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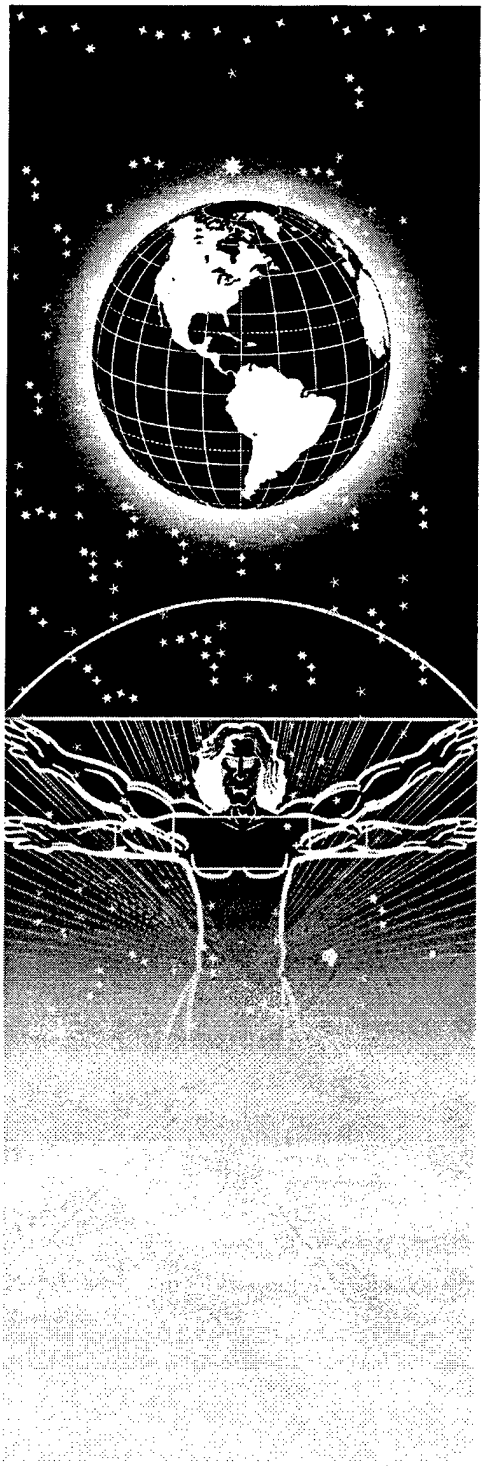

UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

Determination of Partition Coefficients for Trichloroethanol (TCOH) and Chloral Hydrate (CH)-Two Metabolites of Trichloroethylene (TCE)

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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

TERRY A. CHILDRESS, Lt Col, USAF, BSC
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Trichloroethylene (TCE), a common groundwater contaminant, has been shown to be carcinogenic in some animal species. To determine the risk in humans, the use of physiologically-based pharmacokinetic models (PBPK) has become increasingly the method of choice. Formerly it was a common practice to calculate risk estimates on the basis of administered dose-toxicity/tumor incidence, it is now recognized that the dose delivered to the target organ is more accurate. Determination of partition coefficients (the solubility of the chemical in tissue) is one element important in the development of the PBPK model. This study focuses on two metabolites of TCE, chloral hydrate (CH) and trichloroethanol (TCOH). All studies were conducted in $B_6C_3F_1$ mice since this species was used in the NCI/NTP carcinogenicity studies on TCE. Partition coefficients for CH and TCOH were determined using the nonvolatile method of Jepson *et al.*, (Fund. and Appl. Tox 22: 1994). Tissues used in CH studies were pre-treated with 20% lead acetate to insure no metabolism occurred during the incubation period.

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Preface

This is one of a series of technical reports to describe results of experimental laboratory work conducted at the Toxicology Division , Armstrong Laboratory Wright-Patterson AFB in support of a project to construct a physiologically-based pharmacokinetic model (PBPK) for trichloroethylene in B6C3F1 mice. This interim report focuses on the partition coeffcients using the non-volatile method for two major metabolites, Trichloethanol (TCOH) and Chloral Hydrate(CH). The report covers the period from August 1994 to January 1995.

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 the Care and Uses of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, Department of Health and Human Services, National Institute Of Health Publication 385-23, 1986 and the Animal Welfare Act of 1966, as amended.

Table of Contents

List of Figures

List of Tables

Abbrevations

TCOH	Trichloroethanol
CH	Chloral Hydrate
TCE	Trichloroethylene
PBPK	Physiologically-based pharmacokinetic
TCA	Trichloroacetic acid
DCA	Dichloroacetic acid
EPA	Environmental Protection Agency
NCI	National Cancer Institute
NTP	National Toxicology Program
ml	milliliter
°C	degree centigrade
rpm	revolutions per minute
NMWL	nominal molecular weight limit
psi	pounds per square inch
mm	millimeter
M	meter
µg	microgram

INTRODUCTION

Trichloroethylene (TCE) is a solvent that has been used for degreasing of metals. It has also been used for other purposes: a solvent in adhesives, paint stripping, dry cleaning, an anesthetic, and disinfectant. Because of this widespread use of TCE, it has become a common environmental contaminant. It was one of the 10 most commonly detected chemicals at hazardous waste site (1). Exposure of humans to TCE in a variety of settings is of concern primarily because of its carcinogenic potential. TCE has been shown to cause cancer in mice and rats (2-6).

From these studies, it appears that the metabolites of TCE rather than the parent compound are responsible for cytotoxicity and carcinogenicity in the liver and other organs. It has been known that TCE is extensively metabolized in the body to trichloroethanol (TCOH), TCOH-glucuronide, and trichloroacetic acid (TCA). Dichloroacetic acid (DCA) which is also formed and TCA are known inducers of hepatic tumors in mice (7-10). The 1985 EPA health assessment document (11) on TCE noted that the metabolic pathways for TCE appear to be qualitatively similar in mice, rats and humans. The metabolism is quantitatively different with metabolic capacity lower in rats than in mice.

Butler (12) found that chloral hydrate, a hypnotic widely used as an anesthetic and as a drug, was also metabolized to TCOH, TCOH-glucuronide, and TCA. This lead to the hypothesis that chloral hydrate was an intermediate metabolite of TCE. He could not, however, demonstrate the presence of chloral hydrate in plasma and urine. Chloral hydrate has since been found in the plasma and microsomes from rats and humans (13-19).

Thus, the metabolic pathways of TCE (Fig.1) have been studied extensively and reexamined.

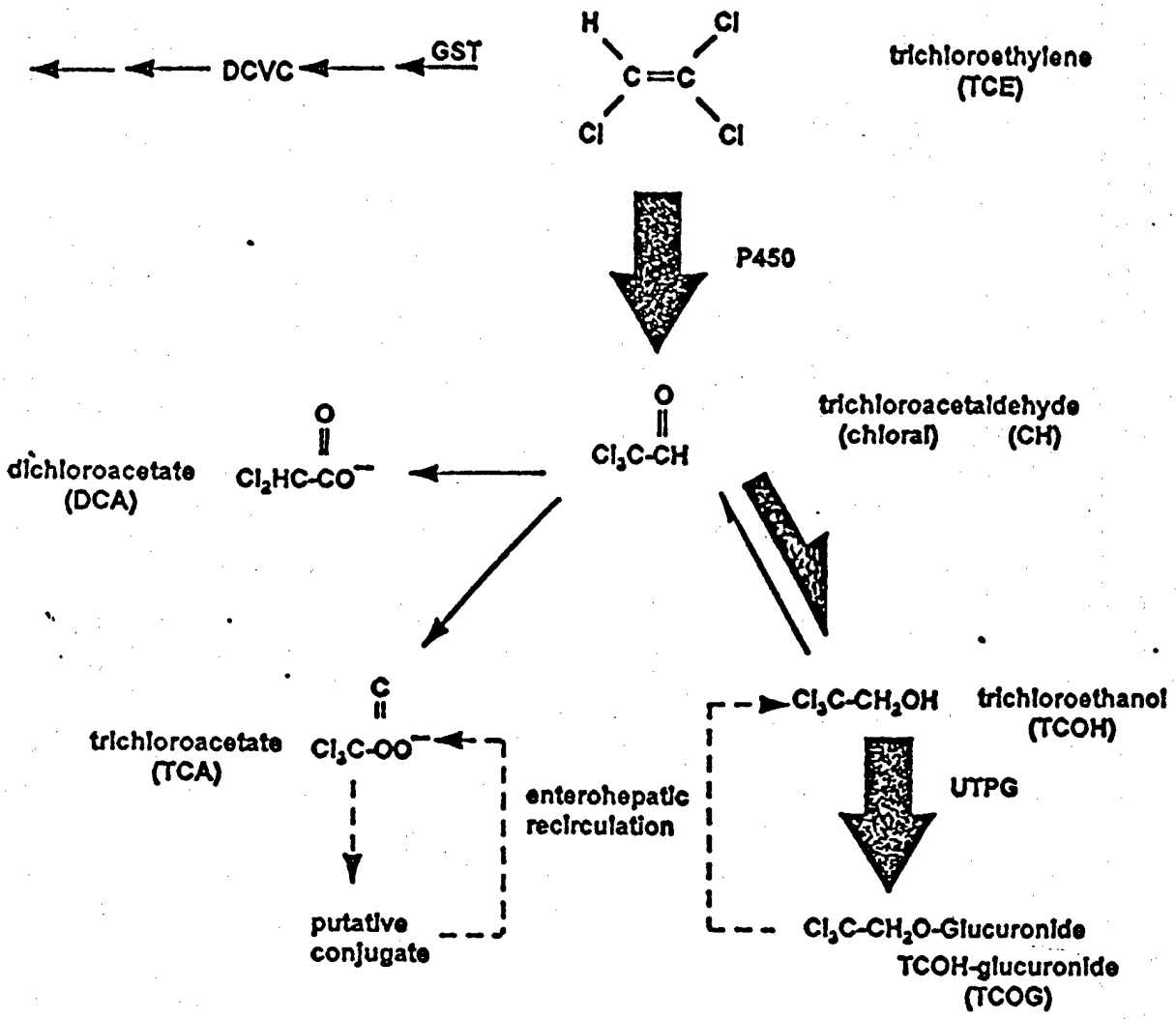

Figure 1. Selected steps in the Metabolism of Trichloroethylene

Development of PBPK models and their utilization

To aid in the understanding of the disposition of a chemical within the body, scientists have developed the physiologically-based pharmacokinetic model (PBPK). Such models support the four important extrapolations: high-low dose, dose route, interspecies and altered exposure patterns. Formerly it was a common practice to calculate risk estimates on the basis of administered dose-toxicity/tumor incidence, it is now recognized that the dose delivered to the target organ is more accurate.

A number of PBPK Models for TCE have been developed. Sato et al. (19) focused on respiratory exposure for humans to TCE. It was a 3 compartmental model, with intercompartmental exchange of TCE governed solely by intertissue diffusion. Metabolic and respiratory excretion was assumed to occur in the richly perfused tissue compartment. Fernandez et al. (20) predicted respiratory elimination of TCE and urinary excretion of TCE metabolites in humans. This model was built on Sato's model with a liver compartment with blood-flow -limited delivery plus a lung compartment for respiratory absorption and elimination. The Ramsey and Andersen model (21) was used by Andersen et al. (22) to predict influence of competitive inhibition on uptake of inhaled TCE in rats. Fisher et al. (23) used the model developed by Andersen and added compartments (mammary tissue, placenta and fetus) to allow for the physiological changes of pregnancy. This model was again modified to account for lactation and nursing (24). The Ramsey and Andersen model with a lung compartment was used by Dallas et al. (25).

Missing, however, from the literature was a PBPK model of TCE in mice. There appears to be a need to develop such a model because many of the positive carcinogenesis studies were carried out in this species.

PBPK models consist of groups of organs (compartments) arranged in anatomical configuration connected by the cardiovascular system. When building the model, 3 groups of parameters are required: (1) organs and tissue volumes, blood flow rates; (2) thermodynamic properties such as partition coefficients; and (3) metabolic constants as well as absorption and excretion . Information about the first group of parameters is available from a variety of literature sources. Partition coefficients or distribution coefficients as a measure of solubility of the chemical in tissue are typically determined experimentally. They represent the ratio of chemical concentration in two interfacing phases when chemical is in equilibrium between 2 phases. For the purposes of PBPK model, the biological phases in which the chemicals are distributed are air, blood, fat, muscle, liver, other tissues and biological fluids. Intravenous dosing and other routes of administration provide the descriptions for parameter 3.

This report focuses of the determination of the partition coefficients for chloral hydrate (CH) and trichloroethanol (TCOH).

Construction of 2 separate models but interlinked for these metabolites that can be used separately or linked with other PBPK models may aid in better understanding the kinetics of parent TRI.

METHODS

Chemicals used: Chloral Hydrate (CAS #302-17-8) and Trichloroethanol (CAS #115-20-8) were obtained from Sigma Chemical. Lead Acetate which was added to inhibit metabolism was obtained from Mallinckrodt. Physiological saline (0.9%) was used as the diluent.

Animals used: B6C3F1 mice 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 Institute of Health, Publication No. 86-23, 1985; and the Animal Welfare Act of 1966, as amended.

Partition coefficients: The method for nonvolatile chemicals developed by Jepson et al. (26). was used. In this method for each run, (3) B6C3F1 mice were sacrificed using CO_2 and tissues harvested and pooled. Partitions for blood, liver, muscle and fat were determined for trichloroethanol and chloral hydrate. Brain was also included in the partitions for chloral hydrate. Tissues and blood were weighed into 3-20 ml scintillation vials capped with teflon/rubber septa to prevent the absorption of the chemical. The appropriate concentration of chemical in 0.9% saline was added to each vial. Typically, 0.5 gram of blood or tissue and 5 mls of chemical in saline were added to each vial. To prevent possible metabolism of chloral hydrate especially in the blood and the liver, 20% lead acetate was added to each vial. Vials were vortexed at medium speed for 18 hours at 37°C. Supernatant was centrifuged at room temperature for 10 minutes at 1500 rpm. The resulting supernatant was filtered through a prewashed Millipore Ultra-PF low-binding cellulose 10,000 NMWL filter. All millipore cells were rinsed with de-ionized water by applying 40 psi of compressed nitrogen. A syringe with plastic tubing was attached to the bottom opening of each cell so that any excess saline was removed. All cells were used within 2 hours of washing to insure that they did not dry out. The supernatant was filtered though each cell by applying 40 psi of pressure and was kept stirring over a magnetic stirrer to prevent clogging of the filter. Triplicate samples of each filtrate were set up for extraction of chemical. Ethyl acetate was the solvent of choice. Samples were vortexed at medium speed for 30 minutes at 37°C and were centrifuged. The ethyl acetate phase was removed and was analyzed by Hewlett Packard 5890 Series II gas chromatography

equipped with a Hewlett Packard 7673A Autosampler. The amount of ethyl acetate added was dependent on the initial concentration of the chemical so each was in the range of the standard curve. Vials, containing the appropriate chemical concentration; but no tissue, served as a reference or blanks. A PE Nelson Turbochrom v.4.03 Analytical System was used to collect and process data. The GC conditions were: Column - Vocol 30 M X 0.53mm, oven temperature 120°C, electron capture detector temperature 300°C, injector temperature 175°C.

RESULTS

The partition coefficients for CH and TCOH determined in this study are listed in Table 1

Tissue : Saline Partition Coefficients

TISSUE	CHLORAL HYDRATE	
	50µG/ml	100µg/ml
BLOOD	1.86 (0.29)	1.82 (0.54)
FAT	0.68 (0.34)	0.88 (0.38)
LIVER	2.55 (0.43)	2.67 (0.29)
MUSCLE	2.28 (0.47)	2.45 (0.47)
BRAIN	2.48 (0.43)	2.53 (0.92)
LUNG	3.07 (1.31)	2.94 (1.64)

TISSUE	TRICHLOROETHANOL	
	50µG/ml	100µg/ml
BLOOD	2.98 (0.25)	3.48 (1.26)
FAT	5.51 (0.64)	5.31 (0.47)
LIVER	3.81 (0.60)	3.68 (1.37)
MUSCLE	3.33 (1.23)	3.86 (1.57)

partitions listed are the mean with coefficient of variation in parenthesis

DISCUSSION

Because of its environmental and toxicological concerns, there is interest in studying of Trichloroethylene and its metabolites. It is known to cause cancer in various animal species; but its relationship and incidence of causing cancer in humans is not clear. From the carcinogenicity studies, it appears that the metabolites of TCE rather than the parent compound are responsible for cytotoxicity and carcinogenicity in the liver and other organs. Thus, we focused on 2 main metabolites chloral hydrate and trichloroethanol. In the experiments, mouse tissue was used because of the incidence of carcinogenicity in this species.

The use of physiologically-based pharmacokinetic modeling has become increasingly common in risk assessment because of it is amenable to interspecies extrapolation necessary to calculate human tissue dose. However, to have a workable model, one must have dependable data. One set of data is the partition coefficients or distribution coefficients. A typical method to determine the partitions is the vial equilibration method in which a sample of headspace chemical is analyzed. Because of the low volatility of these 2 metabolites, this method was not practical. The nonvolatile method developed by Jepson et al. (26) had been shown to be an adequate alternative method. While partitions are only part of the model, these numbers combined with other information included in the PBPK model can help make conclusions about risk.

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