Journal of Chromatography, 612 (1993) 199–208
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6660

Analyses of volatile C_2 haloethanes and haloethenes in tissues: sample preparation and extraction

Xiao Mei Chen, Cham E. Dallas, Srinivasa Muralidhara, V. Srivatsan and James V. Bruckner

Department of Pharmacology and Toxicology, College of Pharmacy, University of Georgia, Athens, GA 30602-2356 (USA)

(First received August 21st, 1992; revised manuscript received October 30th, 1992)

ABSTRACT

A tissue extraction procedure was developed which minimized loss of readily volatilizable compounds for subsequent quantification by headspace gas chromatography, and evaluated for perchloroethylene (PER), 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, and 1,1,2-trichloroethylene. Of the procedures evaluated, joint isooctane and saline tissue homogenization had the most efficient recovery, ranging from 73 to 104% for the four halocarbons from seven different rat tissues. PER concentrations were also determined in tissues of rats following *in vivo* halocarbon administration. Recovery did not appear to be tissue-dependent, but did vary somewhat with test chemical, with the least volatile, most lipophilic compounds exhibiting the highest recovery.

INTRODUCTION

Short-chain aliphatic halogenated hydrocarbons (halocarbons) are a class of volatile organic compounds (VOCs) of increasing concern, due to their widespread occurrence as environmental contaminants and the potential risks they pose to health. Exposure to halocarbons can result in toxic injury of a number of organ systems in animals and humans. Central nervous system (CNS) dysfunction results from overexposure to most halocarbons and other VOCs [1,2]. CNS depressant effects have been directly correlated with the concentration of hydrocarbons in the brain [3,4]. Significant liver and kidney damage can be caused by certain halocarbons [5–7], while some members of this chemical class are carcinogenic in different organ systems in animals [8–10].

Pharmacokinetic studies of halocarbons are needed in order to elucidate target organ uptake, deposition, and elimination of the chemicals. The magnitude of toxic effect in an organ is, of course, dependent upon the amount of chemical present in the tissue. Pharmacokinetic studies conducted to date have primarily involved measurement of concentrations of halocarbons in blood and exhaled breath [11–15]. The limited (*i.e.*, at a single time-point) tissue measurements conducted in some of these studies employed ^{14}C-labeled halocarbons. Measurement of total radioactivity does not delineate between the parent compound, metabolites, and ^{14}C which has entered the body's carbon pool. There have been a limited number of investigations, in which time-courses of tissue deposition of inhaled hydrocarbons have been delineated [3,4,16–18]. In these studies, the tissues were extracted with a solvent and the parent compounds quantified by gas chromatography (GC) or GC–mass spectral

Correspondence to: Dr. Cham E. Dallas, Department of Pharmacology and Toxicology, College of Pharmacy, University of Georgia, Athens, GA 30602-2356, USA.

analysis. No reports of the time-course of uptake and elimination of halocarbons in tissues were found in the literature.

A variety of approaches have been used for analysis of VOCs in blood and tissues. One technique is to simply inject blood and tissue homogenates directly into a GC apparatus [16,19–21]. Major drawbacks of direct injection of biological materials are that the materials cause matrix interferences and that contamination markedly shortens the GC column's lifetime. Solvent extraction is a widely used approach for measuring concentrations of VOCs in blood and tissues. An aliquot of the solvent may be directly injected into the GC column [17,18,22,23]. Since this method typically involves a one-step extraction of the VOC with the solvent, limited sensitivity and interference by other lipophilic compounds can be problematic. In order to circumvent these difficulties, more complex procedures have been employed. One entails evaporation of the solvent extract and trapping of the VOC analyte on a Tenax column [24]. Another involves heating biological samples within a purging device [25–29], with subsequent retention of the analyte on an adsorbent such as Tenax. Such approaches are technically difficult and time-consuming. Headspace analysis has proven to be a sensitive and more efficient means of measuring VOC concentrations in blood samples [14,15,30,31]. No one, however, appears to have reported a suitable technique for quantification of halocarbons or other VOCs in tissues.

In light of the foregoing, it is apparent that there is a need for a rapid, sensitive analytical procedure for reliably measuring the concentrations of halocarbons and other VOCs in different tissues. The overall objective of this project was to adapt the headspace technique previously used for analysis of blood samples for measurement of C_2 halocarbons in different tissues. A major focus of the work was development of a procedure for conservation of the analyte (*i.e.*, minimization of loss by volatilization) during preparation and extraction of the tissue samples. Two C_2 haloalkananes and two C_2 haloalkenes were employed, in order to assess the utility of the procedure for extraction and subsequent analyses of VOCs with different physicochemical properties.

EXPERIMENTAL

Test chemicals and apparatus

1,1,2,2-Perchloroethylene (PER), of 99% purity, and 1,1,2,2-tetrachloroethane (TET), of 97% purity, were obtained from Aldrich (Milwaukee, WI, USA). 1,1,1-Trichloroethane (TRI), of 99% purity, 1,1,2-trichloroethylene (TCE), of 99% purity, and isooctane, of 99.98% purity, were purchased from J. T. Baker (Phillipsburg, NJ, USA). A Sigma Model 300 gas chromatograph equipped with a HS-6 headspace sampler (Perkin Elmer, Norwalk, CT, USA) and a Model 5890 gas chromatograph equipped with a 19395A headspace sampler (Hewlett Packard, Avondale, PA, USA) were used for the analysis of halocarbons. Both the gas chromatographs were equipped with an electron-capture detector. Analyses were carried out on stainless-steel columns (182 cm × 0.317 cm I.D.) packed with 10% FFAP (Alltech Assoc., Deerfield, IL, USA), 3% SP 1000 (Supelco, Bellefonte, PA, USA), or 3% OV-17 (Alltech Assoc.). Tissues were homogenized using the Ultra-Turrax SDT homogenizer (Tekmar, Cincinnati, OH, USA).

Tissue homogenization and extraction procedures

Twelve-week-old male Sprague–Dawley rats were obtained from Charles River Labs. (Raleigh, NC, USA). After two to three weeks, groups of four or eight animals (body weight = 325–375 g) were anesthetized with diethyl ether. Blood samples (1 ml) were withdrawn by closed chest cardiac puncture. Portions (0.5–1 g) of liver, kidney, brain, heart, lung, perirenal fat, and skeletal muscle were removed and placed onto ice. Each tissue was spiked with PER, TET, TCE, or TRI, by injection of 4 μl/g tissue of a solution containing 1 mg halocarbon per ml isooctane. Two homogenization approaches were evaluated using PER. In the first, the tissues were immediately transferred after halocarbon injection to previously chilled 20-ml glass vials containing 4 ml of ice-cold saline. The tissues were allowed to

remain in the tightly capped vials for approximately 30 min, before being homogenized for an established time interval with a Tekmar tissue homogenizer. These times were kept as brief as possible in order to minimize volatilization of the halocarbons. Brain, liver, and fat were the most easily homogenized, requiring only 3–4 s. Kidney, lung and heart required 5–8 s. Skeletal muscle was the most difficult to homogenize, in that it required 20 s. Isooctane (8 ml) was added to the homogenates, which were then vortex-mixed for 30 s and centrifuged at 1800 *g* for 10 min at 4°C in capped vials. An aliquot of the isooctane layer was transferred to capped vials for headspace analysis. In the second approach, tissues were immediately transferred after halocarbon injection to previously chilled 20-ml glass vials containing 2 ml of ice-cold saline and 8 ml of isooctane. Each tissue was then homogenized, vortex-mixed, and centrifuged as before. An aliquot of the isooctane layer was taken for headspace analysis. Only the latter approach (*i.e.*, isooctane homogenization) was subsequently used for determination of TCE, TRI, TET, and PER in tissues, except in the aliquot volume study.

Isooctane aliquot volume study

An experiment was conducted to determine the effect of aliquot volume on the linearity of halocarbon quantification. As in other *in vitro* experiments, a Hamilton gas-tight syringe was used to inject the chemical into the center of the tissue cubes. A 4-μl volume of PER was injected into samples of blood and each of the seven tissues. The tissues were homogenized in saline and subsequently extracted with isooctane as described previously. Aliquots (5–100 μl) of isooctane extract were withdrawn with a pipet and transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal. Each sample vial was then placed into the autosampler unit of the gas chromatograph.

Headspace gas chromatographic techniques

For all the experiments with PER, the GC operating conditions were: headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; column packing, 10% FFAP; flow-rate for argon–methane carrier gas, 60 ml/min. Operating conditions for TET were: headspace sampler temperature, 100°C; injection port temperature, 200°C; column temperature, 150°C; detector temperature, 400°C; column packing, 3% OV-17; flow-rate for argon–methane carrier gas, 60 ml/min. Operating conditions for TRI and TCE were: headspace sampler temperature, 55°C; injection port temperature 150°C; column temperature, 60°C; detector temperature, 400°C; column packing, 3% SP 1000. Except for the isooctane aliquot volume study, all analyses were conducted using a 20-μl aliquot of isooctane in the 8-ml headspace vials. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 s with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column.

The aforementioned conditions resulted in vaporization of the halocarbons in the sample vials, since each VOC was heated to a temperature slightly below its boiling point, and the vial subsequently pressurized and vented into the GC. An experiment was conducted to assess the influence of heating time on quantification of PER. Heating sample vials 5 min or longer resulted in a constant GC detector response (*i.e.*, area under the curve) for a series of known quantities of PER (data not shown).

As saline was utilized in the tissue homogenization procedures, an experiment was conducted to determine whether the presence of saline influenced standard curves. PER was incorporated into four different solvent systems: 8 ml isooctane; 2 ml saline + 8 ml isooctane; 4 ml saline + 8 ml isooctane and 4 ml saline + 8 ml isooctane homogenized for 30 s. Each solution was vortex-mixed for 30 s. Aliquots of 1–25 μl of the isooctane layer, equivalent to 1–25 ng PER, were subjected to GC headspace analysis. Standard curves were generated on the basis of the GC peak area plots. The slopes, intercepts, and correlation coefficients of the curves were compared. As described in the Results section, the standard curves

did not vary significantly from one solvent system to another. Therefore, the simplest system was subsequently employed for preparation of standard solutions of PER, TET, TCE, and TRI (*i.e.*, an appropriate amount of halocarbon was dissolved in isooctane alone). Standard curves for each compound were generated the same day that sample analyses were performed, using the same analytical conditions.

The limit of detection of the GC assay was determined by the method described by MacDougall and Chummett [32]. A signal-to-noise ratio of 3 or greater was considered as the limit of detection. The presence of background noise and any interfering peaks was assessed in air and in isooctane samples. Neither was observed, as the detector baseline was consistently stable. The limit of detection for TRI, TCE, PER, and TET was found to be 1 ng. This amount is equivalent to 8.4, 8.5, 6.7, and 6.6 parts of chemical per billion parts of air for TRI, TCE, PER, and TET, respectively.

In vivo tissue measurements

The concentration of PER was determined in tissues of rats following intra-arterial administration of the compound. Male Sprague–Dawley rats of 325–375 *g* from Charles River Labs. were surgically implanted with an indwelling carotid artery cannula. The cannula exited the body behind the head, so the animal could not disturb the cannula, but have freedom of movement. Food was withheld during an 18-h recovery period before PER administration. PER was incorporated into undiluted polyethylene glycol 400, and a dose of 10 mg PER/kg body weight injected as a single bolus into the arterial cannula. Each animal was anesthetized with diethyl ether 1 h after dosing, and blood taken by closed-chest cardiac puncture. Portions (1 g) of liver, kidney, brain, heart, lung, perirenal fat, and skeletal muscle were excised within 2.5 to 3 min from each animal and immediately placed into chilled vials containing 2 ml of saline and 8 ml of isooctane. The samples were processed for PER analysis using the isooctane homogenization procedure described previously.

Statistics

Comparisons of the percentage recovery of PER from tissues and blood, using the two homogenization procedures, were made using Student's *t*-test. A two-way analysis of variance was utilized to assess the significance of variances among standard curves for the different saline–isooctane mixtures. Values were considered significantly different at $p < 0.05$. The inter-assay variation had a coefficient of variation that did not exceed 12%, and the intra-assay coefficient of variation was less than 10% for all compounds tested.

RESULTS

Results of the study on the effect of isooctane aliquot volume on the linearity of halocarbon quantification are presented in Fig. 1. A very similar pattern was observed for all tissues studied. The quantity of PER increased linearally with increasing aliquot volume up to 25 μl. Use of larger aliquots of isooctane (*i.e.*, 50 and 100 μl) did not result in any further increase in the amount of measurable PER.

Recovery values (%) obtained using the saline and isooctane homogenization approaches for PER are contrasted in Table I. Recoveries were quite good with both procedures, in that values ranged from approximately 72 to 104%. Recoveries of PER from kidney, fat, lung, muscle, and brain were significantly higher when the tissues were homogenized directly in isooctane. Recovery of PER from liver, heart, and blood did not differ significantly for the two procedures.

Recoveries (%) of PER, TET, TCE, and TRI from spiked tissues, utilizing the isooctane homogenization procedure, are tabulated in Table II. Recovery of TET was generally higher than was the case for the other three chemicals. Recovery of TCE was generally the lowest of the four chemicals, with no mean values exceeding 88% for any tissue. Indeed, the lowest recovery of TET (*i.e.*, from fat) was greater than the highest recovery of TCE (*i.e.*, from muscle). The mean recoveries of PER, TET, and TRI from fat were quite similar (within 2%). TCE recovery (73%)

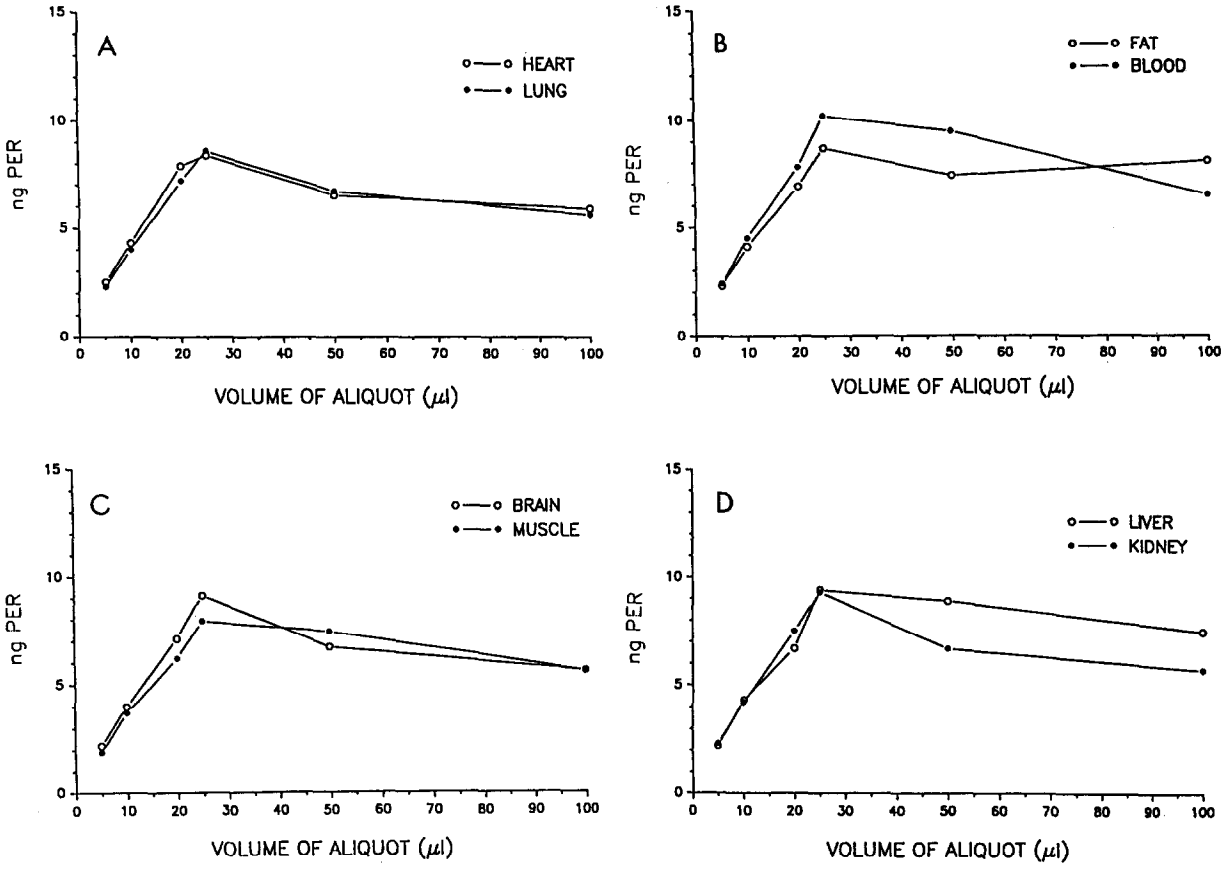

Fig. 1. Effect of isooctane aliquot volume on linearity of PER content in samples from heart and lung (A), fat and blood (B), brain and muscle (C), and liver and kidney (D). Aliquot volumes of 5, 10, 20, 25, 50, and 100 μl were withdrawn from the organic phase of the tissue homogenate and analyzed by GC headspace analysis.

from fat was the lowest for any chemical from any tissue. The range in values for the different tissues was the smallest for TRI (*i.e.*, less than 6%) and the largest for TCE (*i.e.*, 14.9%). The recovery of the four volatile chemicals from tissues with the shortest homogenization time (*i.e.*, liver, fat, and brain) was not substantially different from that from other tissues. Unexpectedly, there was relatively high recovery of all four halocarbons from skeletal muscle, the tissue requiring the longest homogenization time. No tissue consistently exhibited higher or lower recovery values for any of the four chemicals.

Standard curves for PER standards, prepared using four different solvent and saline mixtures, are shown in Fig. 2. The linear regression equations were determined to be $y = 25.9x - 13.3$ for 8 ml isooctane, $25.9x - 8.4$ for the 2 ml saline–8 ml isooctane mixture, $y = 25.8x - 8.0$ for the 4 ml saline–8 ml nonhomogenized isooctane mixture, and $y = 25.2x - 3.6$ for the 4 ml saline–8 ml homogenized isooctane mixture. For all of the data considered together, the linear regression equation was $y = 24.3x - 6.9$. Thus, there was no statistically significant difference between the regression equations for the four solvent systems. Therefore, neither the presence of saline nor homogenization significantly affected standard curves for PER.

Concentrations of PER, measured in tissues of rats 1 h following intra-arterial administration of a single 10 mg/kg dose of PER, are shown in Ta-

TABLE I

EFFECT OF HOMOGENIZATION PROCEDURE ON THE RECOVERY OF PER

Each value represents the mean ± S.E. for recovery of 1,1,2,2-tetrachloroethylene (PER) from tissues of eight rats for isooctane homogenization and four rats for saline homogenization.

Sample	Recovery (%)	
	Saline homogenization	Isooctane homogenization
Liver	95.5 ± 9.9	89.6 ± 3.1
Kidney	69.0 ± 4.9[c]	86.7 ± 1.4[c]
Fat	73.8 ± 4.9[a]	88.2 ± 2.7[a]
Heart	75.8 ± 10.3	81.2 ± 1.2
Lung	78.8 ± 7.1[b]	99.1 ± 2.3[b]
Muscle	80.3 ± 6.3[a]	98.5 ± 2.9[a]
Brain	72.3 ± 5.7[b]	88.6 ± 2.0[b]
Blood	104.8 ± 5.5	95.4 ± 4.1

[a] Significant difference between procedures at $p < 0.05$.
[b] Significant difference between procedures at $p < 0.01$.
[c] Significant difference between procedures at $p < 0.001$.

ble III. PER levels in the fat were an order of magnitude or more higher than in any other tissue sampled. The concentrations of PER measured in the liver, kidney, heart, and lung were relatively consistent (*i.e.*, within 25% of each other). Brain levels of PER were approximately two-fold higher than in these organs, while the blood and skeletal muscle exhibited the lowest concentrations.

DISCUSSION

GC techniques are routinely used to determine levels of VOCs in environmental and biological samples. Wallace *et al.* [33] utilized GC purge- and -trap techniques to conduct large-scale surveys of human exposure to VOCs in drinking water, indoor and outdoor air. GC purge- and -trap techniques have also been used successfully to measure concentrations of VOCs in human blood [27,29,30,34], milk [26,27], and urine [27]. These assays are precise and quite sensitive, as many of the investigators employed GC–mass spectrometric computer analyses. Other investigators have used static GC headspace methods to quantify halocarbons and other VOCs in blood [14,15,30,31]. Such headspace analyses offer the advantages of speed and simplicity, such that large numbers of samples can be assayed daily using a gas chromatograph equipped with an autosampler. Although each of the aforementioned techniques generally work well for air and liquids, little attention has been devoted to adapting them for measurement of VOCs in solid tissues.

TABLE II

RECOVERY OF C_2 HALOALKANES AND HALOALKENES FROM BLOOD AND TISSUES

Values represent the mean ± S.E. for measurement in spiked tissues taken from eight rats. Each spiked tissue was homogenized in 8 ml ice-cold isooctane and 2 ml saline, vortex-mixed, centrifuged at 4°C, and an aliquot of the isooctane assayed by headspace GC as described in Experimental.

Sample	Recovery (%)			
	PER	TET	TCE	TRI
Liver	89.6 ± 3.1	96.1 ± 2.1	86.0 ± 1.3	91.3 ± 4.0
Kidney	86.7 ± 1.4	97.9 ± 1.5	86.9 ± 1.6	88.4 ± 4.9
Fat	88.2 ± 2.7	89.9 ± 1.4	73.0 ± 1.5	88.7 ± 2.2
Heart	81.2 ± 1.2	98.1 ± 1.4	85.9 ± 3.5	89.6 ± 2.4
Lung	99.1 ± 2.3	96.1 ± 0.8	80.0 ± 0.7	89.7 ± 1.9
Muscle	98.5 ± 2.9	97.4 ± 1.1	87.9 ± 1.7	87.6 ± 4.9
Brain	88.6 ± 2.0	100.3 ± 3.1	80.7 ± 2.8	87.6 ± 4.6
Blood	95.4 ± 4.1	97.3 ± 2.1	85.9 ± 2.0	85.5 ± 3.7

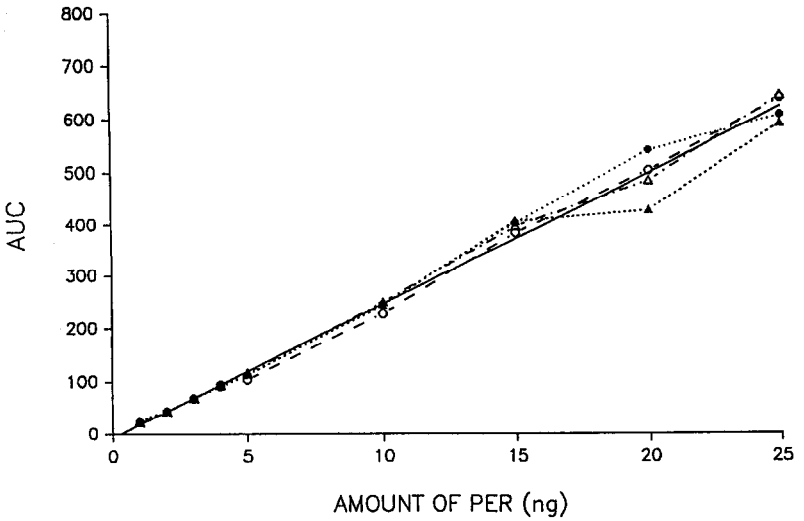

Fig. 2. Standard curves for PER using four solvent and saline combinations and homogenization: (○) 8 ml isooctane; (◆) 8 ml isooctane + 2 ml saline; (△) 8 ml isooctane + 4 ml saline and (▲) 8 ml isooctane + 4 ml saline, homogenized for 30 s. The detector response, presented here as area under the curve (AUC), is plotted against amount of PER. The regression equation for the data presented here is $y = 24.3x - 6.9$.

A practical technique for processing and extracting C_2 halocarbons from tissues for subsequent GC headspace analyses is reported here. Several approaches for determination of VOCs in tissues have been employed previously, with limited success. Direct injections of tissue homogenates or solvent extracts of homogenates have a number of inherent problems, including loss of the VOC by volatilization, GC column contamination, interference by biological matrices and lipophilic macromolecules, and limited sensitivity. One method for measuring toluene in blood and tissues involved extraction with methanol, selective adsorption onto Tenax, and desorption from the Tenax with heat into a gas chromatograph [24]. A significant problem in measuring VOCs in solid tissues is volatization of the analyte during tissue processing. Peterson and Bruckner [24] attempted to overcome this difficulty by crushing the tissues with a rod under methanol within a closed container. This technique was reasonably successful (*e.g.*, 73 and 92% recovery of toluene from liver and brain, respectively), but recoveries from other tissues were limited by incomplete maceration and escape of toluene from the maceration–extraction container. This technique was also labor-intensive and time-consuming, as was a purge- and -trap method described by Lin *et al.* [28] for measuring 1,1- and 1,2-dichloroethylene (1,2-DCE). The procedure of Lin *et al.* [28] involved thermal desorption of halocarbons from previously

TABLE III

TISSUE CONCENTRATIONS OF PER IN RATS FOLLOWING *IN VIVO* EXPOSURE

Each animal was sacrificed 1 h after intra-arterial administration of 10 mg 1,1,2,2-tetrachloroethylene (PER) per kg body weight.

Tissue	PER concentration[a] (μg/g)
Liver	2.4 ± 0.5
Kidney	2.7 ± 0.4
Fat	48.1 ± 4.1
Heart	3.0 ± 0.2
Lung	2.3 ± 0.2
Muscle	1.9 ± 0.2
Brain	4.7 ± 0.4
Blood	1.3 ± 0.1

[a] Values represent the mean ± S.E. for four rats.

minced tissues within a purging device. The chemicals were subsequently trapped on a Tenax column and desorbed with heat into a gas chromatograph. Mean recovery values for 1,2-DCE from liver, kidney, brain, and adipose tissue were 60, 53, 63 and 93%, respectively [28]. The purge- and -trap technique of Pellizzari and co-workers [27,35] also resulted in low recovery (*i.e.*, 13–80%) of a series of halocarbons from adipose tissue. The method involved transfer of 5-g portions of frozen adipose tissue to a 100-ml round-bottom purging flask maintained in an ice bath, addition of the halocarbon dissolved in distilled water, and maceration with a Virtis tissue homogenizer. The flask's contents were then heated to 50°C, stirred and purged with helium for 30 min, in order to transfer the analyte to a Tenax column. These researchers [27,35] attributed their low recovery values and marked inter-sample variability to halocarbon losses during tissue maceration and transfer, as well as retention of the analyte by complex matrices and lipophilic compounds. In contrast, the technique presented in the current paper is quite efficient, in that it involves a homogenization–extraction step and the relative ease and speed of GC headspace analysis. The method was also sensitive (*i.e.*, limit of detection = 1 ng) and efficient, in that recoveries of four different halocarbons from a variety of tissues were quite high (*i.e.*, 73–104%) and consistent (*i.e.*, highest S.E. = 4.9%).

An important factor in the present procedure was the limitation in the volume of aliquot that could be employed in the headspace vials. As standard curve measurements were no longer linear at volumes above 25 μl, a 20-μl aliquot was selected for subsequent use. There was a statistically higher recovery from most tissues when using isooctane homogenization than when using saline homogenization. It appears that homogenization of tissues in an aqueous solution (*i.e.*, saline), with subsequent extraction into isooctane, provided more opportunity for loss of the volatile chemicals through evaporation than did the single step of homogenization in isooctane. Isooctane proved to be superior to a variety of other organic solvents for extraction of all four halocarbons (unpublished data). Other solvents that were employed included methanol, ethyl acetate, *n*-hexane, cyclohexane, and toluene. Some solvents (*e.g.*, ethyl acetate) worked well for one halocarbon, but not for others. Isooctane provided the highest recovery without interfering peaks for all four halocarbons.

The applicability of this approach for analysis of C_2 halocarbons was demonstrated by its use with two haloalkanes and two haloalkenes with differing physicochemical properties. As would be expected, the relative volatility of the chemicals affected the recovery. The boiling points of TRI, TCE, PER, and TET are 74, 86.7, 121, and 140.7°C, respectively [36]. TET, the least volatile chemical, exhibited the highest recovery from most tissues. Recovery of PER was also relatively high from each tissue except the heart. It is noteworthy that PER is the most lipophilic of the halocarbons [37], and therefore should be most efficiently extracted by isooctane. TCE generally exhibited the lowest recovery values, as would be anticipated from its relatively high volatility and low lipophilicity. Recovery of TRI was unexpectedly high from most tissues. One would predict that TRI recovery should also be relatively low, since it was the most volatile and one of the least lipophilic of the four compounds studied.

A basic tenet of toxicology is that of the dose–response relationship (*i.e.*, the magnitude of toxic effect is a function of the administered dose). The concept of dose is now being refined, as it is recognized that the amount of chemical absorbed systemically (*i.e.*, the internal dose) can vary significantly with route of exposure, dosing vehicle and animal species. The blood level over time following exposure has been accepted historically as an index of internal dose, but it often may not accurately reflect concentration of chemicals at sites of action within tissues. Thus, the most logical and precise measures of dose are time integrals of target organ concentrations of bioactive chemicals [38]. Unfortunately, there are a paucity of tissue level *versus* time data sets for VOCs, due largely to the technical difficulties and the inordinate time involved in quantification of these highly volatile compounds in individual samples.

In order to derive appropriate time-course data, tissue concentrations must be measured sequentially during and post exposure in separate groups of animals, necessitating analysis of a large number of samples. A technique is presented here, which allows rapid processing and extraction of C_2 halocarbons from a variety of organs, for subsequent GC headspace analyses. By use of such a procedure, it should be possible to generate comprehensive tissue dose–time-course data to correlate with toxicity data. Recognition and utilization of such information can substantially reduce uncertainties inherent in toxicity and carcinogenicity risk assessments of halocarbons and other VOCs.

ACKNOWLEDGEMENTS

This research was supported by US EPA Cooperative Agreement CR 816258 and by the Air Force Office of Scientific Research, Air Force Systems Command, under Grant AFOSR 910356. The research has not been subjected to EPA review, and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. The US Government's right to retain a non-exclusive royalty-free license in and to the copyright covering this paper, for governmental purposes, is acknowledged.

The authors would like to thank Joy Wilson and Judy Bates for their help in preparation of the manuscript.

REFERENCES

1 T. B. McCarthy and R. D. Jones, *Br. J. Ind. Med.*, 40 (1983) 450–455.
2 E. B. Evans and R. L. Balster, *Neurosci. Biobehav. Rev.*, 15 (1990) 233–241.
3 J. V. Bruckner and R. G. Peterson, *Toxicol. Appl. Pharmacol.*, 61 (1981) 27–38.
4 V. A. Benignus, K. E. Muller, C. N. Barton and J. A. Bittikofer, *Toxicol. Appl. Pharmacol.*, 61 (1981) 326–334.
5 C. D. Klaassen and G. L. Plaa, *Toxicol. Appl. Pharmacol.*, 9 (1966) 139–151.
6 R. J. Jaeger, R. B. Connolly and S. D. Murphy, *Arch. Environ. Health*, 30 (1975) 26–31.
7 G. Bonse and D. Henschler, *CRC Crit. Rev. Toxicol.*, 10 (1976) 395–409.
8 E. K. Weisburger, *Environ. Health Perspect.*, 21 (1977) 7–16.
9 G. H. Laew, E. Kurkjian and M. Rebagliati, *Chem.-Biol. Interact.*, 43 (1983) 33–66.
10 J. V. Bruckner, B. D. Davis and J. N. Blancato, *CRC Crit. Rev. Toxicol.*, 20 (1989) 31–50.
11 D. G. Pegg, J. A. Zempel, W. H. Braun and P. G. Watanabe, *Toxicol. Appl. Pharmacol.*, 51 (1979) 456–479.
12 W. T. Stott, J. F. Quast and P. G. Watanabe, *Toxicol. Appl. Pharmacol.*, 62 (1982) 137–151.
13 A. M. Schumann, T. R. Fox and P. G. Watanabe, *Toxicol. Appl. Pharmacol.*, 62 (1982) 390–401.
14 C. E. Dallas, F. W. Weir, S. Feldman, L. Putcha and J. V. Bruckner, *Toxicol. Appl. Pharmacol.*, 68 (1983) 140–151.
15 C. E. Dallas, J. M. Gallo, R. Ramanathan, S. Muralidhara and J. V. Bruckner, *Toxicol. Appl. Pharmacol.*, 98 (1989) 385–397.
16 P. Bohlen, U. P. Schlunegger and E. Lappe, *Toxicol. Appl. Pharmacol.*, 25 (1973) 242–249.
17 D. E. Rickert, T. S. Baker, J. S. Bus, C. S. Barrow and R. D. Irons, *Toxicol. Appl. Pharmacol.*, 49 (1979) 417–423.
18 J. D. Young, J. C. Ramsey, G. E. Blau, R. J. Karbowski, K. D. Nitschke, R. W. Slauter and W. H. Braun, in W. B. Deichmann (Editor), *Toxicology and Occupational Medicine. Proceedings of the 10th Inter-America Conference on Toxicology and Occupational Medicine, Miami, FL, Oct. 22–25, 1978*, Elsevier/North-Holland, New York, 1979, pp. 297–310.
19 A. Sato, *Med. J. Shinshu. Univ.*, 12 (1967) 299–311.
20 W. D. Collom and C. L. Winek, *Clin. Toxicol.*, 3 (1970) 125–130.
21 M. E. Andersen, H. J. Clewell, M. L. Gargas, M. G. McNaughton, R. H. Reitz, R. J. Nolan and M.J. McKenna, *Toxicol. Appl. Pharmacol.*, 108 (1991) 14–27.
22 J. W. Fisher, M. L. Gargas, B. C. Allen and M. E. Andersen, *Toxicol. Appl. Pharmacol.*, 109 (1991) 183–195.
23 R. H. Reitz, J. N. McDougal, M. W. Himmelstein, R. J. Nolan and A. M. Schumann, *Toxicol. Appl. Pharmacol.*, 95 (1988) 185–199.
24 R. G. Peterson and J. V. Bruckner, *J. Chromatogr.*, 152 (1978) 69–78.
25 B. J. Dowty, L. E. Green and J. L. Laseter, *Anal. Chem.*, 48 (1976) 946–949.
26 E. D. Pellizzari, T. D. Hartwell, B. S. H. Harris, R. D. Waddell, D. A. Whitaker and M. D. Erickson, *Bull. Environ. Contam. Toxicol.*, 28 (1982) 322–328.
27 L. C. Michael, M. D. Erickson, S. P. Parks and E. D. Pellizzari, *Anal. Chem.*, 52 (1980) 1836–1841.
28 S.-N. Lin, F. W.-Y. Fu, J. V. Bruckner and S. Feldman, *J. Chromatogr.*, 244 (1982) 311–320.
29 S. R. Antoine, I. R. DeLeon and R. M. O'Dell-Smith, *Bull. Environ. Contam. Toxicol.*, 36 (1986) 364–371.
30 A. Sato, *Jpn. J. Ind. Health*, 13 (1971) 173–179.
31 A. C. Monster and G. Boersma, *Int. Arch. Occup. Environ. Health*, 35 (1975) 155–163.
32 D. MacDougall and W. B. Chummett, *Anal. Chem.*, 52 (1980) 2242–2249.
33 L. A. Wallace, E. D. Pellizzari, T. D. Hartwell, R. Whitmore, C. Sparacino and H. Zelon, *Environ. Int.*, 12 (1986) 369–387.

34 B. Dowty, D. Carlisle, J. L. Laseter and F. M. Gonzalez, *Biomed. Mass Spectrom.*, 2 (1975) 142–147.
35 E. D. Pellizzari, L. S. Sheldon and J. T. Bursey, *IARC Sci. Publ.*, 68 (1985) 435–444.
36 M. Windholz, S. Budarari, L. Y. Stroumtsos and M. N. Fertig (Editors), *The Merck Index, An Encyclopedia of Chemicals and Drugs*, Merck and Co., Rahway, NJ, 1976, pp. 8905, 9318 and 9319.
37 A. Sato and T. Nakajima, *Arch. Environ. Health*, 34 (1979) 69–75.
38 M. E. Andersen, *Pharmacokinetics in Risk Assessment: Drinking Water and Health*, Vol. 8, National Academy Press, Washington, DC, 1987, pp. 8–26.