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# QUANTITATION OF 1,1- AND 1,2-DICHLOROETHYLENE IN BODY TISSUES BY PURGE-AND-TRAP GAS CHROMATOGRAPHY

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## SUMMARY

A sensitive and accurate method for extraction and quantitation of volatile halocarbons, *i.e.*, 1,1- and 1,2-dichloroethylene (DCE), from body tissues has been developed. The organic volatiles were thermally desorbed from tissues contained within a Tekmar purge device, which was immersed in a stirred water bath at 60°C. In order to avoid foaming, the stream of purging gas (helium) was applied 2 mm above, rather than below the sample surface in the purging device. The purged 1,1- and 1,2-DCE were retained on Tenax-GC (80–100 mesh), then desorbed by heating and vented into a Tracor 560 gas chromatograph. The detection limit of the Hall electrolytic conductivity detector operated in the halogen mode was 50 pg. Recoveries of 1,1- and 1,2-DCE from various animal tissues spiked *in vitro* were greater than 50%. This purge-and-trap technique appears well suited for studies of the uptake and disposition of volatile organics in body tissues.

## INTRODUCTION

Characterization of the uptake and elimination of volatile halocarbons in tissues of animals has a number of inherent problems. Considerable amounts of these volatile compounds are lost by evaporation during normal tissue homogenization and extraction procedures. Most disposition studies to date have involved the use of radiolabeled halocarbons[1–3]. Interpretation of data from such studies is difficult, in that one can not readily distinguish between parent compound and various metabolites in biological samples. Currently, the application of gas-phase analyses of volatile organics is limited primarily to the measurement of concentrations of the compounds in water. Several papers have been published describing the use of this technique to quantitate halocarbons in drinking water[4–7]. However, due to the complexities of the background matrix of biological material, certain modifications of techniques used in water analysis have had to be made in order to work with tissues[8–10]. In determining

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volatile organic compounds in biological samples by purge-and-trap gas chromatography, a major difficulty has been foaming of the sample in the purging device. It has also been reported[11] that certain industrial effluent waters also produce foams in a purging apparatus. The foam tends to enter the transfer line leading to the adsorbent trap and may actually reach the trap itself and deactivate it. There may also be introduction of thermal-decomposition products from labile non-volatile materials, which interfere with the accuracy of quantitative measurements and increase machine "down-time" due to contamination of the highly sensitive analytical detection systems. Although head-space analysis of volatile organics in tissues has been reported[10], this approach is not satisfactory when assaying tissues that contain low concentrations of halocarbons, since the amount of compound that volatilizes and can be injected into the gas chromatograph is below the instrument's detection capability. We undertook to develop a purge-and-trap technique that circumvents these problems, so that pharmacokinetic studies of specific halocarbons (*e.g.*, 1,1- and 1,2-dichloroethylene) in body tissues can be conducted.

## EXPERIMENTAL

### *Materials*

The 1,1- and *trans*-1,2-dichloroethylene (1,1- and *trans*-1,2-DCE) of purity 98% were purchased from Aldrich (Milwaukee, WI, U.S.A.). Glass-distilled isooctane was obtained from Burdick and Jackson (Muskegon, MI, U.S.A.). Water was distilled in a Wheaton autostill 1.5 glass distillation apparatus (Milville, NJ, U.S.A.). To ensure that the isooctane and glass-distilled water were free from contamination that might interfere with 1,1- and 1,2-DCE chromatograms they were pre-purged with a stream of helium (99.995% pure; Linde Div., New York, NY, U.S.A.) for at least 1 h. Sampling vessels were 3.5-ml vials equipped with PTFE-lined screw caps.

### *Instrumentation*

A Tekmar Model LSC-2 purge-and-trap assembly was incorporated with a Tracor GC 560 gas chromatograph (Austin, TX, U.S.A.). The purging unit of the Tekmar LSC-2 was installed with a 5-ml needle sparger kit designed for analyzing tissue samples. However, for convenience, the 5-ml cone-shaped glass vessel of the sparger kit was replaced by a 3.5-ml flat-bottom vial. The volatilized halocarbons were retained by Tenax-GC (80–100 mesh) (Applied Science Labs, State College, PA, U.S.A.) packed into a glass tube in the Tekmar concentrator. Gas chromatographic separation was conducted in a glass column (6 ft × 2 mm I.D.) packed with Carbopak C (80–100 mesh) coated with 0.2% of Carbowax 1500 (Supelco, Bellefonte, PA, U.S.A.). A Hall electrolytic conductivity detector (Tracor) operated in the halogen mode was used with the Tracor GC 560. Gas chromatograms were recorded by a Fisher Recordall series 5000 recorder (Houston, TX), and peak areas were calculated by a HP 3390A integrator (Hewlett Packard, Palo Alto, CA).

### *Animal treatment*

Male Sprague-Dawley rats (200–300 g) served as tissue donors. Some animals received no treatment before being exsanguinated by open-chest cardiac puncture. Tissues needed for various experiments were then rapidly excised. An additional

group of rats was given an intraperitoneal (i.p.) injection of 15 mg of 1,1-DCE per kg of body weight. After 15 min, these animals were also sacrificed by exsanguination, and liver, brain, kidney and adipose-tissue specimens were quickly excised.

*Measurement procedures*

Tissue samples were rapidly and thoroughly minced. Some 4–6 mg of the minced sample were transferred to a 3.5-ml vial containing 200 μl of ice-cold purified water and 1 ng of 1,2-DCE in 30 μl of purified isooctane. After a brief vortexing, the sample vials were attached to the purging device for analyses, or stored in a freezer at −40°C to avoid bacterial contamination and growth. For the analyses, the lower two-thirds of each sample vial was immersed in a stirred water bath at 60°C (unless otherwise specified). A stream of helium at a flow-rate of 15 ml/min into the vial was initiated immediately to deliver the volatilized tissue halocarbons to the trapping device. The penetration of the needle carrying the purging gas was so adjusted that the tip of the needle was *ca.* 2 mm above, rather than below, the sample surface; this arrangement prevented foaming within the sample vial. The sample vials were purged with helium for 3 min so that the breakthrough volumes for 1,1- and 1,2-DCE for the trap concentrator were not exceeded. The Tenax trap in the Tekmar concentrator was maintained at 30°C during this period. Then, a 2-min, 150°C desorption cycle with a helium flow-rate of 35 ml/min was initiated in order to transfer the halocarbons from the trapping device to the gas chromatograph. Gas chromatographic separation of desorbed organic volatiles was carried out with the column oven programmed from 60°–100°C at 2°C/min. Operation conditions of the Hall electrolytic conductivity detector were as follows: hydrogen flow-rate, 35 ml/min; temperature of catalytic reactor, 820°C; 100% isopropanol flow-rate, 0.63 ml/min; attenuation × range of electron meter, 10 × 1. Tissue 1,1-DCE concentrations were determined by comparing ratios of 1,1-DCE/1,2-DCE peak areas with a standard curve.

## RESULTS AND DISCUSSION

Glass-distilled isooctane and water found to contain a number of volatile organic compounds that gave rise to chromatographic peaks interfering with those of 1,1- and 1,2-DCE. Certain contaminants remained even after purification of the water and isooctane by purging with helium. Purge-and-trap chromatograms from 0.5 ml each of purified isooctane and water are shown in Fig. 1A. Since separation of 1,1- and 1,2-DCE from these remaining background peaks was satisfactory (Fig. 1B), further efforts to remove these contaminants and to identify their origin were not made. It should be noted only *trans*-1,2-DCE was utilized here. The GC retention time for *cis*-1,2-DCE was 1.7-fold longer than that for *trans*-1,2-DCE.

Although 1,1- and 1,2-DCE may be markedly different in their biological effects[12–14], their chromatographic properties are very similar. The Hall electrolytic conductivity detector responds similarly to both compounds. Thus, each can be used as an internal standard for the other. The linear dynamic range for quantitative measurements of 1,1- and 1,2-DCE by the Hall detector is determined by injection of various amounts of the compounds directly into the instrument. Thereby, possible sample loss during the purge-and-trap processes is avoided. The responses of the Hall detector, as reflected by peak area calculations, are linear in the concentration range

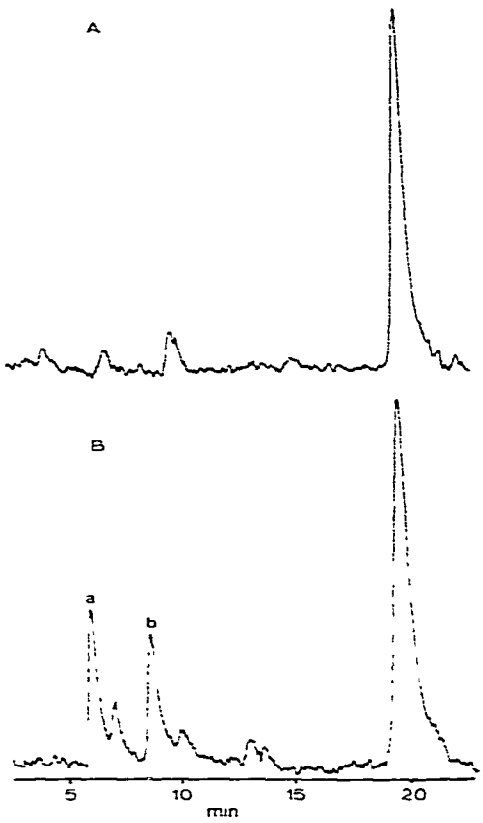


Fig. 1. Representative purge-and-trap gas chromatograms. As can be seen in panel A, certain contaminants remain in a 1-ml mixture of equal parts of glass-distilled water and isooctane, even after purification by purging with helium. As shown in panel B, separation of 1,1-DCE (peak a) and 1,2-DCE (peak b) from the background peaks is satisfactory.

of 50 pg to 10 ng. Therefore, the lowest limit of the linear dynamic range of the detection system is 200 whereas, the lowest detection limit for the compound, based on a signal-to-noise ratio of 5, is 50 pg.

We found that standard solutions of 1,1- and 1,2-DCE in purified isooctane are stable for up to one month, provided that the samples are stored at 0°C. The results of serial assays performed over a 33-day period on 1,1- and 1,2-DCE standards containing 128 pg/$\mu$l are shown in Fig. 2. Although there is a trend towards a gradual decline over the 33-day period in the concentrations of 1,1- and 1,2-DCE in stored standards, substantial losses were not seen until around day 28. The mean peak areas (expressed in arbitrary units $\pm$ S.D.), based upon the first 30 data points in Fig. 2, are 90.7 $\pm$ 10.5 for 1,1-DCE and 71.8 $\pm$ 8.3 for 1,2-DCE. Thus, the response factor of the Hall detector for 1,1-DCE/1,2-DCE is 1.26, when the compounds are injected directly into the chromatograph.

Since foaming of tissues samples is avoided, only those organic volatiles that can be purged from tissues at 60°C are available for GC detection. As 1,1- and 1,2-

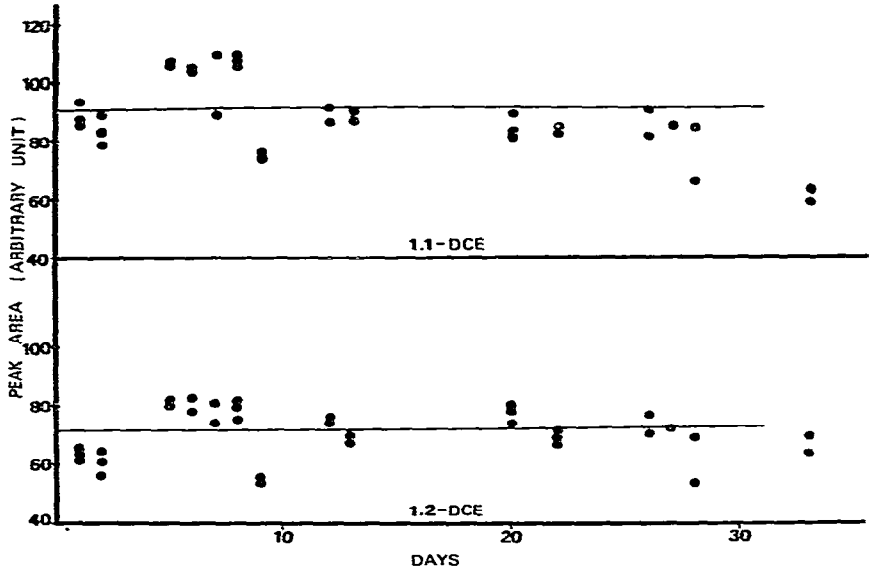


Fig. 2. Influence of storage at 0°C for up to 33 days on the concentration of 1,1- and 1,2-DCE in standards containing 128 pg of 1,1- or 1,2-DCE per μl of isooctane. Concentrations are expressed here as peak areas.

DCE have rather low boiling-points (31.7° and 47.2°C, respectively), one would expect that recovery of these two compounds from tissues samples would be high. An initial experiment was conducted to determine the influence of tissue sample size on recovery of 1,1- and 1,2-DCE. The same quantities of 1,1- and 1,2-DCE (*i.e.*, 0.65 ng in 5 μl of purified isooctane) were added to different amounts of minced liver *in vitro*. Recovery was calculated by dividing the peak areas in the purge-and-trap chromatograms by the areas obtained from standards injected directly onto the gas chromatographic column. Although the recovery (%) of each halocarbon varied indirectly with sample size, recovery of 1,1-DCE was less affected by the quantity of liver present in the purging vessel than was that of 1,2-DCE (see Table I). It is interesting to note that recoveries for 1,1- and 1,2-DCE are comparable when 50 mg or less of liver was utilized. In order to examine the possibility that additional 1,1- or 1,2-DCE might be retrieved by subsequent purging of the liver specimens, the 100-mg liver samples were subjected to a second 3-min purge. No additional 1,1- of 1,2-DCE was recovered as a result of this operation (data not shown). The average response factor of the Hall detector for 1,1-DCE/1,2-DCE was 1.41 ± 0.11 (mean ± S.E. of the 8 determinations shown in Table I on liver samples ≤50 mg) when the compounds were isolated from the spiked liver specimens by the purge-and-trap method. This value is higher than the value (1.26) obtained upon direct injection of the compounds on to the gas chromatographic column. The mean peak areas (expressed in arbitrary units ± S.E.) of 6 determinations per sample for direct injection of 0.65 ng of 1,1- and 1,2-DCE, were 519.4 ± 39.1 and 410.6 ± 24.0, respectively. This phenomenon may indicate that 1,2-DCE has a somewhat higher inherent binding affinity for liver tissue than does 1,1-DCE. The possibility that different halocarbons may have different binding affinities for tissues was also observed by Peoples *et al.*[8]. In determining

TABLE I

EFFECT OF SAMPLE SIZE ON RECOVERY OF 1,1-DCE AND 1,2-DCE FROM LIVER BY PURGE-AND-TRAP GAS CHROMATOGRAPHY

| *Liver* sample size (mg)* | *1,1-DCE*** | | *1,2-DCE*** | | *Response** factor, 1,1-DCE/1,2-DCE* |
|---|---|---|---|---|---|
| | *Peak area (arb. units)* | *Recovery*** (%)* | *Peak area (arb. units)* | *Recovery*** (%)* | |
| 5 | 318 | 61.2 | 235 | 59.7 | 1.35 |
| 10 | 339 | 65.2 | 232 | 56.6 | 1.46 |
| 25 | 316 | 60.7 | 206 | 50.1 | 1.53 |
| 50 | 253 | 48.6 | 191 | 46.4 | 1.32 |
| 100 | 269 | 51.8 | 139 | 33.8 | 1.94 |

* The 1,1- or 1,2-DCE (0.65 ng in 5 μl of isooctane) was added to sealed vials containing 200 μl of water and minced tissue. The contents were then vortexed, kept on ice for 5 min, and purged for 3 min.

** Each value is the average of 2 determinations.

*** Recovery (%) was calculated by dividing the purge-and-trap peak areas by the peak areas obtained upon direct injection of 0.65 ng of 1,1- or 1,2-DCE. The peak areas (expressed as mean ± S.E. of 8 injections) for the standards were 519 ± 39 and 411 ± 24 for 1,1- and 1,2-DCE, respectively.

recoveries of volatile, purgeable halogenated hydrocarbons added to human adipose tissue and serum, these investigators noted that both serum and fat appeared to have greater inherent binding capacity for chloroform than for the other halocarbons tested.

The effect of temperature of the purging vessel on recovery of 1,1- and 1,2-DCE from liver was also determined. As shown in Table II, increasing the temperature of the purging vessel from 60°C to 100°C had no effect on the recovery of 1,1-DCE from 100 mg of liver spiked with 0.65 ng of 1,1-DCE. There was, however, approximately a 10% increase in recovery of 1,2-DCE at the higher temperature. It

TABLE II

EFFECT OF TEMPERATURE OF PURGING VESSEL ON RECOVERY OF 1,1-DCE AND 1,2-DCE FROM LIVER

Each value is the mean of three determinations. Peak areas are expressed ± S.E.

| *Temperature (°C)* | *1,1-DCE** | | *1,2-DCE** | |
|---|---|---|---|---|
| | *Peak area (arb. units)* | *Recovery** (%)* | *Peak area (arb. units)* | *Recovery** (%)* |
| 100 | 251 ± 6 | 52.6 | 186 ± 9 | 50.4 |
| 60 | 251 ± 12 | 52.4 | 146 ± 14 | 39.6 |
| +100*** | 8 ± 6 | 1.7 | 17 ± 16 | 4.5 |

* The 1,1- or 1,2-DCE (0.65 ng in 5 μl of isooctane) was added to sealed vials containing 200 μl of water and 100 mg of minced liver. The contents were then vortexed, kept on ice for 5 min, and purged for 3 min.

** Recovery (%) was calculated by dividing the purge-and-trap peak areas by the peak areas obtained upon direct injection of 0.65 ng of 1,1- or 1,2-DCE. The peak areas (expressed as mean ± S.E. of 8 injections) for these standards were 478 ± 22 and 368 ± 15 for 1,1- and 1,2-DCE, respectively.

*** Following the initial 3-min/60°C purge, a second 3-min purge was carried out at 100°C.

might be noted here that the recovery of 1,2-DCE at 100°C from 100 mg of liver was about the same as that at 60°C from liver samples weighing 25 mg or less. As with the data presented in Table I, the recovery of 1,2-DCE was generally lower than that of 1,1-DCE under each set of conditions in Table II.

Recoveries of 1,2-DCE from portions of extrahepatic tissues spiked *in vitro* are shown in Table III. Recovery of 1,2-DCE is greatest from adipose tissue, recovery from 5 samples ranging from 75 to 104%. Recoveries from spiked samples of brain and kidney are considerably lower, in that the mean recovery from brain is 62.6%, while that from kidney is 53.4%. These findings are in general agreement with those of other investigators who have attempted to measure levels of volatile halocarbons in tissues. Although Peoples *et al.*[8] report excellent recovery of a number of halocarbons from human adipose tissue, Maiorino *et al.*[10] find marked variation in the recovery of volatile halothane metabolites from different tissues. They report the average recovery of 2-chloro-1,1-difluoroethylene from blood, liver and liver micro-

TABLE III

RECOVERY OF 1,2-DCE FROM ADIPOSE TISSUE, KIDNEY AND BRAIN BY PURGE-AND-TRAP GAS CHROMATOGRAPHY

| *Tissue** | *Peak area (arb. units)* | *Recovery*** (%)* |
|---|---|---|
| Adipose | 717 | 104 |
| | 515 | 75 |
| | 713 | 103 |
| | 600 | 87 |
| | 670 | 97 |
| | | Mean ± S.E. = 93.3 ± 12.4 |
| Kidney | 387 | 56 |
| | 375 | 54 |
| | 340 | 49 |
| | 372 | 54 |
| | 359 | 52 |
| | 359 | 52 |
| | 389 | 56 |
| | 363 | 53 |
| | | Mean ± S.E. = 53.4 ± 2.4 |
| Brain | 458 | 65 |
| | 458 | 65 |
| | 445 | 63 |
| | 436 | 62 |
| | 454 | 64 |
| | 411 | 58 |
| | | Mean ± S.E. = 62.6 ± 2.6 |

* To a sealed vial containing 200 µl water and one minced tissue was added 0.91 ng of 1,2-DCE in 5 µl of isooctane. An average of 24 mg of adipose tissue, 8 mg of kidney, and 2 mg of brain was used.

** Recovery was calculated by dividing the tissue purge-and-trap peak areas by the peak areas of a standard. The standard, 0.91 ng of 1,2-DCE, was injected directly onto the column. The peak area of the standards used for adipose tissue and kidney was 690 ± 86 (mean ± S.E. of 5 injections); that used for brain was 709 ± 37 (mean ± S.E. of 4 injections).

somes to be 84, 67 and 43%, respectively. The reason(s) for the pronounced differences in compound recovery from one tissue to another is (are) not clear at this time.

The assay method described herein has been utilized in preliminary studies of the disposition of 1,1-DCE *in vivo*. Specimens of four different tissues were obtained from the rats 15 min after the animals were given an i.p. injection of 15 mg/kg of 1,1-DCE. Chromatograms of control tissues and tissues from the 1,1-DCE-dosed animals are pictured in Figs. 3 and 4. There was good separation of the 1,1- and 1,2-DCE peaks from other volatilized compounds in each tissue, as is apparent in these representative chromatograms. Peoples *et al.*[8] found substantial amounts of chloroform in human serum and adipose samples, and suggested that drinking water might be a major source of this halocarbon. We were unable to detect even trace amounts of chloroform in our tissue specimens on the use of a co-elution gas chromatographic technique. Levels of 1,1-DCE in each tissue were determined by comparing ratios of 1,1-DCE/1,2-DCE peak areas with standard curves obtained by adding known amounts of the compounds to the corresponding control tissue samples (*i.e.*, blanks). It may be seen in Fig. 5 that the ratio of 1,1-DCE/1,2-DCE peak areas *versus* the quantity of 1,1-DCE, from 5-mg control liver specimens spiked *in vitro*, was linear in the range of 50 pg to 1.8 ng of 1,1-DCE. Concentrations of 1,1-DCE in the liver, adipose tissue, kidney and brain 15 min after injection of the 15 mg/kg dose of 1,1-DCE are shown in Table IV. The highest levels of the halocarbon were present in the

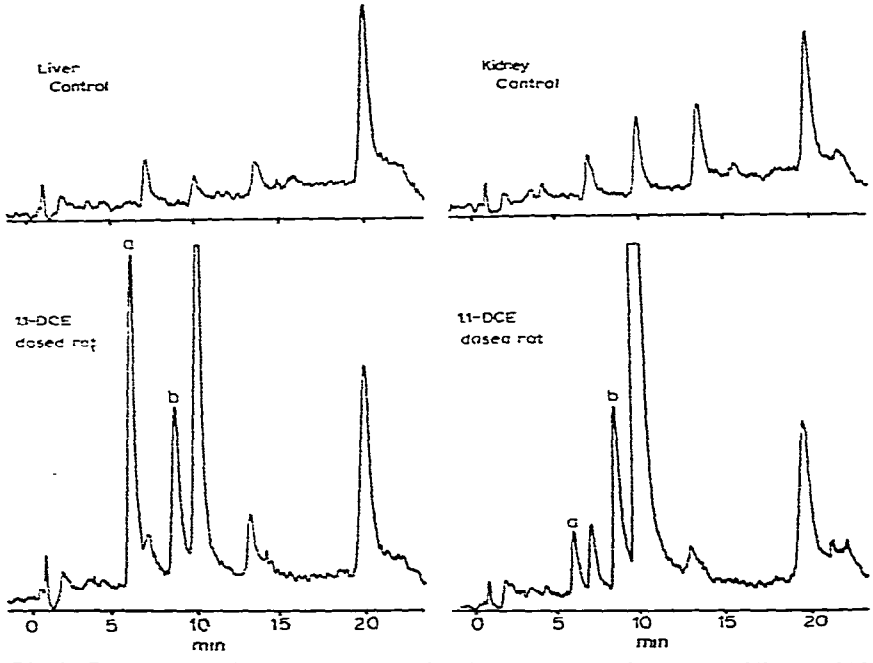


Fig. 3. The upper tracings are representative chromatograms from control liver and kidney specimens. The lower tracings are chromatograms from the corresponding tissues taken from 1,1-DCE-dosed rats 15 min after i.p. injection of 15 mg of 1,1-DCE per kg body weight. There is good separation of 1,1-DCE (peak a) and the internal standard 1,2-DCE (peak b) from background peaks in each tissue.

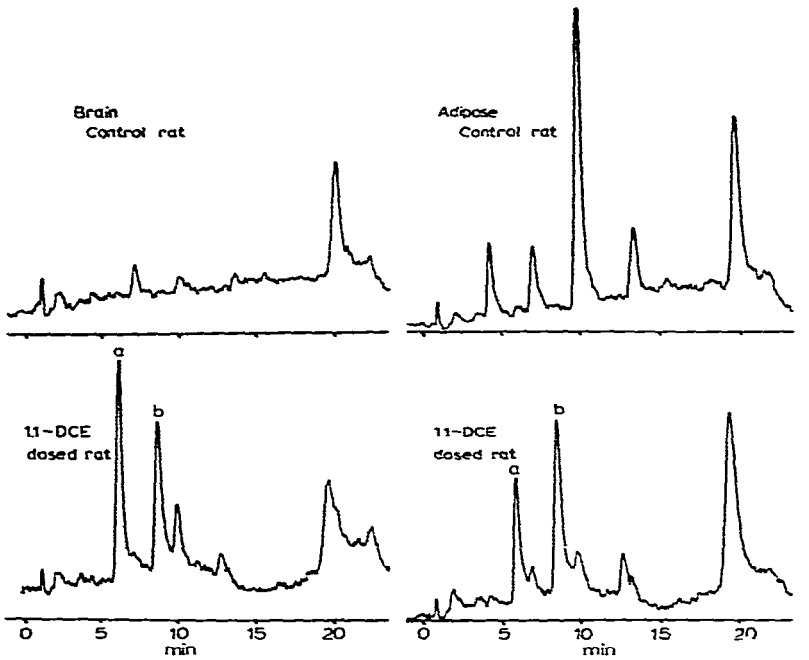


Fig. 4. The upper tracings are representative chromatograms from control brain and adipose tissue specimens. The lower tracings are chromatograms from the corresponding tissues taken from 1,1-DCE-dosed rats 15 min after i.p. injection of 15 mg of 1,1-DCE per kg body weight. There is good separation of 1,1-DCE (peak a) and the internal standard (peak b) from background peaks in each tissue.

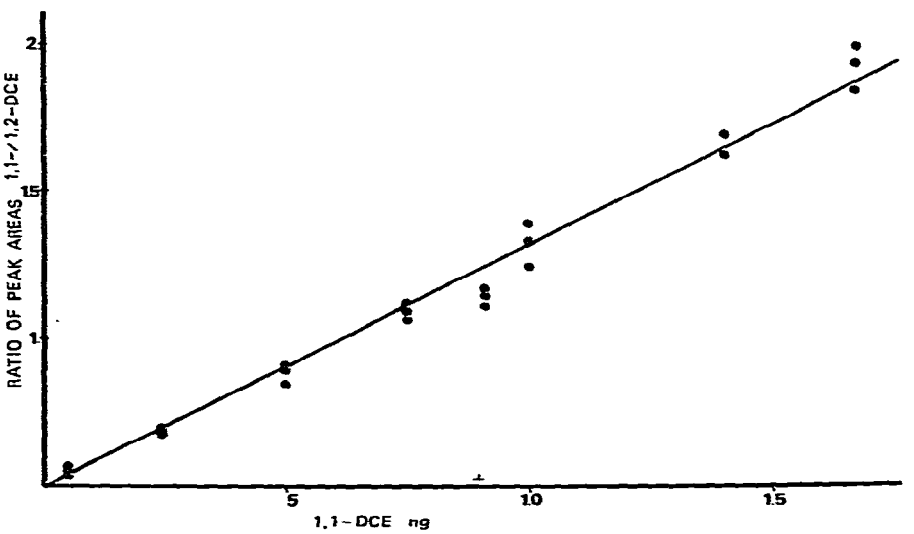


Fig. 5. Standard curve obtained by spiking 5-mg of control liver specimens *in vitro* with 50 pg to 1.8 ng of 1,1-DCE; 1,2-DCE was used as internal standard. The ratios of peak areas of 1,1-DCE/1,2-DCE were plotted against the concentration of 1,1-DCE.

TABLE IV

CONCENTRATIONS OF 1,1-DCE IN TISSUES OF RATS DOSED WITH THE HALOCARBON

All values, in ng of 1,1-DCE per mg of tissue (wet weight), are expressed as the mean ± S.E. of triplicate determinations per sample. Rats were sacrified and tissues were removed 15 min after an i.p. dose of 15 mg/kg of 1,1-DCE.

| *Rat No.* | *Liver* | *Brain* | *Kidney* | *Adipose tissue* |
|---|---|---|---|---|
| 1 | 1.87 ± 0.11 | 0.18 ± 0.01 | 0.56 ± 0.06 | 1.22 ± 0.08 |
| 2 | 1.55 ± 0.12 | 0.14 ± 0.03 | 0.49 ± 0.02 | 1.79 ± 0.27 |
| 3 | 0.87 ± 0.06 | 0.16 ± 0.02 | 0.93 ± 0.04 | 1.05 ± 0.21 |
| 4 | 1.61 ± 0.06 | 0.17 ± 0.01 | 0.93 ± 0.07 | – |
| Mean ± S.E. = | 1.48 ± 0.36 | 0.16 ± 0.03 | 0.78 ± 0.20 | 1.35 ± 0.32 |

adipose tissue and liver, although the kidney also contained a substantial amount. These preliminary findings agree with observations by McKenna and co-workers[1,2] that the liver and kidneys of rats accumulate the greatest amounts of radiolabel following inhalation or ingestion of [$^{14}$C]1,1-DCE by the animals. McKenna and co-workers did not conduct their assays until 72 h after dosing. It is likely that the majority of radiolabel was in 1,1-DCE metabolites at this late time, although one cannot be certain, since measurements of total radioactivity do not distinguish between metabolites and parent compound. In contrast, our currently reported purge-and-trap technique is very sensitive and is specific for the parent halocarbon. This technique appears well suited for studies of tissue uptake and elimination of 1,1-DCE, 1,2-DCE and other volatile organics.

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