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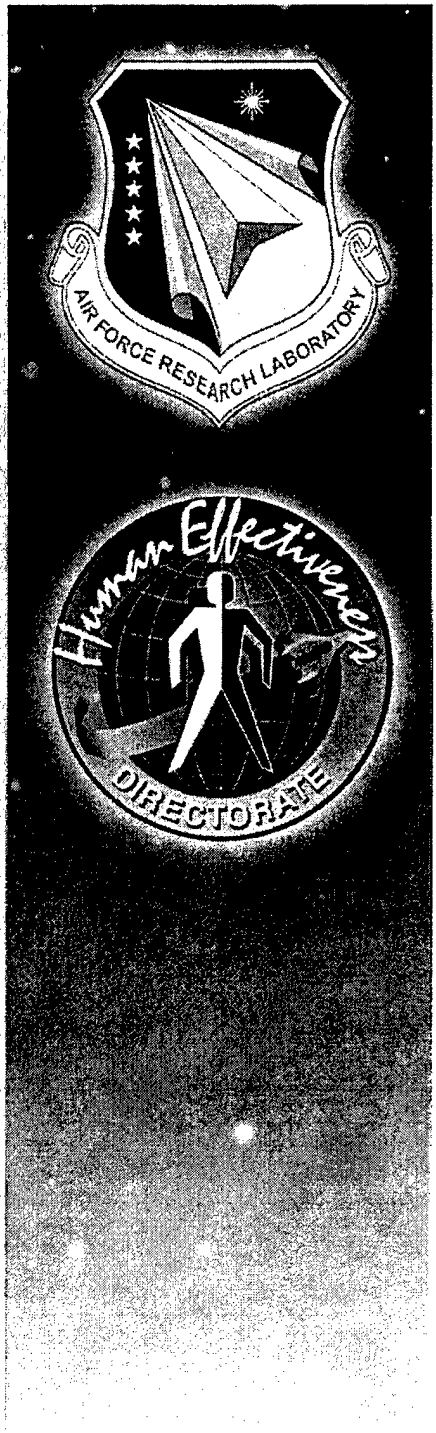

United States Air Force Research Laboratory

Metabolism of Perchloroethylene and 1,1,1,2-Tetrachloroethane After Carbon Tetrachloride Pretreatment in B6C3F1 Mice

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August 2000

FINAL REPORT FOR THE PERIOD AUGUST 1999 TO AUGUST 2000

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Human Effectiveness Directorate
Biosciences and Protection Division
Applied Toxicology Branch
Wright-Patterson AFB OH 45433-7400

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TECHNICAL REVIEW AND APPROVAL

AFRL-HE-WP-TR-2001-0133

The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

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

//SIGNED//

RICHARD R. STOTTS, DVM, Ph D.
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Air Force Research Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2000	Final Report - August 1999 - August 2000

4. TITLE AND SUBTITLE

Metabolism of Perchloroethylene and 1,1,1,2-tetrachloroethane after Carbon Tetrachloride Pretreatment in B6C3F1 Mice

5. FUNDING NUMBERS

Contract F33615-00-C-6060
PE 61102F
PR 4223
TA 42230T
WU 42230T04

6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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Dayton, OH 45437

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

**Air Force Research Laboratory, Human Effectiveness Directorate
Biosciences and Protection Division
Counterproliferation Branch
Wright-Patterson AFB, OH 45433-5707

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

AFRL-HE-WP-TR-2001-0133

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Risk assessments for chlorinated solvents are typically only done for single chemicals, and clean up costs are based on these assessments. However, groundwater and drinking water supplies are usually contaminated with multiple compounds. The most commonly used approach for assessing risks to multiple chemical exposures assumes additivity of response. This study investigates the effect of exposure to mixtures of carbon tetrachloride and 2 other chlorinated solvents, perchloroethylene and 1,1,1,2-tetrachloroethane. Metabolite formation, which is the process thought to be responsible for toxicity is affect carbon tetrachloride. By understanding the alteration in metabolic outcome for perchlorethylene and 1,1,1,2-tetrachloroethane the assessment of risk associated with exposures to these types of mixtures may be more science-based.

14. SUBJECT TERMS			15. NUMBER OF PAGES
Carbon tetrachloride	Trichloroacetic	Perchloroethylene	21
1,1,1,2-tetrachlorethane			16. PRICE CODE

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

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INTRODUCTION

Environmental and occupational exposures of chlorinated solvents are usually to multiple chemicals rather than single chemicals. However, only the risk assessment process of single chemicals has been fairly well developed. In addition, clean-up costs for groundwater and soil contaminated by chlorinated chemicals are driven in part by standards which were established assuming that carcinogens act by a single mode of action and carcinogenic responses are additive (EPA, 1986; Teuschler and Hertzberg, 1995). The most commonly used approach for chemical mixture risk assessment assumes response additivity among components, without accounting for possible interactive effects.

Perchloroethylene (PCE) and 1,1,1,2-Tetrachloroethane (1,1,1,2-TC) are suspected carcinogens commonly contaminating groundwater and soil. Their toxicity appears to be caused by a non-genotoxic mechanism through the action of trichloroacetic acid (TCA), a cytochrome P450 2E1 mediated metabolite of PCE and 1,1,1,2-TC. Carbon tetrachloride (CT) is a common industrial solvent and is found in mixtures with other industrial solvents. CT is also metabolized by cytochrome P450 2E1 (Castillo et al, 1992), forming free radicals which quickly destroy P450 2E1 (Noguchi et al, 1982a, b). Since the first step in the metabolic production of TCA for PCE and 1,1,1,2-TC involves P450 2E1 mediated oxidation, knocking out this pathway with CT may alter the metabolic profile, ultimately changing the carcinogenicity of the chlorinated solvent. This study demonstrates a quantitative method for assessing the metabolic outcome of selected mixtures (delivered as a sequential exposure). The data collected will be used for development and validation of a physiologically based pharmacokinetic model for describing the risks associated with exposure to mixtures of chemicals.

MATERIALS AND METHODS

Oral dosing

Single exposure:

Male B6C3F1 mice (Charles Rivers Laboratory, Raleigh, NC) were weighed and housed 5 per cage with food and water available *ad libitum.* The vehicle for oral dosing was 5% Alkamual (formally Emulphor, Rhodia, Cranbury, NJ), an ethoxylated castor oil (CAS #61791-12-6). The mice were dosed with a single bolus oral gavage. The dose levels for CT were 1, 5 and 20 mg/kg. PCE dose levels were 10 and 100 mg/kg, and 1,1,1,2-TC dose levels were 5, 20, 50, 200 and 400 mg/kg. Animals were serially euthanized from 0.25 to 24 hr after dosing, and blood (drawn from the inferior vena cava) and liver were taken for analysis. An aliquot of blood and a portion of the liver were placed in pre-weighed headspace vials and crimp capped for headspace analysis of CT, PCE or 1,1,1,2-TC. Another aliquot of blood and portion of liver was placed in pre-weighed 4 ml vials containing 20% lead acetate for metabolite analysis.

Co-exposure:
Male B6C3F1 mice were orally dosed with 0, 0.1, 1, 5, 20, 50 and 100 mg/kg CT. After a 1 hr delay the mice were dosed with either 100 mg/kg PCE or 200 mg/kg 1,1,1,2-TC. At 4 hr post challenge dose, the animals were euthanized by CO_2 inhalation, and blood and liver were collected for analysis. An aliquot of blood and a portion of the liver were placed in pre-weighed headspace vials and crimp capped for headspace analysis of CT and PCE or 1,1,1,2-TC. Another aliquot of blood and portion of liver were placed in pre-weighed 4 ml vials containing 20% lead acetate for metabolite analysis. For time course kinetics male mice were orally dosed with 5 mg/kg CT followed 1 hr later by an oral dose of PCE (10 or 100 mg/kg) or 1,1,1,2-TC (20 or 200 mg/kg). Animals were euthanized by CO_2 inhalation at 1, 4, 6, 8, 12, 16, 24 and 30 hr post second dose, and blood and liver were taken, as above.

Gas chromatography
Parent analysis:
CT, PCE and 1,1,1,2-TC standards were prepared in a 5% Alkamual/ 2% methanol solution. Standards and tissue samples were placed in 20 ml headspace vials and immediately crimp sealed. Tissue sample size ranged from 0.1 to 0.6 g. Analyses were performed on an HP 5890 gas chromatograph using an HP headspace autosampler set at 80°C. The gas chromatograph was fitted with a 30m X 0.53mm VOCOL column and used electron capture detection.

Metabolite analysis:
Blood and liver samples were stored in 4 ml vials containing 20% lead acetate at -80°C until analysis. These samples were derivatized with dimethyl sulfate, and the resulting methyl ester products were analyzed by GC-ECD (Abbas and Fisher, 1998).

Partition Coefficient Determination
Partition coefficient values are necessary parameters needed for development of a physiologically based pharmacokinetic model, which will be used later to describe target tissue dose estimates from an administered dose. Partition coefficient values for PCE can be found in the literature (Gearhart et al, 1993). Partition coefficients for CT and 1,1,1,2-TC were determined using the vial equilibration method of Gargas et al. (1989). Naïve mice were euthanized by CO_2 inhalation, and blood, liver, fat and muscle were collected for analysis. Liver, fat and muscle were minced. Approximately 1 g of blood, liver and muscle and 0.1 g of fat were placed in 12.5 ml headspace vials and crimp sealed. Vaporized CT or 1,1,1,2-TC was injected into each headspace vial using a gas tight syringe. The vials were placed in a vortex evaporator (Haake Buchler) and gently vortexed at 37°C for 3 hr to reach equilibrium. Headspace samples were analyzed using gas chromatography with flame ionization detection. Several tissues have metabolic capabilities *in vitro*. To quench the enzymatic metabolism of CT, samples of blood, liver and muscle were treated 1:1 with lead acetate. Fat was not treated. To inhibit the enzymatic metabolism of 1,1,1,2-TC blood was treated 1:1 with lead acetate, while liver was treated 1:1 with lead acetate and heat. Fat and muscle were not treated. Untreated samples of blood, liver and muscle were also used for CT partition coefficient

determination, while untreated samples of blood and liver were used for 1,1,1,2-TC partition coefficient determination.

Statistical Analysis

Where applicable statistical analyses were performed using a 2-sample, 2-tailed t-test ($p \leq 0.05$).

RESULTS

Single exposure kinetics

To determine the unaltered metabolic profiles of PCE and 1,1,1,2-TC over time mice were exposed to single doses of either solvent. The CT data is not shown. The parent levels of PCE and 1,1,1,2-TC were cleared to or near the limits of detection by 4 hr for all doses except 100 mg/kg PCE (Fig 1a,b and 2a,b). Blood and liver levels of TCA formed after single oral exposure to 10 and 100 mg/kg PCE are shown in Figures 3a and b. The appearance of TCA in blood peaks between 1-4 hr post exposure. Blood levels of TCA reached 8.3 ± 1.6 (2 hr) and 48.9 ± 7.7 ug/g (4 hr) for the 10 and 100 mg/kg PCE dose levels, respectively, and was cleared by 10 hr. TCA was still detectable in blood after 8 (10 mg/kg) and 10 hr (100 mg/kg) with levels still at 29.0 ± 4.7 ug/g in the 100 mg/kg PCE dose group.

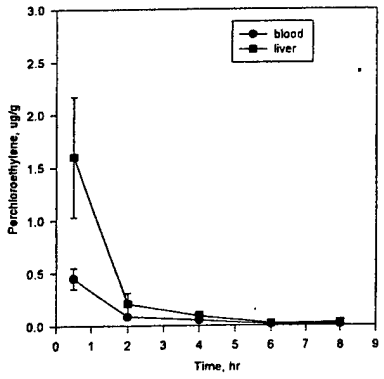

Figure 1a. Clearance of PCE from blood and liver after male B6C3F1 mice were given a single oral dose of 10 mg/kg PCE.

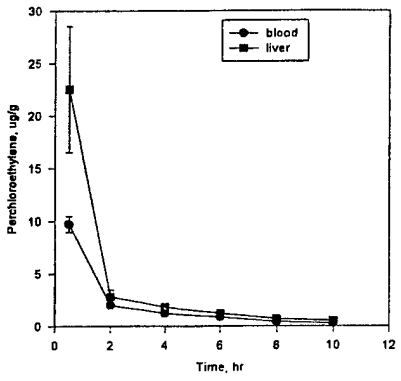

Figure 1b. Clearance of PCE from blood and liver after male B6C3F1 mice were given a single oral dose of 100 mg/kg PCE.

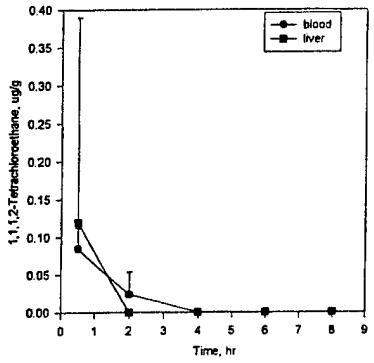

Figure 2a. Clearance of 1,1,1,2-TC from blood and liver of male B6C3F1 mice given a single oral dose of 20 mg/kg 1,1,1,2-TC.

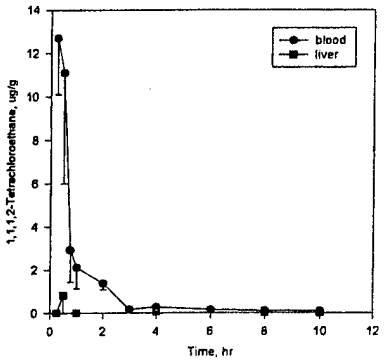

Figure 2b. Clearance of 1,1,1,2-TC from blood and liver of male B6C3F1 mice given a single oral dose of 200 mg/kg 1,1,1,2-TC.

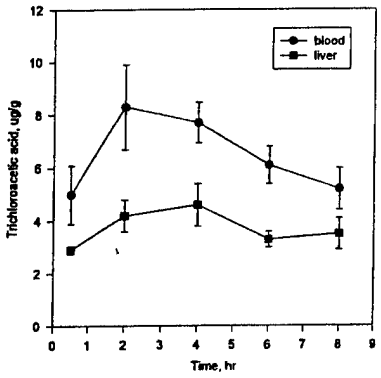

Figure 3a. Blood and liver levels of TCA in male B6C3F1 mice given a single oral dose of 10 mg/kg PCE.

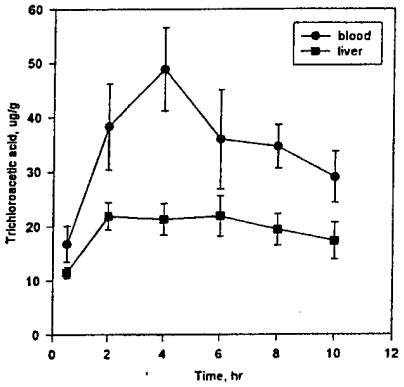

Figure 3b. Blood and liver levels of TCA in male B6C3F1 mice given a single oral dose of 100 mg/kg PCE.

Figures 4a and b show the formation and clearance of TCA and TCOH for 1,1,1,2-TC. After a single oral dose of either 20 or 200 mg/kg 1,1,1,2-TC the formation of TCA peaks between 1-4 hr after dosing. Blood levels of TCA reached 6.9 ± 0.9 and 38.7 ± 3.4 ug/g for the 20 and 200 mg/kg 1,1,1,2-TC dose levels, respectively. While TCOH (from 1,1,1,2-TC) clears rapidly from blood and liver, substantial amounts of TCA are still present in blood and liver after 8 and 10 hr after exposure.

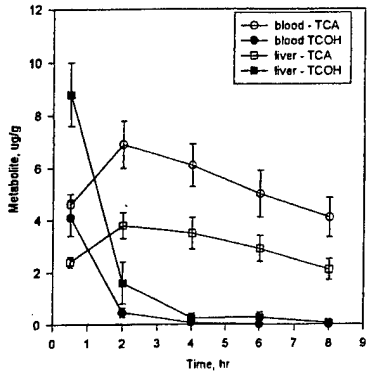

Figure 4a. Blood and liver levels of TCA and TCOH in male B6C3F1 mice given a single oral dose of 20 mg/kg 1,1,1,2-TC.

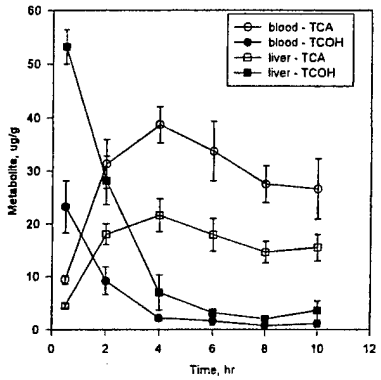

Figure 4b. Blood and liver levels of TCA and TCOH in male B6C3F1 mice given a single oral dose of 200 mg/kg 1,1,1,2-TC.

Co-exposure kinetics

<u>CT/PCE</u>
CT dosimetry

Initial co-exposures to CT and PCE were performed with CT concentrations ranging from 0 to 100 mg/kg. After a 1 hr delay the CT dose was followed by a single oral PCE dose of 100 mg/kg. Tissue collection was completed at 4 hr post PCE dose, and blood and liver levels of PCE were measured (Fig. 5). The concentration of PCE was significantly lower in blood of control animals than in blood of animals pre-treated with 20, 50 or 100 mg/kg CT. The amount of PCE in liver was significantly higher than controls at 50 and 100 mg/kg CT.

The amount of TCA measured in the blood and liver decreased significantly in mice dosed with 1.0 mg/kg CT and higher (Fig 6.). The 0.1 mg/kg CT dose level, however, showed a rise in TCA concentration in both blood and liver with blood levels of TCA being significantly higher.

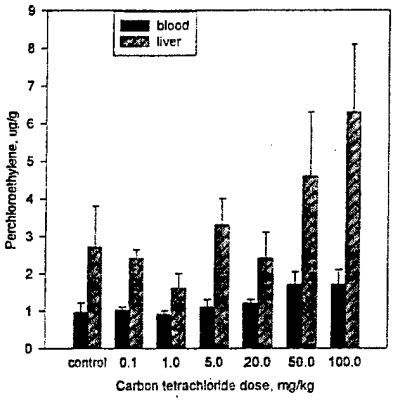

Figure 5. Blood and liver PCE levels 4 hr post 100 mg/kg PCE dose. Male B6C3F1 mice were orally dosed with a series of CT doses 1 hr prior to the oral PCE dose.

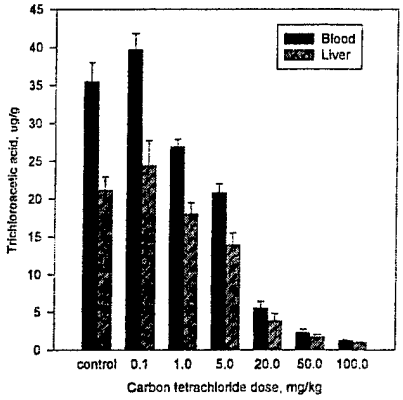

Figure 6. Blood and liver TCA levels 4 hr post 100 mg/kg PCE dose. Male B6C3F1 mice were orally dosed with a series of CT doses 1 hr prior to the oral PCE dose.

Time course kinetics

After exposure to 5 mg/kg CT and subsequent exposure to 10 or 100 mg/kg PCE, blood and liver were collected and analyzed for PCE and TCA over time (Figs 7, 8). The levels of PCE in blood and liver after exposure to CT were not substantially different than the levels found in blood and liver without CT exposure (Figs 1a and 7a, 1b and 7b). However, blood and liver levels of TCA from mice treated with CT were approximately half the blood levels of TCA from untreated mice (Figs 3a and 8a, 3b and 8b). At 4 hr blood levels of TCA peaked at 2.8 ± 0.21 and 15.0 ± 1.7 ug/g for the 10 and 100 mg/kg PCE dose levels, respectively. The concentration of TCA in blood and liver for both doses decreased steadily compared to the untreated animals in Fig 3.

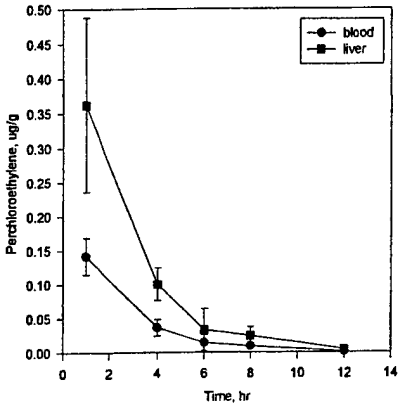

Figure 7a. Clearance of PCE from blood and liver after an oral dose of 10 mg/kg PCE. Male B6C3F1 mice were given a single oral dose of 5 mg/kg CT 1 hr prior to the PCE oral dose.

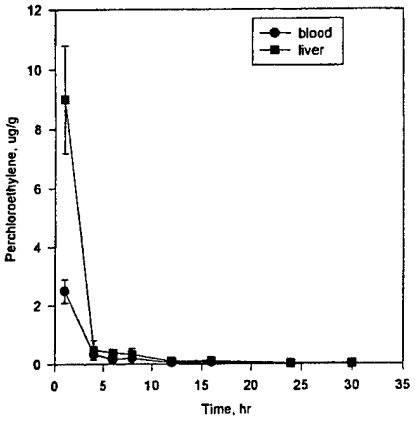

Figure 7b. Clearance of PCE from blood and liver after an oral dose of 100 mg/kg PCE. Male B6C3F1 mice were given a single oral dose of 5 mg/kg CT 1 hr prior to the PCE oral dose

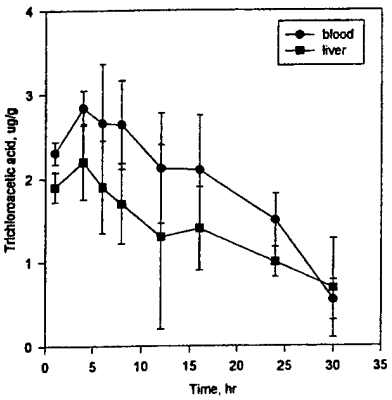

Figure 8a. Levels of TCA in blood and liver after an oral dose of 10 mg/kg PCE. Male B6C3F1 mice were given a single oral dose of 5 mg/kg CT 1 hr prior to PCE dosing.

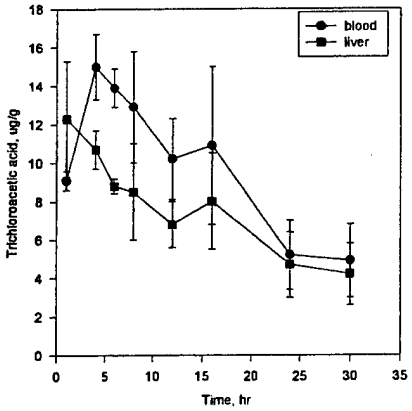

Figure 8b. Levels of TCA in blood and liver after an oral dose of 100 mg/kg PCE. Male B6C3F1 mice were given a single oral dose of 5 mg/kg CT 1 hr prior to PCE oral dosing.

CT/1,1,1,2-TC co-exposures
CT dosimetry

After co-exposure of mice to CT and 200 mg/kg 1,1,1,2-TC, blood levels of 1,1,1,2-TC were slightly lower in the 0.1 mg/kg dose group as compared to control mice (Fig. 9). The concentration of 1,1,1,2-TC was significantly higher in the 5, 20 and 50 mg/kg CT dose groups. 1,1,1,2-TC was not detected at 4 hr post dosing in the liver in any dose group because it clears in 4 hr or less (Fig 2).

When blood and liver were analyzed for TCA, blood showed a slight, though insignificant, increase with a 0.1 mg/kg CT co-dose (Fig. 10). The concentration of TCA in blood was significantly lower than control at 20 and 50 mg/kg CT. TCA levels declined significantly as a function of CT dose for both blood and liver. Trichloroethanol (TCOH) was detected in blood and liver (Fig. 11) but no correlation between TCOH formation and CT dose was evident.

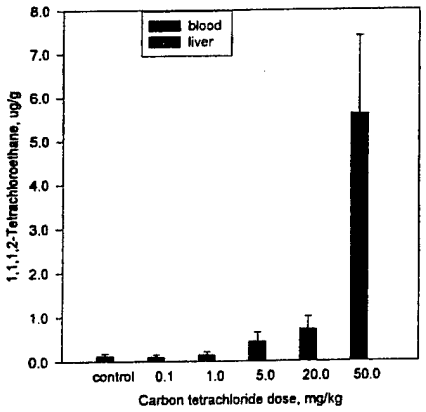

Figure 9. Blood and liver levels of 1,1,1,2-TC 4 hr post 200 mg/kg 1,1,1,2-TC oral dose. Male B6C3F1 mice were orally dosed with CT 1 prior to 1,1,1,2-TC dosing.

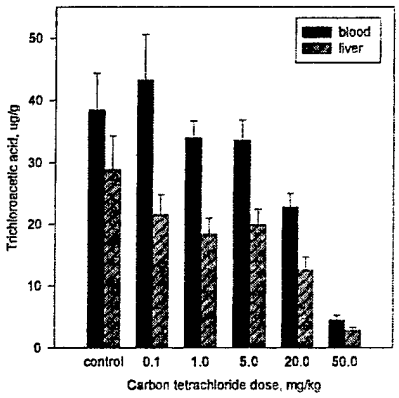

Figure 10. Blood and liver levels of TCA 4 hr post 200 mg/kg 1,1,1,2-TC oral dose. Male B6C3F1 mice were orally dosed with CT 1 hr prior to 1,1,1,2-TC dosing.

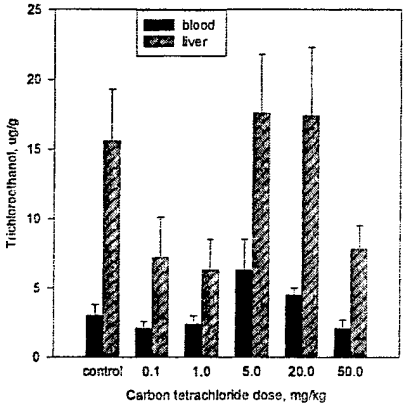

Figure 11. Blood and liver levels of TCOH 4 hr post 200 mg/kg 1,1,1,2-TC oral dose. Male B6C3F1 mice were orally dosed with CT 1 hr prior to 1,1,1,2-TC dosing.

Time course kinetics

1,1,1,2-TC cleared rapidly from blood of mice dosed with 5 mg/kg CT and either 20 or 200 mg/kg 1,1,1,2-TC (Figs 12a, b). The concentration of 1,1,1,2-TC in blood was at or near the limits of detection by 4 hr for both dose levels. 1,1,1,2-TC was detectable in liver only at the earliest time points after dosing.

At 4 hr post dose, TCA peaked in blood at 4.7 ± 0.4 and 29.2 ± 4.4 ug/g for the 20 and 200 mg/kg 1,1,1,2-TC doses, respectively (Figs 13a, b). For the 20 mg/kg dose the amount of TCA in blood and liver was at the limit of detection by 30 hr, while TCA was still detectable in blood and liver after the 200 mg/kg dose.

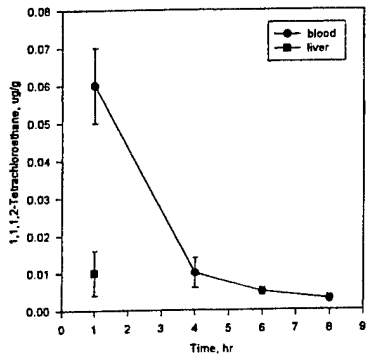

Figure 12a. Clearance of 1,1,1,2-TC from blood and liver after 20 mg/kg 1,1,1,2-TC oral dose. Male B6C3F1 mice were orally dosed with 5 mg/kg CT 1 hr prior to 1,1,1,2-TC dosing.

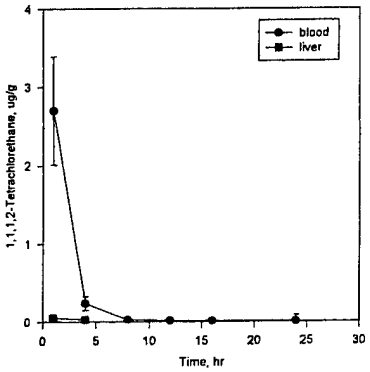

Figure 12b. Clearance of 1,1,1,2-TC from blood and liver after 200 mg/kg 1,1,1,2-TC oral dose. Male B6C3F1 mice were orally dosed with 5 mg/kg CT 1 hr prior to 1,1,1,2-TC dosing.

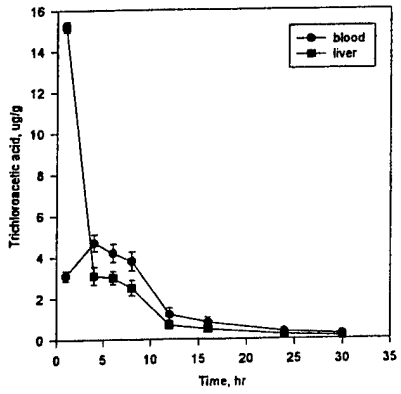

Figure 13a. Levels of TCA in blood and liver after an oral dose of 20 mg/kg 1,1,1,2-TC. Male B6C3F1 mice were given a single oral dose of 5 mg/kg CT 1 hr prior to 1,1,1,2-TC dosing.

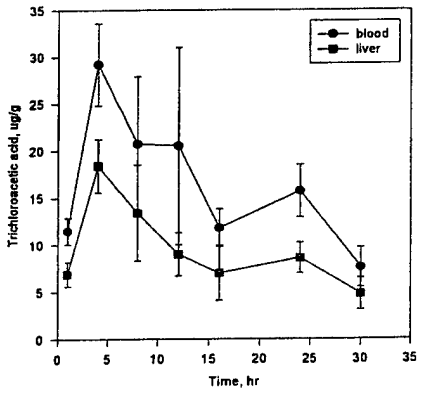

Figure 13b. Levels of TCA in blood and liver after an oral dose of 200 mg/kg 1,1,1,2-TC. Male B6C3F1 mice were given a single oral dose of 5 mg/kg CT 1 hr prior to 1,1,1,2-TC dosing.

Partition Coefficients

Experimentally derived tissue/air partition values for CT and 1,1,1,2-TC are listed in Table 1 and 2, respectively. Fat had the highest tissue/air partition value for both CT and 1,1,1,2-TC at roughly an order of magnitude greater than the liver/air partition. The tissues that were treated with lead acetate or lead acetate and heat showed significantly lower partition values than the untreated tissues for CT. Inhibiting enzymatic activity in blood did not significantly change the 1,1,1,2-TC partition values.

Table 1. Partition coefficients for carbon tetrachloride in male B6C3F1 mouse tissue.

Tissue	95 ppm	467 ppm	950 ppm
Blood			
No treatment	12 ± 1.5	15.8 ± 1.2	12.8 ± 1.9
Lead acetate			3.8 ± 0.2
Liver			
No treatment	66.5 ± 32.6	29.5 ± 6.6	36.3 ± 15.8
Lead acetate			18.3 ± 1.5
Fat			
No treatment	318.5 ± 38	356.4 ± 39.7	347.3 ± 27.5
Lead acetate			
Muscle			
No treatment	21.6 ± 14.3	25.1 ± 8.5	26.8 ± 5.2
Lead acetate			9.6 ± 1.4

Data are presented as mean ± standard deviation. N = 3-6.

Table 2. Partition coefficients for 1,1,1,2-tetrachloroethane in male B6C3F1 mouse tissue.

Tissue	467 ppm	934 ppm
Blood		
No treatment	65.6 ± 4.1	
Lead acetate		64 ± 3.9
Liver		
Lead acetate & heat	145.6 ± 6.5	137.6 ± 13.1
Fat		
No treatment	2964 ± 269	2692 ± 215
Muscle		
No treatment	337 ± 84.8	233 ± 17.6

Data are presented as mean ± standard deviation. N = 4.

DISCUSSION

In the absence of carbon tetrachloride, PCE rapidly clears from blood and liver and is readily metabolized to TCA, even at levels as high as 100 mg/kg. TCA is still present in blood and liver at 8 hr. CT alters the clearance of PCE in a dose dependent manner. TCA production is increasingly inhibited with higher concentrations of CT. However, at the 0.1 mg/kg CT dose level the concentration of TCA is slightly higher than controls, suggesting that the low concentration of CT induces P450 mediated metabolism. At doses higher than 0.1 mg/kg the concentration of CT appears to be high enough to destroy the cytochrome P450 2E1 enzyme, reducing the amount of TCA produced from PCE.

Like PCE, 1,1,1,2-TC is also rapidly metabolized in the absence of CT. However, 1,1,1,2-TC is still readily cleared from the blood even when CT is present at doses up to

and including 20 mg/kg. This confuses the issue as to whether the 2E1 enzyme is inhibited or destroyed. Metabolism of 1,1,1,2-TC is dramatically reduced at 50 mg/kg CT, and the level of TCA in blood and liver at 4 hr post dosing is almost 4-fold less than that of control. At the 0.1 mg/kg CT dose level, the increase in the amount of TCA in the blood also suggests that CT may induce cytochrome P450 2E1. However, liver levels of TCA are decreased when compared to controls. A measurement of the 2E1 enzyme activity could explain or shed light on the degree of inhibition or destruction of enzyme. Chemical exposures are not always limited to single compounds. Groundwater sources, especially those near industrial areas, are routinely contaminated with multiple chemicals. Characterizing the health risks associated with exposure to these chemical mixtures is a complex task. It is possible, even anticipated, that exposure to combinations of chemicals will result in increased toxicity. For example, CT is known to cause liver toxicity. Coupled with nontoxic doses of chlordecone CT causes extensive liver injury and a substantial increase in lethality (Mehendale, 1995). Additionally, co-administration of TCE in rats significantly increased the potentiation of carbon tetrachloride induced hepatotoxicity (Pessayre et al, 1982). However, when co-exposure occurs with CT and chlorinated solvents such as PCE or 1,1,1,2-TC the P450 2E1 mediated metabolism is knocked out, hindering the formation of TCA, the chemical considered to be responsible for toxicity (Bruckner, 1989; Larson and Bull, 1992; Moore and Harrington-Brock, 2000). The kinetic data collected in this study can be used in the development and validation of physiologically based pharmacokinetic models designed to predict the risk of exposure to chemical mixtures. By understanding the alteration in metabolic outcome for PCE and 1,1,1,2-TC, the assessment of risk associated with the exposure to these types of mixtures may be more science based.

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