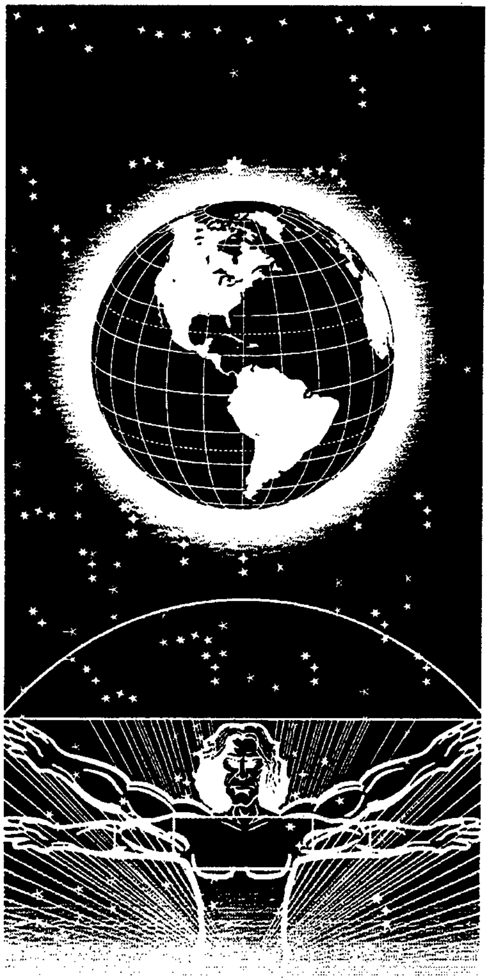
AL/OE-TR-1995-0178



UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

# DEVELOPMENT AND VALIDATION OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL OF CHLORAL HYDRATE AND ITS MAIN METABOLITES

**Janusz Z. Byczkowski**
**Connie S. Seckel**
MAN TECH ENVIRONMENTAL TECHNOLOGY, INC.
P.O. BOX 31009, DAYTON, OHIO 45437

**Richard K. Black**
**Jason R. Creech**
**Brendan L. Garrity**
OCCUPATIONAL AND ENVIRONMENTAL
HEALTH DIRECTORATE TOXICOLOGY DIVISION
ARMSTRONG LABORATORY
WRIGHT-PATTERSON AFB OH 45433-7400

**November 1995**

**Occupational and Environmental Health Directorate**
**Toxicology Division**
**2856 G Street**
**Wright-Patterson AFB OH 45433-7400**

Approved for public release; distribution is unlimited.

19990316 047

## NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Air Force Armstrong Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Service
8725 John J. Kingman Rd., Ste 0944
Ft. Belvoir, Virginia 22060-6218

## DISCLAIMER

This Technical Report is published as received and has not
been edited by the Technical Editing Staff of the Air Force Armstrong Laboratory.

## TECHNICAL REVIEW AND APPROVAL

**AL/OE-TR-1995-0178**

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 DIRECTOR**

**STEPHEN R. CHANNEL**, Maj, USAF, BSC
Branch Chief, Operational Toxicology Branch
Air Force Armstrong Laboratory

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

**1. AGENCY USE ONLY** *(Leave blank)*

**2. REPORT DATE**
November 1995

**3. REPORT TYPE AND DATES COVERED**
Interim Report - April 1994-September 1995

**4. TITLE AND SUBTITLE**
Development and Validation of a Physiologically Based Pharmacokinetic Model of Chloral Hydrate and Its Main Metabolites

**5. FUNDING NUMBERS**
Contract F33615-90-C-0532
PE 63716D
PR 4223
TA 4223OT
WU 4223OT01

**6. AUTHOR(S)**
J.Z. Byczkowski, C.S. Seckel, R.K. Black, J.R. Creech and B.L. Garrity

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Man Tech Environmental Technology, Inc.
P.O. Box 31009
Dayton, OH 45437-0009

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
Armstrong Laboratory, Occupational and Environmental Health Directorate
Toxicology Division, Human Systems Center
Air Force Materiel Command
Wright-Patterson AFB, OH 45433-7400

**10. SPONSORING/MONITORING AGENCY REPORT NUMBER**
AL/OE-TR-1995-0178

**11. SUPPLEMENTARY NOTES**

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

**12b. DISTRIBUTION CODE**

**13. ABSTRACT** *(Maximum 200 words)*
Chloral hydrate is a synthetic sedative/hypnotic drug used in human medicine. It is also generated as a chlorination by-product in drinking water and is produced in the organism from an environmental pollutant - trichloroethylene. Chloral, along with trichloroethylene, are occurring as environmental contaminants at the Air Force, Navy, and Army installations. This report describes the development of an interlinked physiologically based pharmacokinetic model of chloral and its main metabolites, mathematical description of the interlinking between sub-models for each metabolite, model verification with data from the literature and its experimental calibration in B6C3F1 mice. The developed model described successfully the pharmacokinetics of chloral, trichloroethanol and its glucuronide in mice and dogs. It seems that one adequately validated in humans, the developed model might be applied for a computer-aided simulation of body levels of chloral hydrate in a therapeutic situation and for the estimate of toxicokinetics of its active metabolites generated during the environmental pollution scenario.

**14. SUBJECT TERMS**
Pharmacokinetic model; Chloral hydrate; Trichloroacetic acid; Trichloroacetate glucuronide; Computer simulation; Mouse; Dog

**15. NUMBER OF PAGES**
48

**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 |

Standard Form 298 (Rev. 2-89) (EG)
Prescribed by ANSI Std. 239.18
Designed using Perform Pro, WHS/DIOR, Oct 94

## PREFACE

This report describes the results of the development and experimental validation of a mathematical model simulating distribution, metabolism, and disposition of chloral, a derivative of trichloroethylene, an environmental pollutant. This is one of a series of technical reports and publications describing results of a collaborative effort conducted by ManTech Environmental Technology, Inc., Toxic Hazards Research Unit, located at Wright-Patterson Air Force Base, and by Occupational and Environmental Health Directorate, Toxicology Division, and aimed at a pharmacokinetic description of trichloroethylene and its metabolites.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

Research performed by ManTech Environmental was conducted under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F35) and research performed by the Toxicology Division was conducted under Work Unit No. 4223OT01. Funding was provided to the Toxicology Division by the Strategic Environmental Research and Development Program (SERDP).

Lt Col Terry A. Childress, Director of the Toxicology Division, served as Contract Technical Monitor.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

## ABBREVIATIONS

| | |
|---|---|
| CH | Chloral |
| DCA | Dichloroacetic acid |
| g | Gram |
| hr | Hour |
| i.v. | Intravenous |
| kg | Kilogram |
| L | Liter |
| mg | Milligram |
| min | Minute |
| mL | Milliliter |
| PBPK | Physiologically based pharmacokinetics |
| TCA | Trichloroacetic acid |
| TCE | Trichloroethylene |
| TCOH | Trichloroethanol |
| TCOG | Trichloroethanol glucuronide |

# SECTION 1
# INTRODUCTION

Chloral hydrate is one of the oldest of synthetic sedative/hypnotic drugs used in human medicine (Leibreich, 1869). Its popularity has decreased considerably after introduction of barbiturates because barbiturates are much more convenient to administer. Chloral hydrate must be given as a 1 - 2 g oral dose in a flavored solution to disguise its unpleasant taste, or as a retention enema or suppository. Despite administration in huge doses, its toxicity is very low (lethal dose of about 10 g per 70 kg man) and, typically, its only side effect is some gastric irritation after therapeutic oral administration (Goth, 1964). In the organism, chloral hydrate is rapidly metabolized, mainly to trichloroethanol (TCOH) and its glucuronide (TCOG) which is excreted with the urine (Butler, 1948). Both chloral (CH) and TCOH can exert hypnotic activity on the central nervous system (MacKay and Cooper, 1962).

About 11% of the administered chloral hydrate is oxidized to trichloroacetic acid (TCA) (Cabana and Gessner, 1970; Muller et al., 1974), and possibly to a small amount of dichloroacetic acid (DCA) (Davidson and Beliles, 1991). However, it is not clear if DCA is a metabolic product of CH as suggested by Dekant et al. (1984), a product of metabolic reductive dechlorination of TCA as suggested by Larson and Bull (1992), or rather it is an analytical artifact produced by interconversion of TCA in biological samples (Ketcha et al., 1995).

A realization that CH is generated as a chlorination by-product in several municipal drinking water supplies, as well as that it is produced in the organism as an intermediate metabolite from the ubiquitous environmental pollutant - trichloroethylene (TCE) (Cole et al., 1975; Reynolds and Moslen, 1981) rejuvenated interest in the toxicology of CH and its hydrate (Waters et al., 1977). Especially, the recent reports about mutagenic and hepatocarcinogenic potential of chloral hydrate (Daniel et al., 1992) have put its importance in a new light. Chloral and TCE occur as environmental contaminants at Air Force, Navy, and Army installations and for some time these chlorinated compounds have been part of the TriService research initiative. For a rational characterization of the potentially harmful effects of CH and its metabolites, it is crucial to be able to reconstruct its distribution and conversion to its main metabolites: TCOH, TCOG, TCA, and DCA, from the CH exposure dosage. The approach ideally suited for this purpose is the use of a physiologically based pharmacokinetic (PBPK) model (Yang and Andersen, 1994). This report describes development of a PBPK model of CH and its main metabolites, mathematical description of the interlinking between sub-models for each metabolite, model verification with data from the literature and its experimental calibration in B6C3F1 mice.

## SECTION 2
## MATERIALS AND METHODS

A PBPK model was written in ACSL, a Fortran-based continuous simulation language (Mitchell and Gauthier, 1987), and simulations were performed using a SIMUSOLV software package with optimization capabilities (Steiner et al., 1990) on a VAX/VMS mainframe computer (VAX8530, Digital Equipment Corp., Maynard, MA). Parameters were optimized by SIMUSOLV which uses the log likelihood function as the criterion, and either the generalized reduced gradient method for single parameter optimization or the Nelder-Mead search method for multiple parameters optimization to adjust the values.

A method for nonvolatile chemicals, after Jepson et al. (1994), was used for measuring tissue partition coefficients for CH and TCOH. Briefly, 0.5 g of blood or other tissue was added to a 5 mL of solution containing CH or TCOH in an appropriate concentration, 20% of lead acetate, and 0.9% NaCl in 20 mL vial capped with teflon/rubber septum. The vials were equilibrated for 18 hr at 37 °C with vortexing at a medium speed. Equilibrated tissue supernatants were centrifuged at 1500 rpm for 10 min and the resultant supernatants were filtered through prewashed Milipore filters (Ultra-PF, low-binding cellulose, 10,000 NMWL). The ultrafiltrate was extracted with ethyl acetate and analyzed for CH or TCOH by gas chromatography.

B6C3F1 male mice (30-40 g body weight; Charles River, Inc.) were dosed i.v. (by the tail vein) with chloral hydrate or TCOH at an appropriate concentration (10 or 100 mg/kg), dissolved in physiological saline. The injection interval for each mouse was timed. At appropriate times, the mice were sacrificed with $CO_2$, and samples of blood and liver were collected to pre-weighed vials containing 20% lead acetate. The samples were homogenized, extracted with ethyl acetate, and analyzed for CH or TCOH by gas chromatography.

The animals used in this study were handled in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institutes of Health, Publication No. 86-23, 1985; and the Animal Welfare Act of 1966, as amended.

The tissue samples were analyzed for CH and/or TCOH by a Hewlett Packard 5890 Series II gas chromatograph equipped with a 7673 A autosampler. The blank and reference vials contained no tissue but were processed in the same manner as the vials that contained actual tissue sample. The gas chromatograph conditions that separated CH from TCOH were: injection temp. = 175 °C, Electron Capture Detector temp. = 300 °C; oven program (80 °C for 4 min; 25 °C/min to 180 °C for 2.2 min); Vocol 30 M x 0.53 mm column. Data were collected using the Turbochrom Nelson Analytical System v.4.03. Standard curves were made in the appropriate biological matrix, and treated with the same procedures as test samples.

Chloral hydrate and TCOH were obtained from Sigma Chemical Co., Ltd. and lead acetate used to inhibit metabolism in analytical samples was obtained from Mallinckrodt. All other commercial reagents used were of analytical purity.

# SECTION 3
# RESULTS

## PBPK Model Structure

Figure 1 shows a general scheme of an interlinked PBPK model for CH and its metabolites. The main model consisted of three sub-models for CH, TCOH, and TCOG (treated as separate objects) linked by rates of production of TCOH from CH (RAMCH [mg/hr]), glucuronide production from TCOH (RGLUC [mg/hr]), and reabsorption of TCOH from hydrolyzed TCOG (RAGLUC [mg/hr]). Additional outputs from these sub-models were: the amount of DCA produced from CH (ADCA [mg]) with the rate RIDA [mg/hr], the amount of TCA (ATCA [mg]) produced from CH with the rate RTA [mg/hr] and from TCOH with the rate RAMOCH [mg/hr], and the amount of TCOH glucuronide (AGU [mg]) excreted to the urine with the rate RGU [mg/hr]. The output from each sub-model was available for input in subsequent operations of this object-oriented program.

## Rates of Mass Transfer Between Sub-models

i. Production of TCOH from CH:

$$RAMCH = (VMCHOH*CVCHL)/(KMCHOH+CVCHL) + PCTCOH*CVCHL*VL$$

where: VMCHOH [mg/hr] is a pseudo-maximum velocity of TCOH formation from CH, adjusted allometrically to the 0.7 power of the body weight [kg]; * is a multiplication; CVCHL [mg/L] is a CH venous blood concentration leaving the liver; KMCHOH [mg/L] is an apparent Michaelis constant of TCOH formation from CH; PCTCOH [1/hr/animal] is a first order rate constant of TCOH formation from CH, adjusted allometrically to the 0.3 power of the body weight [kg]; VL [L] is a volume of liver, adjusted allometrically to the body weight.

ii. Production of TCOG from TCOH:

$$RGLUC = (VMTCOH*CVOHL)/(KMTCOH+CVOHL)$$

where: VMTCOH [mg/hr] is a pseudo-maximum velocity of TCOG formation from TCOH, adjusted allometrically to the 0.7 power of the body weight [kg]; CVOHL [mg/L] is a TCOH venous blood concentration leaving the liver; KMTCOH [mg/L] is an apparent Michaelis constant of TCOG formation from TCOH.

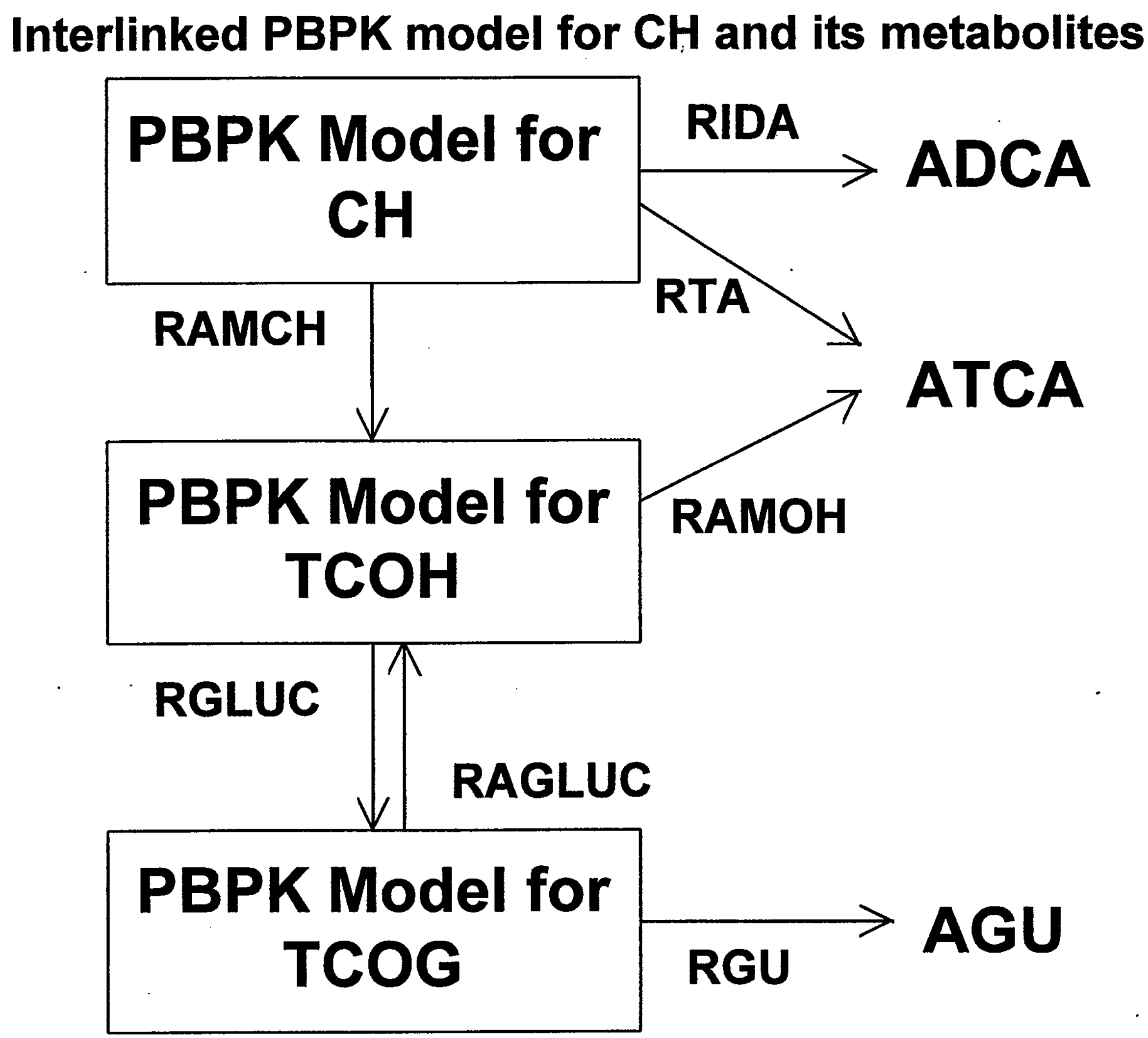



Figure 1. A general scheme of the interlinked PBPK model for chloral and its metabolites.
Symbols used: ADCA - amount of DCA produced from CH [mg]; ATCA - amount of TCA produced from CH and TCOH [mg]; AGU - amount of TCOG excreted to the urine [mg]; RIDA - rate of DCA production from CH [mg/hr]; RTA - rate of TCA production from CH [mg/hr]; RAMOH - rate of TCA production from TCOH [mg/hr]; RGU - rate of TCOG excretion [mg/hr]; RAMCH - rate of TCOH production from CH [mg/hr]; RGLUC - rate of TCOG production from TCOH [mg/hr]; RAGLUC - rate of TCOH reabsorption from hydrolyzed TCOG [mg/hr].

iii. Reabsorption of TCOH from hydrolyzed glucuronide:

$$RAGLUC = KAOHBI*ATGB*e^{-KAOHBI*T}$$

where: KAOHBI [1/hr] is a TCOH uptake from gut rate constant; ATGB [mg/animal] is the amount of TCOH formed by complete hydrolysis of glucuronide in the intestine; T [hr] is the time.

**Rates of Metabolite Mass Output from Sub-models**

i. Formation of DCA from CH:

$$RIDA = CVCHL*PIDA*VL$$

where: CVCHL [mg/L] is a CH venous blood concentration leaving the liver; PIDA [1/hr/animal] is a first order rate constant of DCA formation from CH, adjusted allometrically to the 0.3 power of the body weight [kg]; VL [L] is a volume of liver, adjusted allometrically to the body weight.

ii. Formation of TCA from CH:

$$RTA = CVCHL*PCTCA*VL$$

where: PCTCA [1/hr/animal] is a first order rate constant of TCA formation from CH, adjusted allometrically to the 0.3 power of the body weight [kg].

iii. Formation of TCA from TCOH:

$$RAMOH = CVOHL*PCOTA*VL$$

where: PCOTA [1/hr/animal] is a first order rate constant of TCA formation from TCOH, adjusted allometrically to the 0.3 power of the body weight [kg].

At the input to the next sub-model, the rates (RIDA, RTA, and RAMOH) were adjusted for molecular weight of metabolites.

iv. Excretion of TCOG with the urine:

$$RGU = CBVG*CLGLU$$

where: CBVG [mg/L] is a concentration of glucuronide in ultrafiltrated blood; CLGLU [L/hr] is a renal clearence of TCOG adjusted allometrically to the body weight [kg].

## Compartment Description and Governing Equations

The basic assumption in constructing this PBPK model was that blood flow to the tissue is limiting the metabolite "*m*" delivery. Because any metabolite is retained by the tissue according to its tissue/blood partition coefficient ($P_{mi}$) which may be measured *in vitro*, the concentration of the metabolite in venous blood leaving the tissue ($CV_{mi}$) during the equilibration phase is lower than the concentration in arterial blood ($C_mA$). Therefore, the rate of change of metabolite amount in tissue ($dA_{mi}/dt$), is given by a simple difference between concentration in blood entering and exiting the tissue ($C_mA-CV_{mi}$) multiplied by the blood flow ($Q_i$).

Integrating this equation over a given time, one can calculate the amount of metabolite present in tissue ($A_{mi}$) and, therefore, if the actual volume of tissue ($V_i$) is known, one can calculate the concentration of substance in the tissue ($C_{mi}$) at any time. Using these simple principles, the mass transfer equations describing each compartment building the sub-models for CH, TCOH, and TCOG (schematically shown in Figures 2 and 3) are defined below.

For each well-stirred compartment "*i*" without metabolism or other losses (fat tissue, gut, slowly perfused and rapidly perfused tissues), the rate of change in the amount "A" [mg] of metabolite "*m*" ($dA_{mi}$ over time [hr]) was defined as follows:

$$dA_{mi}/dt = Q_i(C_mA - CV_{mi})$$

where: subscript *i* represents "i-th" compartment; *m* represents the metabolite (CH, TCOH, or TCOG); $Q_i$ [L/hr] represents the blood flow through the i-th compartment; $C_mA$ [mg/L] represents the arterial concentration of metabolite *m*; $CV_{mi}$ [mg/L] represents the venous concentration of metabolite *m* leaving the i-th compartment ($CV_{mi} = C_{mi}/P_{mi}$; where $C_{mi}$ [mg/kg] is a concentration of metabolite *m* in the tissue in i-th compartment and $P_{mi}$ is the tissue/blood partition coefficient of metabolite *m* for i-th compartment. $C_{mi} = A_{mi}/V_i$, where $V_i$ [kg] represents the volume of the i-th compartment).

For the liver compartment a loss term ($RAM_m$ [mg/hr]) was added to the well-stirred compartment description to account for metabolism, and an increment term $RAM_mI$ [mg/hr] to account for production in the previous sub-model ($RAM_m$ and $RAM_mI$ are the rates of mass transfer between sub-models or the rates of mass output from sub-models, adjusted for molecular weight of the substrate). As explained above, depending on the metabolite, rates of mass transfer or mass output followed either the Michaelis-Menten kinetic equation, mixed Michaelis-Menten and first order rate kinetic equation, or the first order rate of metabolism:

$$dA_mL/dt = QL(C_mA - CV_mL) - RAM_m + RAM_mI$$

Because both CH and TCOH (but not TCOG) are slightly volatile, for the lung compartment with two theoretically possible mass inputs (mixed venous blood and inhaled air) and two theoretically possible outputs (arterial blood and exhaled air), at steady state the amount in alveolar air is in equilibrium with the amount in lung blood. Therefore:

$$QP(CI_m - CX_m) = QC(C_mA - C_mV)$$

$$CX_m = C_mA/P_mB$$

where: QP [L/hr] is the air flow through the lungs (alveolar ventilation rate), $CI_m$ [mg/L] is the concentration in inhaled air, $C_mX$ [mg/L] is the concentration in alveolar air, $C_mA$ [mg/L] is the arterial concentration (leaving the lungs), $P_mB$ is blood/air partition coefficient, QC [L/hr] is the blood flow through the lungs (rate of cardiac output), $C_mV$ [mg/L] is the venous concentration (entering the lungs). This equation is solved for $C_mA$.

The rate of change in the amount of CH or TCOH ($RMR_m$ [mg/hr]) in the gastrointestinal tract (after single gavage dosing) was defined analogously to the reabsorption of TCOH from hydrolyzed glucuronide in the gut (as described above):

$$RMR_m = -KA_m*DOS_m*e^{-KAm*T}$$

where: $KA_m$ [1/hr] is a CH or TCOH uptake from gut rate constant; $DOS_m$ [mg/animal] is the total oral dose; T [hr] is the time.

The rate of change in the amount of TCOG remaining in the body (RBODYG [mg/hr]) was defined as a difference between the rate of retention or wash-out from the tissues and the amount excreted with the urine:

$$RBODY = QB*(CGB-CBVG) - RGU$$

where: QB [L/hr] is a blood flow through the body except liver; CGB [mg/L] is a concentration of TCOG in mixed blood; CBVG [mg/L] is a concentration of TCOG in ultrafiltrated blood (in equilibrium with the ultrafiltrate); RGU [mg/hr] is the rate of excretion of TCOG with the urine (defined above).

# PBPK Model for Chloral Hydrate

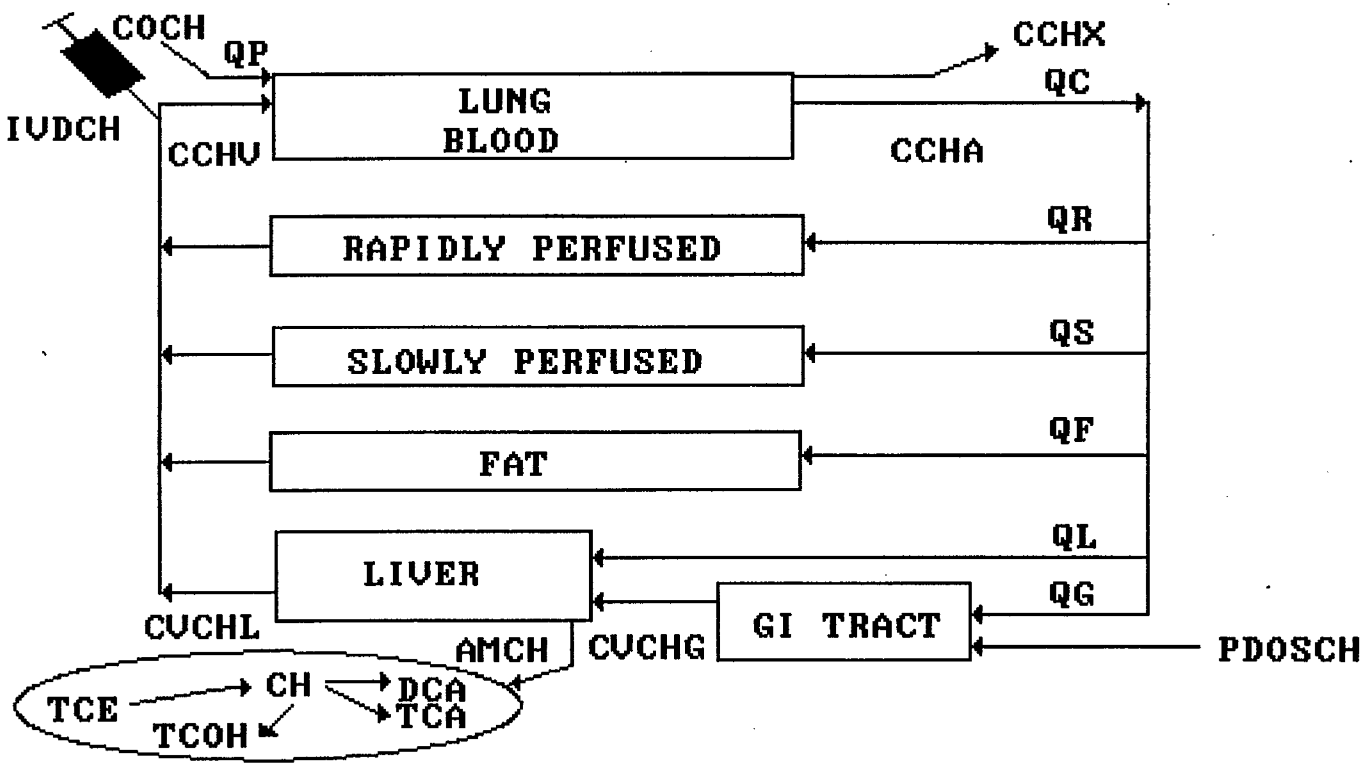



Figure 2. A general scheme of the PBPK sub-model for chloral.
Symbols used: QC - cardiac output [L/hr]; QR - blood flow through rapidly perfused tissues [L/hr]; QS - blood flow through slowly perfused tissues [L/hr]; QF - blood flow through fat [L/hr]; QL - blood flow through hepatic artery [L/hr]; QG - blood flow through gut [L/hr]; PDOSCH - oral dose of CH [mg/kg]; CCHX - concentration of CH in exhaled air [mg/L]; CCHA - concentration of CH in arterial blood [mg/L]; CVCHG - concentration of CH in portal vein [mg/L]; AMCH - amount of CH metabolized [mg]; COCH - concentration of CH in inhaled air [mg/L]; QP - alveolar ventilation [L/hr]; CCHV - concentration of CH in mixed venous blood [mg/L]; CVCHL - concentration of CH in liver venous blood [mg/L]; IVDCH - intravenous dose of CH [mg/kg].

# PBPK Model for Trichloroethanol and Its Glucuronide

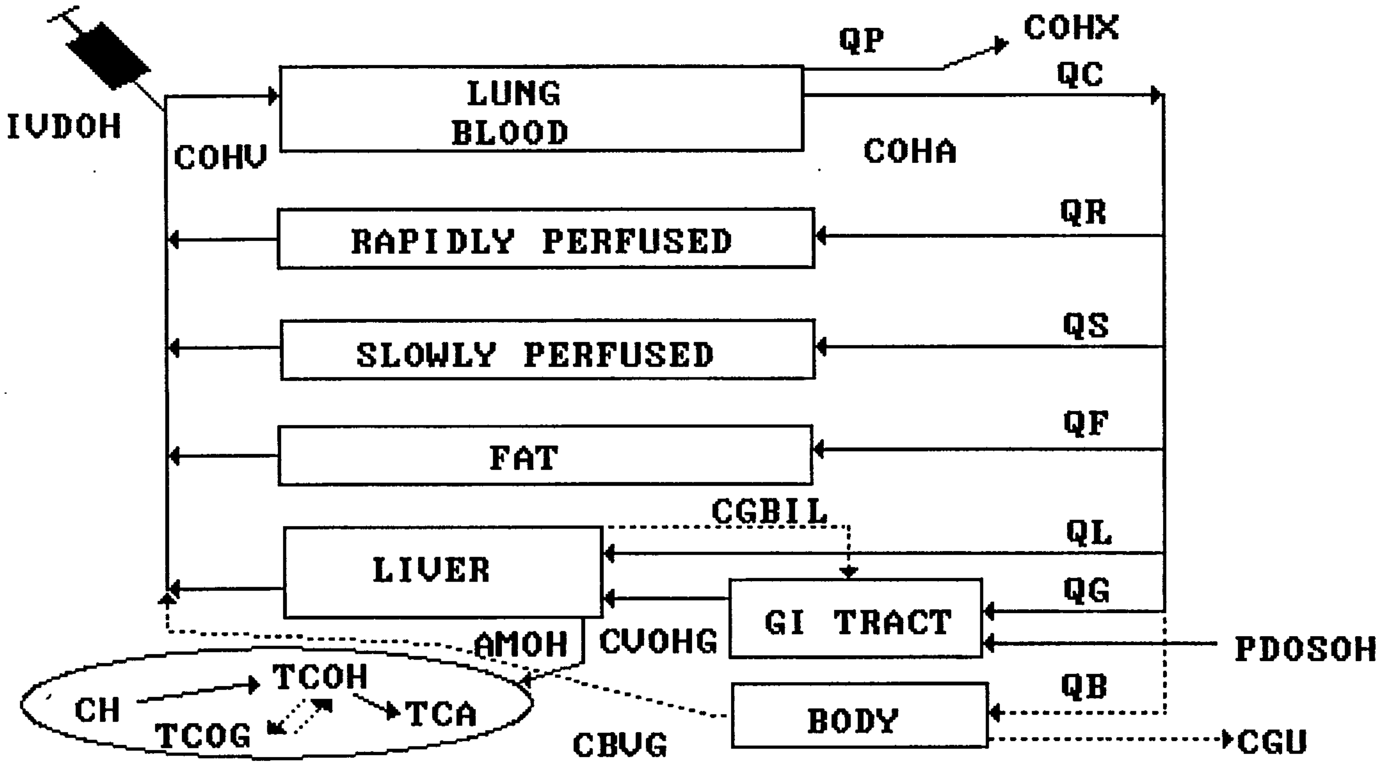



Figure 3. A general scheme of the PBPK sub-models for trichloroethylene and its glucuronide.
Symbols used: QC - cardiac output [L/hr]; QR - blood flow through rapidly perfused tissues [L/hr]; QS - blood flow through slowly perfused tissues [L/hr]; QF - blood flow through fat [L/hr]; QL - blood flow through hepatic artery [L/hr]; QG - blood flow through gut [L/hr]; QB -flow through body except liver [L/hr]; PDOSOH - oral dose of TCOH [mg/kg]; CGU - concentration of TCOG in urine [mg/L]; COHX - concentration of TCOH in exhaled air [mg/L]; COHA - concentration of TCOH in arterial blood [mg/L]; CGBIL - concentration of TCOG in the bile [mg/L]; CVOHG - concentration of TCOH in portal vein [mg/L]; CBVG - concentration of TCOG in mixed venous blood [mg/L]; AMOH - amount of TCOH metabolized [mg]; QP - alveolar ventilation [L/hr]; COHV - concentration of TCOH in mixed venous blood [mg/L]; IVDOH - intravenous dose of TCOH [mg/kg].

## Numerical Values of PBPK Model Constants Used in Simulations

Physiological parameters used in the PBPK model for simulations were adapted from the literature (Compilation by Lindstedt,S.: Unpublished physiological parameters. Physiological Parameters Working Group, ILSI Risk Science Institute; and Arms and Travis, 1988). These parameters included alveolar ventilation (QPC [L/hr/kg]), cardiac output (QCC [L/hr/kg]), tissue blood flows ($Q_i$ [fraction of QCC]), tissue volumes ($V_iC$ [fraction of body weight]), and are listed in Table 1.

TABLE 1. Physiological Parameters Used for PBPK Model Simulations

| Parameter | Description | Value | [Unit] |
|---|---|---|---|
| BW | Body weight | Measured | [kg] |
| QPC | Alveolar ventilation | 30.0 | [L/hr/kg] |
| QCC | Cardiac output | 16.5 | [L/hr/kg] |
| QGC | Blood flow to gut | 0.175 | [ratio] |
| QLC | Blood flow through hepatic artery | 0.24 - QGC | [ratio] |
| QFC | Blood flow to fat | 0.05 | [ratio] |
| QSC | Blood flow to slowly perfused tissues | 0.238 | [ratio] |
| QRC | Blood flow to rapidly perfused tissues | 0.472 | [ratio] |
| QKC | Blood flow to kidney | QRC - 0.252 | [ratio] |
| QUC | Urine flow | 0.0006 | [L/hr/kg] |
| QBILC | Bile flow | 0.00015 | [L/hr/kg] |
| VLC | Volume of liver | 0.05 | [ratio] |
| VFC | Volume of fat | 0.04 | [ratio] |
| VSC | Volume of slowly perfused tissues | 0.558 | [ratio] |
| VRC | Volume of rapidly perfused tissues | 0.031 | [ratio] |
| VGC | Volume of gut tissue | 0.033 | [ratio] |
| VKC | Volume of kidney | 0.018 | [ratio] |
| VBLD | Volume of blood | 0.06 | [ratio] |

Chloral hydrate and TCOH are very soluble in blood so their partitioning between blood and air could not be measured with the method employed. Therefore, their blood/air partition coefficients were estimated from solubilities of radioactive analogues of similar compounds reported in the literature. Similarly, partitioning of TCOG in bile, blood, and solid tissues, as well as renal clearance, were estimated from the glucuronide distribution data available in the literature (Garrett and Lambert, 1973). Partition coefficients of CH and TCOH in other tissues were measured in our laboratory (Seckel et al., 1995). These physicochemical parameters are listed in Table 2. Metabolic parameters and constants for conversions of CH to TCOH, TCOG, TCA, and DCA were initially estimated from the literature (Cabana and Gessner, 1970; Garrett and Lambert, 1973; Larson and Bull, 1992; Templin et al., 1993) and later, fitted and optimized with the PBPK model. The final set of metabolic parameters is listed in Table 3.

TABLE 2. Physicochemical Parameters Used for PBPK Model Simulations

| Parameter | Description | Value | [Unit] |
|---|---|---|---|
| | Partition coefficients for chloral hydrate | | |
| PCHB | Estimated blood/air | 500.0 | [ratio] |
| PCHL | Measured liver/blood | 1.47 | [ratio] |
| PCHF | Measured fat/blood | 0.48 | [ratio] |
| PCHS | Measured slowly perfused/blood | 1.35 | [ratio] |
| PCHR | Measured rapidly perfused/blood | 1.47 | [ratio] |
| PCHG | Measured gut/blood | 1.47 | [ratio] |
| | Partition coefficients for trichloroethanol | | |
| POHB | Estimated blood/air | 5000.0 | [ratio] |
| POHL | Measured liver/blood | 1.06 | [ratio] |
| POHF | Measured fat/blood | 1.53 | [ratio] |
| POHS | Measured slowly perfused/blood | 1.11 | [ratio] |
| POHR | Measured rapidly perfused/blood | 1.06 | [ratio] |
| POHG | Measured gut/blood | 1.06 | [ratio] |
| | Partition coefficients for trichloroethanol glucuronide | | |
| PCBIL | Estimated bile/blood | 70.0 | [ratio] |
| PCGBO | Estimated body/blood | 0.31 | [ratio] |
| CLGLUC | Estimated renal clarence | 0.65 | [L/hr/kg] |
| | Molecular weights | | |
| MWCH | Molecular weight CH | 147.5 | [g/mol] |
| MWTCOH | Molecular weight TCOH | 149.5 | [g/mol] |
| MWGLUC | Molecular weight TCOG | 325.4 | [g/mol] |
| MWTCA | Molecular weight TCA | 163.5 | [g/mol] |
| MWDCA | Molecular weight DCA | 129.0 | [g/mol] |

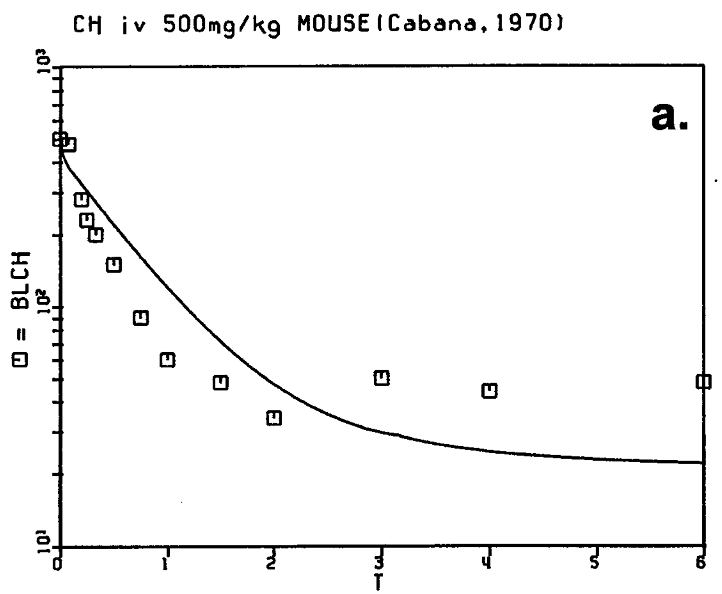
TABLE 3. Metabolic Parameters Used for PBPK Model Simulations

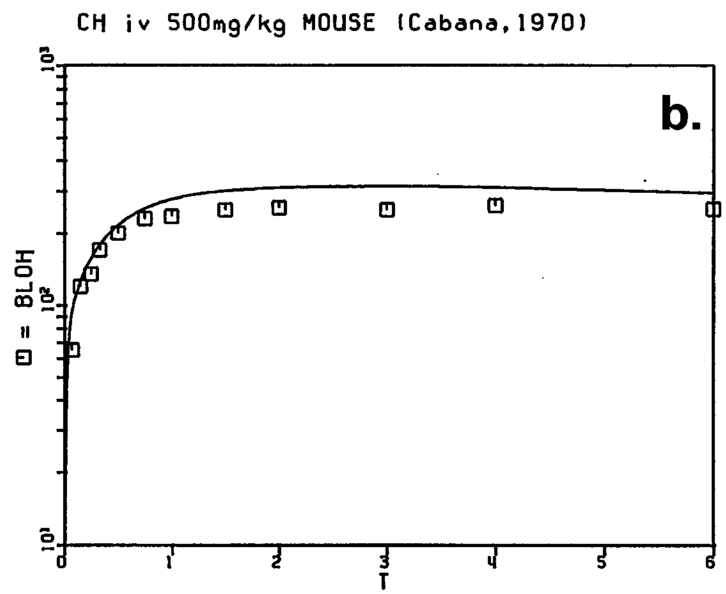
| Parameter | Description | Value | [Unit] |
|---|---|---|---|
| | First order metabolic constants | | |
| PCCOH | Conversion CH->TCOH | 15.0 | [1/hr/kg] |
| PCCA | Conversion CH->TCA | 0.02 | [1/hr/kg] |
| PCIDA | Conversion CH->DCA | 0.3 | [1/hr/kg] |
| KCHC | Metabolic loss of CH by blood | 1.04 | [1/hr/kg] |
| PCOA | Conversion TCOH->TCA | 3.5 | [1/hr/kg] |
| KOHDC | Metabolic loss of TCOH by blood | 7.9 | [1/hr/kg] |
| | Michaelis-Menten metabolic constants | | |
| VMCHOC | Pseudo-$V_{max}$ CH->TCOH | 3.23 | [mg/hr/kg] |
| VMTCOC | Pseudo-$V_{max}$ TCOH->TCOG | 15.0 | [mg/hr/kg] |
| KMCHOH | Apparent Michaelis constant CH->TCOH | 0.0675 | [mg/L] |
| KMTCOH | Apparent Michaelis constant TCOH->TCOG | 1.0 | [mg/L] |

## Experimental Calibration of PBPK Model

Before the experimental calibration in mice, the PBPK model was verified with available literature data. The results of PBPK model computer simulations of historical experimental data by Cabana and Gessner (1970) from mice treated i.v. with 500 mg of CH per kg, are shown in Figure 4. The data set for body levels of remaining CH, shown in Figure 4a, was used to adjust

# Pharmacokinetics of Chloral Hydrate in Mice



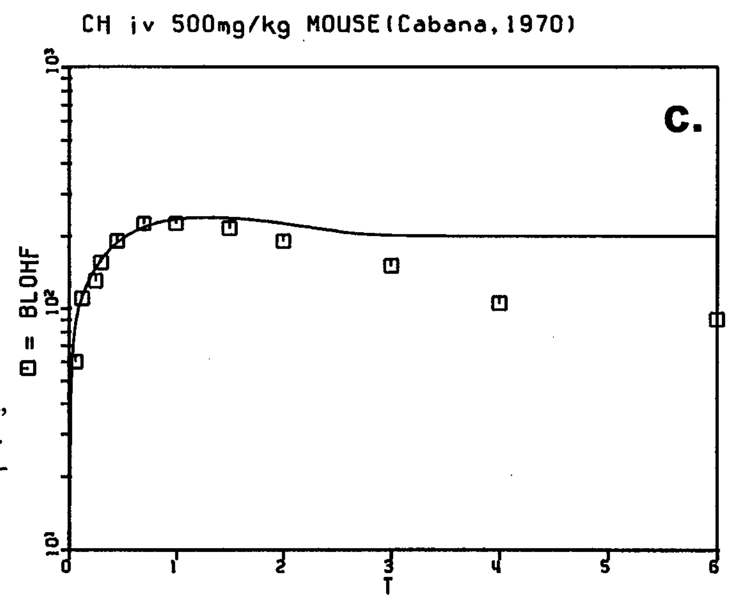




Figure 4. The results of PBPK model computer simulations of historical experimental data by Cabana and Gessner (1970) from mice, treated i.v. with 500 mg of chloral hydrate per kg (small boxes depict means of experimental data for each time-point; continuous lines are the PBPK model simulations).
a. Logarithm of body level of remaining chloral (BLCH [mg/kg]) over time (T [hr]).
b. Logarithm of body level of produced (remaining, glucuronidated, and excreted) trichloroethanol (BLOH [mg/kg]) over time (T [hr]).
c. Logarithm of body level of free (non - glucuronidated) trichloro - ethanol (BLOH [mg/kg]) over time (T [hr]).

initial PBPK model parameters. Once the parameters affecting directly rates of mass output from the CH sub-model (to TCOH, TCA, and DCA) were optimized to fit available literature data (Figure 4a; Larson and Bull, 1992; Templin et al., 1993), the other data from Cabana and Gessner (1970) for body levels of total TCOH (Figure 4b), and free (non-glucuronidated) TCOH (Figure 4c) were poorly predicted by the PBPK model with interlinking rate constants (PCCOH, VMCHOC and KMCHOH) optimized to fit experimental points from Figure 4a. Therefore, the coupling metabolic rate constants linking the CH and TCOH sub-models (affecting directly RAMCH) were estimated as a compromise between values giving statistically "the best" fit to experimental points from Figure 4a, b, and c. These values are listed in Table 3, and the results of computer simulation are plotted on a semi-logarithmic scale as continuous lines in Figures 4a, b, and c.

It was not possible to find in the literature a coherent experimental data set describing TCOH and TCOG distribution in mice, suitable for PBPK simulation. Because of experimental constraints, it was especially difficult to find experimental results on excretion and reabsorption from the bile in mice or other small rodents. However, Garrett and Lambert (1973) have published very detailed experimental data on distribution of TCOH and TCOG in dogs. The physiological parameters used for the dog were scaled allometrically from those presented in Table 1 for the mice. Figures 5 and 6 show the results of PBPK model computer simulations of these experimental data by Garrett and Lambert (1973) from dogs treated i.v. with TCOH or TCOG. The data set for TCOH blood concentrations in dogs treated with 30 mg/kg of TCOH, shown in Figure 5a, was used to adjust the initial model parameters. Thus, we have checked how well the PBPK model simulates distribution of a very high dose of TCOH (Figure 5b), and finally, we have adjusted initial model parameters for TCOG (Figure 6a). Once the parameters directly affecting rates of mass output from the TCOH sub-model (to TCA and TCOG) were optimized to fit the available literature data (Figure 5a), the other data set from Garrett and Lambert (1973) for simultaneous blood concentrations of TCOH (COHV [mg/L]), TCOG (CGB [mg/L]), and the concentration of TCOG in the bile (CGBIL [mg/L]) was simulated without changing the previously estimated parameters (Figure 6b). These parameters are listed in Table 3.

Using the complete set of physiological, physicochemical, and metabolic parameters calibrated with data from the literature (Tables 1, 2, and 3), we have simulated our own experimental data from B6C3F1 mice treated i.v. with 10 or 100 mg/kg of chloral hydrate or TCOH (Figure 7). Each experimental time-point corresponds to the sample collected from one animal.

## Pharmacokinetics of Trichloroethanol in Dogs

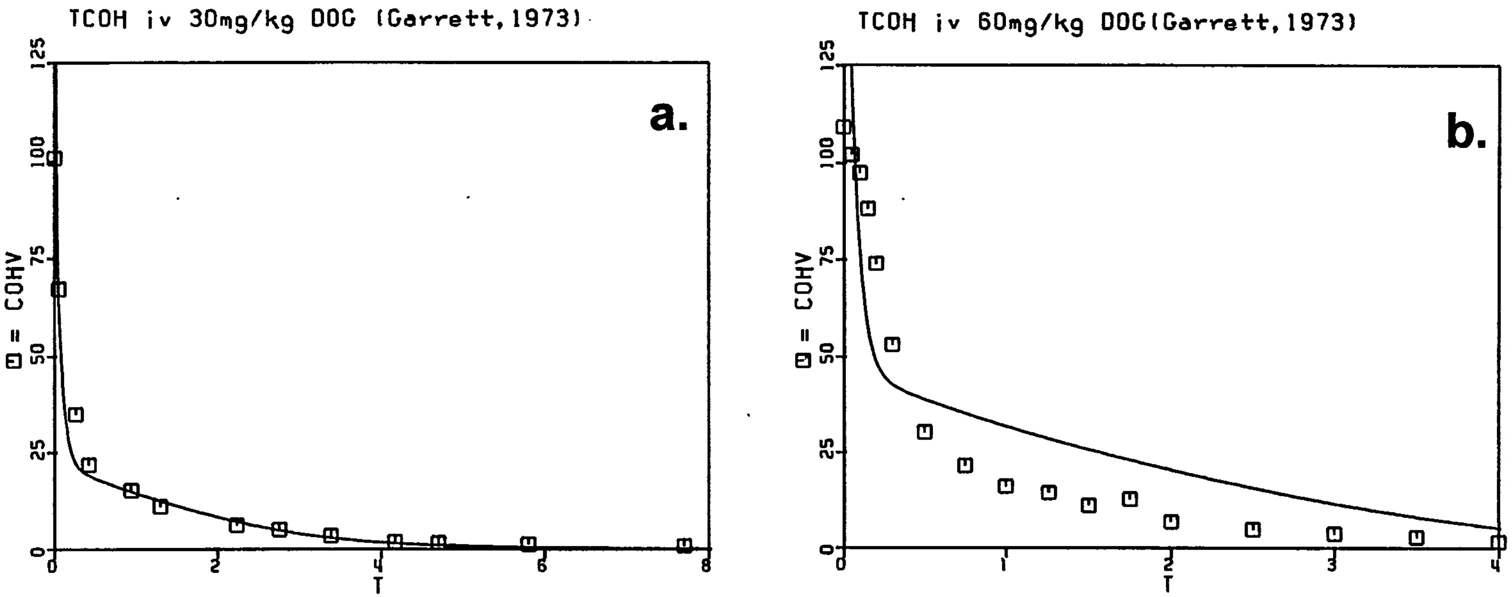



Figure 5. The results of PBPK model computer simulations of historical experimental data by Garrett and Lambert (1973) from dogs treated i.v. with two doses of trichloroethanol (30 and 60 mg of trichloroethanol per kg; small boxes depict means of experimental data for each time-point; continuous lines are the PBPK model simulations). Concentration of trichloroethanol in venous mixed blood (COHV [mg/kg]) over time (T [hr]):

a. After 30 mg of TCOH/kg.

b. After 60 mg/kg.

# Pharmacokinetics of TCOH Glucuronide in Dogs

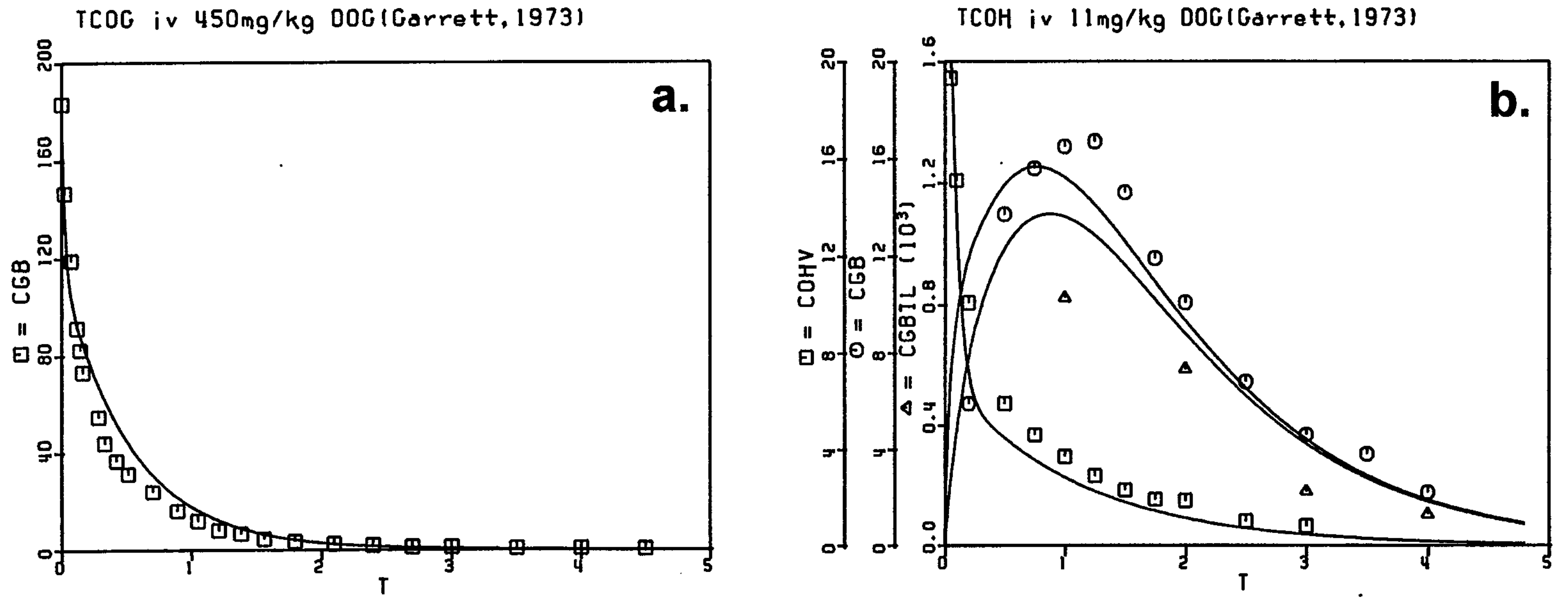



Figure 6. The results of PBPK model computer simulations of historical experimental data by Garrett and Lambert (1973) from dogs treated i.v. with glucuronide of trichloroethanol (TCOG; 450 mg/kg) or a low dose of trichloroethanol (11 mg of TCOH per kg; the symbols depict means of experimental data for each time-point; continuous lines are the PBPK model simulations).

a. Concentration of trichloroethanol glucuronide in venous mixed blood (CGB [mg/L]) over time (T [hr]).

b. Concentrations of trichloroethanol in venous mixed blood (COHV [mg/L]; rectangles), concentrations of trichloroethanol glucuronide in venous mixed blood (CGB [mg/L]; circles), concentrations of trichloroethanol glucuronide in the bile (CGBIL [mg/L]; triangles).

# Pharmacokinetics of Chloral and Trichloroethanol in Mice

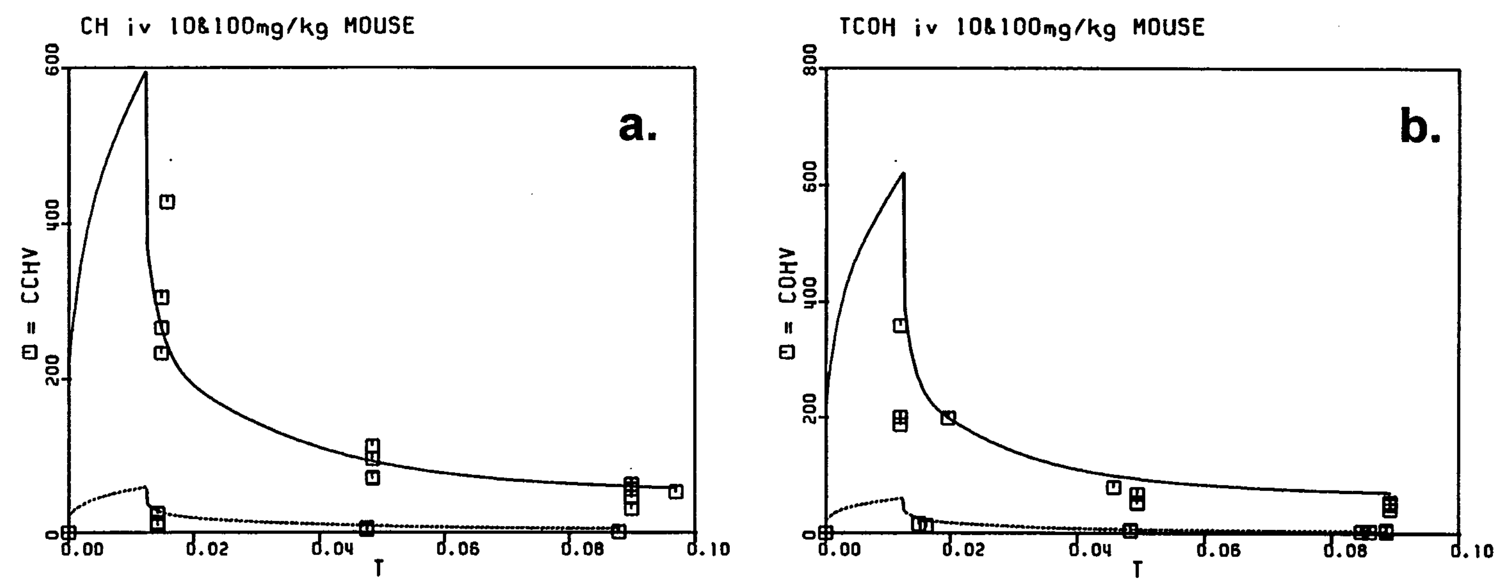



Figure 7. The results of PBPK model computer simulations of experimental data from our laboratory from mice treated i.v. with chloral hydrate or trichloroethanol (10 or 100 mg/kg). Each symbol depicts experimental datum collected from one animal (usually four different animals per time-point); continuous lines are the PBPK model simulations).

a. Concentration of chloral in venous mixed blood (CCHV [mg/L]) over time (T [hr]): after 100 mg of chloral hydrate per kg (upper curve) and 10 mg of chloral hydrate per kg (lower curve).

b. Concentration of trichloroethanol in venous mixed blood (COHV [mg/L]) over time (T [hr]): after 100 mg of trichloroethanol per kg (upper curve) and 10 mg of trichloroethanol per kg (lower curve).

## SECTION 4

## DISCUSSION

The developed interlinked PBPK model for CH and its metabolites represents an attempt to apply an "object oriented" programming strategy (Moniz Barreto et al., 1994) in PBPK modeling. The main model consisted of three sub-models for CH, TCOH, and TCOG. Each sub-model was calibrated separately (as the separate programming "object"), validated with experimental data, and may be used to simulate distribution of each metabolite (Figures 4a, 5a, 5b, 6a, 7a, and 7b). When linked by rates of mass transfer between sub-models, the interlinked PBPK model was validated for sequential production and distribution of the metabolic products resultant from the previous metabolite, and may be used to simulate distribution of each metabolite derived from the parent compound (Figures 4b, 4c, and 6b). This approach may be especially useful for pharmacokinetic exposure characterization and risk assessment when metabolic products are more toxic than their parent compound. This seems to be the case with TCE and CH (Davidson and Beliles, 1991). The existing PBPK models for TCE, described in the literature (Andersen et al., 1987; Dallas et al., 1991), addressed only the distribution of the parent compound (Buben and O'Flaherty, 1985). The PBPK model developed by Fisher et al. (1989; 1990; 1991), however, described also the production of TCE metabolite, TCA. Our model was based on more detailed mechanistic information about production and disposition of intermediate metabolites, CH, TCOH, and TCOG, which have distinct pharmacodynamic properties, different than TCA (Davidson and Beliles, 1991). Thus, our attempt to interlink the sub-models into one functional PBPK model describing the production of several metabolites, may be considered as a novel but consequent endeavor in a pharmacokinetic description of TCE and its main metabolites.

Each of our sub-models was constructed in accordance with the conventional flow rate limited PBPK modeling routine (Yang and Andersen, 1994) and could be allometrically scaled according to the body weight of the animal. As demonstrated in Figures 4b, 5a, and 7b, this allowed us to describe successfully the pharmacokinetics of TCOH in as distant animal species as a mouse and a dog. Obviously, the pharmacokinetic model may be only as good as are the data used for its calibration and validation. Since we were unable to identify in the available literature a coherent data set for pharmacokinetics of chloral hydrate and its main metabolites in humans, it is still to be proved that this model can also adequately describe the distribution of CH in men. On the other hand, PBPK models based on the same mathematical and physiological principles were already used successfully to estimate the exposure to other chemicals in humans under controlled (e.g., Byczkowski and Fisher, 1994) and environmental scenarios (e.g., Fisher and Allen, 1993; Byczkowski and Fisher, 1995). Therefore, it seems that once adequately validated in humans, the developed PBPK model might be applied not only for a computer-aided simulation of body levels of chloral hydrate in a therapeutic situation, but also for the estimate of toxicokinetics of its active metabolites generated during the environmental pollution scenario.

## ACKNOWLEDGMENTS

Part of this material was presented at the Society of Toxicology 34th Annual Meeting Baltimore, MD, in 1995.

This work was performed under Department of the Air Force Contract No. F33615-90-C-0532 and supported in part by SERDP Project No. 4223OT01.

The authors gratefully acknowledge the expert assistance of M. Freedman and P. Parish.

## SECTION 5

## REFERENCES

Andersen, M.E., M.L. Gargas, H.J. Clewell, and K.M. Severyn. 1987. Quantitative evaluation of the metabolic interactions between trichloroethylene and 1,1-dichloroethylene in vivo using gas uptake methods. Toxicol. Appl. Pharmacol. 89:149-157.

Arms, A.D., and C.C. Travis. 1988. Reference physiological parameters in pharmacokinetic modeling. EPA Report EPA/600/6-88/004, Washington, DC.

Buben, J.A. and E.J. O'Flaherty. 1985. Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: a dose-effect study. Toxicol. Appl. Pharmacol. 78:105-122.

Butler, T.C. 1948. The metabolic fate of chloral hydrate. J. Pharmacol. Exptl. Therap. 92:49-58.

Byczkowski, J.Z. and J.W. Fisher. 1994. Lactational transfer of tetrachloroethylene in rats. Risk Analysis 14:339-349.

Byczkowski, J.Z. and J.W. Fisher. 1995. A computer program linking physiologically based pharmacokinetic model with cancer risk assessment for breast-fed infants. Computer Meth. Progr. Biomed. 46:155-163.

Cabana, B.E. and P.K. Gessner. 1970. The kinetics of chloralhydrate metabolism in mice and the effect thereon of ethanol. J. Pharmacol. Expl. Therapeut. 174:260-275.

Cole, W.J., R.G. Mitchell, and R.F. Salamonsen. 1975. Isolation, characterization and quantitation of chloral hydrate as a transient metabolite of trichloroethylene in man using electron capture gas chromatography and mass fragmentography. J. Pharm. Pharmac. 27:167-171.

Dallas, C.E., J.M. Gallo, R. Ramanathan, S. Muralidhara, and J.V. Bruckner. 1991. Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats. Toxicol. Appl. Pharmacol. 110:303-314.

Daniel, F.B., A.B. DeAngelo, J.A. Stober, G.R. Olson, and N.P. Page. 1992. Hepatocarcinogenicity of chloral hydrate, 2-chloroacetaldehyde, and dichloroacetic acid in the male B6C3F1 mouse. Fund. Appl. Toxicol. 19:159-168.

Davidson, I.W.F. and R.P. Beliles. 1991. Consideration of the target organ toxicity of trichloroethylene in terms of metabolite toxicity and pharmacokinetics. Drug Metabol. Rev. 23: 493-599.

Dekant, W., M. Metzler, and D. Henschler. 1984. Novel metabolites of trichloroethylene through dechlorination reactions in rats, mice, and humans. Biochem. Pharmacol. 33:2021-2027.

Fisher, J.W. and B.C. Allen. 1993. Evaluating the risk of liver cancer in humans exposed to trichloroethylene using physiological models. Risk Analysis 13:87-95.

Fisher, J.W., M.L. Gargas, B.C. Allen and M.E. Andersen. 1991. Physiologically based pharmacokinetic modeling with trichloroethylene and its metabolite, trichloroacetic acid, in the rat and mouse. Toxicol. Appl. Pharmacol. 109:183-195.

Fisher, J.W., T.A. Whittaker, D.H. Taylor, H.J. Clewell,III, and M.E. Andersen. 1989. Physiologically based pharmacokinetic modeling of the pregnant rat: a multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. Toxicol. Appl. Pharmacol. 99:395-414.

Fisher, J.W., T.A. Whittaker, D.H. Taylor, H.J. Clewell,III, and M.E. Andersen. 1990. Physiologically based pharmacokinetic modeling of the lactating rat and nursing pup: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. Toxicol. Appl. Pharmacol. 102:497-513.

Garrett, E.R. and H.J. Lambert. 1973. Pharmacokinetic of trichloroethanol and metabolites and interconversions among variously referenced pharmacokinetic parameters. J. Pharm. Sci. 62: 550-572.

Goth, A. 1964. Medical Pharmacology. Second Edition. pp. 221-222, The C.V. Mosby Co., Saint Louis.

Jepson, G.W., D.K. Hoover, R.K. Black, J.D. McCafferty, D.A. Mahle, and J.M. Gearhart. 1994. A partition coefficient method for non-volatile and intermediate volatility chemicals in biological tissues. Fund. Appl. Toxicol. 22:519-524.

Ketcha, M.M., D.A. Warren, C.T. Bishop, and W.T. Brashear. 1995. Factors influencing the conversion of trichloroacetic acid to dichloroacetic acid in biological matrices. Toxicologist 15: 266(1428).

Larson, J.L. and R.J. Bull. 1992. Metabolism and lipoperoxidative activity of trichloroacetate and dichloroacetate in rats and mice. Toxicol. Appl. Pharmacol. 115:268-277.

Larson, J.L. and R.J. Bull. 1992a. Species differences in the metabolism of trichloroethylene to the carcinogenic metabolites trichloroacetate and dichloroacetate. Toxicol. Appl. Pharmacol. 115:278-285.

Leibreich, O. 1869. Das Chloralhydrat, ein neues Hypnoticumund Anaesteticum, Otto Muller's Verlag, Berlin.

MacKay, F.J. and J.R. Cooper. 1962. A study of the hypnotic activity of chloral hydrate. J. Pharmacol. Exptl. Therap. 135:271-274.

Mitchell and Gauthier Associates. 1987. Advanced Continuous Simulation Language (ACSL) Reference Manual. Concord MA.

Moniz Barreto, J.P.C., J.H. Woods, and D.A. Fell. 1994. Object-oriented biochemical simulation: a Monte Carlo method is applied to systems of dioxygen free radical reactions. Modern Trends Biothermokinet. 3:115.

Muller, G., M. Spassovski, and D. Henschler. 1974. Metabolism of trichloroethylene in man. Arch. Toxicol. 32:283-295.

Reynolds, E.S. and M.T. Moslen. 1981. Metabolic activation and hepatotoxicity of trichloroethylene. Adv. Exp. Med. Biol. 136(Part A):693-701.

Seckel, C.S., J.R. Creech, B.L. Garrity, R.K. Black, and J.Z. Byczkowski. 1995. Tissue solubility and kinetics for metabolites of trichloroethylene - chloral and trichloroethanol. Toxicologist 15:198(1059).

Steiner, E.C., T.D. Rey, and P.S. McCroskey. 1990. Reference Guide SIMUSOLV Modeling and Simulation Software. DOW Chemical Co., Midland, MI.

Templin, M.V., J.C. Parker, and R.J. Bull. 1993. Relative formation of dichloroacetate and trichloroacetate from trichloroethylene in male B6C3F1 mice. Toxicol. Appl. Pharmacol. 123: 1-8.

Waters, E.M., H.B. Gerstner, and J.E. Huff. 1977. Trichloroethylene I. An overview. J. Toxicol. Environ. Hlth. 2:671-707.

Yang, R.S.H. and M.E. Andersen. 1994. Pharmacokinetics. In: Introduction to Biochemical Toxicology (Hodgson, E., and P.E. Levi, Eds.). pp. 49-73, Appleton & Lange, Norwalk, CN.

## APPENDIX

Source Codes of BBPD Model Written in ACSL. The *.CSL and *.CMD files should be executed under SIMUSOLV.

*.CSL File

```
PROGRAM: INTERLINKED PBPK MODEL - CHLORAL AND ITS MAIN METABOLITES -
'NOTES: codes by Janusz Z. Byczkowski 03/04/94,revs.09/12/94,02/07/95  '
'---------------------------------------------------------------------'
     '1) ooPBPK CH AND ITS METABOLISM SUB-MODEL - DATA FROM MOUSE,      '
     '   Janusz Z. Byczkowski 02/14/94, rev. 02/07/95, verified with    '
     '   historical data: mouse i.v.- Cabana & Gessner (1970) 06/28/94,'
     '   calibrated: mouse i.v.- Seckel et al. 10/20/94,                '
     '   PCs from mouse: 08/30/94, and Seckel et al. 10/20/94           '
     '2) ooPBPK TCOH AND ITS METABOLISM SUB-MODEL - DATA FROM DOG       '
     '   AND MOUSE,                                                     '
     '   Janusz Z. Byczkowski 02/16/94, rev. 02/07/95, verified with    '
     '   historical data: dog i.v.- Garrett & Lambert (1973), 04/05/94 '
     '   calibrated: mouse i.v.- Seckel et al. 11/17/94,                '
     '   PCs from mouse 08/31/94, and Seckel et al. 10/20/94            '
     '3) ooPBPK TCOG SUB-MODEL - DATA FROM DOG, AND MOUSE,              '
     '   Janusz Z. Byczkowski 02/18/94, rev. 02/07/95, verified with    '
     '   historical data: dog i.v. - Garrett & Lambert (1973), 04/20/94'
     '   calibrated: mouse i.v.- Seckel et al. 11/17/94,                '
     '   PCs for TCOG estimated from dog 04/25/94                       '
     '   OUTPUT OF TCA and DCA verified with historical data: mouse     '
     '   Larson & Bull (1992) and Templin et al. (1993) 09/12/94.       '
INITIAL
     'SCALED TO MICE                                                    '
LOGICAL    CC         $'Flag set to .TRUE. for closed chamber runs      '

' ***      PHYSIOLOGICAL PARAMETERS                                     '

'ANIMAL PARAMETERS                                                      '
CONSTANT   BW = 0.03  $'Body weight (kg)                                '
CONSTANT   QPC= 30.   $'Alveolar ventilation rate (l/hr)                '
CONSTANT   QCC= 16.5  $'Cardiac output (l/hr)                           '
CONSTANT   QGC= 0.175 $'Fractional blood flow to gut                    '
       QLC=0.24-QGC   $'Fractional blood flow through hepatic artery    '
CONSTANT   QFC= 0.05  $'Fractional blood flow to fat                    '
CONSTANT   QSC= 0.238 $'Fractional blood flow to slow                   '
CONSTANT   QRC= 0.472 $'Fractional blood flow to rapid                  '
CONSTANT   QUC= 0.0006$'Urine flow (L/hr*kg-1)                          '
CONSTANT QBILC=0.00015$'Bile flow (L/hr*kg-1)                           '
       QKC=QRC-0.252  $'Fractional blood flow to kidneys                '

CONSTANT   VLC= 0.05  $'Fraction liver tissue                           '
CONSTANT   VFC= 0.10  $'Fraction fat tissue, (0.04 MALE)                '
CONSTANT   VGC= 0.033 $'Fraction gut tissue                             '
CONSTANT   VSC= 0.558 $'Fraction slow tissue                            '
CONSTANT   VRC= 0.031 $'Fraction rapid tissue                           '
CONSTANT   VKC= 0.018 $'Fraction kidney tissue                          '
CONSTANT  VBLD= 0.06  $'Est. fraction venous + arterial blood (1+1)     '

'                     PARTITION COEFFICIENTS                            '
'PCs FOR CH                                                             '
CONSTANT PCHL = 1.47  $'Liver/blood partition coefficient, CH           '
CONSTANT PCHF = 0.48  $'Fat/blood partition coefficient, CH             '
CONSTANT PCHS = 1.35  $'Slowly perfused tissue/blood partition, CH      '
```

```
CONSTANT PCHR = 1.47  $'Richly perfused tissue/blood partition, CH     '
CONSTANT PCHB =500.   $'Blood/air partition coefficient, CH            '
CONSTANT PCHG = 1.47  $'Gut/blood partition, CH                        '
'PCs FOR TCOH                                                           '
CONSTANT POHL = 1.06  $'Liver/blood partition coefficient, TCOH        '
CONSTANT POHF = 1.525 $'Fat/blood partition coefficient, TCOH          '
CONSTANT POHS = 1.11  $'Slowly perfused tissue/blood partition, TCOH   '
CONSTANT POHR = 1.06  $'Richly perfused tissue/blood partition, TCOH   '
CONSTANT POHB =5000.  $'Blood/air partition coefficient, TCOH          '
CONSTANT POHG = 1.06  $'Gut/blood partition, TCOH                      '
CONSTANT CLGLUC=0.65  $'Renal clarence of GLUC (L/hr*kg-1)             '
CONSTANT PCBIL =  70. $'Bile/blood partition, TCOGLUC                  '
CONSTANT PCGBO = 0.31 $'Body/blood partition, TCOGLUC                  '

'                     MOLECULAR WEIGHTS                                 '
CONSTANT MWCH   =147.5 $'Molecular weight CH (g/mol)                    '
CONSTANT MWTCOH =149.5 $'Molecular weight TCOH (g/mol)                  '
CONSTANT MWGLUC =325.4 $'Molecular weight TCOG (g/mol)                  '
CONSTANT MWTCE  =131.5 $'Molecular weight TCE (g/mol)                   '
CONSTANT MWTCA  =163.5 $'Molecular weight TCA (g/mol)                   '
CONSTANT MWDCA  =129.  $'Molecular weight DCA (g/mol)                   '

'                     EXPOSURE PARAMETERS                               '
CONSTANT   CC =.false.$'Default to open chamber                         '
CONSTANT NRATS= 4.    $'Number of mice (for closed chamber)             '
CONSTANT  KLC = 0.05  $'First order loss rate from closed chamber (/hr)'
CONSTANT VCHC = 0.75  $'Volume of closed chamber (L)                    '
CONSTANT SODA = 0.005 $'Volume of soda lime (L)                         '
'CH DOSE                                                                '
CONSTANT PDOSCH= 0.   $'Oral dose CH (mg/kg)                            '
CONSTANT  KACH = 1.   $'Oral uptake rate CH (/hr)                       '
CONSTANT IVDCH = 0.   $'IV dose CH (mg/kg)                              '
CONSTANT  COCH = 0.   $'Concentration of inhaled CH in air (mg/l)       '
'TCOH DOSE                                                              '
CONSTANT PDOSOH= 0.   $'Oral dose TCOH (mg/kg)                          '
CONSTANT  KAOH = 1.   $'Oral uptake rate TCOH (/hr)                     '
CONSTANT KAOHBI= 2.   $'Gut uptake rate from bile (/hr)                 '
CONSTANT IVDOH = 0.   $'IV dose TCOH (mg/kg)                            '
CONSTANT DOSEG = 0.   $'IV dose of glucuronide (mg)                     '
'***  Exposure definition                                               '
IF (CC) RATS = NRATS                  $'Closed chamber simulation       '
IF (CC) KL = KLC
IF (.NOT.CC) RATS = 0.                  $'Open chamber simulation       '
IF (.NOT.CC) KL = 0.
IF (.NOT.CC) SODA = 0.
'(Turn off chamber losses so concentration remains constant)            '
      VCH = VCHC-RATS*BW-SODA              $'Net chamber volume (L)     '
      AICHO = COCH*VCH*MWCH/24450. $'Initial amount CH in chamber (mg) '

IF (PDOSCH.EQ.0.) KACH = 0.                     $'Parenteral CH dosing '
IF (PDOSOH.EQ.0.) KAOH = 0.                     $'Parenteral TCO dosing'

'                     METABOLISM PARAMETERS                             '
'CH METABOLISM                                                          '
CONSTANT RAM   = 0.   $'Proforma rate of TCE->CH metabolism             '
CONSTANT PCCH = 0.999 $'TCE that is converted to CH (%)E-2              '
CONSTANT PCCOH = 27.  $'Fst order constant CH->TCOH (1/hr-1kg)          '
CONSTANT VMCHOC= 0.46 $'Max Velocity CH->TCOH (mg/hr-1kg)               '
CONSTANT KMCHOH= 247. $'Michaelis-Menten const. for CH->TCOH (mg/L)     '
CONSTANT PCCA  = 0.02 $'Fst.order constant CH->TCA (1/hr-1kg)           '
CONSTANT PCIDA = 0.3  $'Fst.order constant CH->DCA (1/hr-1kg)           '
CONSTANT KCHC  = 2.0  $'first order CH loss-metabol. by blood (/hr/kg) '
```

```
'TCOH METABOLISM                                                           '
CONSTANT VMTCOC=45.0  $'Max. velocity of TCOH->TCOG (mg/h/kg)              '
                      'DOG VmTCOC=24.6                                     '
CONSTANT KMTCOH=30.   $'Michaelis-Menten const. for TCOH->TCOG (mg/L)      '
                      'DOG KmTCOH=10.                                      '
CONSTANT  PCOA = 3.5  $'Fst.order constant TCOH->TCA (1/hr-1kg)            '
CONSTANT KOHDC = 5.   $'first order OH loss-metabol. by blood (/hr/kg)     '

'***  Timing commands                                                      '
CONSTANT  DAYS  = 2.    $'SIMULATION DURATION (days)                       '
CONSTANT  TCHNG = 4.0   $'Length of inhalation exposure (hrs)              '
CONSTANT   TINF = 0.01  $'Length of IV infusion (hrs)                      '
CONSTANT POINTS = 500.  $'Number of points in plot                         '
CONSTANT   TGAV = 0.01  $'Length of gavage infusion (hr)                   '
'constant    cint=0.05'

'***  Scaled parameters                                                    '

       QC = QCC*BW**0.74
       QP = QPC*BW**0.74
       QL = QLC*QC
       QF = QFC*QC
       QS = 0.24*QC-QF          $'Values reset as % of slow tissues        '
       QG = QGC*QC
       QR = 0.76*QC-QL-QG       $'Values reset as % of rapid tissues       '
       QK = QR-0.252*QC         $'Fractional blood flow to kidneys         '
                                 'MUST be subtracted from QR               '
       QB = QC-(QL+QG)          $'Blood flow through body except liver     '
       QLB= QL+QG               $'Total flow of blood through liver(L/h)   '
       QU = QUC*BW              $'Flow of urine (L/hr)                     '
      QBIL= QBILC*BW            $'Flow of bile (L/hr)                      '

       VL = VLC*BW
       VF = VFC*BW              $'Volume fat tissue (kg)                   '
       VS = 0.82*BW-VF          $'Values reset as % of slow tissues        '
       VG = VGC*BW
       VR = 0.101*BW-VL-VG      $'Values reset as % of rapid tissues       '
       VK = VKC*BW
       VB = BW-VL               $'Volume of body except liver (kg)         '
    VBLOOD= VBLD*BW             $'Est. volume of venous + arterial blood'

     CLGLU= CLGLUC*BW           $'Renal clarence of TCOG (L/hr)            '
   VMTCOH = VMTCOC*BW**0.7      $'Velocity TCOH->TCOG (mg/hr)              '
     PCOTA= PCOA/BW**0.3        $'Fst.order TCOH->TCA (1/h/mouse)          '
    PCTCOH= PCCOH/BW**0.3       $'Fst.order CH->TCOH (1/h/mouse)           '
    VMCHOH= VMCHOC*BW**0.7      $'Velocity 2nd ord. CH->TCOH (mg/hr)       '
     PCTCA= PCCA/BW**0.3        $'Fst.order CH->TCA (1/h/mouse)            '
      PIDA= PCIDA/BW**0.3       $'Fst.order CH->DCA (1/h/mouse)            '
     IVCHR= IVDCH*BW/TINF       $'Speed IV CH infusion (mg/h)              '
     DOSCH= PDOSCH*BW           $'p.o. dose CH per mouse (mg)              '
     IVOHR= IVDOH*BW/TINF       $'Speed IV TCOH infusion (mg/h)            '
     DOSOH= PDOSOH*BW           $'p.o. dose TCOH per mouse (mg)            '

     KCHD = KCHC/BW**0.3        $'Fst order loss rates (1/h) per mouse     '
     KOHD = KOHDC/BW**0.3

     ' repeated gavage dosing'
INTEGER DAY
       tstop= days*24.
       CINT = tstop/points
       DAY=3.  $'TO START GAVAGE ON MONDAY -1, TUES 0, WEDN 1, ETC.        '
```

```
END     $'End of initial'

DYNAMIC

'***   REPEATED GAVAGE DOSING                                                   '
       'GAV = FEED MICE p.o. YES=1, NO=0.                                       '
DISCRETE CAT1
        INTERVAL CAT = 24.          $'EXECUTE CAT1 EVERY 24 hr                 '
                 DAY=DAY+1
        IF(MOD(DAY,7).GE.5) GOTO OUT
                 GAV = 1.           $'GAVAGE = YES                             '
        SCHEDULE CAT2 .AT. T+TGAV $'SCHEDULE END OF GAVAGE                     '
                 OUT.. CONTINUE
END     $'END OF CAT1'
DISCRETE CAT2
                 GAV = 0.           $'GAVAGE = NO                              '
END     $'END OF CAT2                                                          '

ALGORITHM IALG = 2  $'Gear method for stiff systems                            '
'                     If program hangs-up at long T with low mass input'
'                     change to IALG = 9 at .CSL file (it will use a    '
'                     plenty of computer time to execute).              '
'            It may also help to set during the run time -              '
'                     S DPSITG=.TRUE. - at .CMD file                    '
DERIVATIVE
     '+++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++'
'-----------------------------------------------------------------'
' 1)             ooPBPK SUB-MODEL FOR CHLORAL (CH)                '
'-----------------------------------------------------------------'

      'CICH = Concentration of CH in inhaled air (mg/l)                        '
   CIZONE = RSW((T.LT.TCHNG).OR.CC,1.,0.)
       RAICH= RATS*QP*(CCHA/PCHB-CICH) - (KL*AICH)
        AICH= INTEG(RAICH,AICH0)
        CICH= AICH/VCH*CIZONE
        CPCH= CICH*24450./MWCH
      RINHCH= CICH*QP
      AINHCH= INTEG(RINHCH,0.)

      'CCHA = Concentration of CH in arterial blood (mg/l)                     '
       CCHA = (QC*CCHV+QP*CICH)/(QC+(QP/PCHB))
     AUCCHB = INTEG(CCHA,0.)

      'ACHX = Amount of CH exhaled (mg)                                        '
       CCHX = CCHA/PCHB
     CHXPPM = (0.7*CCHX+0.3*CICH)*24450./MWCH
      RACHX = QP*CCHX
       ACHX = INTEG(RACHX,0.)

      'ACHG = AMOUNT OF CH IN GUT/MOUSE (mg)                                   '
      RACHG = QG*(CCHA-CVCHG)                  $'PARENTERAL ADMINISTRATION '
        ACHG= INTEG(RACHG,0.)
       CVCHG= ACHG/(VG*PCHG)
        CCHG= ACHG/VG
      'single gavage dosing                                                    '
      RMRCH = -KACH*MRCH
       MRCH = DOSCH*EXP(-KACH*T)  $'AMOUNT REMAINING IN STOMACH (mg)          '
      RAOCH = KACH*MRCH
       AOCH = DOSCH-MRCH          $'TOTAL MASS INPUT FROM STOMACH (mg)        '

      'ACHS = Amount of CH in slowly perfused tissues (mg)                     '
      RACHS = QS*(CCHA-CVCHS)
```

```
        ACHS = INTEG(RACHS,0.)
       CVCHS = ACHS/(VS*PCHS)
        CCHS = ACHS/VS

       'ACHR = Amount of CH in rapidly perfused tissues (mg)            '
       RACHR = QR*(CCHA-CVCHR)
        ACHR = INTEG(RACHR,0.)
       CVCHR = ACHR/(VR*PCHR)
        CCHR = ACHR/VR

       'ACHF = Amount of CH in fat tissue (mg)                          '
       RACHF = QF*(CCHA-CVCHF)
        ACHF = INTEG(RACHF,0.)
       CVCHF = ACHF/(VF*PCHF)
        CCHF = ACHF/VF

'ACHL = amount of CH produced in liver per mouse                        '
'metabolism of TCE-->INTERMEDIATE-->CH. Production of intermediate      '
'assumed to be Michaelis-Menten type. Decay of intermediate to yield    '
'CH in liver assumed to be a first order reaction.                      '
        RCH   = ram*PCCH*(MWCH/MWTCE)        $'RATE CONVERSION TCE->CH  '

        'ACHL = AMOUNT OF CH REMAINING IN LIVER/MOUSE (mg)              '
     RCHL=QL*(CCHA-CVCHL)+QG*(CVCHG-CVCHL)+RAOCH+RCH-RKCH-RAMCH-RTA-RIDA
        ACHL  = integ(RCHL,0.)          $'AMOUNT OF CH IN LIVER/MOUSE(mg) '
        CVCHL = ACHL/(VL*PCHL)      $'LIVER VENOUS BLOOD CONCENTRATION CH '
        CCHL  = ACHL/VL                 $'LIVER CONCENTRATION CH   (mg/L) '
        RKCH  = KCHD*CVCHL*VL       $'FST ORDER LOSS RATE-NONSPECIFIC BIND'
     'RATE METABOLIZED TO TCOH (mg/hr): Michaelis-Menten + First order '
        RAMCH =(VMCHOH*CVCHL)/(KMCHOH+CVCHL) + PCTCOH*CVCHL*VL
         RTA  = CVCHL*VL*PCTCA      $'RATE METABOLIZED TO TCA (mg/hr)     '
        RIDA  = CVCHL*PIDA*VL       $'RATE METABOLIZED TO DCA (mg/hr)     '
        AKCH = INTEG(RKCH,0.)
        AMCH = INTEG(RAMCH,0.)
         ATA = INTEG(RTA,0.)
        AIDA = INTEG(RIDA,0.)
       TOTCH = integ(RCH,0.)        $'TOTAL AMOUNT OF CH PRODUCED/MOUSE(mg)'
       BWCH  = TOTCH/BW
       MCH   = BWCH/MWCH            $'MILIMOLES CH PRODUCED (mmoles/kg)    '

       'IVCH = CH Intravenous infusion rate (mg/hr)                     '
        IVCH = IVCHR*(1.-step(tinf))

       'CCHV = Mixed venous blood concentration CH (mg/L)               '
        CCHV = (QF*CVCHF+ (QG+QL)*CVCHL+ QS*CVCHS+ QR*CVCHR+ IVCH)/QC

       'BALANCE OF CH IN MOUSE                                          '
        CHBAL=ACHF+ACHL+ACHS+ACHR+ACHG+AMCH+ACHX+ATA+AKCH+AIDA
       '                                                AMT IN MOUSE (mg)'
        BLCH =(ACHF+ACHL+ACHS+ACHR+ACHG+AKCH)/BW
                                    'BODY LEVEL OF REMAINING CH (MG/KG)'
        CHIN =IVCHR*TINF+AINHCH+AOCH+TOTCH    $'DOSE RECEIVED (mg/mouse)'
'-----------------------------------------------------------------------'
'                 *** END OF CH PROGRAM ***                             '

'-----------------------------------------------------------------------'
' 2)    ooPBPK SUB-MODEL FOR TRICHLOROETHANOL METABOLITE (TCOH)         '
'-----------------------------------------------------------------------'

       'COHA = Concentration of TCOH in arterial blood (mg/l)           '
        COHA = (QC*COHV)/(QC+(QP/POHB))
     AUCOHB = INTEG(COHA,0.)
```

```
       'AOHX = Amount of TCOH exhaled (mg)                                  '
        COHX = COHA/POHB
      OHXPPM = (0.7*COHX)*24450./MWTCOH
       RAOHX = QP*COHX
        AOHX = INTEG(RAOHX,0.)

       'AOHG = AMOUNT OF TCOH IN GUT/MOUSE (mg)                             '
       RAOHG = QG*(COHA-CVOHG)              $'PARENTERAL ADMINISTRATION '
         AOHG= INTEG(RAOHG,0.)
        CVOHG= AOHG/(VG*POHG)
         COHG= AOHG/VG

       'single gavage dosing                                                '
       'MROH = AMOUNT REMAINING IN GUTS (mg)                                '
        MROH = DOSOH *EXP(-KAOH*T)
       RMROH = -KAOH*MROH
       RAOOH = KAOH*MROH
       'TOTAL MASS INPUT FROM STOMACH (mg)                                  '
        AOOH = DOSOH - MROH

       'AOHS = Amount of TCOH in slowly perfused tissues (mg)               '
       RAOHS = QS*(COHA-CVOHS)
        AOHS = INTEG(RAOHS,0.)
       CVOHS = AOHS/(VS*POHS)
        COHS = AOHS/VS

       'AOHR = Amount of TCOH in rapidly perfused tissues (mg)              '
       RAOHR = QR*(COHA-CVOHR)
        AOHR = INTEG(RAOHR,0.)
       CVOHR = AOHR/(VR*POHR)
        COHR = AOHR/VR

       'AOHF = Amount of TCOH in fat tissue (mg)                            '
       RAOHF = QF*(COHA-CVOHF)
        AOHF = INTEG(RAOHF,0.)
       CVOHF = AOHF/(VF*POHF)
        COHF = AOHF/VF

     'TOTCOH = amt. of TCOH produced in liver                               '
'metabolism of CH-->TCOH->TCOG. TCOH production in liver                    '
'assumed to be a first order, and glucuronidation                           '
'is a Michaelis-Menten type, not-limited by UDPGA concentration             '
       RTCOH =CVCHL*VL*PCTCOH*(MWTCOH/MWCH) $'RATE CONVERSION  CH->TCOH '

       'Rate TCOH remaining in liver (mg/hr)                                '
    RTOHL=QL*(COHA-CVOHL)+QG*(CVOHG-CVOHL)+RAOOH+RTCOH-RKTCOH-RGLUC...
          +RAGLUC-RAMOH
   ATCOHL= integ(RTOHL,0.)          $'AMOUNT OF TCOH IN LIVER/MOUSE(mg)'
   CVOHL = ATCOHL/(VL*POHL)      $'LIVER VENOUS BLOOD CONCENTRATION OH '
   CTCOHL= ATCOHL/VL                $'LIVER CONCENTRATION TCOH (mg/L)   '
      RKTCOH = KOHD*CVOHL*VL     $'F-ST ORDER LOSS RATE-NONSPECIFIC BIND'
       RAMOH = CVOHL*PCOTA*VL    $'RATE METABOLIZED TO TCA (mg/hr)      '
        AMOH = INTEG(RAMOH,0.)
      AKTCOH= INTEG(RKTCOH,0.)
'---------------------------------------------------------------------------'
' 3) ooPBPK SUB-MODEL FOR GLUCURONIDE (TCOG) IN LIVER, BILE AND BLOOD      '
'---------------------------------------------------------------------------'
       RGLUC  =(VMTCOH*CVOHL)/(KMTCOH+CVOHL) $'RATE OF GLUCURONIDATION   '
        AGLUC = INTEG(RGLUC,0.)         $'AMT.OF TCOH GLUCURONIDATED (mg)'

           RG = RGLUC*MWGLUC/MWTCOH     $'RATE OF TCOG FORMATION (mg/hr) '
          AGA = AGLUC*MWGLUC/MWTCOH     $'AMOUNT OF TCOG FORMED (mg)      '
```

```
   RGL = QLB*(CGB-CLVG)+RG-RGBIL
  'AMGL = AMOUNT OF TCOG REMAINING IN LIVER (mg)                    '
   AMGL= INTEG(RGL,0.)+DOSEG      $'AMT.OF TCOG REMAIN.IN LIVER(mg)'
   CLVG= AMGL/VL                  $'Pc BLOOD/TISSUE = 1.            '

 'Uniform fast equilibrium with bile                                '
   VBIL = QBIL*(T+1)
  CGBIL = PCBIL*CLVG
  RGBIL = QBIL*CGBIL         $'RATE TCOG EXCRETION IN BILE (mg/hr) '
  AMBIL = INTEG(RGBIL,0.)    $'AMOUNT OF TCOG IN BILE (mg)         '

   ATGB = AMBIL*MWTCOH/MWGLUC  $'AMOUNT OF TCOH FROM TCOG IN BILE '
'MRGLUC= AMOUNT OF TCOH FROM HYDROLYZED TCOG REMAINING IN GUTS (mg)'
  MRGLUC= ATGB*EXP(-KAOHBI*T)
  RAGLUC= KAOHBI*MRGLUC     $'RATE OF ABSORPTION OF HYDROLYZED TCOG'
  AGABS = ATGB - MRGLUC     $'TCOG INPUT FROM GUTS (mg)            '

  TCOHM = AMOH + AGLUC - AGABS  $'TOTAL AMOUNT OF TCOH METABOLIZED'

 TOTCOH= integ(RTCOH,0.)
 bwtcOH= totCOH/bw
 MOTCOH= BWTCOH/MWTCOH       $'MILIMOLES TCOH PRODUCED (mmoles/kg) '
'------------------------------------------------------------------'
'Fast uniform diffusion within the volume of distribution          '
 RBODYG= QB*(CGB-CBVG)-RGU  $'RATE OF TCOG REMAINING IN BODY(mg/h)'
 ABODYG= INTEG(RBODYG,0.)
   CBOG= ABODYG/VB                 $'TISSUE CONCENTRATION OF TCOG  '
   CBVG= CBOG/PCGBO                $'CONC.TCOG IN ULTRAFILTR.BLOOD '
    CGB= (QB*CBVG + QLB*CLVG)/QC   $'CONCENTRATION OF TCOG IN BLOOD'
    AGB= INTEG(CGB,0.)             $'AMOUNT OF TCOG IN BLOOD (mg)  '
'------------------------------------------------------------------'
'  AGU = AMOUNT OF TCOG IN URINE (mg)                              '
    VU = QU*(T+1)
   RGU = CBVG*CLGLU         $'RATE OF TCOG REMOVAL IN URINE (mg/hr)'
   AGU = INTEG(RGU,0.)      $'AMOUNT OF TCOG EXCRETED IN URINE (mg)'
 CGUEX = AGU/VU     $'CUMULATED CONC. TCOG EXCRETED IN URINE (mg/L)'
   CGU = RGU/QU             $'CONCENTRATION OF TCOG IN URINE (mg/L)'

 GLUBAL= AGA + DOSEG - (AMGL + AMBIL + ABODYG + AGU)
'------------------------------------------------------------------------'
'     *** CONTINUE WITH COMMON PART FOR TCOH AND TCOG ***                '
'------------------------------------------------------------------------'

 'IVOH = TCOH Intravenous infusion rate (mg/hr)                    '
  IVOH = IVOHR*(1.-step(tinf))

 'COHV = Mixed venous blood concentration TCOH (mg/L)              '
  COHV = (QF*CVOHF+ (QG+QL)*CVOHL+ QS*CVOHS+ QR*CVOHR+ IVOH)/QC

 'BALANCE OF TCOH IN MOUSE                                         '
 '                                     AMOUNT OF TCOH IN MOUSE (mg)'
 OHBAL = AOHF+ATCOHL+AOHS+AOHR+AOHG+AMOH+AOHX+AGLUC+AKTCOH-AGABS
  BLOH = (AOHF+ATCOHL+AOHR+AOHS+AOHG+AKTCOH+AGLUC-AGABS)/BW
'              BODY LEVEL OF REMAINING AND EXCRETED TCOH (MG/KG)'
 BLOHF = BLOH+(AGABS/BW)-(AGLUC/BW)
'                    BODY LEVEL OF FREE - NON GLUC. TCOH (MG/KG)'
  OHIN = IVOHR*TINF+AOOH+TOTCOH     $'DOSE RECEIVED/MOUSE (mg)     '
'------------------------------------------------------------------------'
'      *** END OF TCOH AND TCOG PROGRAMS ***                             '

'------------------------------------------------------------------------'
'     * RATES AND AMOUNTS OF ACID METABOLITES *                          '
```

```
'-----------------------------------------------------------------------'
       RTCA  =CVCHL*VL*PCTCA*(MWTCA/MWCH)    $'RATE CONVERSION CH->TCA  '

'Additional amount of TCA is generated from TCOH (rate = RAMOH):        '

       RCAOH = RAMOH*(MWTCA/MWTCOH)

       RCHDC=RIDA*(MWDCA/MWCH)       $'RATE CONVERSION OF CH->DCA (mg/h)'
'-----------------------------------------------------------------------'
       ATCA = INTEG ((RTCA + RCAOH), 0.)             $'Amount of TCA (mg)'
       ADCA = INTEG (RCHDC, 0.)                      $'Amount of DCA (mg)'
'-----------------------------------------------------------------------'
   TERMT (T.GE.TSTOP)
END       $'End of derivative                                           '
END       $'End of dynamic                                              '
END       $'End of program                                              '

 '**********************************************************************'
```

## *.CMD File

```
'---------------Cabana and Gessner (1970) Mice: -----------------------'

PROCED Fig4a
prepar t,BLCH,cchv,cchl
'male mouse'
SET TITLE='CH iv 500mg/kg MOUSE(Cabana,1970)'
s symcpl=.true., ivdch=500.,PCCOH=15.,kohdc=7.9
s tchng=0,days=0.25,qlc=.24,VMCHOC=3.23,kmtcoh=1.
s qpc=30,qcc=16.5,vfc=.04,qfc=.05,kchc=1.04,vmtcoc=15
s bw=.03,grdcpl=.f.,wesitg=.f.,KMCHOH=0.0675

DATA
T          BLCH
0.         500.0    INITIAL
0.08       475.0
0.2        280.0
0.25       230.0
0.333      200.0
0.5        150.0
0.75        90.0
1.          60.0
1.5         48.0
2.          34.0
3.          50.0
4.          44.0
6.          48.0
END
START
PLOT BLCH,'log','lo'=10.,'xhi'=6.
END

PROCED Fig4b
prepar t,BLOH,cchv,cchl,cohv
'male mouse'
SET TITLE='CH iv 500mg/kg MOUSE (Cabana,1970)'
s symcpl=.true., ivdch=500.,pccoh=15,kohdc=7.9
s tchng=0,days=0.25,qlc=.24,vmchoc=3.23,kmtcoh=1.
s qpc=30,qcc=16.5,vfc=.04,qfc=.05,kmchoh=0.0675,
```

```
s bw=.03,grdcpl=.f.,wesitg=.f.,kchc=1.04,vmtcoc=15.

DATA
T          BLOH
0.           0.00001    INITIAL
0.07        65.0
0.15       120.0
0.25       135.0
0.33       170.0
0.5        200.0
0.75       230.0
1.         235.0
1.5        250.0
2.         255.0
3.         250.0
4.         260.0
6.         252.0
END
START
PLOT BLOH,'log','lo'=10.,'xhi'=6.
END

PROCED Fig4c
prepar t,BLOHF,cchv,cchl,cohv
'male mouse'
SET TITLE='CH iv 500mg/kg MOUSE(Cabana,1970)'
s symcpl=.true., ivdch=500.,pccoh=15.,kmtcoh=1.
s tchng=0,days=0.25,qlc=.24,vmchoc=3.23,kohdc=7.9
s qpc=30,qcc=16.5,vfc=.04,qfc=.05,kmchoh=0.0675,
s bw=.03,grdcpl=.f.,wesitg=.f.,kchc=1.04,vmtcoc=15.

DATA
T          BLOHF
0.           0.00001    INITIAL
0.063       60.0
0.125      110.0
0.25       130.0
0.3        155.0
0.45       190.0
0.7        225.0
1.         225.0
1.5        215.0
2.         190.0
3.         150.0
4.         105.0
6.          90.0
END
START
PLOT BLOHF,'log','lo'=10.,'xhi'=6.
END

'-------------------END OF MICE DATA--------------------'
'------------Garrett and Lambert (1993) Dogs:-----------'
PROCED Fig5a
prepar t,cohv,cgb
SET TITLE='TCOH iv 30mg/kg DOG (Garrett,1973)'
s bw=20., ivdoh=30.,days=0.4,qcc=18.,qpc=20.,kchc=1.04
s ivdch=0.,vmtcoc=15, kmtcoh=1.,pccoh=15,kmchoh=0.0675
s symcpl=.true.,grdcpl=.f.,wesitg=.f.,vmchoc=3.23
s kohdc=7.9,doseg=0.

DATA
```

```
T           COHV
0.0001      100.8
0.05         67.2
0.26         34.9
0.42         21.8
0.94         15.1
1.3          10.9
2.24          6.1
2.76          4.9
3.39          3.3
4.17          1.8
4.7           1.4
5.8           1.1
7.7           0.7
END
START
PLOT COHV,'lo'=0.,'hi'=125.,'xhi'=8.
END

PROCED Fig5b
prepar t,cohv,cgb
SET TITLE='TCOH iv 60mg/kg DOG(Garrett,1973)'
s bw=10., ivdoh=60.,days=0.2,qcc=18.,qpc=20.,kchc=1.04
s vmtcoc=15, kmchoh=0.0675,kmtcoh=1.,vmchoc=3.23,doseg=0.
s symcpl=.true.,grdcpl=.f.,wesitg=.f.,pccoh=15,kohdc=7.9

DATA
T           COHV
0.0001      109.2
0.05        102.1
0.1          97.4
0.15         88.2
0.2          73.9
0.3          53.1
0.5          30.2
0.75         21.4
1.           16.
1.26         14.3
1.5          11.
1.75         12.6
2.            6.7
2.5           4.7
3.            3.6
3.5           2.7
4.            1.5
END
START
PLOT COHV,'hi'=120,'xhi'=4.
END

PROCED Fig6a
prepar t,cohv,cgb
SET TITLE='TCOG iv 450mg/kg DOG(Garrett,1973)'
s bw=11.85,doseg=450.,days=0.21,ivdoh=0.,pcgbo=0.311
s qcc=18.,qpc=20.,vmtcoc=15,kmtcoh=1.,pccoh=15,kchc=1.04
s symcpl=.true.,wesitg=.f.,grdcpl=.f.,vmchoc=3.23,kmchoh=0.00675

DATA
T           CGB
0.0001      183.
0.02        146.4
0.07        119.
```

```
0.117        91.5
0.14         82.4
0.16         73.2
0.28         54.9
0.33         43.9
0.42         36.6
0.51         31.1
0.7          23.8
0.89         15.9
1.05         11.7
1.21          7.7
1.38          6.4
1.56          4.2
1.8           3.3
2.1           2.3
2.4           1.65
2.7           0.95
3.            0.88
3.5           0.49
4.            0.44
4.5           0.23
END
START
PLOT CGB,'xhi'=4.5
END

PROCED Fig6b
prepar t,cohv,cgb,cgbil
SET TITLE='TCOH iv 11mg/kg DOG(Garrett,1973)'
s bw=13., doseg=0., ivdoh=11.,days=0.2,kchc=1.04
s vmtcoc=15, kmtcoh=1.,vmchoc=3.23,pccoh=15,kohdc=7.9
s symcpl=.t., grdcpl=.f., wesitg=.f.,kmchoh=0.0675

DATA
T          COHV      CGB      CGBIL
0.05       19.3       .         .
0.1        15.1       .         .
0.2        10.1       5.9       .
0.5         5.9      13.7       .
0.75        4.6      15.6       .
1.          3.7      16.5     823.5
1.25        2.9      16.7       .
1.5         2.3      14.6       .
1.75        1.9      11.9       .
2.          1.85     10.1     585.6
2.5         1.        6.8       .
3.          0.8       4.6     179.3
3.5          .        3.8       .
4.           .        2.2     102.5
END
START
PLOT COHV,'hi'=20.,CGB,CGBIL,'xhi'=4.5
END
'----------------------END OF DOGS DATA-----------------------------'
'----------------CH and TCOH IV Mice OUR data-----------------------'
PROCED Fig7a
prepar t,cchv,cchl,clvg,cgb
'male mouse'
SET TITLE='CH iv 10&100mg/kg MOUSE'
s symcpl=.true.,ivdch=100.,ivdoh=0.,POHG=1.06,kchc=1.04,kohdc=7.9
s tchng=0,days=0.0042,qlc=.24,PCHS=1.226,KMCHOH=0.0675,kmtcoh=1.
S PCHR=1.398,PCHG=1.47,POHL=1.06,POHF=1.525,POHS=1.11,POHR=1.06,
```

```
s qpc=30,qcc=16.5,vfc=.04,qfc=.05,tinf=0.0125,PCCOH=15,VMCHOC=3.23
s bw=.042,grdcpl=.f.,wesitg=.f.,PCHL=1.398,PCHF=0.366,vmtcoc=15.

DATA
T           cchv       ivdch
0.           0.        100.    INITIAL
0.0149    304.62          .
0.0149    265.78          .
0.0149    232.88          .
0.0158    427.5           .
0.0485     95.28          .
0.0485     69.36          .
0.0485     70.91          .
0.0485    111.87          .
0.09       56.03          .
0.09       30.18          .
0.09       62.17          .
0.09       45.06          .
0.097      52.01          .
0.           0.          10.     INITIAL
0.0143     24.26          .
0.0143     12.60          .
0.0143      8.85          .
0.0143     11.79          .
0.0476      3.26          .
0.0476      4.75          .
0.0476      5.60          .
0.0476      3.30          .
0.088       0.31          .
0.088       0.75          .
0.088       0.40          .
END
START smooth
PLOT cchv, 'xhi'=0.1
END

PROCED Fig7b
prepar t,cohv,BLOH,BLCH,cgb,ctcohl,clvg
'male mouse'
SET TITLE='TCOH iv 10&100mg/kg MOUSE'
s symcpl=.true.,ivdch=0.,ivdoh=100.,POHG=1.06,vmchoc=3.23,
s tchng=0,days=0.0042,qlc=.24,PCHS=1.35,pccoh=15,kmchoh=0.0675
S PCHR=1.47,PCHG=1.47,POHL=1.06,POHF=1.525,POHS=1.11,POHR=1.06,
s qpc=30,qcc=16.5,vfc=.04,qfc=.05,tinf=0.0125,vmtcoc=15.,kchc=1.04
s bw=.042,grdcpl=.f.,wesitg=.f.,PCHL=1.47,PCHF=0.48,kmtcoh=1.
s kohdc=7.9

DATA
T          COHV       ivdoh
0.          0.        100.    INITIAL
0.012      359.1         .
0.012      187.1         .
0.012      200.7         .
0.0197     198.9         .
0.0458      78.56        .
0.04945     50.43        .
0.04945     67.06        .
0.04945     52.79        .
0.0892      40.28        .
0.0892      48.88        .
0.0892      53.56        .
0.            .          10.    INITIAL
```

```
0.015       14.99        .
0.0162       9.98        .
0.0162      10.39        .
0.0162      12.22        .
0.0484       4.47        .
0.0484       3.09        .
0.0484       3.90        .
0.0484       4.36        .
0.0847       2.70        .
0.0860       2.46        .
0.0886       2.59        .
0.0886       4.85        .
0.0886       1.83        .
END
START smooth
PLOT cohv, 'xhi'=0.1
END

'--------------------END OF OUR MICE DATA----------------------'
```