AAMRL-TR-90-072
Volume II

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

VOLUME II OF VII: TRICHLOROETHYLENE

Clement International Corporation
K.S. Crump Division
1201 Gaines Street
Ruston, LA 71270

December 1990

Final report for the period May 1987 through September 1990

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AAMRL-TR-90-072, Volume II

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 II: TRICHLOROETHYLENE

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)*

This volume describes the design, development and execution of physiologically based pharmacokinetic modeling as they apply to trichloroethylene (TCE) exposure in humans. Emphasis is also on the TCE metabolite, trichloroacetic acid (TCA). The PBPK human model for TCE risk assessment provided a basis for interspecies comparisons and a basis for cancer risk assessment.

14. SUBJECT TERMS	15. NUMBER OF PAGES
trichloroethylene; TCE; pharmacokinetic; model; risk	92
	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 trichloroethylene (TCE). This volume is divided into two parts.

Part 1 is a draft of a document (Allen et al., 1990) that will be submitted for publication. It presents the PBPK modeling work that has been done for TCE and its important metabolite TCA in humans.

Part 2 contains information related to several other issues associated with TCE modeling and risk assessment. The major issues discussed in Part 2 include:

- **Estimation of the risk posed to humans by TCE exposure.** The estimates presented were derived using the PBPK model for female mice developed by Fisher et al. (1990)[1] and the PBPK model for humans developed by Allen et al. (1990). The dose surrogates examined are those potentially related to the induction of liver tumors. Linearized dose-response modeling was used.

- **Alternative approaches for modeling the metabolites of TCE.** These approaches are more complex elaborations of the relatively straightforward approach exemplified by Fisher et al. and Allen et al. Metabolites other than TCA would be modeled and multi-compartment systems would be used for some or all of the metabolites.

- **Alternative dose-response modeling approaches that could be applied for a risk assessment of TCE.** These approaches are related to presumed mechanisms of TCE-induced liver tumors, especially as they relate to peroxisome proliferation.

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[1]Fisher, J., Gargas, M., Allen, B., et al. (1990). Physiologically based pharmacokinetic modeling with trichloroethylene and its metabolite, trichloroacetic acid, in the rat and mouse. Toxicol Appl Pharmacol (submitted).

Mr. Bruce Allen, principal investigator for Clement on this contract, was a co-author of that document, and support for his involvement was provided by this contract.

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PHARMACOKINETIC MODELING OF TRICHLOROETHYLENE AND TRICHLOROACETIC ACID IN HUMANS:

INVESTIGATION OF SPECIES SCALE-UP

A. INTRODUCTION

Pharmacokinetic considerations and, in particular, physiologically based pharmacokinetic (PBPK) modeling provide a rational basis for the comparison of carcinogenicity data among species and for the extrapolation of risks from test species to humans. PBPK modeling is a means by which quantitative estimates of the dose metrics thought to be associated with the occurrence of carcinogenicity (such as concentrations of the ultimate carcinogen at the target sites) can be obtained in various species and used in cancer dose-response modeling. Relevant species differences are explicitly included in terms of species-specific parameter values and can lead to quantitative differences in the dose metric estimates and thus estimated carcinogenic response.

The pharmacokinetics of trichloroethylene (TCE) are of interest because of recent studies showing TCE to be carcinogenic to rodents (NCI, 1976; Bell et al., 1978; NTP, 1990; Maltoni et al., 1986). Mice exposed to TCE by inhalation (Bell et al., 1978; Maltoni et al., 1986) and via gavage (NTP, 1990) developed hepatocellular tumors while rats exposed orally developed kidney tumors (NTP, 1990). A major metabolite of TCE, trichloroacetic acid (TCA), and a minor metabolite, dichloroacetic acid (DCA), have also been shown to cause hepatocellular tumors in mice when administered in drinking water (Herren-Freund et al., 1987). Thus, human exposure to TCE is of concern; TCE has been classified as a B2 carcinogen by the Environmental Protection Agency (EPA, 1985). Species differences in the distribution, elimination, or metabolism of TCE and its metabolites are important determinants of the relevance of the bioassay data for human hazard assessment.

This report presents some aspects of the development of a pharmacokinetic model for TCE that is suitable for use in cancer risk assessment of TCE. The emphasis of the modeling discussed here is on the description of the kinetics of TCA. This emphasis stems from the suggestion that liver cancer in mice may be due to insult caused by TCA rather than by TCE or a reactive epoxide (Green and Prout, 1985). Thus, the model presented extends those PBPK models that have considered only the kinetics of the parent compound, TCE (Bogen, 1988; Koizumi, 1989). The ultimate goal of the pharmacokinetic modeling is the development of appropriate TCE/TCA models for rodents and humans, utilization of those models to estimate dose metrics relevant to hepatocellular tumor development, and completion of a cancer risk assessment for TCE, including dose-response modeling employing the dose metrics so estimated.

The focus of this report is the definition of a TCE/TCA pharmacokinetic model for humans. Of particular interest is the question of scale-up of parameter values. Estimates of parameter values obtained for rodent species have been scaled up for use with humans. The scaled-up values have been compared to estimates derived from the available human literature. Model predictions obtained with the estimates ("scaled" and "human") have been compared to the observations of TCE and TCA concentrations in humans following TCE and TCA exposure. The ability of the estimates to yield adequate model predictions (i.e., reliable estimates of potentially useful dose metrics, especially those related to TCA concentrations) is discussed. Also discussed is the relevance of the resulting models to human cancer risk estimation.

B. METHODS

1. Model Structure

The structure of the human pharmacokinetic model of TCE/TCA that was examined is identical to that used successfully to describe TCE and TCA kinetics in rodents (Fisher et al., 1990; cf. Figure II-1-1). The physiologically based model for TCE consists of four compartments identified with specific tissues or groups of tissues, plus a mechanism for exchange of TCE between the blood and the atmosphere. Metabolism is assumed to occur only in the liver compartment and is allowed to include both first-order and saturable pathways. No other means of TCE elimination are included. (Enzyme inhibition, a feature found necessary to model the male mouse data (Fisher et al., 1990), was not incorporated into the human model.)

The physiologically based TCE model is coupled to a one compartment model for TCA. TCA distributes in its volume of distribution and is eliminated by a first-order mechanism. The input term for TCA is defined in terms of the saturable metabolism of TCE: a fixed proportion of TCE that is metabolized via that pathway is converted to TCA.

The parameters needed to define the TCE/TCA model are listed in Tables II-1-1 and II-1-2. Mathematical equations describing the system are presented in Appendix II-1-A.

2. Parameter Estimation

Given the structure of the model described above, the remaining work consisted of estimating values for the parameters. The parameters used to define the human TCE/TCA model can be classified into two groups. The

physiological parameters are those that describe the body of the organism, such as compartment volumes and blood flow rates (Figure II-1-1). Such parameters are independent of the chemical under investigation. The physicochemical parameters are chemical dependent and describe the interactions a particular compound has with the body compartments and the rates of its elimination and metabolism. The physicochemical parameters include partition coefficients and kinetic constants.

The sequence followed in the course of parameter estimation was:

1. Physiological parameters and partition coefficients were estimated and fixed.

2. The kinetic parameters -- parameters defining the kinetics of TCA (volume of distribution and elimination rate) and the metabolism of TCE (including the proportion of metabolized TCE that produces TCA) -- were estimated by scale-up of the corresponding rodent values.

3. The human experimental literature was examined and, in light of relationships among the kinetic parameters, direct estimates of kinetic parameter values were obtained, or starting values and bounds were determined for use in optimization.

4. Optimization of a subset of parameters was completed, where the data used in the optimization were obtained from the human experimental literature.

Details of each step are presented here.

Physiological Parameters and Partition Coefficients. Values of compartment volumes (liters per kg body weight) were taken from Arms and Travis (1987). Similarly, blood flows to the compartments (defined in terms of the proportion of total cardiac output directed to the compartments) were from Arms and Travis (1987). Alveolar ventilation and cardiac output rates were scaled according to body weight raised to the 0.74 power (cf. Reitz and Nolan, 1986; Andersen et al., 1987). Data from Astrand et al. (1973) and Monster et al.

(1979) were used to estimate alveolar ventilation rates and the relationship between alveolar ventilation and cardiac output.

Experimental determinations of a human blood-to-air partition coefficient are available (Sato et al., 1977; Arms and Travis, 1987; Gargas et al., 1989). The value used in the model discussed here was the average of the reported estimates. The fat-to-air partition coefficient was that reported by Sato et al. (1977). The remaining tissue-to-air partition coefficients were estimated from vial equilibration experiments that used rat tissues as surrogates for human tissues (Koizumi, 1989); rat tissues were assumed to be equivalent to human tissues for partition coefficient estimation (Andersen et al., 1987; Reitz et al., 1988). Each tissue-to-blood coefficient was estimated by dividing the corresponding tissue-to-air coefficient by the blood-to-air coefficient.

The physiological parameters and partition coefficients were fixed at the values estimated in the manner just described. They remained constant in all the subsequent work related to estimation of other parameters of the human model. Their values are displayed in Table II-1-1.

Kinetic Parameter Scale-Up. The kinetic parameters of the TCE/TCA model are (1) Kf, Vmax, and Km (defining metabolism of TCE); (2) PO (determining the percentage of TCE metabolized via the saturable pathway that produces TCA); and (3) Vd and Ke (the volume of distribution and first-order elimination rate of TCA, respectively). Values for each of these parameters were estimated by scaling up the values estimated for mice and rats (Fisher et al., 1990).

The first approach to this scaling was accomplished as follows. PO (unitless) and Km (mg/liter) were assumed to be invariant across species and

body weights. The remaining parameters were assumed to scale according to some power on body weight (bw):

$$(1a) \quad Vmax = Vmax_c * bw^{0.7} \ (mg/hr),$$

$$(1b) \quad Kf = Kf_c * bw^{-0.3} \ (hr^{-1}),$$

$$(1c) \quad Vd = Vd_c * bw^{1.0} \ (liters/kg \ bw),$$

$$(1d) \quad Ke = Ke_c * bw^{-0.3} \ (hr^{-1}).$$

These were the same powers on body weight used by Fisher et al. (1990) for intraspecies scaling. Scale-up was completed separately from mice and from rats (averaging the scaling constants across sexes within each species; cf. footnotes to Table II-1-3 in Fisher et al., 1990).

A second approach to scale-up was used for the parameters Vmax and Km. For that approach, *in vitro* data from Elcombe (1985) were examined. Those data represented the conversion of TCE to TCA in cultured hepatocytes of mice, rats, and humans. It was assumed that the maximum *in vitro* rates (MR) of that conversion (per number of cells) were proportional to ($Vmax_c$ * PO) and that the *in vitro* Michaelis constants (MC) were proportional to Km. Then, with subscripts M, R, and H representing mice, rats, and humans, respectively,

$$(2a) \quad MR_M/(Vmax_c * PO)_M = MR_H/(Vmax_c * PO)_H,$$

$$(2b) \quad MC_M/Km_M = MC_H/Km_H,$$

and similarly for rats. All the MR and MC values were given (Elcombe, 1985); $Vmax_c$, Km, and PO values for rodents were available from Fisher et al. (1990). Thus, $Vmax_c$ and Km values for humans were estimated from equation (2), once

estimates for PO in humans were obtained (either assuming, as described above, that PO is the same in humans as in the rodent species under consideration or by deriving an estimate of PO in humans from the human literature, discussed below).

Kinetic Parameter Estimation from Human Data. As in the case of scale-up, the parameters that were estimated from the human data were the scaling constants corresponding to each kinetic parameter. The same dependence on body weight shown in equation (1) was assumed. It was convenient to work from "back to front" for the parameter estimation described here: the volume of distribution and elimination rate for TCA were addressed first, followed by examination of PO, and finishing with the estimation of the TCE metabolic parameters.

Vd_c and Ke_c. Paykoc and Powell (1945) administered sodium-TCA by iv drip and observed the TCA plasma concentrations and amounts of TCA eliminated in the urine over time. It was assumed that excretion of TCA in urine represents a fixed proportion, PU, of the total elimination of TCA. Muller et al. (1974) suggested that PU may be as small as 0.5. After calculating the amount of TCA administered to each of three individuals (correcting an apparent error of Paykoc and Powell), urinary excretion data for two of those individuals were utilized to establish relationships represented as follows:

$$(3) \qquad Vd = X - (Y/PU),$$

where X and Y have specific values for each of the two individuals, based on amounts of TCA administered, amounts eliminated and TCA plasma concentration. For the third individual, for whom Paykoc and Powell estimated Vd (apparently

with the same incorrect calculation of amount of TCA administered) but for whom no urinary excretion data were presented, a correction to the Vd estimate was determined based on the corrections to the other two individuals: the correction factors were very consistent for those two and an average correction factor was used to estimate the corrected Vd for the third individual. Vd_c values were determined by dividing the Vd estimates by the corresponding body weights. The Vd_c estimates depended on the value of PU, as shown in equation (3).

An apparent dependence of Vd_c on body weight was found. That dependence was incorporated by utilizing an equation of the form

$$(4) \qquad Vd_c = \alpha + (\beta * bw). \qquad \boxed{}$$

The values of α and β were determined by linear regression through the three points corresponding to the three individuals examined by Paykoc and Powell. Because the Vd_c values depended on PU, so too did the estimates of α and β.

Muller et al. (1974) noted that the apparent half-life of TCA in the plasma (and therefore the apparent elimination rate constant for TCA) varies depending on the compound administered. The half-life after oral TCA dosing was shortest (50.6 hours) while after inhalation of TCE the half-life was longer, between 85.6 and 99 hours. In recognition of these differences, three elimination rate scaling constants, corresponding to different exposure situations, were estimated and used as appropriate in subsequent calculations.

The first situation for which an elimination rate was estimated is that following TCA administration. The rate constant in that case, $Ke_{c\text{-}TCA}$, was estimated from plasma TCA concentration data of Paykoc and Powell (1945) and

of Muller et al. (1974). Muller et al. employed oral dosing with sodium-TCA. In both cases, regression of log-transformed plasma concentrations on time yielded Ke_{TCA} estimates, converted to $Ke_{c\text{-}TCA}$ estimates when adjusted for body weight. Body weights were given for the three individuals studied by Paykoc and Powell; the three individuals tested by Muller et al. were assumed to average 69.5 kg (Muller et al., 1975). Because the calculations were based on plasma concentrations directly, the estimates of $Ke_{c\text{-}TCA}$ did not depend on PU.

The second situation arose when there existed an apparent steady-state with respect to TCA plasma concentrations. In that case, $Ke_{c\text{-}SS}$ was estimated from renal clearance data presented by Marshall and Owens (1954):

$$(5) \qquad Ke_{c\text{-}SS} = RC/(24 * Vd * PU)/bw^{-0.3},$$

where RC is renal clearance (liters per day). The estimate of $Ke_{c\text{-}SS}$ was based on the average of the body weights (73.3 kg) and clearances (2.7 liters/day) for 18 individuals studied by Marshall and Owens. Calculation of $Ke_{c\text{-}SS}$ depended on the value of PU, directly as shown in equation (5) and indirectly through dependence of Vd on PU.

The third situation for which a TCA elimination rate needed to be calculated corresponds to the scenario of greatest interest, that in which humans are exposed to TCE and TCA steady-state need not occur. The elimination rate scaling constant for this situation, $Ke_{c\text{-}TCE}$, could be estimated in four ways:

1. Directly from TCA plasma concentration data following TCE exposure, regressing log-transformed concentrations on time (Muller et al., 1974).

2. From TCA plasma concentration data following chloral hydrate (CH) administration, regressing log-transformed concentrations on time (Muller et al., 1974).

3. By adjusting the $Ke_{c\text{-}TCA}$ estimate by a factor equal to the ratio of the apparent half-life of TCA in plasma following TCA administration to the apparent half-life of TCA following TCE inhalation (between 50.6/85.6 and 50.6/99.0; Muller et al., 1974).

4. By adjusting the $Ke_{c\text{-}TCA}$ estimate by a factor equal to the ratio of the apparent half-life of TCA in plasma following TCA administration to the apparent half-life of TCA following CH administration (50.6/62.4; Muller et al., 1974).

Reasons for interest in the results of CH administration are presented in the Discussion section. In any case, because of the variability in the results obtained by these four approaches (see the Results section below), the parameter $Ke_{c\text{-}TCE}$ was estimated by optimization (see below).

PO. PO was estimated from the human literature by considering all the pathways by which TCE can be converted to TCA. TCA is produced from TCE when the intermediate CH is oxidized and, indirectly, when CH is reduced to trichloroethanol (TCOH) which can then be oxidized to TCA (Figure II-1-2). The overall proportion of TCA formed by P-450 metabolism of TCE is dependent on the proportion of CH formed from TCE oxidation, the proportion of CH oxidized to TCA, and the proportion of TCOH oxidized to TCA, and can be expressed quantitatively in the following form:

$$(6) \qquad PO = P(TCE_{ox},CH) * [P(CH,TCA) + (1-P(CH,TCA))*P(TCOH,TCA)],$$

where TCE_{ox} is TCE that is oxidized by P-450, and P(x,y) is the proportion (probability) of x producing y. Use of this equation assumes that all CH not transformed to TCA is transformed to TCOH, so that P(CH,TCOH) = (1-P(CH,TCA)). The terms of equation (6) were estimated as follows.

Following oral CH administration to human subjects, plasma concentrations of TCA were determined (Marshall and Owens, 1954, Table V). It was assumed that at half an hour after ingestion: (1) all the CH was absorbed and metabolized, (2) the TCA observed was formed directly from CH without TCOH as an intermediate, and (3) no TCA was eliminated. Then,

$$(7) \qquad P(CH,TCA) = ([TCA] * Vd)/(D * bw)$$

where [TCA] was the plasma concentration of TCA observed half an hour after administration of the CH dose, D, expressed in mg-equivalents of TCA per kg body weight. The estimation of P(CH,TCA) depended on the value of PU, through the dependence of Vd on PU.

Estimation of P(TCOH,TCA) involved the data in Tables III and IV in Owens and Marshall (1955), describing the steady-state plasma concentrations and urinary elimination of TCA following TCOH administration in two subjects. Using the Vd_c and $Ke_{c\text{-}SS}$ estimates discussed above, total TCA elimination in 24 hours could be estimated by the following equation:

$$(8) \qquad 24 * (Ke_{c\text{-}SS} * bw^{-0.3}) * [TCA] * Vd,$$

where [TCA] was the steady-state plasma TCA concentration. Under steady-state conditions, the amount eliminated equals the amount produced, and thus the proportion of the 1094 mg-equivalents of TCA given daily that was converted to TCA was determined. The average of the values so estimated for the two individuals was taken as an estimate of P(TCOH,TCA). Clearly, this estimate

depended on the value of PU, since both $Ke_{c\text{-}ss}$ and Vd were defined in terms of PU.

As an alternative, the second term on the right-hand side of equation (6), the term in square brackets, expressing the overall proportion of CH that becomes TCA, was estimated as an entity itself. From Marshall and Owens (1954, Table I) one could obtain proportions of CH doses appearing as TCA in urine. Those proportions, divided by PU, represent the proportions of the CH doses eliminated as TCA and thus the proportions of CH converted to TCA, because at steady-state the amount of TCA produced equals the amount eliminated. From Owens and Marshall (1955, Tables I and II), one could obtain estimates of the total amount of CH administered over a period of days (D, in mg-equivalents of TCA), the total amount of TCA eliminated in the urine during that time (EU, in mg), and the final TCA plasma concentration ([TCA]). Then, the proportion of CH converted to TCA was estimated by:

$$(9) \qquad (EU/PU + [TCA] * Vd)/D.$$

Finally, from Owens and Marshall (1955, Table V), it was possible to obtain estimates of the steady-state concentrations of TCA in plasma ([TCA]) following repeated administration of fixed CH doses (D, in mg-equivalents of TCA) and thereby estimate the proportion of CH converted to TCA by:

$$(13) \qquad (24 * Ke_{c\text{-}ss} * bw^{-0.3} * [TCA] * Vd)/D,$$

because of the equivalence of input and output at steady-state. An estimate of the overall proportion of CH converted to TCA was obtained by taking a weighted average of the estimates derived from the three sources, where the weighting depended on the number of individuals contributing to each estimate.

The literature presents, at best, only indirect data for the estimation of $P(TCE_{ox},CH)$. Thus, this parameter was estimated by optimization (see below).

Vmax, Km, and Kf. These parameters describing the metabolism of TCE could not be estimated directly from the human literature. In fact, no data relevant to the estimation of Kf were found. For all parameter sets based on the human literature, Kf has been set to zero.

Three estimates of Km were selected for investigation. The first is that value used by Fisher et al. (1990) for both rats and mice (0.25 mg/L). The other two were values suggested by the *in vitro*-based scaling discussed above.

Estimates of $Vmax_c$ were obtained solely by optimization (see below).

Optimization. In the description of the literature-based estimation of parameters, three model parameters were noted as being subject to optimization, Ke_{c-TCE}, $P(TCE_{ox},CH)$, and $Vmax_c$. In addition, the parameter PU was optimized, because of the dependence of many of the model parameters on the value of PU. All four of these parameters were optimized together using the software package SCoP[1]. The system of differential equations defined and solved by SCoP included all the parameter interdependencies presented above

[1]Simulation Control Package, National Biomedical Simulation Resource, Duke University Medical Center, Durham, North Carolina.

(e.g., the dependence of P(TCOH,TCA) on $Ke_{c\text{-}SS}$ and PU; cf. equation 8). Optimization was accomplished by minimizing the squared differences between log-transformed model predictions and corresponding log-transformed reference data points.

The reference data points consisted of time-course data on TCE concentrations in blood, TCE concentrations in exhaled breath, TCA concentrations in plasma, and cumulative urinary excretion of TCA. The reference data were obtained from Monster et al. (1976), Monster et al. (1979), Muller et al. (1974, 1975), and Stewart et al. (1970). These data sets provided data following single and repeated exposure to TCE over a range of atmospheric concentrations from 50 to 200 ppm. (The data are displayed in figures discussed in the Results section.) Starting points and bounds for each parameter subject to optimization were determined in the following manner.

For PU, Muller et al. (1974) suggested that perhaps as little as one-half of the TCA administered was recovered in the urine. On the other hand, one individual studied by Paykoc and Powell (1945) excreted nearly 90% of the administered TCA dose. Theoretically, all elimination of TCA may occur via urinary excretion (PU = 1). Consequently, the selected starting point for PU optimization was 0.9 with bounds, 0.5 and 1.0.

As discussed above, four approaches to estimating $Ke_{c\text{-}TCE}$ were examined. The bounds for optimizing that parameter were set at the minimum and the maximum of the resulting values. A starting point was selected that was intermediate between the remaining two values.

The proportion of oxidized TCE producing CH, $P(TCE_{ox},CH)$, was determined in preliminary analyses to be close to 1; the starting value selected for

optimization was 0.95. If that variable assumed the value 1, then all the TCE metabolized by the P-450 system would end up as CH in humans; no evidence was uncovered to suggest that this is impossible and so an upper bound for $P(TCE_{ox},CH)$ was set to 1.0. The value 0.76 was selected as a lower bound. Soucek and Vlachova (1960) estimated that 19% of the TCE retained after inhalation exposure was excreted in the urine as TCA. Assuming that TCA excreted in the urine is a fixed percentage (PU) of the total TCA eliminated, regardless of the compound administered, then the ratio of TCA urinary excretion after TCE administration (U_{TCE}) to TCA urinary excretion after CH administration (U_{CH}) should indicate the fraction of CH that is formed during TCE oxidation. As presented above, Marshall and Owens (1954) suggested an average value of 25% for the proportion of CH excreted in the urine as TCA. Thus, the ratio U_{TCE}/U_{CH} was estimated to be (0.19/0.25) = 0.76. This was considered a lower bound because 19% was among the smallest reported estimates of the percent of retained TCE appearing as TCA in urine (Monster et al., 1976; Smith, 1978) and, perhaps more importantly, because that percentage was based on total TCE retained, not on the TCE that was oxidized. Because of the saturation of P-450 metabolism, alternative routes of TCE metabolism (Dekant et al., 1986), and the possibility of elimination of TCE in exhaled breath, all TCE retained need not be oxidized. Therefore, the amount of TCA in urine as a percentage of the oxidized portion would be greater than 19%.

For $Vmax_c$, no direct evidence suggested starting values or bounds. Consequently, manual adjustment of the fit of the model to the data was used to select starting values for $Vmax_c$ (dependent on the value of Km) and bounds were selected so that optimization was assured to occur with $Vmax_c$ within those bounds. In this sense, $Vmax_c$ was the least constrained of all the

parameters, having no well-defined bounds (other than an implicit lower bound of zero) restricting the optimization.

C. RESULTS

1. TCA Volume of Distribution and Elimination Rate

The values of Vd_c obtained for rats and mice were 0.378 and 0.206, respectively. Similarly, Ke_c values in rats and mice were 0.046 and 0.074, respectively (Fisher et al., 1990). The value of $Ke_{c\text{-}TCA}$ (the scaling constant for elimination of TCA following TCA administration) estimated from the human literature was 0.040. The equation for Vd_c (cf. equation 4) determined from the investigation of the human literature (dependent on the optimized value of PL which was 0.934) was estimated to be

(11) $$Vd_c = 0.341 - 0.0034 * bw.$$

Figure II-1-3 displays predictions of plasma TCA concentrations and cumulative urinary excretion of TCA following TCA administration for each of the three pairs of Vd_c and Ke_c estimates. The predictions corresponding to the scaled rodent values do not match observed data; it appears that the scaled Vd_c estimates are too large. Moreover, the scaled elimination rate constants may be too large, especially the constant scaled from mice. Note that the scaled elimination rates were found to be appropriate in rodents following TCE exposure, whereas the human-based estimate is that relevant to TCA administration. However, if the rodents are similar to humans, the rodent TCA elimination rate constant pertinent to TCA administration would be larger than

the constants used here (Muller et al., 1974). Thus, if rodent scale-up was based on estimated elimination rates pertaining to TCA administration, discrepancies between observed TCA concentrations and predictions based on rodent scale-up would be even larger than the discrepancies displayed in Figure II-1-3.

The estimates of $Ke_{c\text{-}TCE}$ that could be derived from the human literature were as follows (see Methods for a description of the means by which these estimates were derived):

1. Directly from TCA plasma concentration data following TCE exposure, 0.027 (average of estimates following two exposure scenarios);

2. From TCA plasma concentration data following CH administration, 0.040;

3. By adjusting the $Ke_{c\text{-}TCA}$ estimate by a factor equal to the ratio of the apparent half-life of TCA in plasma following TCA administration to the apparent half-life of TCA following TCE inhalation (between 50.6/85.6 and 50.6/99.0), 0.022;

4. By adjusting the $Ke_{c\text{-}TCA}$ estimate by a factor equal to the ratio of the apparent half-life of TCA in plasma following TCA administration to the apparent half-life of TCA following CH administration (50.6/62.4), 0.032.

Thus, the bounds for optimization of $Ke_{c\text{-}TCE}$ were 0.022 and 0.040, and the starting value was 0.030. The optimization returned a value of 0.028 for $Ke_{c\text{-}TCE}$. [The optimized parameters implied a value of 0.063 for $Ke_{c\text{-}SS}$, a value determined from the human literature but depending on the values of Vd_c and PU.]

2. Metabolic Constants and PO

Table II-1-2 displays the estimates of the parameters when scaled from mice or from rats. The mouse and rat "scale-up" values are directly from Fisher et al. (1990), averaged over the two sexes of each species.

The results of incorporating the data from *in vitro* experiments relevant to estimation of $Vmax_c$ and Km are also shown in Table II-1-2. The various values of $Vmax_c$ displayed there are the result of different assumptions about the relationship between human and rodent PO values. The larger $Vmax_c$ values for each species were derived assuming that the human PO is the same as that in the species under consideration. The values in brackets result from using the optimized human PO of 0.33, discussed below. Since the optimized human PO is larger than PO determined for either rodent species, $Vmax_c$ estimated using that PO value is smaller than that obtained assuming equal PO across species: less TCE is required to be metabolized to produce the same amount of TCA when PO is larger.

Figures II-1-4 and II-1-5 reveal that the direct mouse and rat scale-ups and the extrapolations based on *in vitro* results fail to match the observed behavior of TCE and TCA in humans. With the direct scale-ups, the TCE blood concentrations are fairly well predicted but the TCA plasma concentrations are clearly underpredicted. The *in vitro*-assisted extrapolations predict neither the TCE nor TCA concentrations at all well. For the predictions shown in Figures II-1-4 and II-1-5, the optimized Vd_c and Ke_{c-TCE} were used because the scaled-up values of those parameters were shown to be inappropriate (Figure II-1-3). The predictions based on the *in vitro* results used Kf_c equal to zero.

The parameters optimized against the human experimental literature were obtained as follows. Two approaches were investigated for estimating the proportion of CH that produces TCA (see the description following equation (6) above); the one that examined the contributions of the direct CH-to-TCA pathway and the CH-to-TCOH-to-TCA pathway implied a value of 0.346 while the approach that considered the conversion of CH-to-TCA as a whole implied a value of 0.260 (both approaches depended on the estimates of PU, Vd_c, and $Ke_{c\text{-}SS}$). Then, optimization of the parameter $P(TCE_{ox},CH)$ yielded estimates of PO, via equation (6). When this was done, the first approach returned values for $P(TCE_{ox},CH)$ that were below the upper limit of 1.0. With the second approach, the upper limit was returned every time and the error associated with the fit of the model using the optimized parameter values was always larger than the error associated with the values obtained with the first approach. It was judged that: 1) the estimate of 0.26 for the proportion of CH converted to TCA was too small; 2) the first approach, with its larger estimate of that proportion (0.346), was a more suitable basis for optimizing the parameters; and 3) the $P(TCE_{ox},CH)$ estimate based on the first approach (0.947) yielded a reasonable estimate for PO (0.33).

Considered in the optimization of the parameters was the value of Km. Because Km is a difficult parameter to estimate, the three values determined by rodent scale-up or *in vitro* relationships (0.25, 0.278, or 1.5 mg/L) were used in three different optimizations. Naturally, this resulted in different values for the optimized parameters, the most notable being values of $Vmax_c$ (which were 6.9 for Km = 0.25, 7.2 for Km = 0.278, and 14.9 for Km = 1.5). The values of Ke_{c_TCE}, $P(TCE_{ox},CH)$, and PU were relatively constant over the choices of Km. However, the choice of Km = 1.5 for the value in the optimized

parameter set (Table II-1-2) was justified on the basis of the error in the fit of the model to the data: with that value of Km, the resulting optimized parameters provided the smallest error of the three optimizations.

Figures II-1-6 through II-1-10 display observed TCE and TCA data and the predictions of those data using the optimized parameter set. The data shown were the basis for the optimization. An excellent fit to the data was obtained, especially for TCA plasma concentrations after repeated exposure to TCE and for TCA urinary excretion.

D. DISCUSSION

1. Model and Parameter Acceptability

In general, the model discussed above with parameter values scaled from rodents did not yield predictions that matched the experimentally observed behavior of TCE and TCA in humans. On the other hand, the model with parameters estimated from the literature concerning human exposures to TCE and TCA did provide predictions that were in good agreement with the observations (including observations not shown here), especially for TCA plasma concentrations following repeated TCE exposure (Figures II-1-7 and II-1-9). It appears, therefore, that the model structure is an adequate basis for predicting TCE and TCA kinetics in humans, but that scaling up of parameters from rodents to humans was not successful for TCE and TCA.

Certain caveats related to the success of the model in predicting TCE/TCA kinetics may be warranted however. These caveats relate to the prediction of TCA following single TCE exposures.

TCA Production. As shown in Figures II-1-6b and II-1-8b, the TCA concentrations predicted by the model increase rapidly during exposure, as do the observed TCA concentrations. Following exposure, however, the predicted concentrations do not continue to increase for as long a time as the observations. This discrepancy is related to the production of TCOH, which the model ignores. In reality TCOH is produced and stored (Muller et al., 1974) and thus is available for continued TCA production even after TCE has been eliminated. The model, on the other hand, treats TCA as if it were instantaneously produced from TCE. The methods described above take into account the various pathways for TCA production as they relate to total amounts of TCA produced. The model does not account for the kinetics of those pathways. In the model, when TCE is eliminated, TCA production ceases. Consequently, TCA concentrations following single exposures to TCE are generally underpredicted.

TCA Elimination. A related concern is TCA elimination. As noted above, it has been observed that apparent rates of TCA elimination differ depending on the administered compound (Muller et al., 1974). This, too, is probably due to the formation of TCOH as a product of TCE metabolism. The store of TCOH following TCE exposure acts as a source of TCA production that continues to serve as an input while at the same time TCA is being eliminated. The difference between the rates of elimination and input is the apparent rate of loss of TCA, and so, with TCOH as a source of continued TCA input, the apparent rate of TCA elimination is smaller when TCOH can be formed (following TCE or CH administration) than when no TCOH is formed (after TCA administration).

The difference among apparent rates of TCA elimination, dependent on the administered compound, has been incorporated into the proposed model via consideration of the three separate parameters Ke_{TCA}, Ke_{SS}, and Ke_{TCE}. The value of Ke_{TCE}, estimated by optimization, apparently appropriately accounts for the input due to TCOH oxidation: the slopes of the TCA plasma concentration curves are well predicted, even following single TCE exposure (Figures II-1-6b, II-1-7a, II-1-8b, and II-1-9b).

As mentioned in the Methods section, the elimination rates of TCA following CH administration were considered pertinent to the estimation of Ke_{TCE}. This is the case because the model accounts for the persistence of TCE in the various body compartments, so that continued production of TCA from TCE stores should be addressed. Thus, in order to obtain an elimination rate pertinent to TCE exposure, it may be inappropriate to adjust the apparent elimination rate following TCA administration by a factor determined by the ratio of half-lives following TCE and TCA exposure. If that was done, the contribution to that difference due to storage of TCE would be "double counted," once implicitly through use of the ratio of those half-lives and once explicitly through the model's ability to correctly predict TCE storage. CH, however, is a relatively short-lived intermediate of TCE metabolism. By examining the elimination rate following CH administration, the effects of TCOH storage would still be addressed, without the confounding of TCE storage with which one need not be concerned (because of the stated capabilities of the model).

In fact, the optimized value for Ke_{c-TCE} (0.028) was somewhat larger than predicted directly from TCA plasma concentration data following TCE exposure and substantially larger than predicted by adjusting Ke_{c-TCA} according to the

ratio of TCA half-lives (Muller et al., 1974). The optimized value was not as large as predicted based on TCA elimination following CH exposure.

Extensions of the model proposed here could address some of the issues related to the kinetics of TCA production and TCA elimination. The first step in that direction would be to explicitly consider TCOH and its distribution, storage, elimination, and conversion to TCA (cf. Nolan et al., 1984). One would expect, if such an extension was adequately defined, that TCA exposures after single TCE exposures could be better predicted and that some of the ambiguity regarding TCA elimination rates could be eliminated.

It is worth emphasizing, however, that the TCA concentrations were predicted quite well in situations in which TCE was encountered repeatedly. This is due to the diminished importance of the timing of TCOH conversion to TCA as TCA stores accumulate. For all of the repeated exposure scenarios examined, TCA accumulates during the five days of exposure, it is not eliminated completely during two days of nonexposure, and thus a pool of TCA remains that, at later times, substantially drowns out the contribution of TCA newly produced from TCOH (at least in terms of more or less subtle differences in the timing of that new production). The model appears to be satisfactory to predict TCA concentrations for chronic TCE exposure, even when there are some periods of nonexposure.

2. Interspecies Parameter Comparisons

Because the scale-up from rodents to humans was generally unsuccessful, it is of interest to compare the estimates of the parameter values in humans to those in rodents. The species differences imply different behaviors of TCE

and TCA in humans, compared to rats and mice, and the kinetic differences may have some direct bearing on risk assessments for TCE.

There is substantially less metabolism of TCE per body weight in humans than in mice (cf. estimates in Table II-1-2). Both male and female mice have a larger estimated $Vmax_c$ and a smaller estimated Km than humans. Compared to rats, the optimized $Vmax_c$ in humans is somewhat larger, but Km for humans is larger; as a result the metabolic rate at low concentrations will be about four and one-half times greater in rats than in humans.

On the other hand, it appears that more of the metabolized TCE is converted to TCA in humans than in rodents. In mice, 7% to 14% of the oxidized TCE becomes TCA. Rats are comparable at 12%. In contrast, about one-third of the TCE that is metabolized by humans is converted to TCA. Thus, considering this information and the metabolic rate differences, the production of TCA (at low TCE concentrations) in mice is about three times greater, and in rats about one and one-half times greater, than in humans, on a body weight basis.

The volume of distribution for TCA is smaller in humans than in rodents. The proportion of the body into which TCA distributes was found to vary for different sized humans; this is probably related to the fact that TCA is hydrophilic and the increase in body size in larger individuals is often due to increased fat content. For 60 to 80 kg humans, optimized volumes of distribution ranged from about 14% to 7% of total body size. In contrast, TCA distributes into between 25% and 51% of the total weight of a rat and between 18% and 24% of total weight in mice (Fisher et al., 1990). Thus, human TCA plasma concentrations will be higher than those for rodents if the same amount of TCA is in the organism. The smaller volume of distribution for humans may

be related to the relatively extensive binding of TCA to plasma proteins in humans (Marshall and Owens, 1954; Sellers and Koch-Weser, 1971). It may be the case that binding to plasma proteins is not as extensive in rodents as in humans.

Binding differences may also be responsible for differences in TCA elimination rates which were higher in the rodent species than in humans. The elimination rate scaling constants for rats and male mice were relatively constant at about 0.045; for female mice the scaling constant was more than two times larger, 0.104 (Fisher et al., 1990). Human TCA elimination was best described with a scaling constant of 0.028. TCA is retained for a considerably longer period in humans than in rodents.

Comparing humans to rodents, then, more of the TCE that is metabolized becomes TCA, the volume of TCA distribution is smaller, and TCA is eliminated more slowly. Because of these factors, area under the plasma TCA concentration-time curve tends to be greater in humans than in rodents. This is the case even though the metabolic capacity of mice greatly exceeds that of humans and the low concentration metabolic rate in rats is somewhat larger than in humans.

3. Issues and Implications for Cancer Risk Assessment

It has been suggested that hepatocellular tumor responses observed in some bioassays of TCE are closely related to TCA production (Green and Prout, 1985). This relationship may be mediated through the peroxisome proliferating activity of TCA (Rao and Reddy, 1987) or peroxisome proliferation may be a marker for another TCA effect manifested by increased DNA synthesis and cell proliferation (Goldsworthy and Popp, 1987; Popp et al., 1989). In any case,

an appropriate approach to the assessment of liver cancer risks associated with TCE exposure must include, at the very least, some consideration of TCA kinetics and their differences across species and across dose levels. The TCE/TCA model for humans described here, and the corresponding models for rodents (Fisher et al., 1990), provide the basis for consideration of TCA by providing a means of estimating dose- and species-specific dose surrogates that are defined in terms of TCA concentration.

The implications of the human modeling discussed here on risk assessment for TCE are interesting. The current regulatory procedure for risk assessment entails expressing dose in terms of average daily TCE exposure per body weight or surface area. Alternatively, PBPK modeling could be employed to estimate dose surrogates more closely related to the dose at the target tissue(s). Metabolism is believed to be necessary to create the ultimate carcinogen; consequently, dose surrogates based on metabolized TCE should be examined. If one stops at the point of examining amounts of TCE metabolized and defining dose surrogates based on those amounts scaled according to body weight or compartment volume (cf. Bogen, 1988), then humans will appear to be much less susceptible, because the metabolic capacity of mice is much greater than that of humans (compare the estimates of $Vmax_c$ and Km in Table II-1-2). However, with the capability of the model proposed here to track TCA concentrations *per se*, and as a result of the values of the parameters controlling TCA kinetics, the purported reduction in susceptibility disappears. That is, the slower TCA elimination rate and smaller volume of distribution for TCA in humans, compared to mice, offset the difference in metabolic capacities, if dose surrogates based on TCA concentrations are used. In fact, preliminary analyses have suggested that the risks (based on mouse liver tumors) estimated

using areas under TCA plasma concentration-time curves as the dose surrogates are actually greater than those one would obtain using administered doses scaled to body weight.

The results cited are preliminary. Note that although TCA concentrations have been used to define dose surrogates believed to be more closely related to the action of TCE with respect to liver tumors, those concentrations pertain to plasma. The model does not predict TCA concentrations in the liver, the actual site of action. A logical extension of the modeling reported here would be to include other compartments for describing TCA kinetics, including the liver. (This is also supported by the observation of Paykoc and Powell (1945) that the kinetics of TCA elimination may not be best described by a single compartment.) The TCA concentration in the liver should, however, be related to the plasma concentration, except insofar as binding tends to restrict TCA to the plasma.

It may also be worth considering using peroxisome proliferation as the basis for defining dose surrogates. TCA induces peroxisome proliferation, at least in rodent species (Elcombe, 1985). However, it is not known if peroxisome proliferation is causally related to hepatocellular tumor induction. It has been suggested that peroxisome proliferators as a class induce liver tumors by causing hepatocytes to generate reactive oxygen species that would cause mutations in DNA (Rao and Reddy, 1987). However, recent work (cf. Goldsworthy and Popp, 1987; Popp et al., 1989) suggests that peroxisome proliferation *per se* may not be causing the liver tumors. Rather, an effect manifested by increased DNA synthesis and cell proliferation, leading to promotion of initiated cells, is indicated. The peroxisome proliferative

effect of TCA might still be of interest in that case as a marker of promotional activity.

To utilize peroxisome proliferation in the assessment of liver cancer risks, some suitable TCA-based metric must be linked to a variable representing peroxisome content or peroxisomal enzyme activity in the kinetic models. Functional relationships for representing the link may be estimable from data in the literature (cf. Elcombe, 1985). It may also be possible to represent peroxisomal enzyme activity, for example, by a differential equation that could be added to the system of such equations already developed for the TCE/TCA model discussed here. That equation would relate changes in peroxisomal enzyme activity to TCA concentrations and homeostatic mechanisms that ordinarily maintain the level of such activity. Species differences in susceptibility to peroxisome proliferation could be represented with species-specific estimates for the parameters of the equation.

Finally, the emphasis of the modeling discussed here has been on TCA and liver cancer risk. There are other routes and products of TCE metabolism that may be relevant to assessing the cancer risk that humans may face when they are exposed to TCE.

Dichloroacetic acid (DCA), another product of TCE oxidation (Figure II-1-2), has been shown to be a liver carcinogen in mice (Herren-Freund et al., 1987). It is possible that the effect of TCE on the liver may be the result of an interaction of TCA and DCA. Although no direct evidence is available to assess the amount of DCA produced in humans, the parameterization of the model discussed allows indirect estimation of that amount. The parameter $P(TCE_{ox},CH)$ determines the proportion of oxidized TCE that is converted to CH; the optimized value of that parameter was about 95%. That means that about 5%

of the oxidized TCE is converted to DCA and other products. This information could be of value in determining species differences in susceptibility to TCE if it is determined that DCA is relevant to the production of liver tumors and if the extent of DCA production in rodents following TCE exposure can be estimated.

Also of interest are the kidney tumors that have been observed in rats (NTP, 1990). Dekant et al. (1986) have identified metabolites of TCE in the urine that would arise from the conjugation of TCE with glutathione and that could indicate a pathway producing kidney carcinogens. Thus, while the modeling work presented here is important and relevant to the estimation of liver tumor hazards, extensions of that modeling will be necessary to address all of the issues that might arise in the course of assessing TCE cancer risks in general.

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Table II-1-1

Physiological Parameters and Partition Coefficients
Used in the Human TCE Model[a]

Compartment Volumes (liters):	
Fat (vf)	.19 * bw[b]
Rapidly perfused (vr)	.05 * bw
Slowly perfused (vs)	.62 * bw
Liver (vl)	.026 * bw
Alveolar Ventilation (qp; liter/hr):	$12.9 * bw^{.74}$
Cardiac Output (qc; liter/hr)	$15.0 * bw^{.74}$
Compartment Blood Flows (liter/hr):	
Fat (qf)	.05 * qc
Rapidly perfused (qr)	.44 * qc
Slowly perfused (qs)	.25 * qc
Liver (ql)	.26 * qc
Partition Coefficients:	
Fat/blood (pf)	73.3
Rapidly perfused tissue/blood (pr)	6.8
Slowly perfused tissue/blood (ps)	2.3
Liver/blood (pl)	6.8
Blood/air (pb)	9.20

[a]Compartment volumes and compartment blood flows from Arms and Travis (1987). Alveolar ventilation and cardiac output from Astrand et al. (1973) and Monster et al. (1979). Partition coefficient estimates from Sato et al. (1977), Arms and Travis (1987), Gargas et al. (1989), and Koizumi (1989) [see Methods].

[b]bw = body weight in kg.

Table II-1-2

Estimates of Metabolic Constants and PO

Parameter	Mouse Scale-up[a]	Rat Scale-up[a]	In vitro, Mouse[b]	In vitro, Rat[b]	Optimized
$Vmax_c$ (mg/hr/kg)	35.2	10.99	2.47 [0.51 (0.73)[d]][e]	3.25 [0.57][e]	14.9
Km (mg/liter)	0.25	0.25	1.5	0.278	1.5
Kf_c (hr^{-1}/kg)	0	2.1	---[f]	---	---
PO (unitless)	0.7 (0.14)[g]	0.12	---	---	0.33

[a]Values averaged over sex of each rodent species (Fisher et al., 1990).
[b]Values scaled from males (Fisher et al., 1990) via *in vitro* relationships (Elcombe, 1985).
[c]Optimized values determined from fits to human data.
[d]Value obtained with higher PO value from low-dose mouse experiment (Fisher et al., 1990; cf. footnote g).
[e]*In vitro* $Vmax_c$ values could be obtained assuming same PO for humans and rodents (values outside brackets) or by assuming species-specific PO values (values inside brackets).
[f]A "---" indicates that no data were available from that source to estimate the corresponding parameter.
[g]In parentheses is the PO value estimated for male mice at the lowest administered dose (Fisher et al., 1990).

FIGURE LEGENDS

II-1-1. TCE/TCA Pharmacokinetic Model

II-1-2. Metabolic Pathways of TCE

II-1-3. Observed and predicted TCA behavior after TCA administration. A: TCA plasma concentration, average of three individuals (data from Muller et al., 1974). B: TCA plasma concentration for one individual (Paykoc and Powell, 1945). C: Cumulative urinary TCA excretion, average of three individuals (Muller et al., 1974). D: Cumulative urinary TCA excretion for one individual. TCA volume of distribution and elimination rate estimated from mouse data (curve 1) rat data (2), and human data (3). Proportion of eliminated TCA that is excreted in urine (93%) is the same for all curves.

II-1-4. Observed and predicted TCE (A) and TCA (B) concentrations (five 4-hour exposures to 70 ppm; Monster et al., 1979). $Vmax_c$, K_m, Kf_c, and PO scaled directly from mice (curve 1), from mice using *in vitro* relationships and assuming humans have same PO as mice (2), and from mice using *in vitro* relationships and assuming species-specific PO (3): see value in Table II-1-2. Optimized human TCA volume of distribution and elimination are used throughout.

II-1-5. Observed and predicted TCE (A) and TCA (B) concentrations (five 4-hour exposures to 70 ppm; Monster et al., 1979). $Vmax_c$, K_m, Kf_c, and PO scaled directly from rats (curve 1), from rats using *in vitro* relationships and assuming humans have same PO as rats (2), and from rats using *in vitro* relationships and assuming species-specific PO (3): see value in Table II-1-2. Optimized human TCA volume of distribution and elimination are used throughout.

II-1-6. Observed and predicted TCE and TCA behavior during and after single, 6-hour exposure to 100 ppm TCE (data from Muller et al., 1974 ("x") and Muller et al., 1975 ("+"). Predictions based on optimized human parameters.

II-1-7. Observed and predicted plasma concentration (A) and cumulative urinary excretion during and after five, 6-hour exposures to 50 ppm TCE (data from Muller et al., 1975). Predictions based on optimized human parameters.

II-1-8. Observed and predicted TCE and TCA behavior during and after single, 4-hour exposure to 140 ppm TCE (data from Monster et al., 1976). Predictions based on optimized human parameters.

II-1-9. Observed and predicted TCE and TCA behavior during and after five, 4-hour exposures to 70 ppm TCE (data from Monster et al., 1979). Predictions based on optimized human parameters.

II-1-10. Observed and predicted concentrations of TCE in exhaled breath (A) and cumulative urinary TCA excretion (B) during and following five, 7-hour exposures to 200 ppm TCE (3 hours of exposure, half an hour break, then 4 more hours of exposure; data from Stewart et al., 1970). Predictions based on optimized human parameters.

Figure II-1-1
TCE/TCA Pharmacokinetic Model

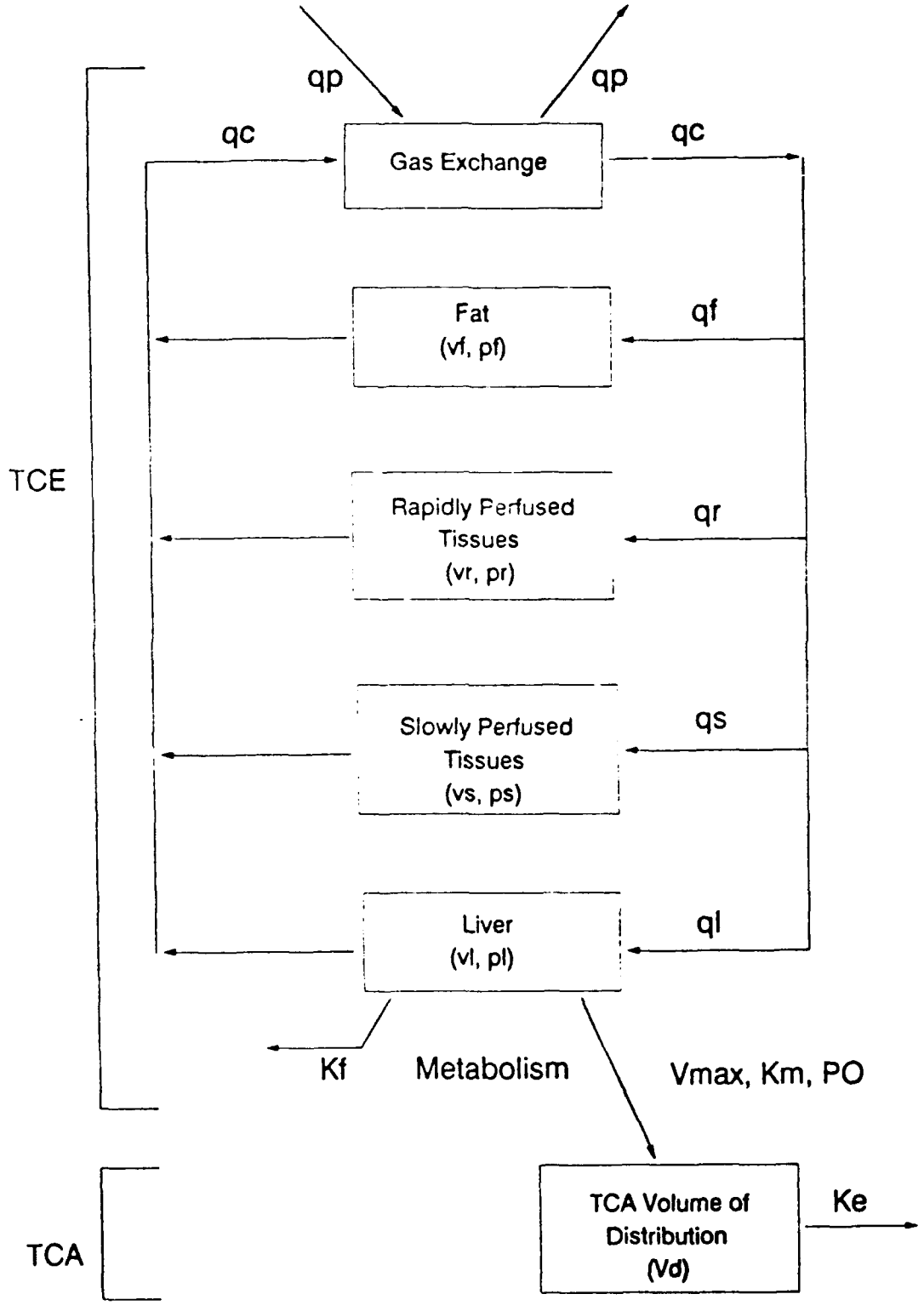


Figure II-1-2

Metabolic Pathways of TCE

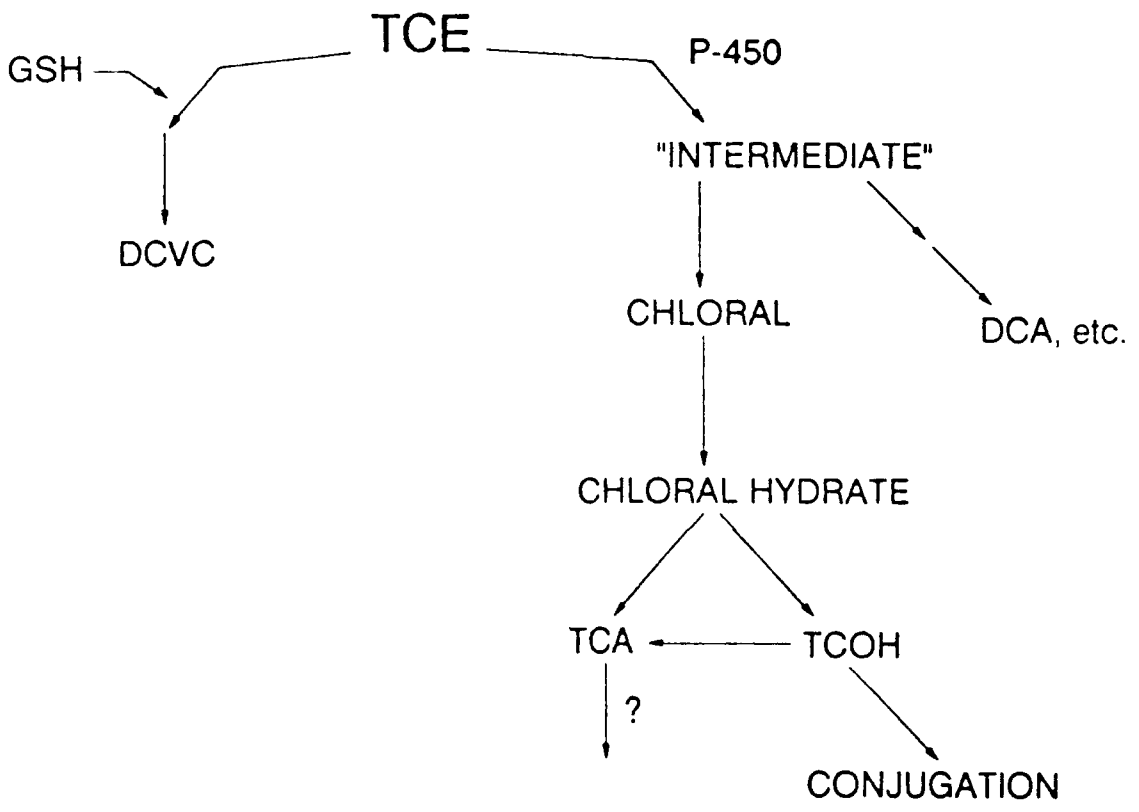


Figure II-1-3

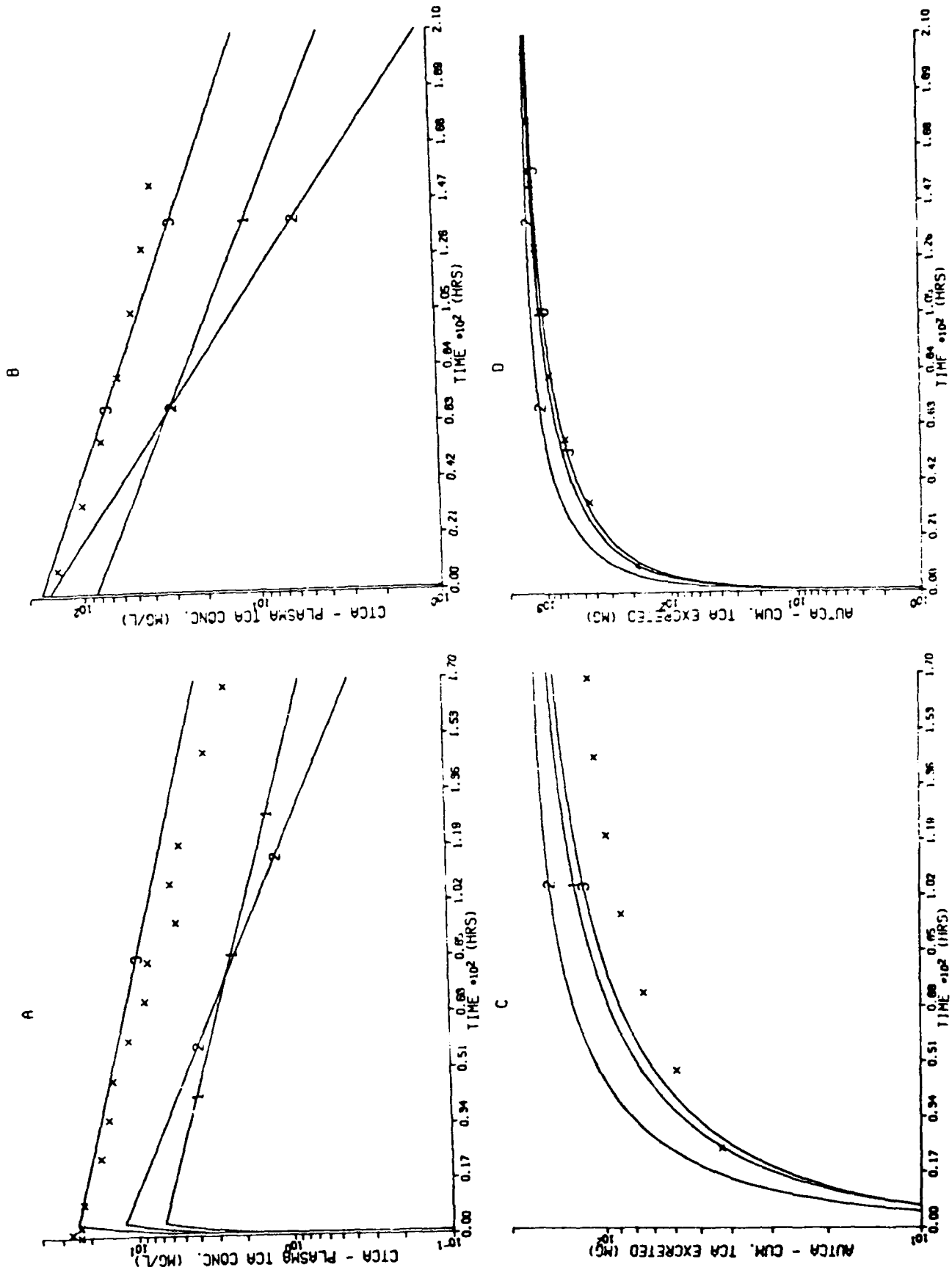

A
B
C
D
TIME *10² (HRS)
CTCA - PLASMA TCA CONC. (MG/L)
AUTCA - CUM. TCA EXCRETED (MG)

Figure II-1-4

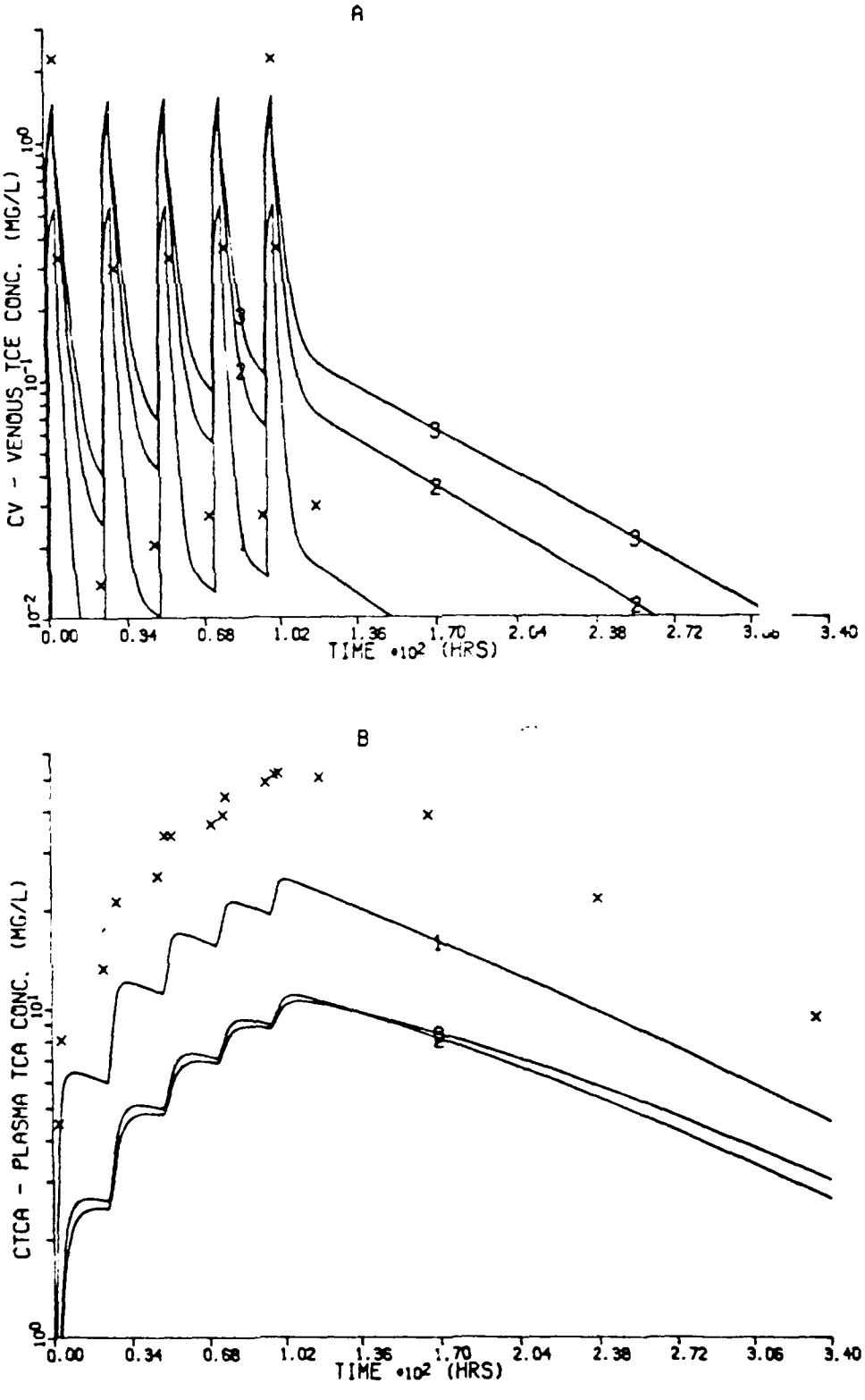

A
CV - VENOUS TCE CONC. (MG/L)
10^{0}
10^{-1}
10^{-2}
0.00
0.34
0.68
1.02
1.36
1.70
2.04
2.38
2.72
3.06
3.40
TIME $\cdot 10^{2}$ (HRS)
B
CTCA - PLASMA TCA CONC. (MG/L)
10^{1}
10^{0}
0.00
0.34
0.68
1.02
1.36
1.70
2.04
2.38
2.72
3.06
3.40
TIME $\cdot 10^{2}$ (HRS)

Figure II-1-5

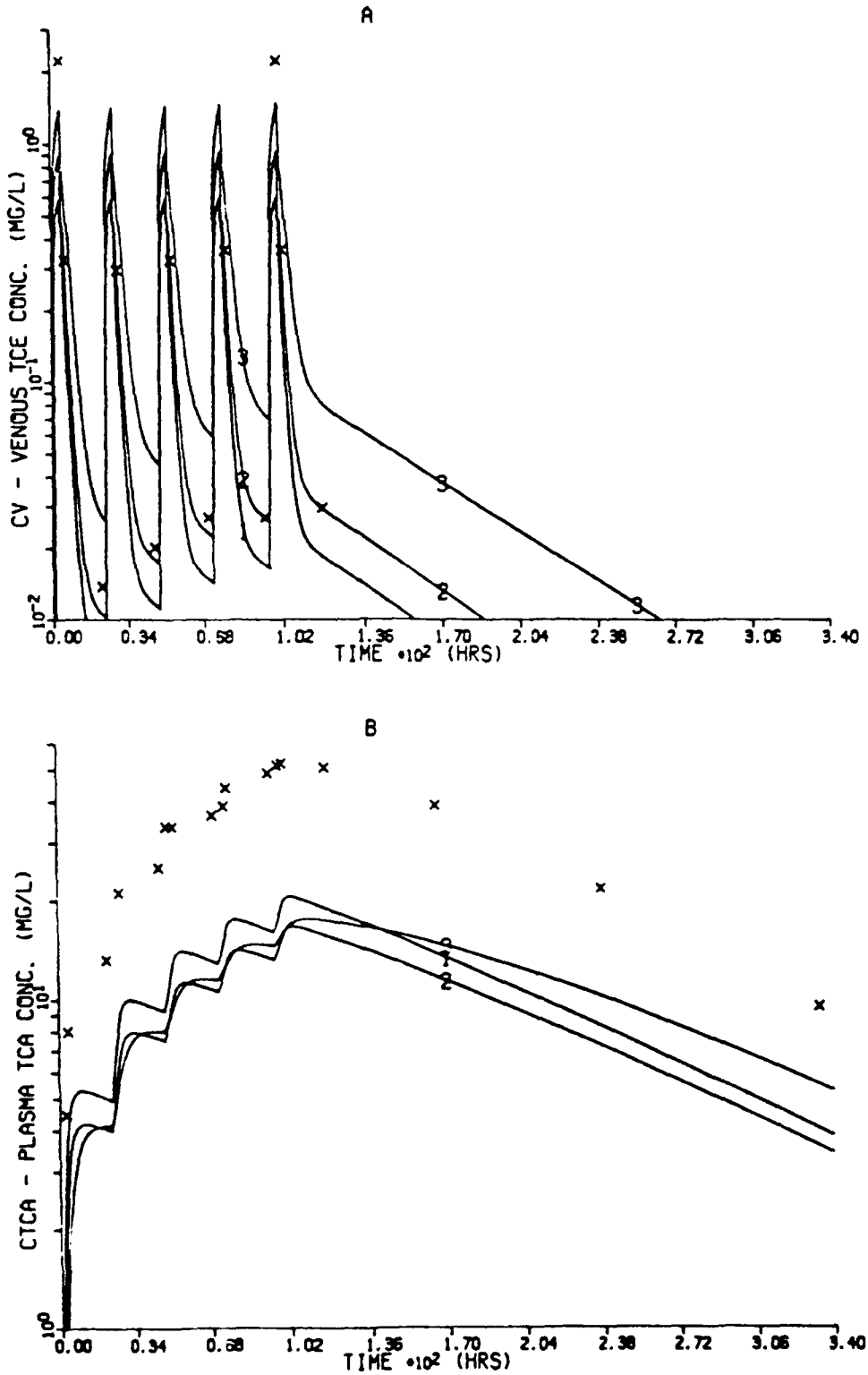

A
CV - VENOUS TCE CONC. (MG/L)
10^{0}
10^{-1}
10^{-2}
0.00
0.34
0.68
1.02
1.36
1.70
2.04
2.38
2.72
3.06
3.40
TIME $\cdot 10^{2}$ (HRS)
B
CTCA - PLASMA TCA CONC. (MG/L)
10^{1}
10^{0}
0.00
0.34
0.68
1.02
1.36
1.70
2.04
2.38
2.72
3.06
3.40
TIME $\cdot 10^{2}$ (HRS)

Figure II-1-6

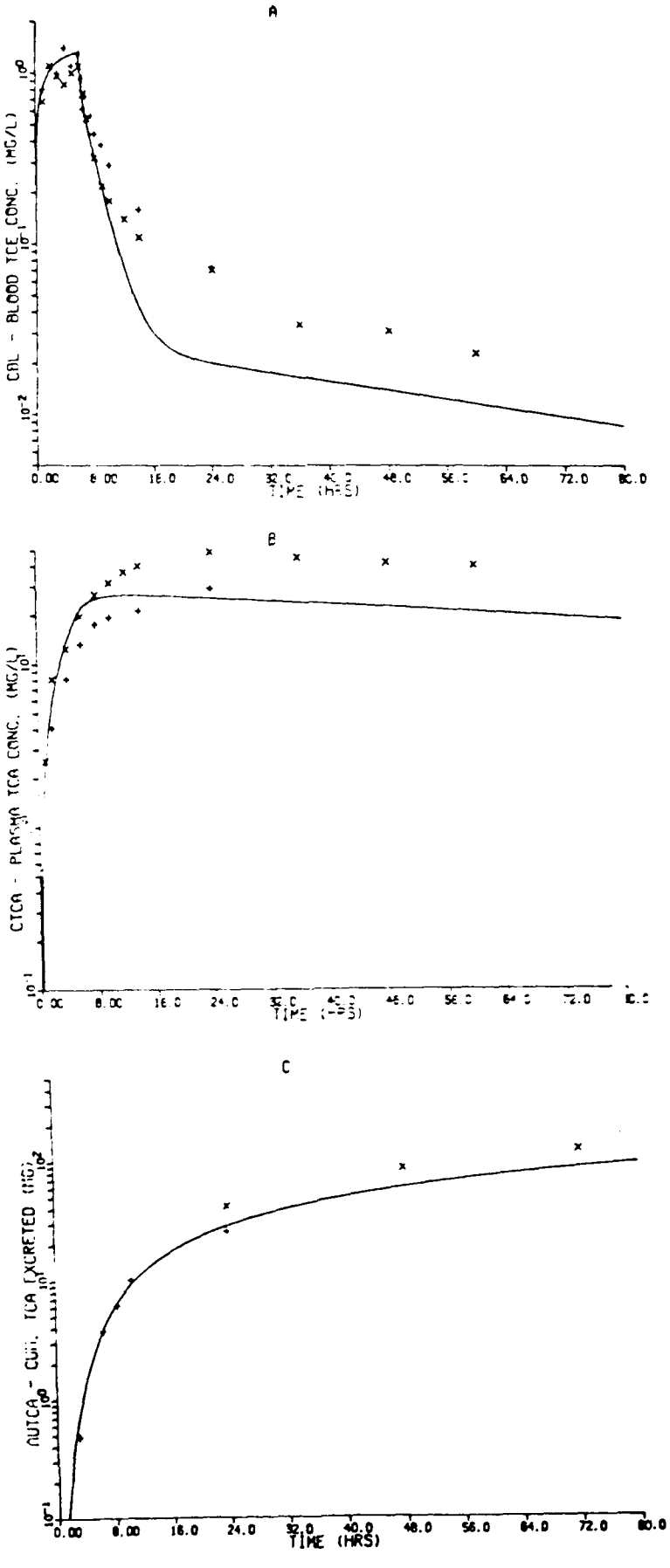

A
CBL - BLOOD TCE CONC. (MG/L)
TIME (HRS)
B
CTCA - PLASMA TCA CONC. (MG/L)
TIME (HRS)
C
AUTCA - CUM. TCA EXCRETED (MG)
0.00
8.00
16.0
24.0
32.0
40.0
48.0
56.0
64.0
72.0
80.0
TIME (HRS)

Figure II-1-7

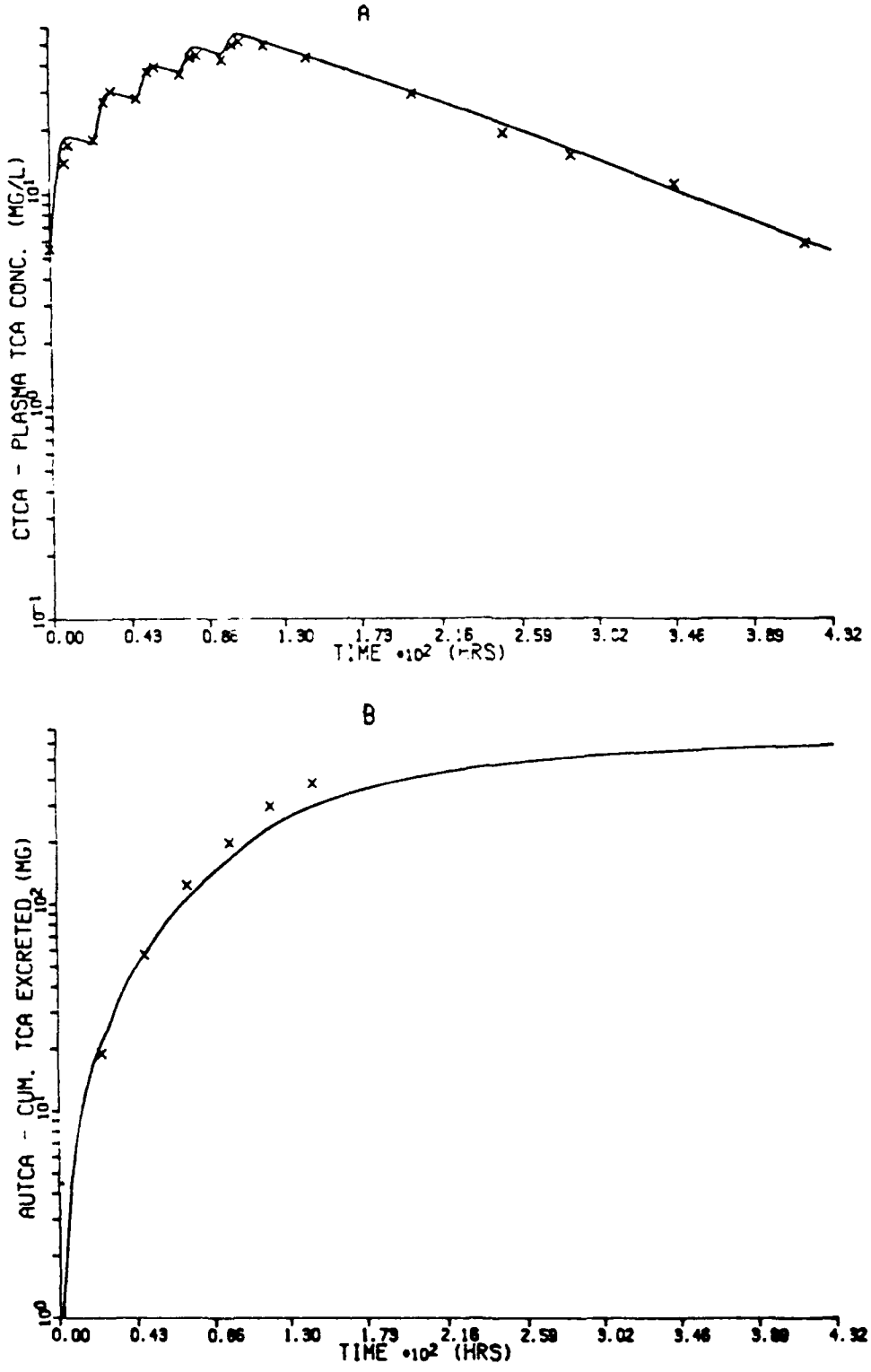

A
CTCA - PLASMA TCA CONC. (MG/L)
10^1
10^0
10^{-1}
0.00
0.43
0.86
1.30
1.73
2.16
2.59
3.02
3.46
3.89
4.32
TIME $\cdot 10^2$ (HRS)
B
AUTCA - CUM. TCA EXCRETED (MG)
10^2
10^1
10^0
0.00
0.43
0.86
1.30
1.73
2.16
2.59
3.02
3.46
3.89
4.32
TIME $\cdot 10^2$ (HRS)

Figure II-1 8

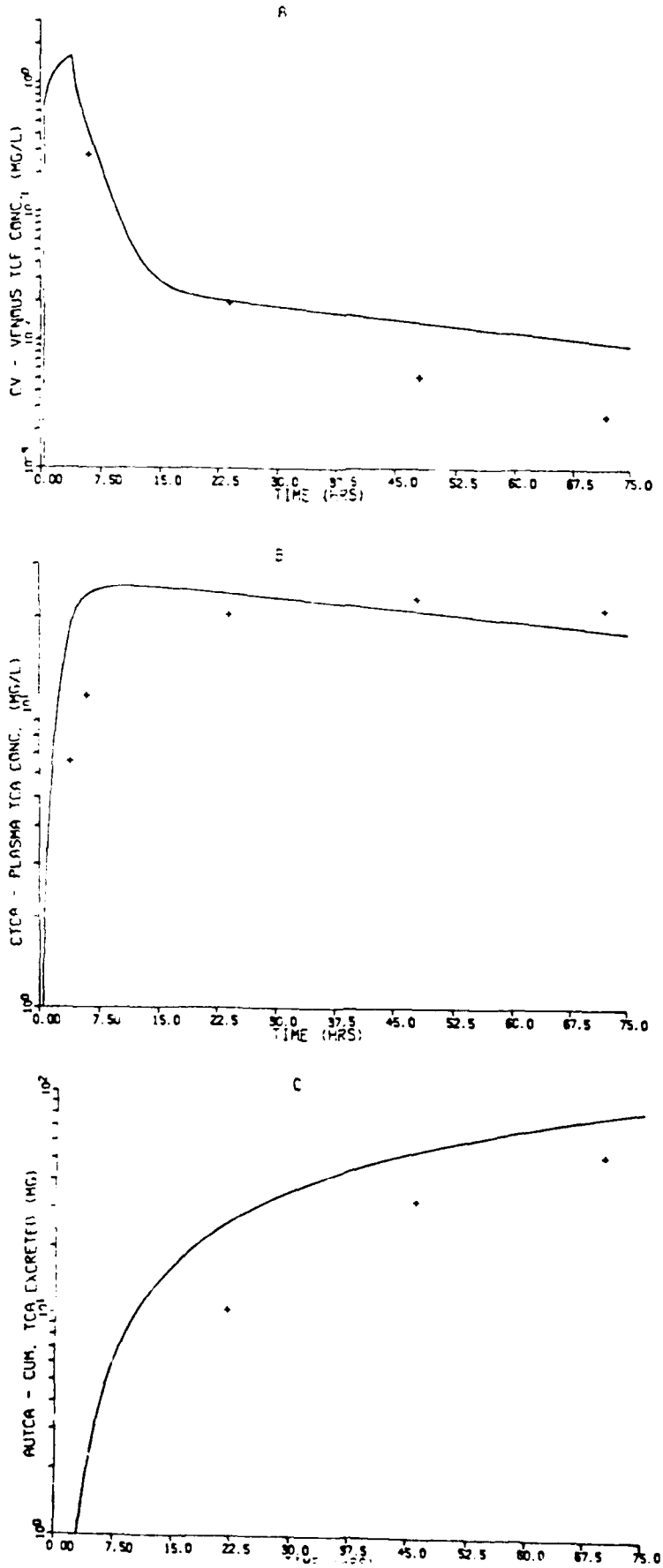

A
CV - VENOUS TCE CONC. (MG/L)
TIME (HRS)
B
CTCA - PLASMA TCA CONC. (MG/L)
TIME (HRS)
C
AUTCA - CUM. TCA EXCRETED (MG)
0.00
7.50
15.0
22.5
30.0
37.5
45.0
52.5
60.0
67.5
75.0

Figure II-1-9

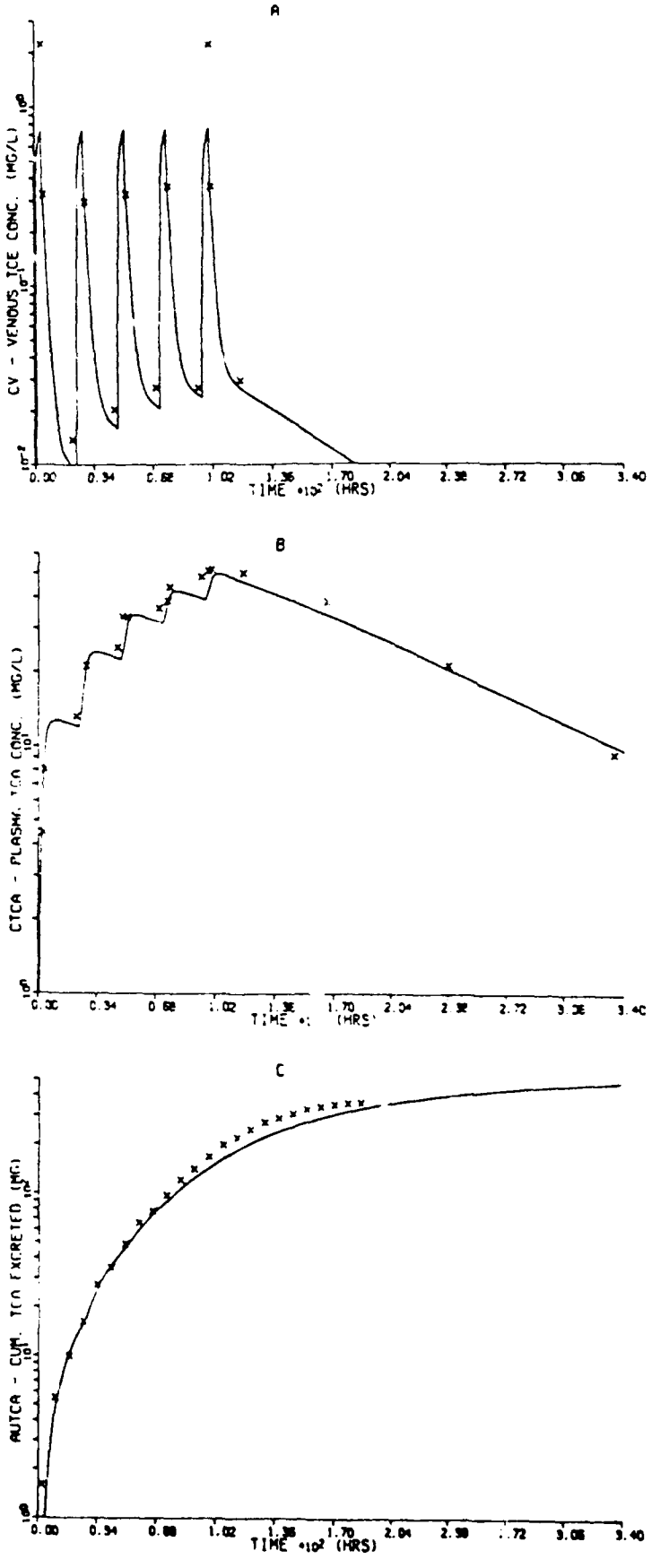
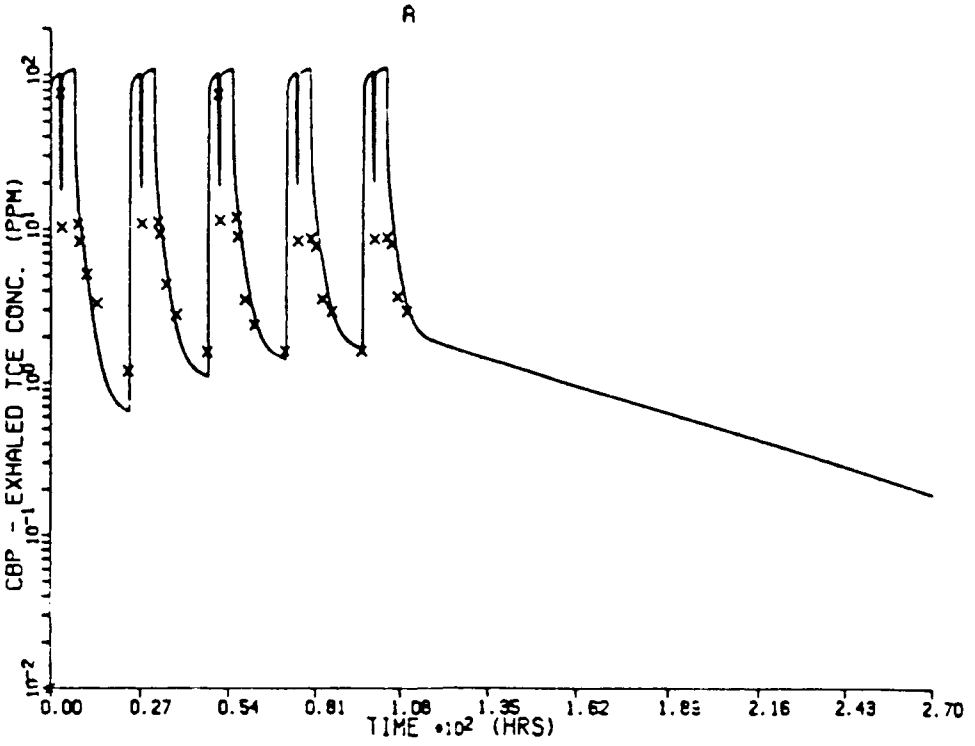
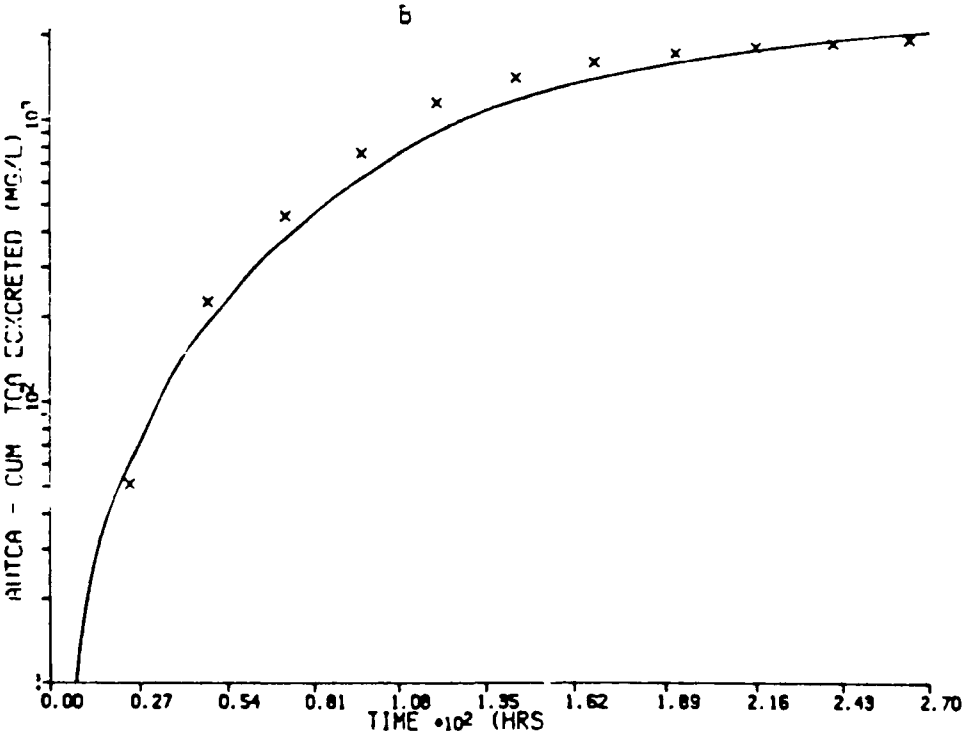

A
CV - VENOUS TCE CONC. (MG/L)
TIME *10² (HRS)
B
CTCA - PLASMA TCA CONC. (MG/L)
TIME *10² (HRS)
C
AUTCA - CUM. TCA EXCRETED (MG)
TIME *10² (HRS)

Figure II-1-10





APPENDIX II-1-A

EQUATIONS DEFINING THE TCE/TCA PBPK MODEL

APPENDIX II-1-A

EQUATIONS DEFINING THE TCE/TCA PBPK MODEL

TCE

Gas Exchange Compartment

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

Fat Compartment

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

Rapidly Perfused Tissue Compartment

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

Slowly Perfused Tissue Compartment

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

Liver Compartment

dCL/dt = QL*(CA-CVL)/VL - dCL1/dt - dCL2/dt + (DRINK+GAV)/VL

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

dCL2/dt = Kf*CVL

Mixed Venous Blood

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

TCA

dCTCA/dt = PO*(dCL1/dt)*VL*(MWTCA/MWTCE)/Vd - Ke*CTCA

dUTCA/dt = PU*Ke*CTCA*Vd

$CTCA_B$ = 0.6*CTCA

Ci - Concentration of TCE in i

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

CVi - Concentration of TCE in venous blood leaving compartment i (i=L, F, R, S); CVi=Ci/pi

CL1 - Virtual concentration of TCE metabolized via MFO pathway

CL2 - Virtual concentration of TCE metabolized via first-order pathway

DRINK - Rate of TCE introduction into liver compartment via drinking water

GAV - Rate of TCE introduction into liver compartment via gavage

Vmax - $Vmaxc*bw^{0.7}$

Kf - $Kfc*bw^{-.3}$

CTCA - Concentration of TCA in plasma

UTCA - Cumulative amount of TCA eliminated in urine

$CTCA_2$ - Concentration of TCA in blood

MWTCA - Molecular weight of TCA

MWTCE - Molecular weight of TCE

VOLUME II

PART 2 OF 2 PARTS

ISSUES ASSOCIATED WITH MODELING AND RISK ASSESSMENT FOR TCE

A. RISK ASSESSMENT

The PBPK models developed for mice (Fisher et al., 1990) and humans (Allen et al., 1990) yield estimates of delivered doses (dose surrogates) that may be related to the production of liver tumors. Such tumors have been observed in mice (NCI, 1976; Bell et al., 1978; NTP, 1990). The potential for human liver cancer risk associated with exposure to TCE can be evaluated in light of the mouse results.

The dose surrogates that were considered for an assessment of liver cancer risks were average daily values of 1) the amount of TCE 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 their potential relationship to mechanisms of liver tumor production.

The surrogate based on the amount of TCE metabolized could be related to liver tumor production if a short-lived, reactive intermediate was responsible for the induction of liver tumors. In that case, Andersen et al. (1987) have 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 TCE-induced liver tumors (Green and Prout, 1985), the corresponding dose surrogate has been included in the analyses discussed here.

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 TCE-induced liver tumors (Green and Prout, 1985).[1] 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 TCE 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 like area under the concentration curve that the reactions responsible for tumor induction can occur at any time that TCA is present.

Risk estimates for TCE were derived from liver tumor incidences (hepatocellular adenomas and carcinomas) for female B6C3F1 mice (Table II-2-1). Gavage exposures (NCI, 1976; NTP, 1990) were represented in the PBPK model as direct inputs to the liver that lasted for 2 hours, at which time all

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[1]For an opposing opinion, see Hathway (1980). Hathway proposes that DCA is the product associated with the liver tumors.

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.

Male mice were not examined. It was determined (Fisher et al., 1990) that male mice exhibited enzyme inhibition after a single exposure to TCE. No repeated exposure studies have been conducted that address this issue. Hence, it was not known how this enzyme inhibition would affect the rates of metabolism (and therefore the values of the three dose surrogates) for the chronic exposure situations exemplified by the carcinogenicity bioassays. That is, the estimation of dose surrogate values for use in the dose-response modeling was not sufficiently well-defined by the single exposure results used to define the male mouse model.

No enzyme inhibition was detected in female mice. Thus, it appeared reasonable to estimate dose surrogates for chronic exposure based on the model defined using single-exposure studies in female mice.

Risks were not estimated from rat carcinogenicity data. Rats do not develop liver tumors in response to TCE 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 TCE exposure (NTP, 1990), the dose surrogates estimated by the model are not relevant to such tumors. Additional PBPK modeling would be necessary to derive appropriate dose surrogate values (see Section B below).

The results of the risk estimation are presented in Tables II-2-2 and II-2-3. 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 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 per day. The body weight assumed for the calculations was 70 kg.

The concentrations 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 amount of TCE metabolized yielded the largest concentrations associated with the specified risks, i.e., indicated the lowest potential for TCE induced liver tumors in humans. The assessment based on the area under the TCA concentration curve dose surrogate yielded the smallest concentrations. The observation that the "allowable" concentrations associated with TCE metabolism were larger than those associated with TCA production is due to the fact that the metabolism of TCE in humans produces more TCA than that in mice (33% of TCE metabolized becomes TCA in humans as opposed to 7% in mice). Area under the TCA concentration curve yielded even smaller concentrations for the specified risk levels than did production of TCA because TCA is eliminated more slowly in humans than in mice and because the volume of distribution is smaller (per body weight) in humans than in mice.

For comparison, when the standard EPA analysis was completed (without consideration of pharmacokinetic differences, assuming mice and humans are equally sensitive when dose is expressed as mg/surface area per day), the atmospheric concentrations associated with 10^{-3} risk ranged between 0.8 and 0.33 ppm; and the atmospheric concentrations associated with 10^{-6} risk ranged between 8×10^{-5} and 3×10^{-4} ppm. The drinking water concentrations determined by

that method were between 2.9 and 12 mg/L for a 10^{-3} risk; they were between 2.9 and 12 μg/L for a 10^{-6} risk.

The 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, TCE may be acting through the metabolite, TCA, which in turn may be acting through its effects on peroxisomes (see Section C below). Humans may be less susceptible to the peroxisome proliferating effects of TCA (Elcombe, 1985).

B. ALTERNATIVE PBPK MODELING APPROACHES

In conjunction with early work on the development of a rodent PBPK model, effort was expended on an investigation of products of TCE metabolism other than TCA. In particular, interest was focussed on 1,2-dichlorovinyl-L-cysteine (DCVC), dichloroacetic acid (DCA), trichloroethanol (TCOH) and chloral hydrate (CH).

DCVC is a product of the pathway that starts with conjugation of TCE and glutathione (GSH). DCVC may be related to the production of kidney tumors (Dekant et al., 1989). Appendix II-2-A provides a short discussion of this metabolic pathway and its relationship to nephrotoxicity.

DCA is an alternative product of TCE metabolism that arises when the P-450 metabolism of TCE yields dichloroacetyl chloride (DCC) rather than chloral as the product after the first intermediate (which may or may not be a free epoxide -- see Miller and Guengerich, 1982, 1983). DCA is the first stable

product formed from DCC. DCA was of interest because it has been associated with liver tumor induction when given to mice in drinking water (Herren-Freund et al., 1987).

CH is the first relatively stable product formed from chloral. CH is a precursor in the formation of TCA. TCOH is also a product of CH metabolism, but it can itself be oxidized to TCA. Thus, the motivation for interest in CH and TCOH was the belief that better characterization of the kinetics of TCOH and CH, in addition to those of TCE and TCA, would yield a more accurate model.

In the course of the examination of DCA, TCOH, and CH the following ideas were developed.

1. TCE Metabolism

The metabolism of TCE could be modeled according to the scheme depicted in Figure II-2-1. In that diagram the various rates can be expressed as follows:

(1) $dM1/dt = Vmax_{TCE} * CVL / (Km_{TCE} + CVL)$,

(2) $dM2/dt = k_{gsh} * CVL * Vl$;

(3) $dM1a/dt + dM1b/dt = dM1/dt$:

(3a) $$dM1a/dt = \begin{cases} \alpha * dM1/dt & \text{for } dM1/dt \leq TH \\ dM1/dt - (1-\alpha)*TH & \text{for } dM1/dt > TH. \end{cases}$$

(3b) $$dM1b/dt = \begin{cases} (1-\alpha)*dM1/dt & \text{for } dM1/dt \leq TH \\ (1-\alpha)*TH & \text{for } dM1/dt > TH. \end{cases}$$

Here, CVL is the concentration of TCE in the venous blood leaving the liver; $Vmax_{TCE}$ and Km_{TCE} are the parameters defining saturable (Michaelis-Menten) metabolism of TCE; k_{gsh} is the first-order rate constant for TCE conjugation with glutathione; and Vl is the volume of the liver. Figure II-2-2 depicts the rates of production of TCA/TCOH and DCA predicted by those equations.

The assumptions underlying the use of the equations shown above are as follows:

- The pathway involving conjugation with GSH can be modeled as though it were a first-order pathway; that is, the depletion of GSH that might occur is not serious enough to require modeling of cofactor depletion and/or second-order metabolism for that pathway. Rouisse and Chakrabarti (1986) saw only a 29% decrease in GSH concentrations following intraperitoneal administration of TCE to rats (11.15 mmoles/kg). However, these rats were pretreated with phenobarbital, which might decrease the amount of TCE metabolized via the GSH pathway, if the pretreatment enhances MFO metabolism.

- The "intermediate" compound [i.e., TCE epoxide in the P-450/microsomal environment or a TCE-P-450 complex (Miller and Guengerich 1982, 1983)], as well as the precursors in TCOH and TCA production (chloral, chloral hydrate) and the precursors in DCA production (free epoxide, DCC) are short-lived and do not leave the liver. Thus, this modeling approach need not include these compounds *per se*; only the disappearance of TCE (with rates dM1/dt and dM2/dt) and the appearance of DCA (rate dM1a/dt) and of TCOH and TCA (rate dM1b/dt) are included in the model.

- A spillover model, as discussed by Hathway (1980), is appropriate to model the production of DCA. Hathway assumed that DCA is produced only when the capacity of P-450 to catalyze the reaction of the "intermediate" to chloral is exceeded (in equation 3a, when dM1/dt is greater than the value TH with α equal to zero). Equations 1 through 3 relax that assumption, allowing for some "leakage" of epoxide from the P-450 environment. That free epoxide can degrade to form DCC as well as other observed metabolites (such as oxalic acid and HAAE; cf. Dekant et al., 1986). The leakage is represented in equations 3a and 3b by a positive value for α. Since there appears to be little or no information regarding values of α (except possibly some information to indicate upper limits for that parameter's values), the application of this approach in a risk assessment context might require examination of various assumptions regarding the value of α (and of TH too, to some extent) in order to determine the impact on risk estimates. This may be especially important if DCA is considered to be responsible for the hepatocellular tumors observed (as Hathway suggests).

- Actually, since free epoxide that gets out of the P-450/microsomal environment can be further metabolized to products other than DCC, the modeling of DCA formation might be expressed in terms of some (large) fraction of dMla/dt. It is not known at this time if that fraction would be constant for all TCE liver concentrations (all rates dMla/dt).

- As an alternative to the spillover model for production of DCA, it may be reasonable to represent DCA production as a saturable pathway separate from the one assumed to produce TCOH and TCA. If that approach were adopted, the Km for the DCA-producing pathway would probably be substantially larger than that for the production of TCOH and TCA, representing a low affinity process that may only become important and noticeable at high exposure levels. This would correspond to the observations of Hathway (1980) in which DCA appeared only at high doses.

2. TCA Modeling

An alternative approach to the modeling of TCA distribution might be represented as shown in Figure II-2-3. That approach is intermediate between a full physiologically based model (such as that employed for the modeling of TCE) and the single compartment modeling for TCA that was developed by Fisher et al. (1990) and Allen et al. (1990). For each persistent metabolite of TCE, the model contains two compartments, one representing the liver and the other representing the extra-hepatic volume of distribution. This approach is attractive because (1) the primary focus for TCA is its effect in the liver and (2) data required for a full physiologically based approach for TCA (such as data regarding partitioning, binding, and further metabolism via conjugation) do not appear to be available for all species.

Equations representing this system are formulated as follows:

$$(4)\quad dTCOH_l/dt = (1-f_l)*dMlb/dt + k_{12e}*TCOH_d/Vd_e - TCOH_l/Vl * (k_{conj} + k_{tcohtca} + k_{21e});$$

$$(5)\quad dTCOH_d/dt = k_{21}*TCOH_l/Vl - TCOH_d/Vd_e * (k_{12} + k_{ae} + k_{ue});$$

$$(6)\quad dCTCOH_l/dt = k_{conj}*TCOH_l/Vl + k_{12c}*CTCOH_d/Vd_c - CTCOH_l/Vl*(k_{21c} + k_{lc});$$

$$(7)\quad dCTCOH_d/dt = k_{21c}*CTCOH_l/Vl - k_{12c}*CTCOH_d/Vd_c - k_{uc}*CTCOH_d/Vd_c;$$

$$(8)\quad dTCA_l/dt = f_l*dMlb/dt + k_{tcohtca}*TCOH_l/Vl + k_{12a}*TCA_d/Vd_a - TCA_l/Vl*(k_{21a} + k_{la});$$

$$(9)\quad dTCA_d/dt = k_{21a}*TCA_l/Vl - k_{12a}*TCA_d/Vd_a - k_{ua}*TCA_d/Vd_a.$$

In these equations, $TCOH_i$, $CTCOH_i$, and TCA_i are the amounts, expressed in mmoles, in compartment i of free trichloroethanol, conjugated trichloroethanol, and trichloroacetic acid, respectively, where i is either l (for liver) or d (for the extrahepatic volume of distribution). The three compounds are assumed to have different extrahepatic volumes of distribution because of differences in partitioning to organs and tissues, binding, etc. The rate terms (k_{ij}, in L/hr) are defined such that e as the final letter in a subscript refers to TCOH, c to CTCOH, and a to TCA. Other letters or numbers in the subscripts refer to route or pla[illegible] of elimination (u is in urine, a is in expired air [for TCOH], l is from the liver [biliary excretion]) and the 1's and 2's refer, as in classical models, to the first (extrahepatic) and second (liver) compartments and transfer between the two. Alternative equations dealing with TCA in the liver can be formulated:

(10) $$dTCA_l/dt = f_l*dMlb/dt + k_{tcohtca}*TCOH_l/Vl + (k_{32a}+k_{12a})*TCA_d/Vd_a - TCA_l/Vl*(k_{21a} + k_{23a});$$

(11) $$dTCA_b/dt = k_{23a}*TCA_l/Vl - k_{32a}*TCA_b/Vb;$$

where Vb is the volume of a third compartment (bile) that gets TCA from the liver and returns it there. This corresponds to suggestions by Green and Prout (1985) that a labile TCA conjugate (not a glucuronide conjugate) is excreted into the bile but that it dissociates there. The dissociated TCA returns to the liver via enterohepatic circulation. The schematic for this part of the model is depicted in Figure II-2-4.

The assumptions underlying equations 4 through 11 are as follows:

- Only conjugated TCOH is excreted in the bile. Some perfused rat liver studies (Kawamoto et al., 1987a) have found small amounts of free TCOH in bile. Some studies in dogs (Hobara et al., 1986a; Hobara et al., 1987a) have also found small (no more than 1%) amounts of free TCOH in bile. Extensive biliary excretion of TCOH is unlikely given the low molecular weight of TCOH.

- Both TCOH and CTCOH, as well as TCA, are excreted in the urine. Many studies have found TCOH and CTCOH in urine. But Green and Prout (1985) have suggested that only CTCOH is excreted in the urine.

- TCOH is somewhat volatile and can therefore be lost through exhaled air. There is not much information regarding this point in the literature on animal studies. However, at least one human study has used TCOH in exhaled air as a gauge of TCE exposure (Hubner et al., 1987), although the blood/air partition coefficient for TCOH suggested in that work is on the order of 8700, implying that TCOH does not partition out of blood to air very much at all.

- The extrahepatic volumes of distribution are at least as large as the volume of the plasma. They will be larger to the extent that TCA, TCOH, and CTCOH distribute to the organs and red blood cells, bind to proteins or other macromolecules, and concentrate in certain tissues.

All of the approaches outlined in the discussion above are quite complex and require a great deal of data. Some effort was expended on developing such

a detailed PBPK model for rats and dogs. Although dogs have not been subject to carcinogenicity testing of TCE, we had hoped to develop and validate a dog model, as well as a similar model in at least one rodent species and humans. The interest in dogs stemmed at least in part from a desire to study the "scalability" of PBPK parameters across species. Typically, such scaling has been investigated from one rodent species to another or from rodents to humans (see, for example, Reitz et al., 1987). The development of a PBPK model for dogs would have provided an interesting alternative for examining parameter scaling, especially since dogs are a species of intermediate size (between rodent and humans) and they are neither rodents nor primates.

There exist several reports in the literature documenting exposure of dogs to TCE, CH, TCOH, and TCA (Barrett et al., 1939; Garrett and Lambert, 1973; Marshall and Owens, 1954; Owens and Marshal, 1955; Hobara et al., 1982, 1986a, 1986b, 1987a, 1987b, 1987c, 1988a, 1988b). Moreover, several studies of TCE and its metabolites with perfused rat liver have been reported (Bonse et al., 1975; Kawamoto et al., 1987a, 1987b, 1987c, 1988). Nevertheless, the data required for development of the detailed models discussed above may not be readily available. In part because of those data gaps, and also because of apparently conflicting results in the literature, the development of detailed rat and dog models was given low priority by the Clement team and was not satisfactorily completed. The progress made, however, would allow a quick start-up and rapid progress toward complete characterization of the kinetics of TCE, TCA, and other metabolites (notably TCOH) in dogs and rats should interest at a later date dictate such a need.

C. ALTERNATIVE APPROACHES TO DOSE-RESPONSE MODELING

The risk assessment reported in Part 2, Section A of this volume was conducted using the standard regulatory dose-response model, the linearized multistage model (Howe et al., 1986). These were completed using dose surrogates based on TCE metabolism, TCA production, and TCA area under the concentration curve. In this section, alternative dose surrogates and dose-response models are suggested for use in future TCE risk assessments.

With respect to the choice of dose surrogates, the primary emphasis should be on extending the pharmacokinetic models so that they reflect explicitly TCA's effect on the proliferation of peroxisomes. In essence, this approach would incorporate some dose-effect considerations, where the dose is a metric based on TCA concentration and the effect is peroxisomal enzyme activity. Thus, an extended model could include a term such as

$$(12) \qquad EP = f[CTCA(t)]$$

where EP is a measure of peroxisome-associated enzyme activity, CTCA(t) is the concentration of TCA at time t, and f(x) is a function relating those two variables. The form of f(x) may be determined by reference to *in vitro* experiments on peroxisome proliferation in hepatocytes from various species exposed to TCA (Elcombe, 1985). The literature on other peroxisome proliferators may also be relevant, if it can help to determine behavior that may be common to peroxisome proliferating chemicals and that should then be represented by the function f(x). It is possible that a threshold type of model would be appropriate if it is determined that some minimum concentration

of a peroxisome proliferator is required before effects on enzyme activities are observed.

Moreover, as *in vivo* data on peroxisome proliferation during and after TCA exposure become available (DeAngelo and Daniel, 1990; DeAngelo et al., 1989) it may be possible to model the relationship between peroxisomal enzyme activity and instantaneous TCA concentration in the framework of differential equations (in keeping with the remainder of the pharmacokinetic models). One form of differential equation representation of the effect of TCA is given by

$$(13) \qquad dEP(t)/dt = Kd * CTCA(t) + Ks * (1+A) * EP_0 / (EP(t) + A*EP_0) - Ks$$

where Kd, Ks, and A are kinetic parameters to be estimated from the time course data on enzyme activity, and EP_0 is the steady-state peroxisomal enzyme activity in the absence of exposure. The first term on the right side of equation (13) represents TCA's effect on the change in enzyme activity; the second and third terms describe the propensity of the system to return to base-line conditions. When CTCA(t) is zero, the enzyme activity will return to EP_0 at a rate determined by Ks and A. Species-specific values for the parameters in equation (13) could account for apparent species differences in peroxisome proliferation response to TCA exposure (Elcombe, 1985).

These approaches to modeling peroxisome proliferation (as reflected primarily by changes in peroxisomal enzyme activities) within the framework of pharmacokinetic modeling provide opportunities for refinement of the dose surrogates that can be estimated and related to the incidence of hepatocellular tumors.

If increased peroxisomal enzyme activity results in increased free radical production and therefore increased probability of DNA damage (Rao and Reddy, 1987), then peroxisome proliferation would be expected to increase the probability (rate) of mutation. Dose surrogates such as an average EP value or area under the EP-time curve may be appropriate, because those measures are considered to be closely related to effects that are irreversible (Gillette, 1987).

On the other hand, recent work suggests that peroxisome proliferation may actually represent a marker of the action of TCA (Goldsworthy and Popp, 1987; Popp et al., 1989). Under this assumption, peroxisome proliferation is not the proximate cause of tumor induction; it is not related to increased mutation rates. In that case, dose surrogates based on EP(t) may still be valuable as indicators of the carcinogenic potential, but in this case one might be more interested in some other surrogates, such as maximum or minimum EP(t) values, depending on the mechanisms for which EP is assumed to be a marker (Gillett, 1987).

A reasonable alternative dose-response modeling approach is based on the two-stage model of cancer (Moolgavkar and Knudson, 1981). That model could be used in conjunction with the dose surrogates estimable by the models developed by Fisher et al. (1990) and Allen et al. (1990) or with the peroxisome-based surrogates discussed above.

Clement personnel have implemented a computer version of the two-stage model that can be used with the response rates from carcinogenicity bioassays to estimate parameters and derive risk estimates. The form of the model is as follows:

$$P(d,t) = 1 - \exp\{-F(d)*(\exp\{G(d)*t\} - G(d)*t - 1)/G(d)^2\} \quad (14)$$

where P(d,t) is the probability of a tumor at time t, when exposed to dose d, and

$$F(d) = x_1 + x_2*(d - d_0) + x_3*(d - d_0)^2, \quad (15)$$

$$G(d) = x_4 + x_5*(d - d_1)^w, \quad (16)$$

and the x_i's, d_0, d_1, and w are parameters to be estimated.

Briefly, the function F(d) represents the effect of dose on the mutation rates from normal to first-stage and from first-stage to malignant cells. G(d) represents the effect of dose on the proliferation of first-stage cells. The terms x_1 and x_4 are background rates for mutation and proliferation of first-stage cells, respectively. The terms d_0 and d_1 represent dose thresholds below which the action of the chemical is assumed to be zero. Versions of this model have been discussed in the literature (Thorslund et al., 1987; Portier and Bailer, 1989).

For TCE exposure and subsequent TCA formation, the model could be implemented in at least two ways, depending on the assumptions made about the mechanism of action of TCA with respect to liver tumors.

First, if it is assumed that peroxisome proliferation induced by TCA increases the production of reactive oxygen species that then attack DNA (Reddy and Lalwani, 1983), then the effect of the dose surrogate would be modeled through the function F(d). In that case the parameter x_5 would be set to zero, since it would be assumed that cell proliferation is not affected by exposure. The parameters x_2, x_3, and d_0 would have to be estimated. The

background terms, x_1 and x_4, could be estimated from historical control data (Portier and Bailer, 1989) or, for x_1, from experiments specifically designed to measure mutation rates of hepatocytes.

Alternatively, the relationship between TCA and liver tumors may be mediated through a cell proliferation effect. This has been suggested to be the case for other peroxisome proliferators (Marsman et al., 1988). Then, the parameters x_2 and x_3 would be set to zero and x_5, d_1, and w could be estimated. Values for the background terms would again be required.

In connection with the second alternative (the effect of TCA being on first-stage cell proliferation), one specific example of a risk assessment approach can be presented. The steps for such an assessment are listed here:

1. Estimate values for the background terms x_1 and x_4. Portier and Bailer (1989) have estimated values for the background terms for hepatocellular tumors in B6C3F1 mice. A similar estimation for human background terms could be based on the age-specific rates of liver tumors in the general population.

2. Select a dose surrogate. The surrogate used to define the term "d" in equations (14)-(16) should be selected so as to be as closely related to the proliferation of first-stage cells as possible. The surrogate might be based on TCA concentration, either in its volume of distribution (as estimable now from the models of Fisher et al. or Allen et al.) or in the liver (as estimable from models like those discussed in Section B above). The surrogate might be based on peroxisome proliferation, in which case such proliferation would be related to TCA concentration via equation (12) or (13).

3. Complete the dose-response modeling. Use the results from carcinogenicity bioassays (and possibly other experiments) to estimate the terms x_5, d_1, and w. These parameters define the relationship between the selected dose surrogate (step 2) and first-stage cell proliferation in the test species. Time-to-response data are necessary for estimation of the parameters.

4. Extrapolate to humans. Using the human background terms (step 1) and the parameters estimated in step 3, calculate the human risk of liver tumors. Such a calculation will also employ the relationship between TCE exposures and dose surrogate values that was developed for humans (via the human PBPK modeling and, perhaps, the human relationship between TCA and peroxisome proliferation). However, the relationship that was estimated from the bioassay data, i.e., the relationship

between the dose surrogate and first-stage cell proliferation, is assumed to be the same for all species and can therefore be extrapolated from the test species to humans.

The assumption given in step 4 above is the key assumption and is present in one form or another in all risk assessments that are based on experiments on nonhuman species. The standard regulatory approach (extrapolating from test species to humans without use of pharmacokinetic data and assuming that multistage model parameters estimated when doses are expressed in mg/surface area/day) makes that assumption at the level of administered dose, on the one hand, and overall response rate on the other. With the addition of pharmacokinetics (but retaining use of the multistage model) the assumption is made at the level of delivered dose and overall response rate. In the example defined by steps 1 through 4 above, the assumption is made at the level of delivered dose and first-stage cell proliferation rate. The hope is that by redefining the level at which the assumption is made, i.e., by getting more specific about the dose and the response to which the cross-species extrapolation applies, that assumption is more realistic.

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Table II-2-1

Dose-Response Data for Bioassays of TCE in Mice

Bioassay	Doses[a] Experimental	$[TCE]_m$	$[TCA]_p$	TCA-AUC	Liver Tumor Response Rate[b]
NTP (1990)	0	0	0	0	6/48
Gavage	1000	4873.5	424.22	350.29	22/49
Female					
NCI (1976)	0	0	0	0	0/20
Gavage	753	4133.08	359.77	266.02	4/50
Female	1507	[illegible]2.07	461.53	341.29	11/47
Bell et al. (1978)	0	0	0	0	8/99
Inhalation	100	2303.77	200.54	153.05	9/100
Female	300	6065.86	528.02	431.46	10/94

[a] Experimental doses are reported in mg/kg body weight for gavage studies and in ppm air for concentration inhalation studies. $[TCE]_m$ is amount of TCE 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 II-2-2

Inhalation Risk Assessment Results:
Female Mice Exposed to TCE

Bioassay	Risk[c]	Associated Dose Surrogate Values[a] $[TCE]_m$	$[TCA]_p$	TCA-AUC	Estimated Human Air Concentrations (ppm)[b] $[TCE]_m$	$[TCA]_p$	TCA-AUC
NTP (1990)	1E-03	6.82E0	5.94E-01	4.90E-01	3.2E-01	6.9E-02	1.0E-02
Gavage	1E-06	6.82E-03	5.94E-04	4.90E-04	3.2E-04	6.9E-05	9.7E-06
NCI (1976)	1E-03	2.58E+01	2.24E0	1.66E0	1.2E0	2.6E-01	3.4E-02
Gavage	1E-06	2.58E-02	2.24E-03	1.66E-03	1.2E-03	2.6E-04	3.4E-05
Bell et al.	1E-03	5.71E+01	4.97E0	3.98E0	2.7E0	5.7E-01	8.2E-02
(1978)	1E-06	5.71E-02	4.97E-03	3.98E-03	2.7E-03	5.7E-04	8.0E-05

[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 II-2-3

Drinking Water Risk Assessment Results:
Female Mice Exposed to TCE

Bioassay	Risk[c]	Associated Dose Surrogate Values[a]			Estimated Human Drinking Water Concentrations (mg/L)[b]		
		$[TCE]_m$	$[TCA]_p$	TCA-AUC	$[TCE]_m$	$[TCA]_p$	TCA-AUC
NTP (1990)	1E-03	6.82E0	5.94E-01	4.90E-01	7.4E0	1.5E0	2.3E-01
Gavage	1E-06	6.82E-03	5.94E-04	4.90E-04	7.6E-03	1.6E-03	2.3E-04
NCI (1976)	1E-03	2.58E+01	2.24E0	1.66E0	2.8E+01	5.9E0	7.8E-01
Gavage	1E-06	2.58E-02	2.24E-03	1.66E-03	2.9E-02	6.1E-03	7.9E-04
Bell et al.	1E-03	5.71E+01	4.97E0	3.98E0	6.2E+01	1.3E+01	1.8E0
(1978)	1E-06	5.71E-02	4.97E-03	3.98E-03	6.2E-02	1.3E-02	1.9E-03

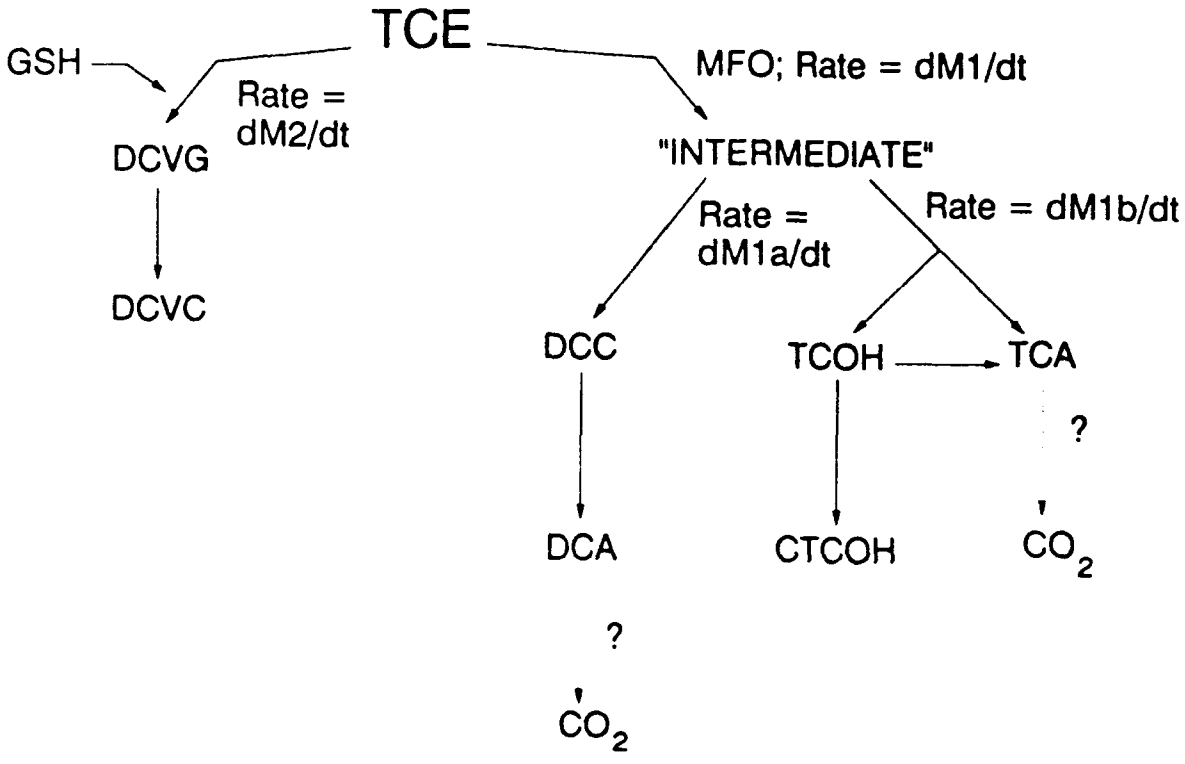
[a] The values of the dose surrogates estimated from the bioassay to correspond to the stared level of risk.

[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 II-2-1

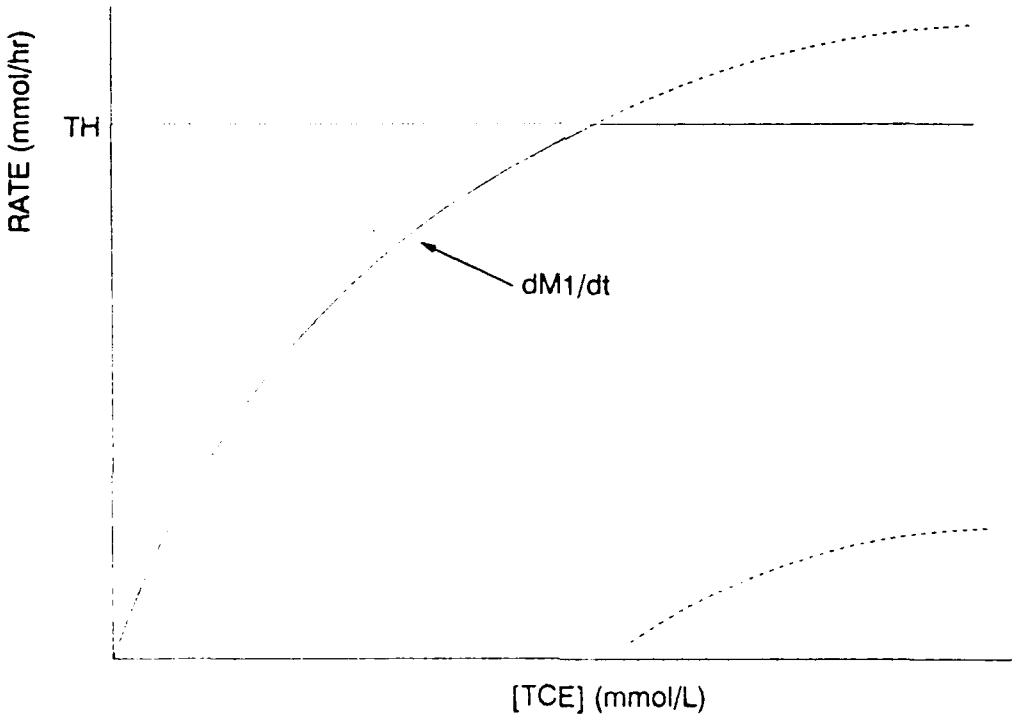
TCE Metabolism



Schematic of the pathways of TCE metabolism showing rates of reaction.

Figure II-2-2

Rates of Production of TCA/TCOH and DCA

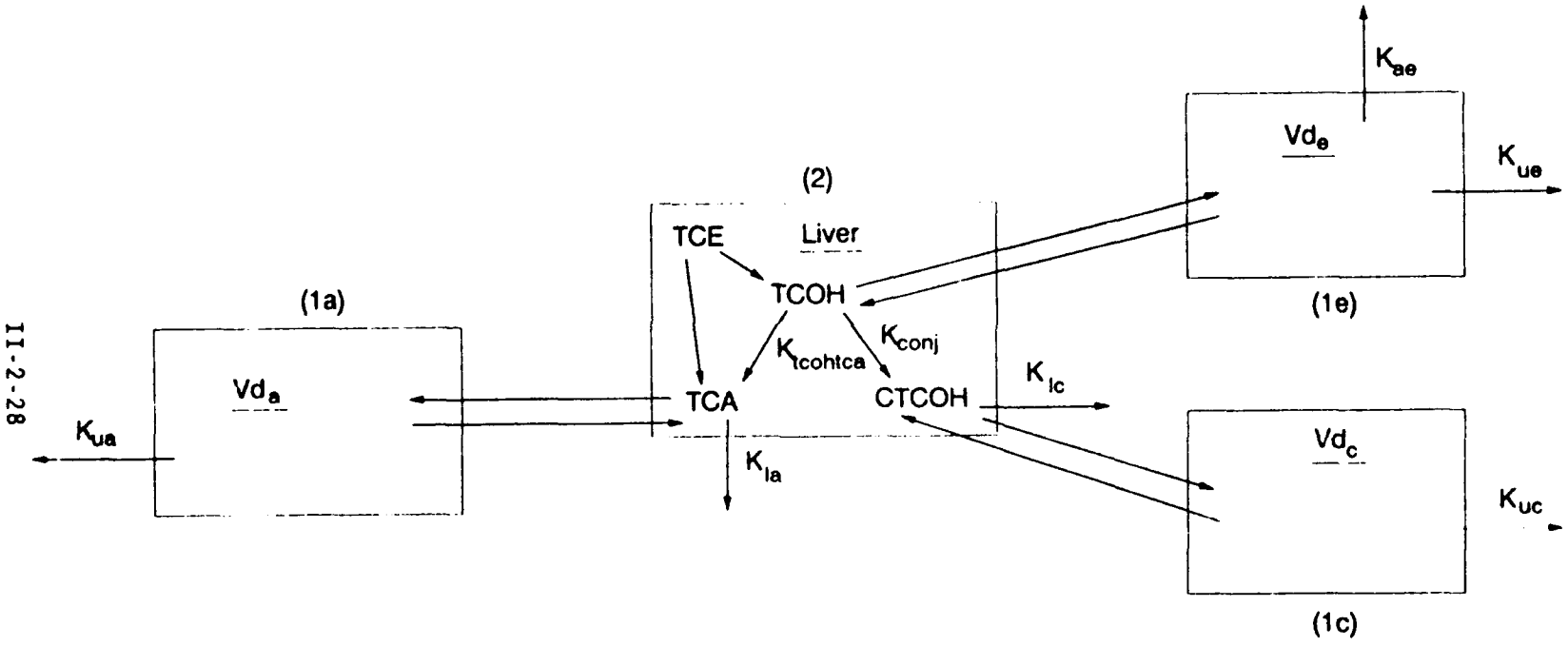


TCA/TCOH [——]; DCA [·····]

[α = 0]

TCA/TCOH and DCA production consistent with text equations (1) - (3), when α = 0.

Figure II-2-3
Schematic of Possible Model for TCE Metabolites

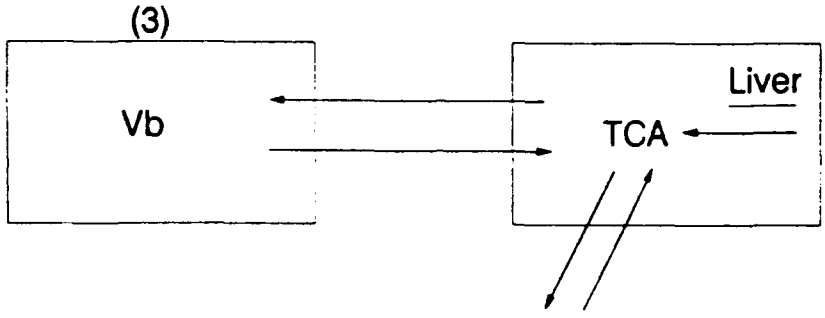


Alternative schemes for modeling TCE metabolites that explicitly consider the the liver and extrahepatic volumes of distribution.

See text equations (4) - (9).

Figure II-2-4

Schematic for an Alternative Treatment of TCA in the Liver and Bile



[Vb = Volume of Bile]

Possible treatment of TCA excretion into bile and reabsorption with subsequent return to liver. Other aspects of TCA kinetics would be as shown in Figure II-2-3. See text equations (10) - (11).

APPENDIX II-2-A

OVERVIEW OF TCE CONJUGATION WITH GLUTATHIONE AND ITS RELATIONSHIP TO NEPHROTOXICITY

APPENDIX II-2-A

OVERVIEW OF TCE CONJUGATION WITH GLUTATHIONE
AND ITS RELATIONSHIP TO NEPHROTOXICITY

A glutathione conjugation pathway for metabolism of trichloroethylene (TCE) has been proposed to explain the nephrotoxicity observed in rats, but not in mice, following exposure to high concentrations of trichloroethylene (Dekant et al., 1986). This metabolic pathway becomes more important as the cytochrome P-450 metabolic pathway for trichloroethylene becomes saturated.

The initial step in this pathway is the conjugation of TCE, facilitated by glutathione-S-transferase, in the liver to form a glutathione-S-conjugate, S-(1,2-dichlorovinyl)glutathione (DCVG) (Dekant et al., 1989). DCVG is secreted from the hepatocytes into the plasma and is delivered to the kidney (Dekant et al., 1989). A small percentage of DCVG is delivered to the bile (Anders et al., 1988) and may be excreted in the feces (Dekant et al., 1989). The kidney metabolizes 70% of the glutathione-S-conjugates in the plasma (Dekant et al., 1989), therefore most of the DCVG in the plasma would be metabolized by the kidney.

Once in the kidney, DCVG reacts with gamma-glutamyltransferase, which is localized on membranes of the renal proximal tubules (Anders et al., 1988). This reaction yields glutamate and S-(1,2-dichlorovinyl)-L-cysteinylglycine (DCVCG) (Elfarra et al., 1986; Lock, 1988; Anders et al., 1988). DCVCG is then acted upon by cysteinyl glycine dipeptidase to yield glycine and a cysteine-S-conjugate, S-(1,2-dichlorovinyl)-L-cysteine (DCVC) (Dekant et al., 1989; Lock, 1988; Anders et al., 1988).

DCVC may then be metabolized in the kidney by one of three pathways resulting in detoxification, recirculation, or production of the ultimate

nephrotoxin (Dekant et al., 1989). The detoxification pathway involves the N-acetylation of DCVC by N-acetyltransferase to form S-(1,2-dichlorovinyl)N-acetyl-cysteine (N-Ac-DCVC), a mercapturic acid, which is excreted in the urine (Dekant et al., 1989). DCVC may also be recirculated to the liver via peritubular circulation (Dekant et al., 1989). In the liver DCVC may be acetylated to form N-Ac-DCVC which can then be transported back to the kidney where it may be excreted in the urine, or may be deacetylated to reform DCVC (Dekant et al., 1989).

The final pathway, which may result in the ultimate nephrotoxin, involves the action of β-lyase on DCVC (Anders et al., 1988; Dekant et al., 1989; Lock, 1988). β-lyase, which is present in high concentrations in the proximal tubular cells of the rat kidney (Vamvakas et al., 1987), cleaves DCVC to yield pyruvate, ammonia and a reactive sulfur-containing fragment, 1,2-dichlorovinylthiol (Dekant et al., 1989; Green and Odum, 1985; Anders et al., 1988). 1,2-Dichlorovinylthiol is generally considered to be the metabolite causing the nephrotoxicity observed in rats following exposure to high concentrations of trichloroethylene (Anders et al., 1988; Dekant et al., 1989). 1,2-Dichlorovinylthiol has been shown to interact with glutathione, DNA and protein and may inhibit mitochondrial respiration (Green and Odum, 1985). It is through these interactions that 1,2-dichlorovinylthiol may cause kidney toxicity. 1,2-Dichlorovinylthiol may be further metabolized to yield chloroacetic acid; however, the mechanism by which it is produced has not been reported (Dekant et al., 1989).

β-lyase has been reported to occur in the rat kidney (Green and Odum, 1985); the occurrence of β-lyase in the kidney of mice has not been reported. Because the toxicity of DCVC may be due to its metabolic activation by

β-lyase, the (possible) lack of β-lyase in the kidney of the mouse may explain the occurrence of kidney tumors in rats and not in mice following exposure to high concentrations of trichloroethylene. The probability that the cytochrome P-450 pathway for TCE metabolism may be saturated at lower concentrations in rats than in mice may also explain the occurrence of kidney tumors in rats (Elcombe et al., 1982). The glutathione pathway would be utilized to a greater extent following exposure to lower concentrations in the rat than in the mouse.

U S Government Printing Office 1991—548-018/20238