.e., a two-fold increase in dose increased uptake by a factor of 2.1. The addition of two 30-minute work periods (bicycle) during exposure to 142 ppm PERC resulted in a

40% increase in estimated uptake. The amount of PERC recovered from expired air decreased with level of activity, indicating that physical activity resulted in increased uptake. Percent recovery was as follows: 95% (72 ppm), 92% (144 ppm), 78% (142 ppm with work load). Only an estimated 2% of PERC, in the form of primary metabolite, was excreted in the urine. The authors hypothesized that the reduced recovery after work load was due to either overestimation of uptake, existence of an unknown metabolic pathway, or excretion of PERC by another route.

APPENDIX III-1-B

PERC PBPK MODELS OF REITZ AND NOLAN (1986)

AND HATTIS ET AL. (1986)

APPENDIX III-1-B

PERC PBPK MODELS OF REITZ AND NOLAN (1986) AND HATTIS ET AL. (1986)

1. Reitz and Nolan model

Gas Exchange Compartment

CA = (QC * CV + QP * CI)/(QC + QP/PB)

Liver Compartment

dCL/dt = QL * (CA - CVL)/VL - dCL1/dt + (DRINK + GAV)/VL

dCL1/dt = Vmax * CVL/(VL * (Km + CVL))

Fat Compartment

dCF/dt = QF * (CA - CVF)/VF

Richly Perfused Compartment

dCR/dt = QR * (CA - CVR)/VR

Slowly Perfused Compartment

dCS/dt = QS * (CA - CVS)/VS

Mixed Venous Blood

CV = (QL * CVL + QF * CVF + QR * CVR + QS * CVS)/QC

2. Hattis et al. model

Gas Exchange Compartment

CA = (QC * CV + QP * CI)/(QC + QP/PB)

Liver Compartment

dCL/dt = QL * (CA - CVL)/VL - dCL1/dt + (DRINK + GAV)/VL

dCL1/dt = Vmax * CL/(VL * (Km + CL))

Fat Compartment

dCF/dt = QF * (CA - CVF)/VF

Richly Perfused Compartment

dCR/dt = QR * (CA - CVR)/VR

Slowly Perfused Compartment

dCS/dt = QS * (CA - CVS)/VS

Muscle Compartment

dCM/dt = QM(CA - CVM)/VM

Mixed Venous Blood

CV = (QL * CVL + QF * CVF + QR * CVR + QS * CVS + QM * CVM)/QC

Ci - Concentration of PERC in i

i - L for liver
F for fat
R for richly perfused
S for slowly perfused
M for muscle
A for arterial blood leaving gas exchange compartment
V for mixed venous blood
I for inhaled air

CVi - Concentration of PERC in venous blood leaving compartment i (i - L, F, R, S, M); CVi - Ci/Pi
CLl - Virtual concentration of PERC metabolized via MFO pathway
DRINK - rate of PERC introduction into liver compartment via drinking water
GAV - rate of PERC introduction into liver compartment via gavage

In the Hattis et al. model applied to humans, the flow rate parameters (QP, QC, QL, QF, QR, QS, QM) were time-dependent so that for 16 hours per day they assumed their "active" values and for 8 hours per day they assumed their "sleeping" values (cf. Table III-1-2).

In the Hattis et al. model applied to rodents, no slowly perfused compartment was used. The parameters for the muscle compartment are such that that compartment contains all slowly perfused tissue.

VOLUME III

PART 2 OF 2 PARTS

EXTENDED PBPK MODELING AND REVISED RISK ASSESSMENT

A. INTRODUCTION

This part of Volume III presents extensions to pharmacokinetic models discussed in Part 1. Dose-response modeling and a risk assessment for PERC are presented. The risk assessment takes advantage of the dose surrogates that can be estimated with the extended PBPK models and that are relevant to the assessment of the liver cancer risk posed by PERC.

The emphasis in this part of the document is on the liver tumors observed in mice exposed to PERC (NCI, 1977; NTP, 1986). Thus, the extensions to the PBPK models previously published are such that one can predict concentrations of the metabolite that is thought to be responsible for those tumors, trichloroacetic acid (TCA) (Herren-Freund et al., 1987, Green and Prout, 1985). Because liver tumors have not been observed in rats exposed to PERC (NTP, 1986), discussion will be limited to modeling of mice and humans.

B. REVIEW OF PUBLISHED PBPK MODELS OF PERC

In addition to the PBPK models for PERC discussed in Part 1 of this volume (Reitz and Nolan, 1986; Hattis et al., 1986), more recent versions of PERC models have been published (Ward et al., 1988; Koizumi, 1989). Ward et al. (1988) presented versions for mice, rats, and humans; their mouse model differed in structure from that of Reitz and Nolan (1986) only because it included a first-order pathway as well as a saturable pathway for PERC metabolism, while the Reitz and Nolan model included only a saturable pathway. Thus, the equation for the rate of metabolic elimination of PERC from the liver (dCLl/dt; see Appendix III-1-B) is altered in the Ward et al. model to be:

$$(1) \qquad dCL1/dt = Vmax * CVL/(VL * (Km + CVL)) + Kf * CVL.$$

Table III-2-1 presents the values of the parameters (or of the scaling constants used to derive the parameter values) for the published mouse models[1]. The parameter values shown in that table have been set so that they apply to the equations shown in Appendix III-1-B for the Reitz and Nolan model; i.e., the values of the parameters presented by Hattis et al. have been converted so that they apply to the somewhat different equations used by Reitz and Nolan. As can be seen in Table III-2-1, values of the parameters estimated by the various investigators differ, sometimes greatly, even though much the same data have been used by all investigators to derive parameter estimates.

Table III-2-2 presents the parameter sets that have been published for human PERC models. The model presented by Hattis et al. (1986) (discussed in detail in Part 1 of this volume) was not considered here. That model divided the body into compartments defined slightly differently than the compartments of the other three human models and all of the mouse models. In any case, predictions of dose surrogate values derived from the Hattis et al. model did not differ greatly from those predicted by the Reitz and Nolan human model, especially with respect to amount of PERC metabolized (see Table III-1-6).

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[1]In Table III-2-1, two entries are recorded for Reitz and Nolan (1986). Those authors had developed two sets of parameter values for mice; Part 1 of this volume discussed the set that Reitz and Nolan labeled "Mouse 1." Because we were interested for this work in refining and extending PERC PBPK modeling for mice, all published parameter sets were considered as starting points for parameter estimation, including Reitz and Nolan's "Mouse 2" parameter set.

C. EXTENSION OF THE PBPK MODELS

The extension to these models that is discussed here allows tracking of the major PERC metabolite, TCA (Figure III-2-1). In essence, the PERC PBPK model was linked to a single compartment model for TCA via the P-450 metabolism of PERC. In this extension a certain proportion (PO) of PERC metabolized by the MFO pathway is converted to TCA. Short-lived precursors in TCA production are ignored in this approach. According to the extended model, TCA is eliminated from its volume of distribution at a first-order rate, Ke. Work with trichloroethylene (for which TCA is also a metabolite) provided the basis for this representation of TCA pharmacokinetics and its link to the PBPK model of the parent compound (Fisher et al., 1990; Allen et al., 1990 - Volume II, Part 1). The equations that describe the extension to the models are displayed in Appendix III-2-A.

1. Fitting a Mouse Model

The data from several literature sources were used as reference points for fitting a mouse PERC/TCA model. Those sources include the following:

Schumann et al. (1980). This study included results of experiments in which B6C3F1 mice (assumed to average 24.5g body weight) were exposed to PERC either through inhalation (10 ppm for 6 hours) or via gavage (500 mg/kg). The authors reported amounts of PERC exhaled unchanged (post-exposure) and equivalents of PERC transformed and appearing in the exhaled breath, urine, feces, or carcass, thus allowing estimation of amount of PERC metabolized.

One difficulty with this study, for the inhalation exposure, is that the measurement of indicators of metabolism started only after the end of

exposure, so that the amounts measured were probably less than the total amount metabolized. On the other hand, the measured amount was probably greater than the amount metabolized after exposure stopped, because fecal, urinary, and (probably) pulmonary elimination captured post-exposure undoubtedly included some metabolites formed during the exposure period. Thus, for estimating parameters, the measurements of PERC exhaled unchanged were given greater weight when the inhalation results were considered. The reported amount metabolized was considered to be a lower limit for the total amount metabolized. In the gavage study, these difficulties did not exist, so the parameter estimation discussed here considered the PERC exhalation data and the metabolism data equally.

Dekant et al. (1986). This study reported results of a gavage experiment with female NMRI mice (assumed average body weight of 25g). The dose of PERC was 800 mg/kg; exhaled PERC and indicators of metabolism were measured; the proportion metabolized and the proportion exhaled unchanged were reported.

Mitoma et al. (1985). Radiolabeled PERC was given to male B6C3F1 mice (average body weight assumed to be 20g) in gavage doses of 225 and 900 mg/kg. PERC in the expired air and radioactivity measured in expired air, excreta, and the carcass for 48 hours after dosing were reported. In the case of the 900 mg/kg dose, recovery accounted for less than 80% of the administered dose. The percent recovery of the lower dose was not reported.

Buben and O'Flaherty (1985). Groups of male Swiss-Cox mice averaging 40g in weight were given a range of gavage doses repeatedly (5 days per week for 6 weeks). The authors reported the amount of urinary metabolite recovered per day. TCA was the only urinary metabolite observed. The authors also

noted that the TCA concentration in the urine increased over the course of a week, but that pattern did not appear to change from week to week. This is indicative of retention of TCA from one day to the next (increasing TCA excretion during the latter part of a week) but elimination sufficiently fast to dispose of TCA during the 2 weekend days of no exposure.

Odum et al. (1988). This study reported the concentration of TCA in blood during and following a 400 ppm inhalation exposure. The authors used B6C3F1 mice with an average weight of 25g. One potential difficulty with the presentation of the TCA results is that the sex of the mice used in the determination of the TCA concentrations was not specified.

Ikeda and Ohtsuji (1972). This study included an intraperitoneal (ip) exposure and an inhalation exposure of female DD mice. The ip dose was 2.78 mmol/kg and the atmospheric concentration for the inhalation exposure was 200 ppm for 8 hours. Urine was collected for 48 hours from the beginning of exposure in either case, and the amount of TCA in the urine was recorded. Trichloroethanol (TCOH) was also observed in the urine, unlike the results obtained by Buben and O'Flaherty (1985).

The first step in finding a model to use for estimating dose surrogate values in mice was to fix the physiological parameters. As shown in Table III-2-1, the volumes of the compartments and the flow rates (as a percentage of cardiac output) did not differ very much from model to model. Thus the values of the volumes and volume-specific flow rates used for the model fitting were averages of those used in the published models. The alveolar ventilation rate and the cardiac output rate that were used (22.9 and 15.9 L/hr, respectively) were derived from data in Arms and Travis (1987). These

physiological parameter values were fixed for the remainder of the model fitting process.

The next step in finding a suitable mouse model was to compare the predictions of the published models (with the physiological parameters set at the values discussed in the preceding paragraph) to the observations from the experiments discussed above. Some of those comparisons are shown in Table III-2-3. The results in Table III-2-3 do not depend on the values of the parameters needed to define the model extension (the TCA extension); i.e., they are the observations that can be predicted using only the parameters presented with the published models.

In addition to the experiments that measured total amounts metabolized and expiration of PERC, several of the studies reported urinary excretion of the metabolites, especially TCA. These data are particularly valuable for defining the parameters in the extension to the models and were used to select one model for use from among the published models. The data of Buben and O'Flaherty and Odum et al. were particularly useful in that regard.

The results of the Odum et al. study were used to define the elimination rate of TCA, Ke. The results suggested a value of 0.025 for Kec, the scaling constant which is multiplied by body weight raised to the (-0.3) power to give Ke. The value for volume of distribution was set at 24% of the total body size, in accordance with the value suggested for TCA following trichloroethylene administration (Fisher et al., 1990).

Then, using those two parameter values and setting PO and PU (the proportion of metabolized PERC that becomes TCA and the proportion of eliminated TCA that appears in the urine, respectively) to 1.0, the predicted values of the amount of TCA excreted in urine in a day could be matched to the

observations of urinary TCA presented by Buben and O'Flaherty. The predictions should be overestimates if either PO or PU are less than 1, i.e., if not all metabolized PERC becomes TCA or if some of the TCA eliminated does not appear in the urine. (The model predictions of daily urinary TCA are directly proportional to the product of PO and PU.) The results of the comparison of observed and predicted urinary TCA are shown in Table III-2-4.

Combining the results shown in Tables III-2-3 and III-2-4, it was determined that the parameter values specified by Reitz and Nolan (1986) for their "Mouse 1" model were the best basis for further refinement of the parameters. This decision was reached because:

- The supposed upper bounds on the amount of TCA excreted in the urine failed to reach the observed urinary TCA values presented by Buben and O'Flaherty for high doses when either the Hattis et al. model or the Ward et al. model was used. Both of the versions presented by Reitz and Nolan apparently provided true upper bounds for urinary TCA, in the sense that at all dose levels the predicted amounts exceeded the observed amounts.

- Both of the two Reitz and Nolan versions overestimated metabolism at mid-range dose levels, as compared to the observations of Schumann et al. (oral dosing) and Dekant et al. This suggested that a refinement of the models would be to decrease the rate of metabolism by adjusting Vmax or Km. Such a refinement would also tend to reduce the urinary TCA outputs. It was apparent from the Buben and O'Flaherty observations and corresponding predictions that most of that reduction should occur at the lower dose levels, since the degree of overestimation of urinary TCA was greater at the lower levels than at the higher levels (Table III-2-4).

- However, if metabolism was to be reduced, especially at the lower dose levels, then that would tend to increase the amount of PERC expired unchanged. The "Mouse 2" model of Reitz and Nolan was already overpredicted the amount of expired PERC, so the suggested changes would make that prediction even worse. The "Mouse 1" model, on the other hand, underpredicted the amount of PERC expired unchanged, so the suggested refinement would tend to improve that prediction.

- The "Mouse 1" model also had the best prediction of amount metabolized as observed by Mitoma et al. at their lower dose level. (Predictions from all of the models were low.) The suggested refinement would tend

to worsen this prediction, however. All of the models clearly underpredicted the amount metabolized observed by Mitoma et al. at the higher dose level, 900 mg/kg.

Thus, based on the above considerations, the "Mouse 1" model of Reitz and Nolan was selected and refinements to this model were investigated. Increasing Km to (7.0) provided the improvements sought. The overestimation of urinary TCA at low doses was more consistent with the overestimation of that variable at higher doses (that is, before refinement of PO and PU, the factor by which urinary TCA was overestimated at low doses was more comparable to the factor by which that variable was overestimated at higher doses). Moreover, the predictions of amount metabolized were decreased, making them more consistent with the observations of Dekant et al. and of the oral dosing experiment of Schumann et al., without severely affecting the predicted amount metabolized for the inhalation exposure of Schumann et al. or for the gavage exposure of Mitoma et al.

The end result of this stage of refinement is shown in Table III-2-5. The model prediction of amount metabolized following the low-level inhalation exposure of Schumann et al was below the lower limit represented by their observation, as were the predictions of the amounts metabolized for the Mitoma et al. experiments. However, the observed amounts metabolized for the oral experiments of Schumann et al. and of Dekant et al. were overestimated by the model. The urinary TCA data of Buben and O'Flaherty were fairly well predicted, once the product of PO and PU was set equal to 0.52, the average factor across all dose groups by which the predicted urinary TCA values (with PO and PU both equal to 1) overestimated the observed values.

Reexamining the blood TCA concentration data of Odum et al., and recalling that Vd for TCA was set at 24% of the body, and that Kec (the

scaling constant for Ke, which determines the rate of elimination of TCA) was estimated to be 0.025, a value for PO was derived. Other than the metabolic constants (which were derived as discussed above), Ke, and Vd, PO is the only parameter that affects the time course of TCA concentration in the blood. Since the product of PO and PU was determined to be 0.52 from consideration of the Buben and O'Flaherty data, a lower limit for PO was 0.52 (since PU, as a proportion, cannot exceed 1.0). The optimal value for PO based on the Odum et al. data was the lower limit just mentioned, 0.52 (Figure III-2-2). As seen in Figure III-2-2, the TCA concentration predictions were slightly high when TCA concentration was at its peak and for some time thereafter. However, the overall rate of disappearance of TCA from the blood was in good agreement with observed values.

From the discussion in the previous paragraph, it is apparent that the estimate of PU must be 1.0. This value is in accordance with the assumption that TCA is eliminated in the urine only, and that TCA is not metabolized (primarily to TCOH) to any great extent. This assumption is consistent with the observation of Buben and O'Flaherty (1985) that no TCOH was detected in the urine. However, Dekant et al. (1986) did observe some TCOH, as did Ikeda and Ohtsuji (1972).

In fact, the data of Ikeda and Ohtsuji (1972) on urinary TCA were compared with predictions of the model with PU=1.0. The model predicted amounts of urinary TCA far in excess of those observed by Ikeda and Ohtsuji. The model predicted about 2.0 mg of TCA excreted in urine 48 hours after an inhalation exposure of 200 ppm for 8 hours and about 1.1 mg excreted in that time following an ip dose of 2.78 mmol/kg, whereas Ikeda and Ohtsuji observed only about 0.5-0.6 mg for such exposures. It may be the case that PU for the

strain of mice used by Ikeda and Ohtsuji (DD) is different from that for the strains studied by Buben and O'Flaherty (Swiss-Cox) or by Dekant et al. (NMRI). Actually, there is not enough information contained in any one of those three studies to determine if PO, PU, the metabolic constants, or a combination of those parameters may differ across strains. However, as long as the concentration of TCA in the blood (plasma) can be accurately predicted by the model, then it is not important for risk assessment purposes how much TCA is eliminated in the urine. It appears that, for the strain of mice used in the cancer bioassays (B6C3F1), the model can predict TCA blood concentrations, as assessed by the fit of the model predictions to the observations of Odum et al. (1988).

The values of the parameters that define the mouse model are shown in Table III-2-6.

2. Fitting a Human Model

The values of the parameters for the human PERC/TCA model are also displayed in Table III-2-6. The values shown there were derived as follows.

The human models presented by Reitz and Nolan (1986), Ward et al. (1988), and Koizumi (1989) were used as the starting points for derivation of a human PERC/TCA model. The values of the parameters used in those three models are displayed in Table III-2-2.

The first step in determining parameter values for the human PERC/TCA model was the specification of the physiological parameters. As in the case of the mouse model, those parameter values were set equal to the average of the values presented in the published models. In the case of the alveolar ventilation rate and the cardiac output rate, however, a slightly different

approach was adopted. Based on observations reported in the literature (Astrand, 1973; Monster et al, 1979a; Hattis et al., 1986), the alveolar ventilation rate was allowed to vary according to the time of day. The diurnal variation was set so that during waking hours (16 hours per day) the alveolar ventilation rate scaling parameter (qpc) was set to 29.4, representing moderate activity levels, with a corresponding cardiac output scaling parameter (qcc) of 20.5. During sleeping hours, the value of qpc was set to 12.4 and qcc to 15.8. The model was designed so that periods of rest (sitting, or with minimal activity, such as during some of the experimental exposures discussed below) could also be simulated; during such periods, qpc = 17.3 and qcc = 17.1.

The partition coefficients and the metabolic rates, as well as the parameters defining TCA kinetics, were adjusted based on comparisons of model predictions to observed outcomes. Starting values for such parameters were taken from the published models (for partition coefficients and metabolic constants) or from Allen et al. (1990) (for the parameters defining TCA kinetics). The study by Monster et al. (1979b) was particularly useful for the purposes of parameter estimation.

Monster et al. (1979b) exposed individuals to PERC (72 ppm or 144 ppm) for 4 hours and measured concentrations of PERC in exhaled air and blood as well as TCA in blood and urine for almost 170 hours after the end of exposure. All of the published human models predicted the exhaled air and PERC blood concentrations fairly well. The Ward et al. (1988) predictions of PERC blood concentrations tended to be low, for both exposure levels. This may have been due to a low-dose metabolic rate that was greater than that of the other two models (compare the metabolic constants in Table III-2-2). Moreover, the

Monster et al. (1979b) results indicated little saturation of metabolic capacity at the two dose levels employed. This is consistent with a larger value of Km, representative of linear behavior for a wider range of (relatively) low doses. Based on these two features, the Ward et al. model was no longer considered.

Of the remaining two models, the one presented by Reitz and Nolan had the larger Km, yet the one presented by Koizumi predicted PERC blood and exhaled air concentrations slightly better. Since those concentrations are largely determined by the partition coefficients (in particular, the blood-to-air partition coefficient), the partition coefficients from Koizumi were selected and were paired with the metabolic constants from Reitz and Nolan, for further testing.

Exhaled PERC concentration data from three additional studies were used to validate the choices for parameter values that were discussed above. The studies were conducted by Fernandez et al. (1976), Opdam and Smolders (1986), and Stewart et al. (1970).

Opdam and Smolders (1986) exposed individuals to very low concentrations of PERC (0.5 to 9 ppm) and measured alveolar concentrations shortly after exposure started (20 to 40 minutes). The model predictions of alveolar concentration tended to be slightly high in comparison with the observations of Opdam and Smolders. The observed alveolar concentrations ranged from about 20 to 25% of the atmospheric concentrations for all the exposure levels during the time period of interest. The model predicted alveolar concentrations that were 25 to 30% of the atmospheric concentrations.

Stewart et al. (1970) exposed individuals to an average atmospheric concentration of 101 ppm in 7-hour blocks and measured alveolar concentrations

following the exposures. The model predictions matched very well the observed concentrations for up to 5 hours after the end of exposure, but tended to be low (1 to 2 ppm vs. the observed values of 2 to 4 ppm) 1 to 5 days after the exposure.

Fernandez et al. (1976) exposed humans to concentrations of PERC ranging from 100 to 200 ppm for variable lengths of time (1 to 8 hours). Only for the 100 ppm exposure were alveolar concentrations measured during exposure. For an 8-hour exposure to 100 ppm, the model predictions for alveolar concentrations matched the observations at first, but then quickly exceeded the observations. The pattern observed by Fernandez et al. (steady increase in alveolar concentration throughout exposure) was not predicted by the model. The pattern predicted by the model was a rapid increase in concentration that leveled off about midway through the 8-hour exposure. The predicted alveolar concentration at the end of exposure was only slightly greater than that observed by Fernandez et al.

During the post-exposure period for all atmospheric concentrations (100, 150, and 200 ppm), the model predictions of alveolar concentrations tended to be somewhat higher than those observed by Fernandez et al. immediately after exposure. This was particularly true of the 100 ppm exposure level. For all exposure levels, the predictions at 2 to 3 hours after the end of exposure were in good agreement with the observations.

The studies by Opdam and Smolders (1986), Stewart et al. (1970), and Fernandez et al. (1976) were limited, but they all indicated that the model parameter values selected, especially the values of the partition coefficients, were satisfactory. Except for a tendency for alveolar concentrations to be overestimated during exposure, the agreement between

observed and predicted alveolar concentrations was adequate. Overprediction of alveolar concentrations in the immediate post-exposure period was relatively minor, as was the under prediction of such concentrations several days after a day of exposure.

When attention was turned to the TCA concentrations in the blood, it was determined that the metabolic rate implied by the selected parameters was insufficient to yield the observed concentrations, even if all metabolized PERC produced TCA. This determination was made under the assumption that the volume of distribution for TCA would be the same in this case as in the case of TCE exposure (i.e., that TCA kinetics are independent of the parent compound). The determination was also made after derivation of an elimination rate for TCA that provided the correct slope during the period in which TCA concentrations were decreasing. That elimination rate was determined by setting the scaling constant, Kec, equal to 0.045.

To correct for the inadequate production of TCA predicted by the model, the maximal rate of metabolism was increased. As part of that process it was determined that Km could be increased slightly to yield a better fit and that the parameter PO could be set to 0.95, a value suggested by the analysis of TCA production from TCE (Allen et al., 1990). That value of PO is the proportion of TCE that yields chloral hydrate (CH) in humans (Allen et al., 1990); in the case of PERC, it is the proportion of metabolized PERC that produces TCA, since TCA, not CH, is the first stable product of PERC metabolism. The estimates of Vmaxc and Km resulting from this stage of the parameter estimation procedure were 0.61 and 4.9, respectively. The predictions of the PERC/TCA model with these parameter values are shown in

Figures III-2-3 through III-2-5, in comparison with the observations from Monster et al. (1979b).

At that point, all that remained to be specified for the human PERC/TCA model was a value for PU, which determines the percentage of eliminated TCA that appears in the urine. Several studies presented data relevant to estimation of PU, but in general the results of the studies were somewhat inconsistent and did not necessarily correspond to the pattern of TCA excretion predicted by the model.

Monster et al. (1979b) observed the pattern of TCA excretion for 70 hours following inhalation exposures. Ogata et al. (1971) also exposed individuals to inhalation exposures of PERC (87 ppm for 3 hours) and monitored TCA excretion for nearly 70 hours. In both cases, the observed pattern of TCA excretion corresponded to a higher rate of elimination in the first 22 hours than in the two subsequent 24-hour periods. In fact, the Ogata et al. data suggested peak rates of excretion about 6 hours after the beginning of exposure. The model predictions did not match that pattern. Regardless of the value of PU selected, the rate of TCA excretion in the first 22 to 24 hours was relatively constant (after an initial rise lasting for 3 to 4 hours) and that rate was maintained at roughly the same level for up to 70 hours. Thus, the model predicted more excretion than was observed at later periods after the end of exposure. Monster et al. (1979b) stated that the relatively large measured value of urinary TCA during the first 22 hours may have been due to another compound (that they could not identify) so that their determination of TCA in urine during that time may be artifactual.

Despite the possible difference in pattern of excretion, the data from Monster et al. (1979b) on total TCA excretion, combined with that of Fernandez

et al. (1976) and Ikeda et al. (1972) can be used to suggest values for PU. The estimation of that parameter resulted in a value of 0.70. With that value, the predicted total amounts of TCA excreted following the exposures that were studied by Monster et al. (1979b) (72 and 144 ppm, for 4 hours, with TCA in urine monitored for 70 hours) were about 9 and 15 mg, respectively, whereas the observed values were about 6 and 11 mg, respectively. Similarly, TCA excretion was slightly overestimated for the Fernandez et al. (1976) exposure (150 ppm for 8 hours, with monitoring of TCA excretion for 80 hours after the start of exposure). The observed average for two human volunteers and model-predicted cumulative amounts of TCA excreted after the end of exposure are shown here:

Time (hrs)	Observed (mg)	Predicted (mg)
16	3.8	4.1
32	11.6	12.3
56	21.0	23.5
80	24.6	31.5

Conversely, the model-predicted TCA excretion for the occupationally exposed individuals studied by Ikeda et al. (1972) were less than those observed. This comparison is relatively rough given the variability of the results shown in Ikeda et al. and the fact that data points had to be approximated from graphical presentations. However, representative results are as follows (these are urinary TCA concentrations assuming 2 liters of urinary output per day, estimated for the afternoon of the second Friday of a simulated 2 week exposure, where exposure lasts 8 hours per day, 6 days per week). For 10 ppm, 30 ppm, 100 ppm, and 300 ppm exposures, the predicted urinary concentrations were 3.4 mg/L (observed average was about 8 mg/L), 8.8

mg/L (observed was about 23 mg/L), 21.8 mg/L (observed was about 50 mg/L), and 38.8 mg/L (observed was about 50 mg/L), respectively.

Thus, balancing the underestimation of the Ikeda et al. observations with the overestimation of the Monster et al. and Fernandez et al. observations, the value for PU suggested above, 0.70, appeared to give an adequate compromise. It should be noted that the predictions for the Ikeda et al. exposures could be increased if either less than 2 liters of urine are excreted per day (which may be the case, since a body weight of 61 kg, as opposed to the standard 70 kg, was assumed for the Japanese workers in that study) or the simulations were carried out for longer periods of time. In the latter case, the predicted TCA concentrations would increase because a single day of no exposure (the exposed individuals worked 6 days per week) was not sufficient to clear TCA from the body, thereby resulting in accumulation from week to week. Longer simulations showed that the accumulation did not entail increased urinary TCA concentrations more than about 10% above the predicted values cited above.

Two other observations are pertinent to the Ikeda et al. study and the TCA results. Ikeda et al. observed TCOH as another urinary metabolite of PERC. In fact, the maximal concentration suggested by those authors was around 25 mg/L, about half as much as for TCA. The presence of TCOH is a consequence of the metabolism of TCA. The presence of so much TCOH suggests that a substantial fraction of TCA was eliminated by metabolism and not by urinary excretion. This observation argues against increasing PU as a means of increasing the TCA concentrations in order to provide a better match to the Ikeda et al. TCA observations (and in this sense is consistent with the results of Monster et al. (1979b) and Fernandez et al. (1976)).

The second additional observation about the Ikeda et al. TCA results is that they suggest saturation of metabolism at a lower dose than is implied by the value of the Km parameter (4.9 mg/L) selected for the human model. This implication has not been given much weight in light of the excellent experimental results of Monster et al. (1979b) (including such results as TCA blood concentrations as well as TCA urinary output). However, the results from Monster et al. (1979b) were obtained from individuals who were not chronically exposed; the individuals studied by Ikeda et al. were exposed occupationally. Perhaps an induction of PERC metabolism (resulting in a decrease in Km) results from long-term exposure to PERC.

As discussed in the case of the mice, the specific value of PU is relatively unimportant, as long as the model can predict concentrations of TCA in the blood and plasma. On the basis of the correspondence between the observed and predicted TCA blood concentrations obtained for the Monster et al. (1979b) exposures (Figure III-2-5), it appears that the model with the parameter values displayed in Table III-2-6 is suitable.

D. RISK ASSESSMENT

The PBPK models developed above for mice and humans yielded estimates of delivered doses (dose surrogates) that were related to the production of liver tumors. Such tumors have been observed in mice (NCI, 1977; NTP, 1986). The potential for human liver cancer risk associated with exposure to PERC can be evaluated in light of the mouse results.

The dose surrogates that were considered in the assessment of liver cancer risks were the average daily values for 1) the amount of PERC

metabolized per liver volume, 2) the amount of TCA produced per liver volume, and 3) the area under the TCA concentration curve. Each of these dose surrogates is of interest because of its potential relationship to mechanisms of liver tumor production.

The surrogate based on the amount of PERC metabolized could be related to liver tumor production if a short-lived, reactive intermediate was responsible for the induction of liver tumors, as discussed in Part 1 of this volume. In that case, Andersen (1981) has shown that the amount metabolized per liver volume is a reasonable surrogate for representing the total exposure of the liver to the reactive intermediate. Although a mechanism mediated through a reactive intermediate is not generally considered to be responsible for PERC-induced liver tumors, the corresponding dose surrogate has been included in the analyses discussed here. This dose surrogate was considered in the preliminary risk assessment presented in Part 1.

The dose surrogates based on TCA (production or area under the concentration curve) are more closely associated with the product thought to be responsible for PERC-induced liver tumors. TCA is considered to be a liver carcinogen that may act through its effect on peroxisome proliferation (see below). Such proliferation has been observed in response to xenobiotics only in the liver.

TCA production per liver volume provides a measure of TCA specific to the liver, prior to its introduction into the systemic circulation. If the action of TCA that induces tumor production is relatively rapid, then the long-term kinetics of TCA may not be as important as the rate at which it is being produced. Alternatively, such a dose surrogate could be relevant if TCA

does not easily return to the target sites (within the liver or within the cell) once it has left the liver.

Area under the TCA concentration curve is based on the concentration of TCA in its volume of distribution. Thus, this measure is not associated specifically with the liver. However, it does provide an indication of the persistence of TCA; unlike PERC metabolism or TCA production, area under the concentration curve provides a measure relevant to products, like TCA, that are long-lived and are therefore present for extended periods of time. It is assumed with a dose surrogate such as area under the concentration curve that the reactions responsible for tumor induction can occur at any time that TCA is present.

Risk estimates for PERC were derived from liver tumor incidences (hepatocellular adenomas and carcinomas) for male and female B6C3F1 mice (Table III-2-7). Gavage exposures (NCI, 1977) were represented in the PBPK model as direct inputs to the liver that lasted for 2 hours, at which time all administered dose was absorbed. The linearized multistage modeling approach that is the standard dose-response procedure for regulatory agencies (e.g., the EPA) was used.

Risks were not estimated from rat carcinogenicity data. Rats do not develop liver tumors in response to PERC exposure. As discussed above, the dose surrogates that were estimated from the models are related to liver tumors. Although rats developed kidney tumors in response to PERC exposure (NTP, 1986), the dose surrogates estimated by the model are not relevant to such tumors. Rats also developed mononuclear cell leukemia (NTP, 1986); it is not known if TCA area under the curve may be a suitable dose surrogate for such a response. Additional PBPK modeling would be necessary to derive

appropriate dose surrogate values for the kidney tumors and perhaps also for the leukemias. Section B, Part 2 of Volume II of this document discussed the modeling of metabolites formed through glutathione conjugation and their possible relationship to kidney tumors. The considerations presented in that discussion are relevant to PERC also.

The results of the risk estimation are presented in Tables III-2-8 and III-2-9. The results are expressed in terms of concentrations (atmospheric or drinking water) that are associated with two levels of extra risk (10^{-6}, one in a million, and 10^{-3}, one in a thousand) when exposures to PERC at those concentrations last the entire lifetime. Drinking water exposure was represented in the PBPK model as continuous input to the liver, assuming 100% absorption and an intake of 2 liters of water per day. The body weight assumed for the calculations was 70 kg.

The concentrations of PERC associated with either of the two levels of risk depended on the dose surrogate selected for low-dose and species-to-species extrapolation. The assessment based on the amount of PERC metabolized dose surrogate yielded the largest concentrations associated with the specified risks, i.e., indicated the lowest potential for PERC induced liver tumors in humans. The assessment based on the area under the TCA concentration curve dose surrogate yielded the smallest concentrations, although those concentrations were not much less than those predicted using TCA production as the dose surrogate. The observation that the concentrations associated with PERC metabolism were larger than those associated with TCA production is due to the fact that the metabolism of PERC in humans produces more TCA than that in mice (95% of PERC metabolized becomes TCA in humans as opposed to 52% in mice). Area under the TCA concentration curve yielded

slightly smaller concentrations for the specified risk levels because the TCA volume of distribution was estimated to be smaller in humans than in mice (on a body weight basis). The smaller volume of distribution in humans entailed higher concentrations per amount of TCA produced, i.e., relatively larger dose surrogate values for humans compared to mice. The more rapid elimination in humans (Kec in humans was 0.045 and in mice it was 0.025) could not compensate for the smaller volume of distribution.

For comparison, the atmospheric and drinking water concentrations associated with 10^{-6} and 10^{-3} risks derived from standard EPA analyses (without consideration of pharmacokinetic differences, assuming mice and humans are equally sensitive when dose is expressed as mg/surface area per day) were calculated. Atmospheric concentrations associated with 10^{-3} risk averaged 18 ppb; those associated with 10^{-6} risk averaged 18 ppt. Drinking water concentrations associated with 10^{-3} risk averaged 0.84 mg/L; those associated with 10^{-6} risk averaged 0.84 µg/L.

For each set of dose-response data (Table III-2-7) the concentrations associated with 10^{-3} or 10^{-6} risk determined by the EPA approach were smaller than the corresponding concentrations calculated using any of the dose surrogates. In the case of PERC, pharmacokinetic considerations suggested that chronic exposure is not associated with as much risk as estimated in the standard EPA approach.

The PERC concentrations estimated to be associated with the levels of risk discussed above should be considered to be lower bounds. That is, higher concentrations may yield risks no greater than those given. This is the case because, in accordance with standard regulatory procedure, the doses reported were the 95% lower bounds predicted by the multistage model. In addition,

PERC may be acting through the metabolite, TCA, which in turn may be acting through its effects on peroxisomes. Humans may be less susceptible to the peroxisome proliferating effects of TCA (Elcombe, 1985). Section B, Part 2 of Volume II of this report discussed the issues associated with peroxisome proliferation, including the PBPK modeling extensions that may be necessary to derive appropriate dose surrogates and the use of such information in dose-response modeling.

E. UNCERTAINTIES IN THE TETRACHLOROETHYLENE RISK ASSESSMENT

The introduction to this document (Volume I) discussed the preliminary considerations about uncertainties that are associated with risk assessment practices and those associated with the use of PBPK modeling in risk assessment contexts. The uncertainties were grouped into two major categories, model uncertainties and extrapolative uncertainties. In this section those uncertainties are briefly reviewed and related to the risk assessment presented above for PERC.

The model uncertainties were those associated with values selected for the parameters and the structure of the model. In the risk assessment presented above, parameter uncertainty was not explicitly addressed. Such uncertainty can be considered, and distributions of risk estimates obtained, via the analyses that are presented in Volume VI of this report. The software that accompanies this document (PBPK_SIM) can implement the necessary analyses. That software is fully compatible with the extended PERC model discussed above.

Uncertainties are also associated with the structure of the model. As discussed in Volume II (Section B, Part 2), alternative representations for the kinetics of TCA are possible. In particular, the inclusion of a liver compartment (and at least one other compartment to represent the extrahepatic distribution of TCA) would enhance the ability of the model to estimate a dose surrogate related to liver carcinogenicity. Further extension to include consideration of peroxisome proliferation might also reduce uncertainties, especially because of apparent species differences in response to peroxisome proliferation (Elcombe, 1985), if peroxisome proliferation is indeed related to the liver carcinogenicity of PERC.

On the other hand, the structure of the extended model discussed in Section B of this volume represents an improvement over the models that have been published. The ability to predict long-lived metabolites such as TCA reduces uncertainties to the extent that those metabolites contribute to PERC's carcinogenicity and to the extent that the dose surrogates estimable from the model are closely related to those associated with cancer-causing processes.

The extended modeling and the risk assessment discussed above may also be considered to have reduced the extrapolative uncertainties (those related to prediction of results outside the range of observation). Modeling of TCA, in and of itself, obviates the necessity to base dose surrogates on parent compound concentrations and to rely completely on amount of PERC metabolized for metabolite-based dose surrogates. As discussed, a surrogate based on amount metabolized per liver volume may be suitable to characterize effective dose for a reactive intermediate, but it may not be suitable when the action is not associated with such an intermediate.

Two factors contribute greatly to uncertainty in PBPK-assisted risk assessments. The first is the possiblity of cell differences, differences from tissue to tissue and differences across species. The second concerns site-specific dose surrogates and the use of those surrogates to extrapolate across species, i.e., the characterization of risks for humans based on specific tumors in animals (Volume I, Part 1, Section B). In the assessment above, the effects of cell differences have been minimized and the meaning of the risk estimates clarified as much as possible by focussing on one tumor type. The hepatocellular tumors observed in mice are probably linked to TCA. Thus, the assessment used dose surrogates that are probably relevant to the selected tumor type. Moreover, it was emphasized that the human risks that were being estimated were those related to human liver cancer. Thus, concern about the human tissues or organs to which the mouse-based risk estimates apply has been avoided.

It was still assumed that human liver is as sensitive as mouse liver (when exposures yield equal values of the dose surrogate under consideration). That is, it was assumed that a mouse exposure yielding dose surrogate value X is associated with the same probability of liver cancer as a human exposure also yielding the dose surrogate value X. Because it is not known whether or not additional cross-species scalings are appropriate (scalings that might be used to account for sensitivity differences), this assumption is still uncertain.

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Table III-2-1

Parameter Values from Published Mouse PERC Models

Parameters	Hattis[a]	Reitz and Nolan[b] 1	Reitz and Nolan[b] 2	Ward[c]
Alveolar Ventilation; qp/bw$^{.74}$				
qpc (l/hr/kg$^{.74}$)	14.5	28.0	28.0	21.0
Cardiac output; qc/bw$^{.74}$				
qcc (l/hr/kg$^{.74}$)	17.5	28.0	28.0	21.0
Blood flow rates/qc				
qlc	0.25	0.25	0.25	0.25
qfc	0.05	0.05	0.05	0.09
qrc	0.51	0.51	0.51	0.51
qsc	0.19	0.19	0.19	0.15
Tissue volumes/bw				
vlc	0.06	0.04	0.04	0.06
vfc	0.10	0.05	0.05	0.10
vrc	0.05	0.05	0.05	0.05
vsc	0.70	0.78	0.78	0.70
Partition coefficients (tissue-to-air)				
pb	18.9	16.9	24.4	16.9
pla	70.3	50.9	98.9	70.3
pfa	2060.0	816.3	2359.0	2060.0
pra	70.3	50.3	112.6	70.3
psa	20.0	43.8	127.0	20.0
Metabolic constants				
Vmaxc (mg/hr/kg$^{.7}$)	1.58	8.34	3.60	1.50
Km (mg/l)	0.71	4.56	2.22	0.40
Kfc (hr^{-1}/kg$^{-.3}$)	0.0	0.0	0.0	0.60

[a] From Hattis et al. (1986).
[b] From Reitz and Nolan (1986). Reitz and Nolan discussed two sets of mouse parameters -- "Mouse 1" and "Mouse 2."
[c] From Ward et al. (1988).

Table III-2-2

Parameter Values from Published Human PERC Models

Parameters	Reitz and Nolan[a]	Ward[b]	Koizumi[c]
Alveolar Ventilation; qp/bw$^{.74}$			
qpc (l/hr/kg$^{.74}$)	15.0	15.0	11.5
Cardiac output; qc/bw$^{.74}$			
qcc (l/hr/kg$^{.74}$)	15.0	16.0	14.9
Blood flow rates/qc			
qlc	0.24	0.25	0.25
qfc	0.05	0.05	0.05
qrc	0.52	0.51	0.53
qsc	0.19	0.19	0.18
Tissue volumes/bw			
vlc	0.0314	0.04	0.026
vfc	0.231	0.20	0.195
vrc	0.05	0.05	0.031
vsc	0.621	0.62	0.524
Partition coefficients (tissue-to-air)			
pb	10.3	10.3	11.0
pla	60.6	70.3	45.7
pfa	1225.7	1638.0	1301.0
pra	60.6	70.3	45.7
psa	31.9	80.0	19.7
Metabolic constants			
Vmaxc (mg/hr/kg$^{.7}$)	0.256	0.18	0.528
Km (mg/l)	4.56	0.3	1.0
Kfc ($hr^{-1}/kg^{-.3}$)	0.o	0.0	0.0

[a] From Reitz and Nolan (1986).
[b] From Ward et al. (1988).
[c] From Koizumi (1989).

Table III-2-3

Observed Values from PERC Literature
and Corresponding Predictions from Four Published Models

	Predictions			
		Reitz and Nolan[b]		
	Hattis[a]	1	2	Ward[c]
Schumann et al. (1980)				
Inhalation exposure - 10 ppm				
Expired PERC: 0.048 mg	0.051	0.039	0.071	0.041
Amount metabolized: 0.36 mg	0.30	0.30	0.33	0.33
Oral exposure - 500 mg/kg				
Expired PERC: 82.6%	87.8	77.2	76.7	84.7
Metabolized: 17.4%	12.2	22.8	23.3	15.3
Dekant et al. (1986) - 800 mg/kg				
Expired PERC: 85.1% (88.6%)[d]	90.9	82.6	82.0	87.8
Metabolized: 10.9% (11.4%)[d]	9.1	17.4	18.0	12.2
Mitoma et al. (1985)				
225 mg/kg				
Amount metabolized: 1.66 mg	0.87	1.56	1.52	1.00
900 mg/kg				
Expired PERC: 10.3 mg	16.5	15.0	15.0	16.0
Amount metabolized: 4.0 mg	1.5	2.7	3.1	2.1

[a] From Hattis et al. (1986).
[b] From Reitz and Nolan (1986). Reitz and Nolan discussed two sets of mouse parameters -- "Mouse 1" and "Mouse 2."
[c] From Ward et al. (1988).
[d] Values in parentheses are corrected for the amount of radioactivity not recovered. It was assumed that unrecovered radioactivity was distributed in the same proportions as recovered radioactivity.

Table III-2-4

Comparison of Observed and Predicted Urinary TCA

	Urinary TCA (mg/kg/day)[a]				
			Reitz and Nolan Predicted[d]		
Gavage dose (mg/kg)	Observed[b]	Hattis[c]	1	2	Ward[e]
20	0.16	0.38	0.49	0.49	0.40
100	0.72	1.1	1.8	1.7	1.1
200	1.2	1.5	2.9	2.7	1.6
500	2.5	2.3	4.3	4.4	2.3
1000	3.1	2.9	5.8	5.9	2.9
1500	3.8	3.2	6.7	6.7	3.2
2000	4.2	3.4	7.4	7.2	3.3

[a]Observations were from the "latter part of the week." Thus, predictions were equal to the simulated amount of TCA excreted in urine on a Friday. No differences were observed from one Friday to another, so only one week of exposure was simulated. PO and PU were both equal to 1 for this simulation.
[b]Observations estimated from data in Buben and O'Flaherty (1985).
[c]From Hattis et al. (1986).
[d]From Reitz and Nolan (1986). Reitz and Nolan discussed two sets of mouse parameters -- "Mouse 1" and Mouse 2."
[e]From Ward et al. (1988).

Table III-2-5

Observed Variables and Predictions from "Optimized" PERC/TCA Model

Variable	Units	Observed	Predicted
Expired PERC			
Schumann et al.			
10 ppm inhalation	mg	0.048	0.052
500 mg/kg gavage	%	82.6	79.0
Dekant et al.			
800 mg/kg gavage	%	85.1 (88.6)[a]	83.9
Mitoma et al.			
900 mg/kg gavage	mg	10.3	15.2
Metabolism			
Schumann et al.			
10 ppm inhalation	mg	0.36	0.25
500 mg/kg gavage	%	17.4	20.9
Dekant et al.			
800 mg/kg gavage	%	10.9 (11.4)[a]	16.1
Mitoma et al.			
225 mg/kg gavage	mg	1.66	1.4
900 mg/kg gavage	mg	4.0	2.8
Urinary TCA, gavage			
Buben and O'Flaherty			
20 mg/kg	mg/day	0.16	0.42 (0.22)[b]
100 mg/kg	mg/day	0.71	1.6 (0.82)[b]
200 mg/kg	mg/day	1.2	2.4 (1.3)[b]
500 mg/kg	mg/day	2.5	4.0 (2.1)[b]
1000 mg/kg	mg/day	3.1	5.4 (2.8)[b]
1500 mg/kg	mg/day	3.8	6.3 (3.3)[b]
2000 mg/kg	mg/day	4.2	6.9 (3.6)[b]

[a]The percentages in parentheses are adjusted for less than total recovery (96% recovery). The adjustment assumes that unrecovered radioactivity is distributed in the same proportions (between unchanged PERC and metabolized PERC) as recovered radioactivity.

[b]In parentheses are the predictions obtained by setting PO*PU = 0.52. The values in parentheses are those ultimately predicted by the fully defined mouse model, for which PO = 0.52 and PU = 1.0.

Table III-2-6

Values of Parameters for Final Mouse and Human PERC/TCA PBPK Models

Parameter	Mouse Value	Human Value
qpc	22.9	17.3[a]
qcc	15.9	17.1[a]
qpc	0.25	0.243
qfc	0.07	0.05
qrc	0.51	0.52
qsc	0.17	0.187
vlc	0.05	0.032
vfc	0.075	0.209
vrc	0.05	0.044
vsc	0.74	0.588
pb	16.9	11.0
pla	50.9	45.7
pfa	816.3	1301.0
pra	50.9	45.7
psa	43.8	19.7
Vmaxc	8.34	0.61
Km	7.0	4.9
Kfc	0.0	0.0
PO	0.52	0.95
Vdc	0.24	0.341-(0.0034*bw)
Kec	0.025	0.045
PU	1.0	0.70

[a] These values are for resting individuals. Rates increase during activity and decrease while sleeping.

Table III-2-7

Dose-Response Data for Bioassays of PERC in Mice

Bioassay	Doses[a]				Liver Tumor Response Rate[b]
	Experimental	$[PERC]_m$	$[TCA]_p$	TCA-AUC	
NTP (1986)	0	0	0	0	17/49
Inhalation	100	1049.57	537.72	1666.61	31/49
Male	200	1667.37	854.22	2647.74	41/50
NTP (1986)	0	0	0	0	4/48
Inhalation	100	1100.16	563.63	1656.63	17/50
Female	200	1743.08	893.01	2624.93	38/50
NCI (1977)	0	0	0	0	2/17
Gavage	464.44	1309.10	670.68	1971.23	32/49
Male	928.89	1762.93	903.18	2654.63	27/48
NCI (1977)	0	0	0	0	2/20
Gavage	334	1129.60	578.72	1631.84	19/48
Female	669	1556.41	797.38	2248.48	19/48

[a] Experimental doses are reported in mg/kg body weight for gavage studies and in ppm air for concentration inhalation studies. $[PERC]_m$ is amount of PERC metabolized per liver volume (mg/L); $[TCA]_p$ is amount of TCA produced per liver volume (mg/L); TCA-AUC is area under the TCA concentration curve (mg*hr/L).

[b] Number of mice with hepatocellular adenomas or carcinomas per number of mice examined.

Table III-2-8

Inhalation Risk Assessment Results·
Mice Exposed to PERC

Bioassay	Risk[c]	Associated Dose Surrogate Values[a]			Estimated Human Air Concentrations (ppm)[b]		
		$[PERC]_m$	$[TCA]_p$	TCA-AUC	$[PERC]_m$	$[TCA]_p$	TCA-AUC
NTP (1986)	1E-03	1.12E0	5.73E-01	1.78E0	6.2E-01	3.4E-01	2.4E-01
Inhalation	1E-06	1.12E-03	5.73E-04	1.78E-03	6.2E-04	3.4E-04	2.4E-04
Male							
NTP (1986)	1E-03	2.82E0	1.45E0	5.29E0	1.6E0	8.7E-01	7.3E-01
Inhalation	1E-06	2.83E-03	1.45E-03	5.30E-03	1.6E-03	8.5E-04	7.2E-04
Female							
NCI (1977)	1E-03	1.47E0	7.52E-01	2.21E0	8.2E-01	4.4E-01	3.0E-01
Gavage	1E-06	1.47E-03	7.52E-04	2.21E-03	8.1E-04	4.4E-04	3.0E-04
Male							
NCI (1977)	1E-03	2.36E0	1.21E0	3.41E0	1.3E0	7.2E-01	4.6E-01
Gavage	1E-06	2.36E-03	1.21E-03	3.41E-03	1.3E-03	7.1E-04	4.6E-04
Female							

[a] The values of the dose surrogates estimated from the bioassay to correspond to the stared level of risk.

[b] The atmospheric concentrations to which humans would have to be exposed for a lifetime in order to obtain average daily dose surrogate values equaling those corresponding to the stated level of risk. Thus, the atmospheric concentrations are those estimated by each bioassay and dose surrogate combination to yield the stated level of risk.

[c] Extra risks $[(P(d)-P(0))/(1-P(0))]$.

Table III-2-9

Drinking Water Risk Assessment Results:
Mice Exposed to PERC

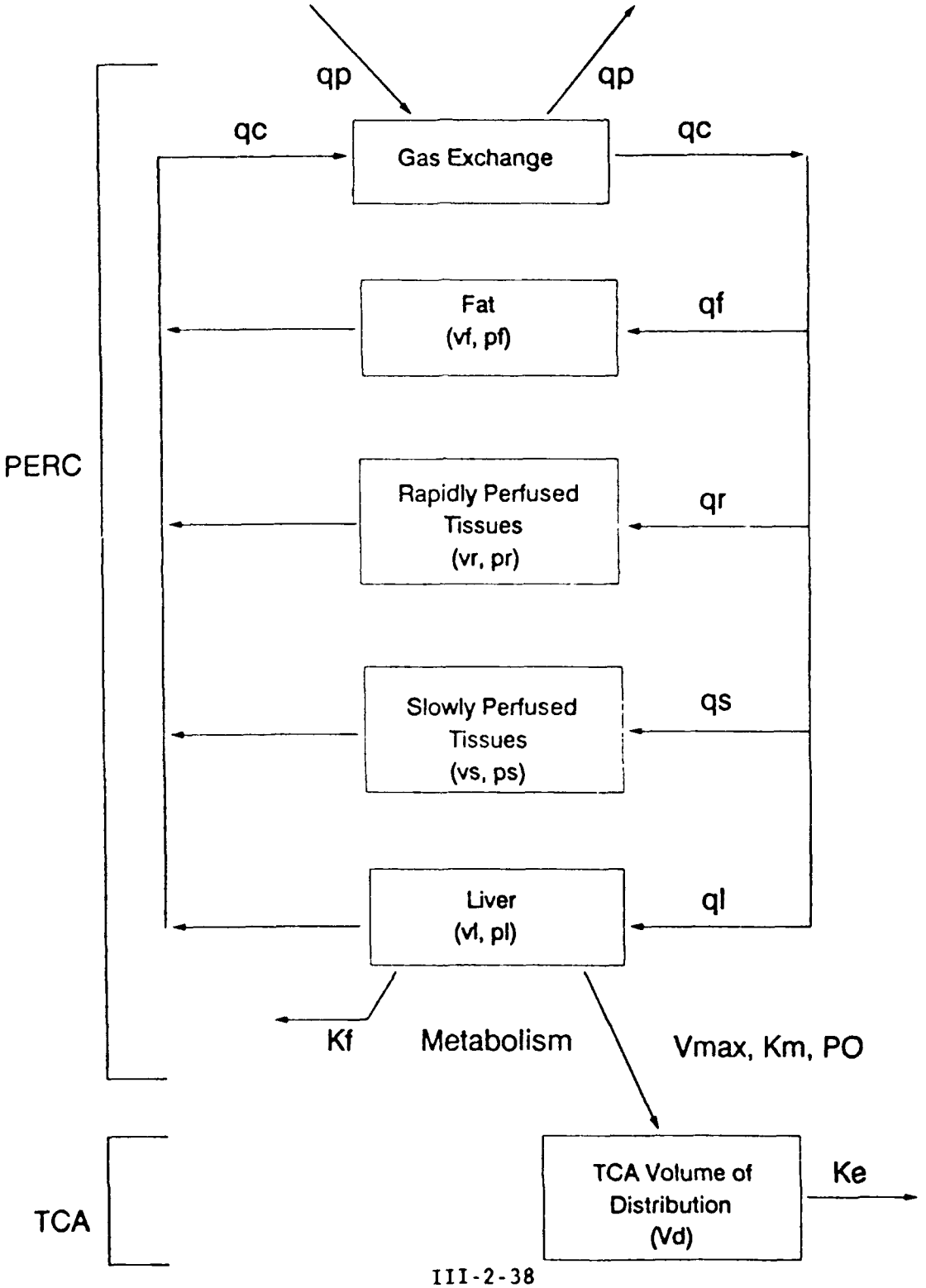
Bioassay	Risk[c]	Associated Dose Surrogate Values[a]			Estimated Human Drinking Water Concentrations (mg/L)[b]		
		$[PERC]_m$	$[TCA]_p$	TCA-AUC	$[PERC]_m$	$[TCA]_p$	TCA-AUC
NTP (1986) Inhalation Male	1E-03	1.12E0	5.73E-01	1.78E0	1.8E+01	9.8E0	7.0E0
	1E-06	1.12E-03	5.73E-04	1.78E-03	1.7E-02	9.8E-03	6.9E-03
NTP (1986) Inhalation Female	1E-03	2.82E0	1.45E0	5.29E0	4.6E+01	2.5E+01	2.0E+01
	1E-06	2.8E-03	1.45E-03	5.30E-03	4.5E-02	2.4E-02	2.0E-02
NCI (1977) Gavage Male	1E-03	1.47E0	7.82E-01	2.21E0	2.3E+01	1.2E+01	8.7E0
	1E-06	1.47E-03	7.52E-04	2.21E-03	2.3E-02	1.2E-02	8.6E-03
NCI (1977) Gavage Female	1E-03	2.36E0	1.21E0	3.41E0	3.8E+01	2.1E+01	1.3E+01
	1E-06	2.36E-03	1.21E-03	3.41E-03	3.7E-02	2.0E-02	1.3E-02

[a] The values of the dose surrogates estimated from the bioassay to correspond to the stared level of risk.

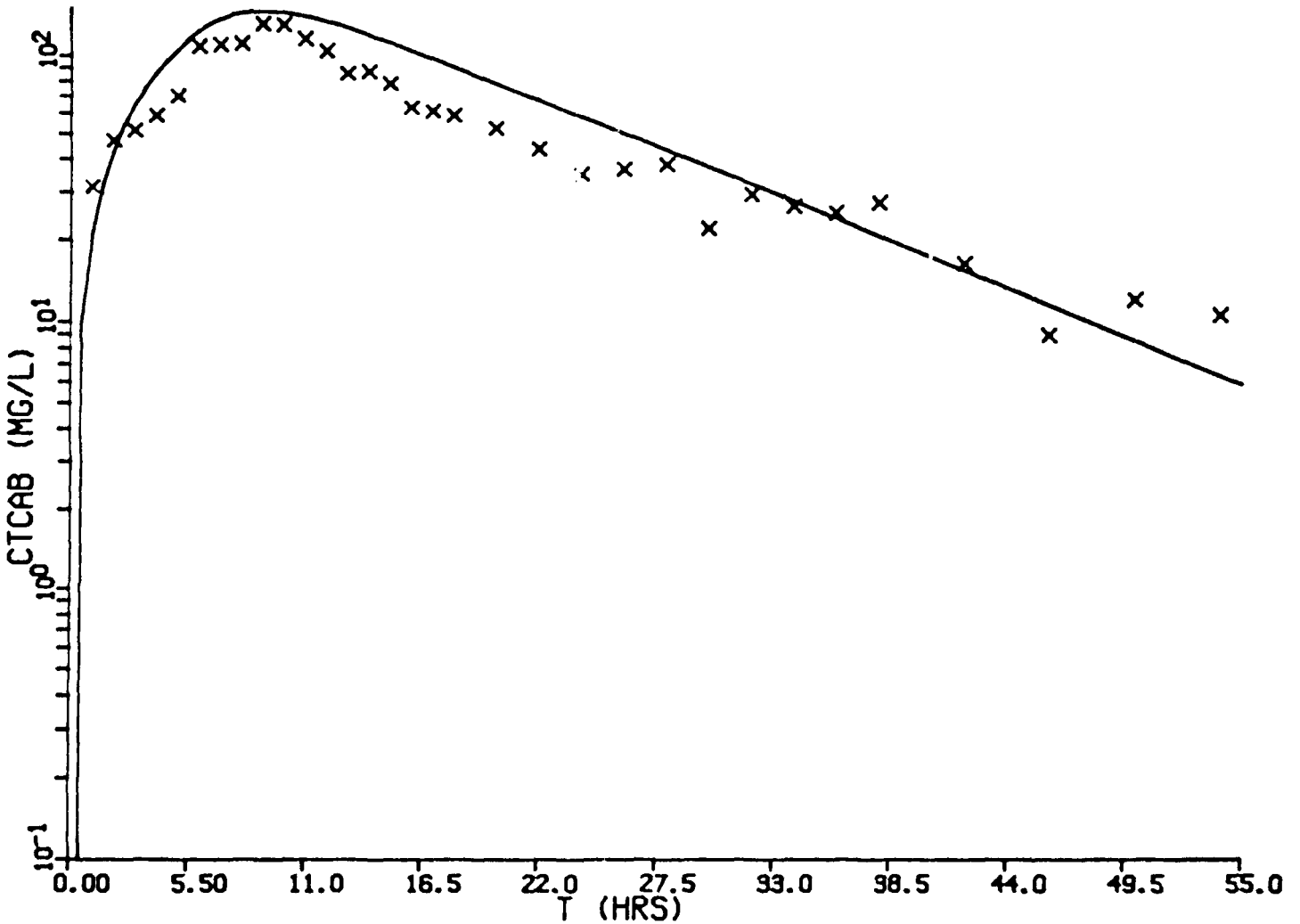
[b] The drinking water concentrations to which humans would have to be exposed for a lifetime in order to obtain average daily dose surrogate values equaling those corresponding to the stated level of risk. Thus, the drinking water concentrations are those estimated by each bioassay and dose surrogate combination to yield the stated level of risk.

[c] Extra risks $[(P(d)-P(0))/(1-P(0))]$.

Figure III-2-1
PERC/TCA Pharmacokinetic Model



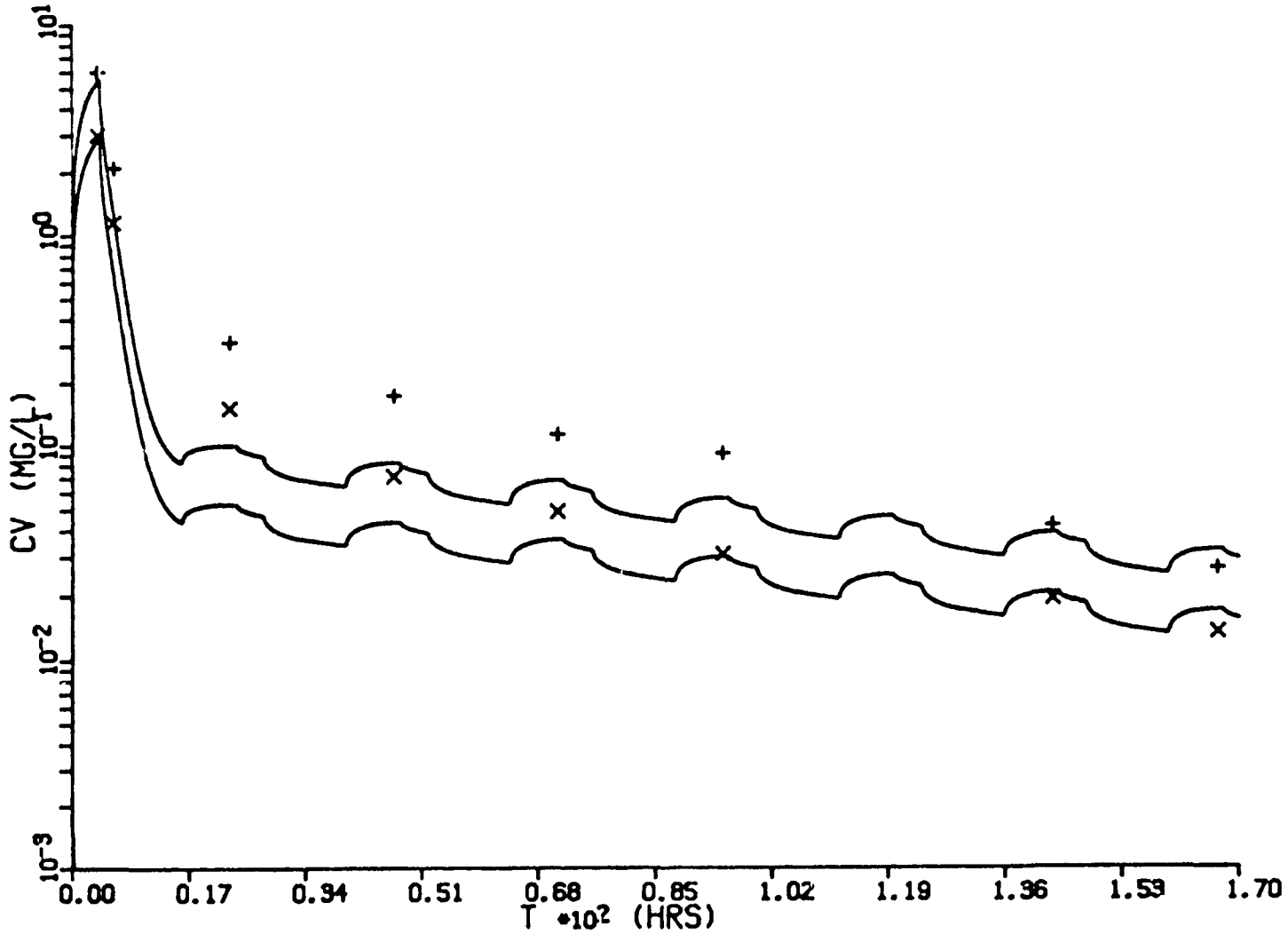
Observed and Predicted TCA Blood Concentrations in Mice



TCA blood concentrations in mice during and following a 6-hour exposure to 400 ppm PERC (Odum et al., 1988). Observations are represented by x's; model predictions are represented by the solid line.

Figure III-2-3

Observed and Predicted PERC Venous Blood Concentrations in Humans



Observed PERC concentrations in venous blood following single 4-hour exposures to 72 ppm (x) or 144 ppm (+) (Monster et al., 1979b). Model predictions are represented by the solid lines.

Figure III-2-4

Observed and Predicted Concentrations of PERC in Exhaled Breath

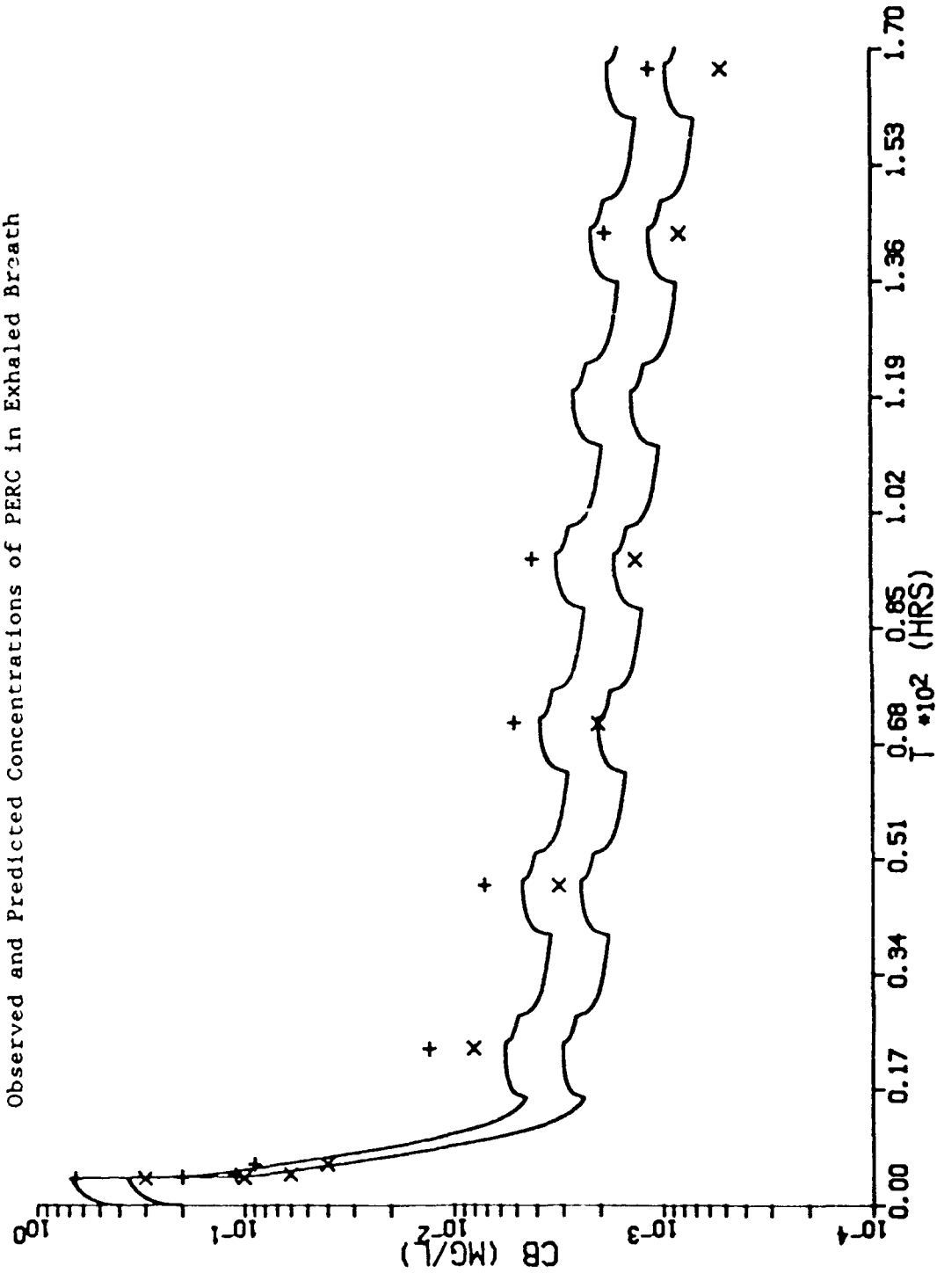
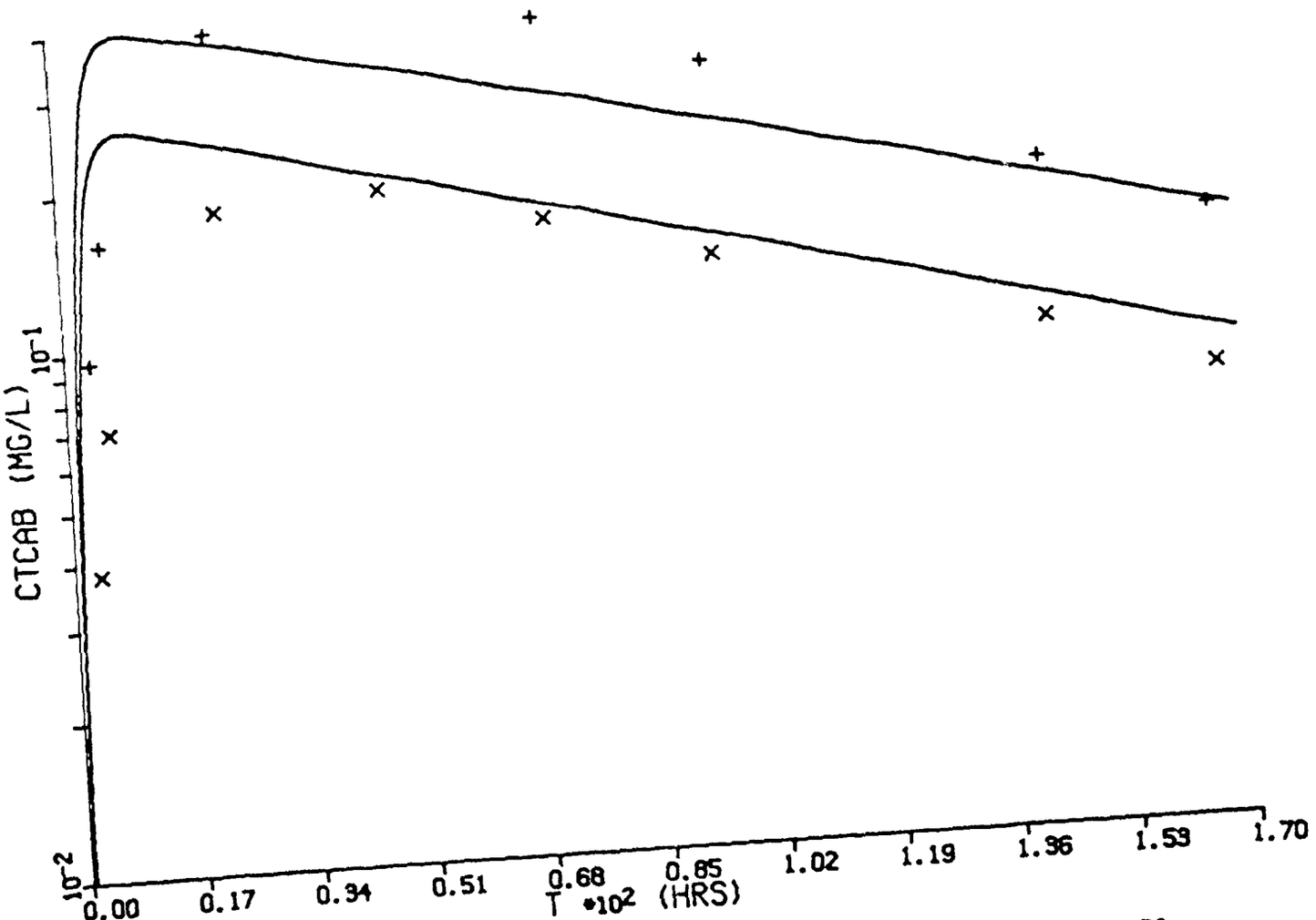


Figure III-2-5

Observed and Predicted TCA Blood Concentrations in Humans



Observed blood concentrations following single 4-hour exposures to PERC, 72 ppm (x) or 144 ppm (+) (Monster et al., 1979b). Model predictions are represented by the solid lines.

APPENDIX III-2-A

EQUATIONS DESCRIBING THE KINETICS OF TCA

Appendix III-2-A

Equations Describing the Kinetics of TCA

$$(dCTCA/dt)*Vd = PO*Vmax*CVL/(Km+CVL)*(MWTCA/MWPERC)-Ke*CTCA*Vd$$

$$dUTCA/dt = PU*Ke*CTCA*Vd$$

$$CTCA_B = 0.6*CTCA$$

CTCA - Concentration of TCA in plasma

Vd - Volume of distribution for TCA

VL - Volume of liver

CVL - Concentration of PERC in blood leaving the liver

MWTCA - Molecular weight of TCA

MWPERC - Molecular weight of PERC

UTCA - Cumulative amount of TCA eliminated in the urine

$CTCA_B$ - Concentration of TCA in blood

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