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Journal of Chromatography B, 732 (1999) 145–153

JOURNAL OF CHROMATOGRAPHY B

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Simple method for rapid measurement of trichloroethylene and its major metabolites in biological samples

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Received 24 September 1998; received in revised form 25 May 1999; accepted 11 June 1999

Abstract

A simple and rapid, yet sensitive technique was developed for concurrent measurement of trichloroethylene (TCE) and its major metabolites (i.e., trichloroacetic acid, trichloroethanol and dichloroacetic acid) in blood and in solid tissues. The method involves addition of an esterizer (water, sulfuric acid, methanol; 6:5:1; v/v/v) to blood or tissue homogenate in sealed vials, and subsequent gas chromatographic headspace analysis. The procedure should be useful in medical monitoring of TCE exposure as well as in experimental work, notably pharmacokinetic and pharmacodynamic studies pertaining to TCE carcinogenesis.

Keywords: Trichloroethylene

1. Introduction

Trichloroethylene (TCE) is a volatile organic chemical that has been widely used as a fumigant, metal degreaser, dry cleaning agent and solvent in other commercial operations. It has been estimated that as many as 3.5 million persons have been exposed occupationally to TCE in the USA [1]. TCE and other halogenated hydrocarbons enter the environment and are commonly found as contaminants in air and water. Thus, large segments of the population are exposed to very low levels of these chemicals. There is concern that such exposures may result in an increased risk of cancer. High doses of TCE have been shown to produce cancers in mice and rats [2]. The causal agents are believed to be certain TCE metabolites, notably trichloroacetic acid (TCA) and dichloroacetic acid (DCA) [3–6].

Quantitation of TCE and its major metabolites in experimental animals and human subjects is necessary in medical monitoring and in toxicity and carcinogenesis studies. A basic tenet of toxicology is that the magnitude and duration of adverse effects are functions of the dose and the length of time the chemical is present. The concept of dose is being refined from administered dose to absorbed dose (e.g., blood concentration of parent compound) to target tissue dose of bioactive moieties (e.g., DCA and TCA). The most logical and precise way to express internal dose is as a time integral of the target organ concentration of bioactive chemical(s) [7]. In order to derive appropriate time-course data, tissue concentrations must be measured sequentially during and post exposure in an adequate number of subjects. As TCE exposure levels may be quite low, a sensitive analytical procedure is needed which will

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0378-4347/99/$ – see front matter
PII: S0378-4347(99)00282-0

allow rapid but accurate measurement of TCE and its metabolites in large numbers of blood and tissue samples.

A number of procedures have been developed to quantify TCE metabolites in biological specimens. These methods include spectrophotometric determination using the Fujiwara reaction [8] and gas chromatographic (GC) techniques [9–13]. The basic approach to analysis of polar TCE metabolites is to volatilize them for GC analysis. The most common way to achieve this has been to produce a methyl ester of the compound of choice. Most esterification techniques involve complicated, time-consuming procedures for processing biological samples, as well as the use of poisonous and explosive chemicals. DeBaere et al. [14], for example, used diethyl ether as an extractant and diazomethane as a derivatizing agent. Some methods require freezing samples overnight, thawing and incubating them, extraction of the metabolites into an organic phase and direct injection of an aliquot into a GC for analysis [12,13]. Ohara et al. [10], however, employed a relatively simple esterification procedure, which allowed GC quantitation of TCA and trichloroethanol in urine. The objective of the current study was to adapt and optimize the method of Ohara et al. [10] for concurrent measurement of TCE and its major metabolites in blood and solid tissues.

2. Experimental

2.1. Chemicals and animal procedures

All chemicals were analytical grade. 1,1,2-Trichloroethylene (TCE) was obtained from Aldrich Chemicals (St. Louis, MO, USA). Trichloroacetic acid (TCA), trichloroethanol (TCEOH) and dichloroacetic acid (DCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sulfuric acid and methanol came from J.T. Baker (Phillipsburg, NJ, USA). The esterification mixture, or esterizer, was made of distilled water, concentrated sulfuric acid and methanol in a ratio of 6:5:1 (v/v/v). Alkamuls®, a polyethyloxated vegetable oil, was bought from Rhone Poulenc (Cranbury, NJ, USA). Male Sprague-Dawley rats and B6C3F1 mice (Charles River Laboratories, Raleigh, NC, USA) were used for dosing with TCE and for subsequent collection of blood and tissue samples. The rats and mice received 100 mg TCE/kg bw in a 5% aqueous Alkamuls® emulsion by gavage. At selected intervals after the oral dosing, groups of animals were sacrificed by cervical dislocation and blood obtained by closed chest cardiac puncture. Tissues, including the liver, kidneys and lungs, were quickly removed after opening the thoracic and abdominal cavities. A portion (0.15–1.0 g) of each tissue was rapidly blotted to remove excess blood and transferred to a 20-ml scintillation vial containing 4 ml of ice-cold 0.9% saline. The tissues were then homogenized in less than 20 s with a polytron homogenizer (Ultra Turrax, Tekmar, Cincinnati, OH, USA). A 5–100 μl aliquot of blood or homogenate was transferred to a 20-ml vial containing 200 μl of esterizer. These vials were capped with PTFE-lined rubber septa, crimped tightly, vortexed, and placed into the GC headspace autosampler for analysis.

2.2. Headspace gas chromatography

A Perkin-Elmer Model 8500 gas chromatograph (GC) fitted with an electron capture detector (ECD) and a HS-101 headspace autosampler (Perkin Elmer, Norwalk, CT, USA) were utilized. Analyses were carried out on a 10″×1/8″ OD stainless steel column packed with 10% OV-17 on Supelcoport® (Supelco, Bellefonte, PA, USA). The temperatures for analyses were as follows: column, run isothermal at 150°C; injector 200°C; and detector 360°C. Nitrogen was used as the carrier gas at 60 ml/min. Samples were heated at 110°C in a thermostat-controlled autosampler chamber for 30 min before being vented into the GC. Each run was for 8 min. All compounds were well resolved (Fig. 1).

2.3. Standard curve

Standard solutions of 10 μg TCE/ml isooctane and 10 μg of TCA, TCEOH and DCA/ml distilled water were prepared from freshly made stock solutions of 1 mg/ml of each compound. Aliquots (1–50 μl containing 10–500 ng chemical) were transferred to 20-ml scintillation vials containing 200 μl of esterizer, in duplicate. The vials were then capped with PTFE-lined rubber septa and aluminum caps,

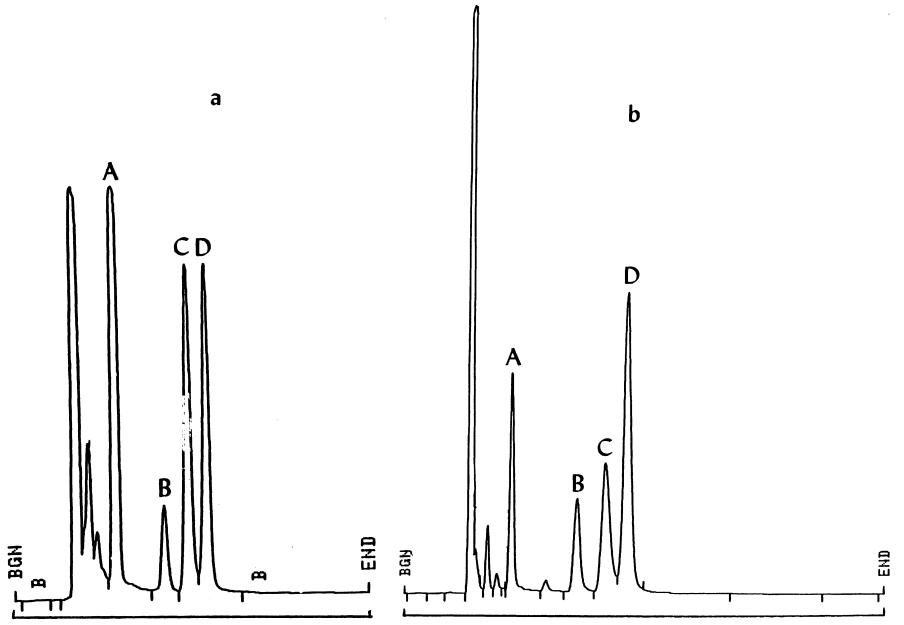

Fig. 1. Representative chromatograms of: (a) standard solution containing 100 ng of TCE, TCA, TCEOH and DCA; and (b) lung of a rat dosed orally with 100 mg/kg of TCE. A: TCE; B: DCA; C: TCEOH; D: TCA.

crimped tightly, vortexed and analyzed by headspace GC as described above. Blank esterizer (200 μl) was used as the control. Detector responses (i.e. peak areas) were plotted against the known amounts of each compound and linear regression equations derived. Good linearity was observed in the range of 10–200 ng for TCE and each metabolite. The response factor was higher for trichloro than for dichloro compounds. The limits of detection (LODs) were determined by a standard procedure [15]. A signal-to-noise ratio of 3 or greater was considered as the LOD. Neither background noise nor interfering peaks were observed, as the detector baseline was consistently stable. The LOD for TCA and TCEOH was ~5 ng/ml (ppb), while that for DCA was ~10 ng/ml.

2.4. Effect of esterizer volume

Different volumes of the esterizer, ranging from 50 to 600 μl, were employed to determine the appropriate volume to use in the analyses. A constant amount (100 μg) of each test compound (i.e. 10 μl of a 10-μg/ml solution) was pipetted into vials containing 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml of esterizer, in duplicate. The vials were tightly capped, vortexed and analyzed by headspace GC in order to ascertain the peak areas for each compound and esterizer volume.

2.5. Coefficient of variation

All standard solutions were tested for coefficient of variation. A 10-μl aliquot of each chemical standard containing 100 ng of chemical was mixed with 200 μl of esterizer. These solutions were capped, vortexed and analyzed by headspace GC as described previously. Eleven replicates were run.

2.6. Percent recovery assessment

The recoveries of DCA, TCA and TCEOH were determined from spiked rat blood and tissue samples. One ml of blood and multiple 1-g portions of liver, kidney and lung were obtained from undosed rats and transferred to scintillation vials containing 4 ml of chilled saline. A series of stock solutions containing from 0.05 to 10 mg TCA, TCEOH and DCA/ml of distilled water were prepared. Five μl of each solution were injected directly into four differ-

ent portions of blood and each tissue. The blood and tissues were homogenized within the scintillation vials for approximately 20 s by use of a polytron. Twenty-μl duplicates of each homogenate were transferred to 20-ml headspace vials containing 0.2 ml of the esterizer. All vials were capped with Teflon®-covered rubber septa and aluminum caps, tightly crimped and vortexed for 30 s to achieve esterification. Reference standards were prepared by injecting 5 μl of each of the stock solutions into scintillation vials containing only 4 ml of cold saline. Duplicates of these reference standard solutions were processed and analyzed with the spiked blood and tissue samples by headspace GC. The amount of analyte in each sample was determined by measuring the peak area, including the peak area in the appropriate linear regression equation, and solving the equation. Percentage recovery was calculated by simply dividing the amount of analyte found in the biological sample by that in the reference standard and multiplying×100.

3. Results

The analytical procedure resulted in good resolution of TCE and its major metabolites. Representative chromatograms of a standard solution and lung are presented in Fig. 1. The peaks were well separated from one another, facilitating quantification of the parent compound (TCE) and metabolites (TCA, TCEOH and DCA). Retention times for these substances were: TCE – 1.7 min; DCA – 2.9 min; TCEOH – 3.3 min; and TCA – 3.7 min. No interfering peaks were found in chromatograms of blood or of any tissue. Intrasample variability was modest. Coefficients of variation were as follows: TCE – 7.4%; TCA – 7.1%; TCEOH – 2.2%; and DCA – 2.6%.

Standard calibration curves of peak areas versus amounts of TCE, TCA, TCEOH and DCA were linear in the range of 10 to 200 ng (data not shown). There was a non-linear response for quantities of each compound greater than 200 ng. The linear regression equations were determined to be: TCE: $y=2.714x\pm(-6.169)$; TCA: $y=1.322x\pm(9.958)$; TCEOH: $y=1.18x\pm(-0.55)$; and DCA: $y=0.3754x\pm(7.533)$.

The volume of esterizer was found to affect the quantitation of TCE and its metabolites. It was necessary to use the esterizer to convert TCA and DCA to methyl esters which could be volatilized. This was not necessary for TCE and TCEOH. It can be seen in Fig. 2 that the TCE and TECOH peak areas are greatest when no esterizer is present. Stepwise addition of larger volumes of the esterizer resulted in progressive decreases in the TCE and TCEOH peak areas. This phenomenon is the result of reduced volatilization of TCE and TCEOH due to the increasing volumes of liquid. An aim of this experiment was also to determine which volume of esterizer resulted in the maximum detector response for DCA and TCA, the two carcinogenic metabolites of TCE. Relatively high peak areas for both DCA and TCA were seen at 200 μl, so this volume of the esterizer was utilized in subsequent analyses.

There was good recovery of TCE metabolites from blood and most tissues studied. Percentage recoveries of TCA and TCEOH ranged from 57 to 100% (Table 1). Recovery of TCA, TCEOH and DCA was most efficient from lung and blood. Recovery of all three metabolites was least efficient from the liver. This was particularly true for DCA. DCA levels measured in spiked liver samples were very low or nondetectable. DCA recovery from kidney was substantially higher than from liver, but still significantly lower than from blood and lung. These results may be indicative of DCA catabolism, particularly in the liver. TCA and TCEOH recoveries from kidney were usually somewhat less than from blood and lung. Recovery of TCEOH and DCA did not appear to be concentration-dependent in the range examined here. An exception was recovery of DCA from kidney, which generally increased with increasing DCA concentration. In contrast, TCA recovery was relatively independent of both concentration and tissue. Previous experiments (data not shown) revealed that recovery of TCE from spiked blood and tissues was quite good (i.e. 81–91%).

Time-courses of TCE and its metabolites in blood and tissues of mice and rats were readily determined using the assay. Concentrations of TCE and metabolites in the blood of rats, following oral dosing, are shown in Fig. 3. The blood levels of TCE diminish over time as the parent compound is metabolized. High concentrations of TCA accumulate in the rats'

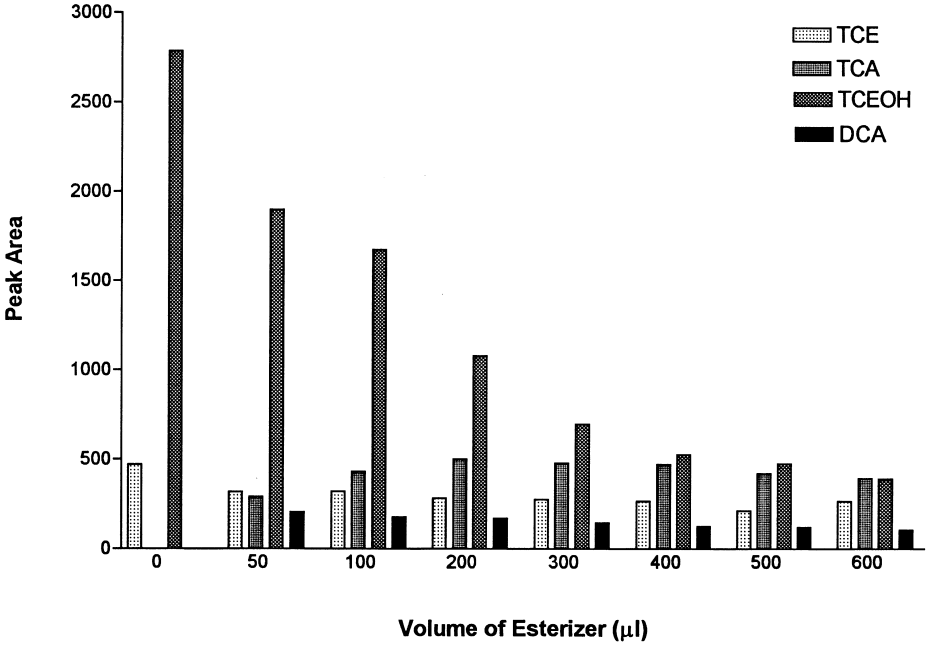

Fig. 2. Effect of volume of esterizer on the quantitation of TCE and its metabolites. GC detector responses are represented by peak areas.

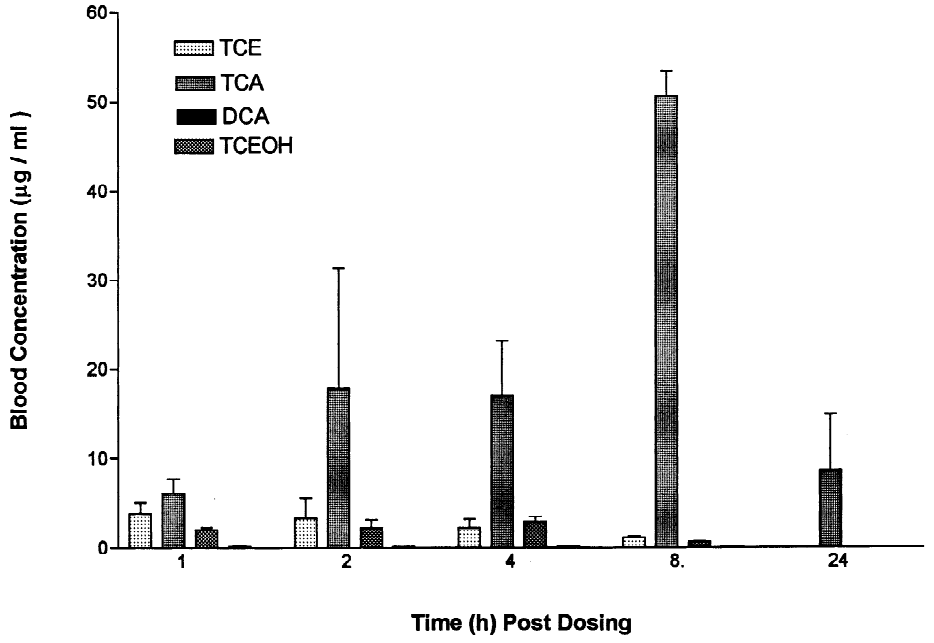

Fig. 3. Time-course of TCE and its metabolites in the blood of rats dosed orally with 100 mg TCE/kg bw. Mean blood levels for groups of 6 rats are expressed as μg/ml±SD.

Table 1
% Recovery of TCE metabolites from spiked rat blood and tissues[a]

Compound	Concn. (mg/ml)	Blood	Liver	Kidney	Lung
TCA	0.05	100	76	77	87
	0.1	90	57	78	98
	0.25	87	87	86	86
	0.5	76	79	64	64
	1.0	89	71	78	86
	5.0	85	77	63	72
	10.0	81	71	65	72
TCEOH	0.05	75	57	65	74
	0.1	78	57	78	88
	0.25	84	71	76	88
	0.5	78	63	74	85
	1.0	98	75	86	87
	5.0	68	68	77	88
	10.0	70	70	79	85
DCA	0.05	52	7	15	42
	0.1	58	ND[b]	34	61
	0.25	67	12	24	66
	0.5	68	2	29	73
	1.0	78	1	43	76
	5.0	70	ND	65	81
	10.0	61	ND	63	72

[a] Values are expressed as mean percentage recovery for 4 samples.
[b] ND – Not detected.

blood, reaching a maximum 8 h after dosing. Mice gavaged with the same dose (100 mg/kg) of TCE initially have higher concentrations of TCA in their bloodstream, though the TCA levels diminish more rapidly (Fig. 4A). In contrast to rats, the mice also have high blood levels of TCEOH and DCA. DCA is barely detectable in the blood of the rats (Fig. 3). TCA, TCEOH and DCA are present in all three tissues of mice at each time-point (Figs. 4 and 5). Unexpectedly, the lungs exhibit quite high concentrations of these metabolites during the 24 h monitoring period (Fig. 5B). Very high levels of TCEOH are evident in kidney at 1 h, though the levels steadily decline thereafter (Fig. 5A).

4. Discussion

The analytical technique described here is sensitive, rapid and relatively simple. Most published procedures for quantifying TCE and its metabolites in biological specimens are complex and time-consuming [8,9,11–13]. In the search for a more time-efficient method, the publication of Ohara et al. [10] was discovered. These investigators were able to measure TCA and TCEOH in esterified urine samples by headspace GC. Our study objective was to determine whether their approach could be optimized and used to simultaneously quantify both TCE and its metabolites in complex biological matrices (i.e., blood and solid tissues).

The procedure outlined in the publication of Ohara et al. [10] proved to be adaptable to blood and tissue analyses. Their limits of detection were 2 ng/ml (ppb) for TCA and 5 ng/ml for TCEOH. The limit of detection for these metabolites in the current study was 5 ng/ml. Ohara and co-workers [10] used 100 μl of urine. We routinely used from 5 to 100 μl of blood or tissue homogenate, depending upon the concentration of analyte present. Use of such small volumes of blood allowed serial sampling from the same animal without depletion of its blood supply. Ohara et al. [10] measured TCA and TCEOH, while both the parent compound (TCE) and its metabolites (TCA, TCEOH and DCA) were assayed by the currently described procedure. Proper handling of blood and tissue samples and GC headspace analysis made it possible to minimize TCE volatization and loss during sample processing. We utilized a packed GC column rather than a capillary column and a smaller volume of esterizer, comprised of water, sulfuric acid and methanol (6:5:1). Humbert et al. [11] and DeBaere et al. [14] utilized capillary columns to quantify chloral hydrate, TCEOH and TCA in human samples. A capillary column can certainly be used, though we found that its relatively long retention times substantially increased the time required to analyze large numbers of samples.

The analytical procedure described here should be quite useful in medical monitoring and in experimentation involving TCE and its metabolites, especially for pharmacokinetic and pharmacodynamic studies. The headspace technique is quite sensitive and accurate. It offers the advantages of speed and simplicity, such that numerous samples can be collected and subsequently assayed with a GC equipped with an ECD and a multicompartment autosampler. A headspace GC technique was previ-

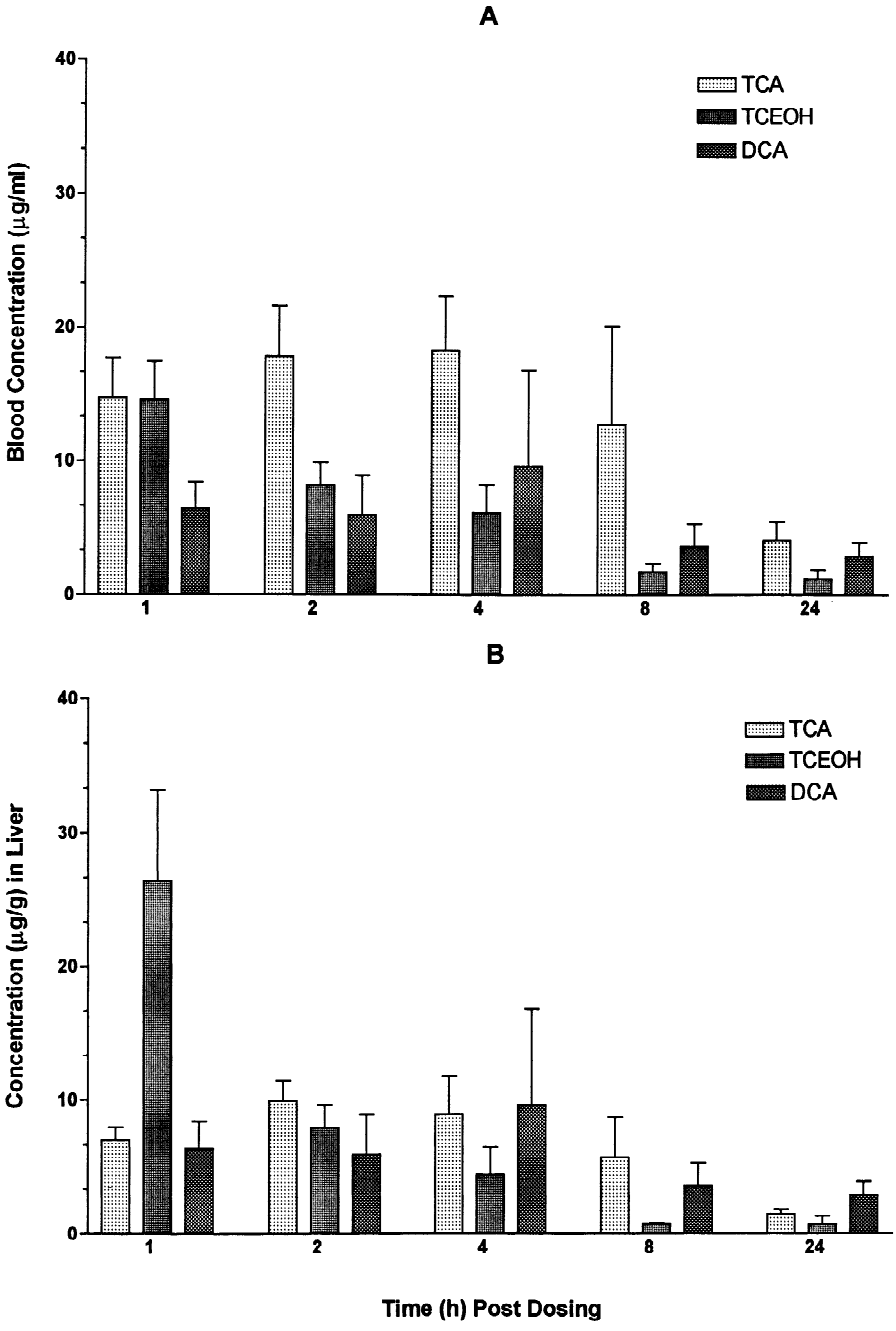

Fig. 4. Concentrations of TCE metabolites over 24 h in the blood (A), and liver (B), of mice given 100 mg TCE/kg bw orally. Mean tissue and blood concentrations for groups of 6 mice are expressed as μg/g±SD.

ously developed in our laboratory [16] for measurement of TCE and other volatile halocarbons in blood and tissues. Metabolites, however, could not be assayed with this method. Pharmacokinetic and pharmacodynamic studies frequently require serial sampling from multiple subjects, in order to clearly

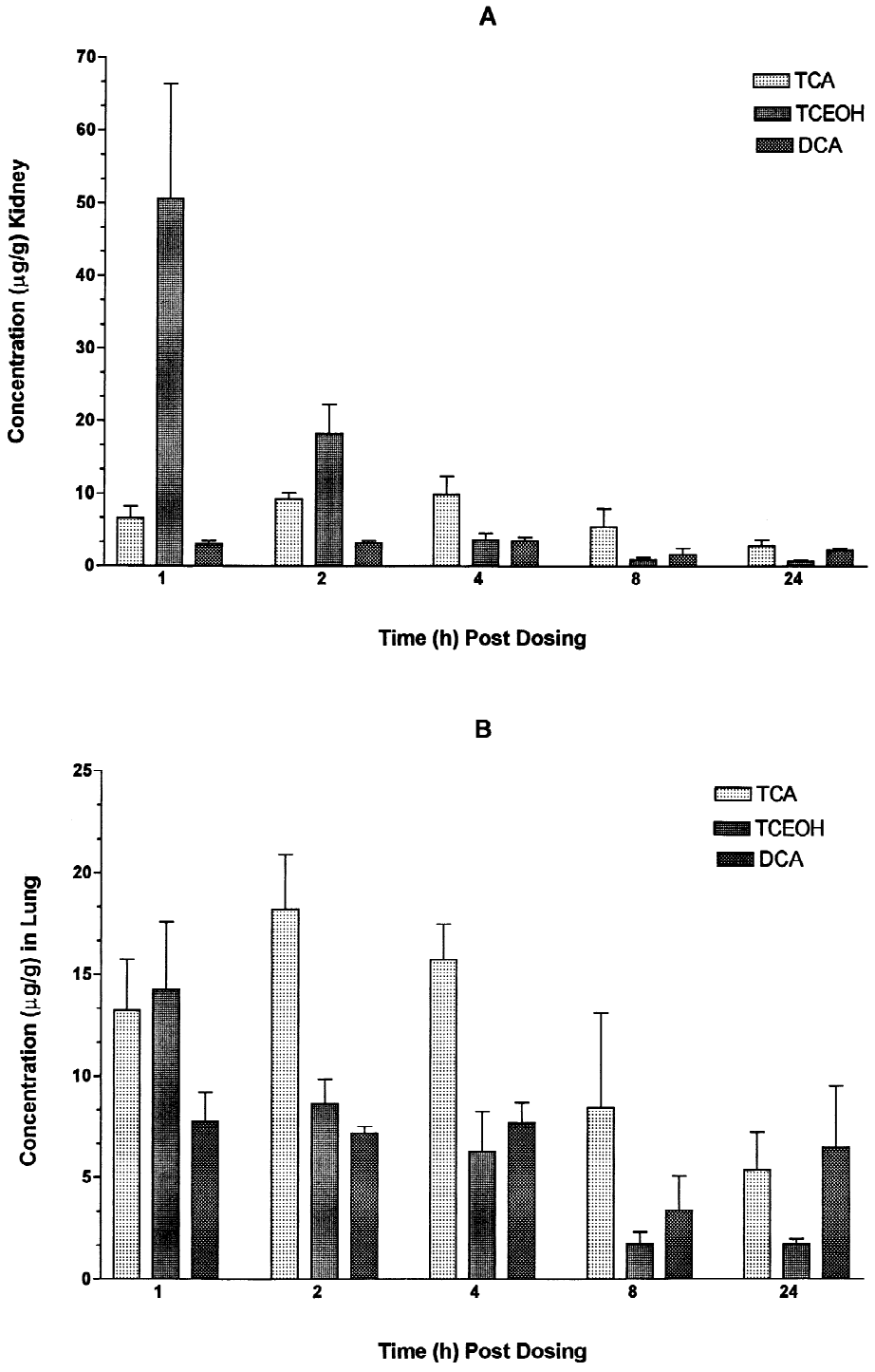

Fig. 5. Concentrations of TCE metabolites over 24 h in kidney (A) and lung (B) of mice given 100 mg TCE/kg bw orally. Mean tissue concentrations for groups of 6 mice are expressed as mg/g tissue±SD.

delineate the uptake, distribution and elimination profiles of parent compound and metabolites in different parts of the body.

Representative time-courses of TCE and its metabolites are presented here. Following TCE ingestion, TCE levels in the blood of rats diminish with time, though TCA levels progressively increase (Fig. 3). TCE levels drop even more rapidly in mice, falling so low by 1 h after dosing that they are not detectable in blood or tissues. In contrast, blood TCA concentrations in mice and rats progressively increase over time, reaching a maximum 8 h post dosing in the rats. Concentrations of TCEOH and DCA are much higher in the blood of mice than rats (Figs. 3 and 4A). The relatively rapid decrease in parent compound and large increase in all three metabolites are indicative of the higher rate and extent of TCE metabolism by mice [17,18]. Although TCE metabolism is quantitatively quite different in mice, rats and humans, it is thought to be qualitatively similar [19]. DCA appears to be an exception, as we found it in substantial amounts only in the mouse. The high concentrations of DCA and TCA in the liver and lungs of mice may contribute to the susceptibility of these organs to cancer in this species.

Acknowledgements

This work was supported by U.S. Department of Energy Grant #DE-FC02-98CH1092 and U.S. Agency for Toxic Substances and Disease Registry Contract #9869172.

The authors would like to thank Ms. Joy Wilson and Ms. Mary Eubanks for their skillful preparation of this manuscript.

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