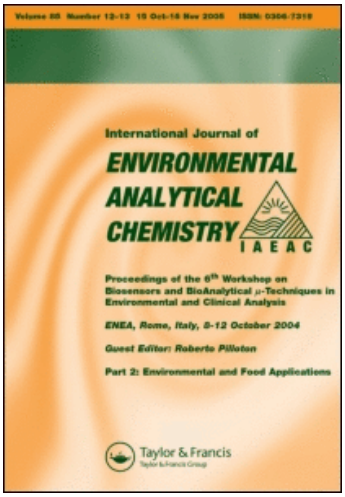


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# Distribution of Volatile Halogenated Organic Compounds Between Rat Blood Serum and Adipose Tissue

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A 31-day rat-dosing sequence was used to study some aspects of the physiological distribution, metabolism, storage and rate of elimination of chloroform, trichloroethylene, carbon tetrachloride and bromodichloromethane from rat adipose tissue and blood serum. Data points were collected nine times during the dosing period and twice after dosing had ceased. Purge/trap/desorb methodology was employed using a liquid sample concentrator, a gas-liquid chromatograph and a Hall electrolytic conductivity detector in series. Compound identities were confirmed using a GC/MS analytical system.

For these volatile compounds, tissue levels fluctuated but did not indicate increased storage with time. Adipose tissue to blood serum levels never differed by more than a factor of three. Within 3–6 days after dosing was terminated, practically all of the halogenated compounds had left the examined tissues. Metabolic conversion of trichloroethylene and carbon tetrachloride into chloroform and/or a chloroform precursor was observed; bromodichloromethane-dosed animals did not have serum chloroform levels exceeding those observed for the control animals.

KEY WORDS: Rat blood serum, rat adipose tissue, volatile halogenated hydrocarbons, chemical storage.

## INTRODUCTION

The human organism distributes ingested organochlorine pesticides (OC's) and polychlorinated biphenyls (PCB's) to many of its various tissues and biofluids. OC residues such as p,p'-DDT {1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane} and its major metabolite, p,p'-DDE {1,1-dichloro-

2,2-bis(p-chlorophenyl)ethylene}, have been detected in subcutaneous adipose,[1-3] liver and brain tissue[4,5] and milk of lactating women.[6,7] PCB residues have been reported to be present in similar tissues.[6-10] More volatile halogenated organic compounds have also been detected in human fat. In a review covering some of the volatile purgeable halogenated hydrocarbons (VPHH's), Fishbein[11] included data on the presence of trichloroethylene (TCE),[12] tetrachloroethylene (PCE; perchloroethylene)[12] and carbon tetrachloride[12] in human adipose.

As one facet of a larger project involving determination of VPHH's in human blood serum and adipose tissue, it became necessary to re-examine[13-15] how certain halogenated organic compounds are metabolically transformed and distributed between these same two tissues in Sprague-Dawley rats. The results of this investigation are based on a 31-day dosing (gavage) sequence. The appropriate analytical methodology was recently published by Peoples, Pfaffenberger, Shafik and Enos.[16] It is based on a purge/trap/desorb procedure reported in 1974 by Bellar and Lichtenberg[17] for use in analyzing municipal drinking water samples. No extraction or clean-up is required, the cost per analysis is reasonable and the time required per analysis is short.



## EXPERIMENTAL

### Apparatus

A Tekmar Model LSC-1 liquid sample concentrator was interfaced to a Tracor Model 222 gas chromatograph (GC) equipped with a Hall electrolytic conductivity detector which was operated in the halide specific mode. The chromatographic column was a 2-m × 4-mm I.D. glass U-tube containing *n*-octane on 100-120 mesh Porasil C packing. The GC operating conditions included: a nitrogen carrier gas flow-rate of 30 ml/min, an inlet temperature of 140°, and a transfer line temperature of 210°. The Hall detector furnace was maintained at 900° with a hydrogen flow-rate of 40 ml/min and a solvent (1:1 *n*-propanol:distilled water) flow of 0.4 ml/min.

After a 15-min purge, the sample was desorbed for 6 min at 150° to the 60° GC column which was then programmed 7°/min to 140°.

A Finnigan Model 4000 gas chromatograph/mass spectrometer (GC/MS) analytical system interfaced to a Tekmar liquid sample concentrator was used to confirm the identities of the compounds quantified by the gas chromatographic procedure.

Both the GC and the GC/MS systems utilized a hot plate stirrer and a glycerol bath to heat the sample in the Tekmar purging device.

## Solvents and reagents

Chloroform, carbon tetrachloride and hexane were Pesticide Grade from Fisher Scientific Co., U.S.A. Trichloroethylene, 1,2-dichloroethane and bromoform were from Aldrich Chemical Co., U.S.A.; bromodichloromethane and dibromochloromethane, from Columbia Organic Chemical Co., U.S.A. Dow Corning Antifoam Emulsion B was from Fisher Scientific Co., U.S.A., and the *n*-octane on 100–120 mesh Porasil C chromatographic packing was purchased from Supelco, Inc., U.S.A.

Hexane solutions of external VPHH standards were prepared with final concentrations ranging from 0.5 to 2.4 μg/l.

## Thirty-one day dosing sequence

Male Sprague-Dawley rats (108) were housed in pairs in clean stainless-steel cages and observed for several days as they ate and drank *ad libitum*. No ill animals were found. Then each day at 8:00 A.M. for 25 days (see Table I) twelve rats in each of nine dosage groups were given by gavage

TABLE I

Days on which rats were dosed (by gavage with a VPHH in corn oil) and sacrificed

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dosing Schedule: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
| ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | | | | | | |
| ↑ | | ↑ | | | ↑ | | | ↑ | | | ↑ | | | | ↑ | | | ↑ | | | ↑ | | ↑ | | ↑ | | | ↑ | | ↑ |
| Sacrifice schedule: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

the dosages of chloroform, carbon tetrachloride, trichloroethylene (TCE) and bromodichloromethane (BDCM) indicated in Table II. One animal from each group was sacrificed 2 hr after dosing on the 1st, 4th, 8th, 11th, 15th, 18th, 21st, 23rd, 25th, 28th and 31st days of the sequence. Whole blood was collected by cardiac puncture using a syringe and allowed to clot. Blood serum was separated from the clot and stored at 4° prior to being analyzed. Adipose tissue was surgically removed and frozen in clean glass containers in a freezer devoid of halogenated organic vapors. Analysis of the serum and fat for VPHH content was initiated immediately after each round of sacrifice. Most samples were analyzed within 48 hr, but the serum obtained from the rats which were dosed with trichloroethylene was stored under refrigeration and analyzed at the end of the experiment. All analyses were performed according to the method of Peoples, Pfaffenberger, Shafik and Enos.[16] Results are summarized in Tables II and III.

TABLE II

Average blood serum and adipose tissue levels during the 25 days VPHH's were administered[a]

| VPHH | Dosage (mg/day) | $CHCl_3$ | | $CCl_4$ | | TCE | | BDCM | |
|---|---|---|---|---|---|---|---|---|---|
| | | Serum[b] | Fat[c] | Serum | Fat | Serum | Fat | Serum | Fat |
| Control | — | 10 | 3 | | | | | | |
| $CHCl_3$ | 0.5 | 12 | 99 | | | | | | |
| $CHCl_3$ | 5 | 69 | 12000 | | | | | | |
| $CCl_4$ | 1 | ND[d] | 140 | 11 | 1900 | | | | |
| $CCl_4$ | 10 | 59 | 2600 | 310 | 18000 | | | | |
| TCE | 1 | 1600 | 100 | | | ND | 280 | | |
| TCE | 10 | 9300 | 480 | | | ND | 20000 | | |
| BDCM | 0.5 | ND | ND | | | | | 1 | 51 |
| BDCM | 5 | ND | ND | | | | | 23 | 1800 |

[a]Values were obtained by averaging 9 determinations.
[b] = μg/l.
[c] = ng/g of hexane extractable fat.
[d]ND = less than 1 μg/l serum or 1 ng/g hexane extractable fat.



TABLE III

Average blood serum and adipose tissue levels of VPHH's several days after dosing had ceased[a]

| VPHH | Dosage (mg/day) | $CHCl_3$ | | $CCl_4$ | | TCE | | BDCM | |
|---|---|---|---|---|---|---|---|---|---|
| | | Serum[b] | Fat[c] | Serum | Fat | Serum | Fat | Serum | Fat |
| Control | — | 8 | 5 | | | | | | |
| $CHCl_3$ | 0.5 | 7 | 5 | | | | | | |
| $CHCl_3$ | 5 | 8 | 2 | | | | | | |
| $CCl_4$ | 1 | ND[d] | 6 | ND | 14 | | | | |
| $CCl_4$ | 10 | 16 | 17 | ND | 168 | | | | |
| TCE | 1 | 28 | 6 | | | ND | 1 | | |
| TCE | 10 | 60 | ND | | | 6 | 1 | | |
| BDCM | 0.5 | ND | ND | | | | | 1 | 4 |
| BDCM | 5 | ND | ND | | | | | 1 | 3 |

[a]Values were obtained by averaging the VPHH levels remaining 3 days and 6 days after dosing by gavage had been terminated.
[b] = μg/l.
[c] = ng/g of hexane extractable fat.
[d]ND = less than 1 μg/l serum or 1 ng/g hexane extractable fat.

As indicated in Table I, dosing was terminated on day 25, but two additional rounds of sacrifice were performed on days 28 and 31. The averages of the values obtained from these analyses are given in Table III.

## GC/MS component confirmation

The identities of the components quantified by the LSC/GC/HECD method were confirmed by using an LSC/GC/MS analytical system. All confirmations were based on both relative retention values (GC data) and mass fragmentation data, *viz.*, *m/z* values and isotopic rations (Table IV).

TABLE IV

Values[a] used in performing LSC/GLC/MS confirmatory analyses

| Molecular formula | Molecular weight[b] | Parent peak intensity[c] | Eight most intense ions/relative abundances | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| $CHCl_3$ | 118 | | 83 | 85 | 47 | 87 | 48 | 49 | 50 | 82 |
| | | 2.4 | 100 | 65 | 20 | 11 | 10 | 7 | 3 | 3 |
| $C_2HCl_3$ | 130 | | 95 | 130 | 132 | 97 | 60 | 35 | 134 | 25 |
| | | | 100 | 99 | 95 | 64 | 56 | 31 | 30 | 28 |
| $CCl_4$ | 152 | | 117 | 119 | 47 | 35 | 82 | 121 | 84 | 49 |
| | | 0.0 | 100 | 96 | 48 | 44 | 30 | 30 | 19 | 15 |
| $CHBrCl_2$ | 162 | | 83 | 85 | 129 | 47 | 127 | 87 | 48 | 79 |
| | | 0.6 | 100 | 66 | 17 | 16 | 13 | 11 | 11 | 7 |

[a]From reference 22.
[b]The molecular weights are calculated using the most abundant isotopes.
[c]When the parent ion is not one of the eight most intense ions, the relative intensity of the parent ion is listed in this column.



# RESULTS AND DISCUSSIONS

## LSC/GC/HECD analyses

The methodolgy presented in the Experimental indicated use of a Hall electrolytic conductivity detector (HECD). When operated in the halide specific mode, rather good limits of detectability are obtained: 0.05 $\mu$g/l for chloroform and carbon tetrachloride, 0.1 $\mu$g/l for bromodichloromethane and *ca.* 5 $\mu$g/l for trichloroethylene.[18] Inasmuch as the HECD is relatively insensitive to non-halogenated species when operated in the halide-specific mode, the resulting chromatogram usually has only a few components. (During LSC/GC/MS confirmatory analyses, many more components are detected by the mass spectrometer). LSC/GC/HECD charts are quantified using the external standards described in the Experimental. In this work, quantification was accomplished using peak heights instead of the often-employed triangulation procedure.

## LSC/GC/MS confirmatory analyses

It is preferable to confirm the identities of the compounds quantified using LSC/GC/HECD analyses by LSC/GLC/MS methodology. All confirmations can be based on both relative retention data (somewhat different

for the two analytical systems), mass fragmentation data ($m/z$ values), and ratios of the fragments which contain the different isotopes of chlorine and bromine. The cluster with $m/z=83$, 85 and 87 corresponding to the respective positively charged fragments $CH^{35}Cl_2$, $CH^{37}Cl^{35}Cl$ and $CH^{37}Cl_2$ is particularly useful in confirming the presence of chloroform in biologic materials. Pertinent data for the mass spectral confirmatory work are included in Table IV.[19]

## Thirty-one day dosing sequence

This sequence has been described in the Experimental. Table I indicates both the dosing and the sacrifice schedules followed. Dosing was daily for 25 consecutive days. Two of the sacrifice periods were scheduled three and six days after the final round of dosing had taken place. Results are summarized in Tables II and III. The values reported in Table II were obtained by averaging nine determinations for each dosage level of each VPHH. The range of values was sometimes rather large; no steady increase in VPHH levels in either blood serum or adipose tissue was noted. This implies that VPHH's are not bioconcentrated in rat adipose to the extent that organochlorine pesticides are. For example, in the dosing sequence using 5 mg of chloroform, a relatively high serum level of 92 ppb was obtained on day 1; a low level of 33 ppb, on day 8; and on day 25, the serum level of chloroform was 84 pbb, very close to the level on day 1. Fat levels of chloroform on these three days were 14.3, 7.7 and 11.7 ppm, respectively. These values correspond to fat/serum ratios of 155, 232 and 139, respectively.

Carbon tetrachloride (1 mg), probably because of its lower volatility, gave more consistent results. Thus on days, 1, 11 and 25, the respective serum carbon tetrachloride levels were 12, 11 and 10 ppb with corresponding fat levels of 1.40, 2.15 and 1.64 ppm. These values correspond to fat/serum ratios of 117, 195 and 164. At the lower carbon tetrachloride dosage level (1 mg), a maximum of 0.2 ppm of chloroform metabolite was observed in fat; none was detected in serum. At the higher dosage level (10 mg), chloroform metabolite was detected in both serum (*ca.* 59 ppb) and fat (*ca.* 2.62 ppm), with average $CCl_4/CHCl_3$ ratios of 5 for serum and 7 for fat.

Chloroform was detected in both the serum and fat of TCE-dosed rats, and even when relatively high serum levels of chloroform were noted, no TCE was detected in the serum. Thus the ratio of serum $TCE/CHCl_3$ is in marked contrast to the whole-number ratio obtained for serum $CCL_4/CHCl_3$. The average adipose tissue ratios for $TCE/CHCl_3$ were 2.8 and 41 respectively, for low and high dosages of trichloroethylene.

In the case of TCE-dosed rats, the following sequence of events presumably leads to the observed distributions of TCE and $CHCl_3$. TCE is absorbed in the gut and transported to the liver via the portal vein. Because the administered dose exceeds the capacity of the liver to completely metabolize it to trichloroethanol and trichloroacetic acid (TCA) during the initial pass through the liver, the TCE which bypasses the liver is sequestered by the fat tissue. Evidently before the sacrifice period is reached (2 hr after dosing), the level of TCE in the serum falls below 1 ppb. However, relatively large amounts of TCA accumulate in the serum, decomposing only slowly to form chloroform which is also sequestered by the adipose. Most of the TCA exists as circulating TCA, which decomposes under our analytical conditions to give an indication of relatively high serum levels of chloroform. The polar nature of TCA would presumbably prevent its accumulation in fat; only relatively low levels of chloroform are found in the adipose samples.



## Storage of OC's and VPHH's in Adipose tissue

DDT and one of its major metabolic products, DDE, have high fat:water partition coefficients and, therefore, tend to accumulate in adipose tissue. Studies[1-7] in both man and laboratory animals have indicated that there is a log-log relationship between the daily intake and the residues of DDT and DDT-derived material in depot fat. At a constant rate of intake, however, the concentration of the insecticide in adipose tissue reaches an equilibrium and thereafter remains relatively constant. Following cessation of exposure, DDT is slowly eliminated from the body. Elimination has been estimated by Hayes[20] to occur at a rate of approximately one percent of stored DDT excreted per day. During the years of its most extensive use (U.S.A.) in the late 1950's and early 1960's, the average storage of DDT in fat was about 5 ppm. Total storage of DDT-derived material was about 15 ppm (primarily DDT and DDE). With declining use of DDT, there has been a gradual reduction in these levels so that the average adipose level for man in the late 1960's was 1–2 ppm of DDT and a total of about 9 ppm of total DDT-derived materials. The levels continued to decrease, and Hayes[20] estimated that the average amount of DDT that an adult in the U.S.A. obtained from ingested food went from approximately 0.2 mg in 1958 to about 0.04 mg per day in 1970.

One of the objectives of this study was to determine if laboratory rats would accumulate VPHH's in their adipose tissue during a regular dosing sequence and then, after dosing had been terminated, slowly eliminate the materials as was known to occur for stored DDT and DDE. The Experimental results (Table III) indicate that VPHH's are very rapidly

eliminated from the adipose tissue of the rats. The VPHH levels obtained from serum and fat samples taken three and six days after dosing had been stopped were low and virtually equivalent. The values reported in Table III are therefore the averages of these values for pairs of rats taken from each dosage group.

The chloroform levels in chloroform-dosed rats were essentially the same as those obtained from control animals. TCE and BDCM levels in TCE- and BDCM-dosed rats were negligible after 3–6 days. At the end of six days, carbon tetrachloride-dosed rats had eliminated over 99% of their stored carbon tetrachloride and essentially all of their stored chloroform. TCE-dosed rats had also eliminated most of their stored trichloroethylene and chloroform.

Table V contains some physical properties of the VPHH's used in this investigation. These help to explain why VPHH's are eliminated so much more rapidly than organochlorine pesticides (OC's) are. The four VPHH's all have boiling points below 100°; their highly volatile nature is also reflected in their corresponding vapor pressures, all above 40 mm Hg at 20°. The physiological temperature of the laboratory rat is itself sufficient to enhance the process of VPHH elimination.



TABLE V

Physical properties of VPHH's used in the 31-day rat dosing experiments[a]

| | Boiling point (°C) | Vapor pressure (mm Hg @20°C) | Solubility in water (@20°; ppb w/w) | Partition coefficient (water/air @20°C; w/v per w/v) |
|---|---|---|---|---|
| Chloroform | 61.3 | 150.5 | 8200 | 8.6 |
| Carbon tetrachloride | 76.8 | 90.0 | 785 | 1.1 |
| Trichloroethylene | 87.0 | 57.9 | 1100 | 2.7 |
| Bromodichloromethane | 90.2 | 42.3 | 420 | 0.6 |

[a]From reference 11.

In contrast, OC's have much higher boiling points and considerably lower vapor pressures. Also, they are very insoluble in water, whereas the VPHH's are somewhat water soluble as indicated in Table V. Finally, VPHH's are readily eliminated via expiration. It is not uncommon for over 90% of an administered VPHH to be exhaled by the lungs unchanged.[15] This route of elimination is not followed by OC's. The last column in Table V indicates the water/air partition coefficients of the

VPHH's. The implication is that perhaps increased elimination via expiration may result from decreased water/air partitioning.

The overall conclusion is that although VPHH's are readily absorbed by rat adipose tissue, when exposure (dosage) is removed, the volatile compounds are quickly eliminated from the organism.

## Alternate sources of serum chloroform

It is well established[13-15, 21, 22] that the major metabolites of trichloroethylene (TCE) are trichloroacetic acid (TCA) and trichloroethanol (Figure 1). Trichloroethanol is rapidly eliminated in the urine. TCA is not

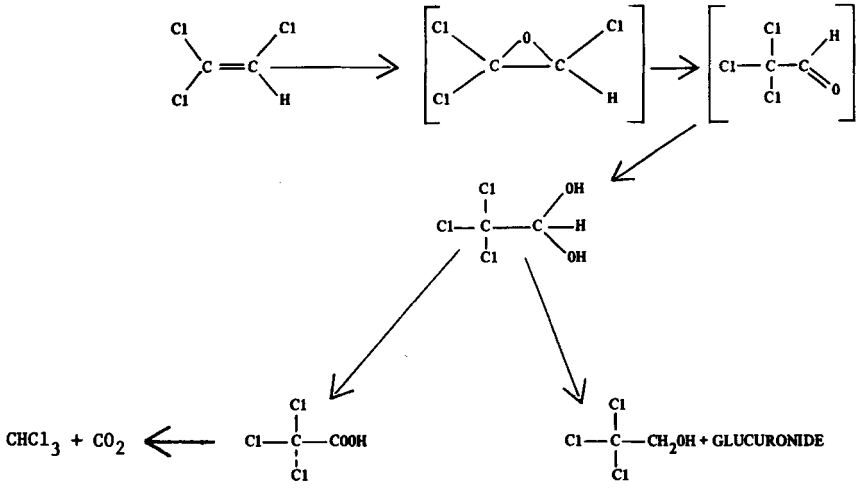


FIGURE 1 Metabolic route by which trichloroethylene is converted into trichloroethanol and trichloroacetic acid. As indicated, trichloroacetic acid decomposes into carbon dioxide and chloroform under the analysis conditions given in the text.

as readily eliminated, and under the conditions of our analyses, TCA is readily decomposed to carbon dioxide and chloroform as indicated in Figure 1. Therefore, if an organism has been exposed to TCE, serum chloroform analyses will be confounded by the presence of TCA. This was demonstrated in the TCE-dosed rats: no serum TCE was found but high levels of chloroform were. We are currently developing a method by which true serum chloroform can be distinguished and quantified in the presence of serum TCA. This study also indicates that if serum chloroform is derived from TCA, TCE will be found in adipose tissue taken from the same animal. We are now examining paired samples of blood serum and

adipose tissue acquired from the same human subjects on the same day. If one source of serum chloroform in the samples from humans is TCA, intact TCE will be found in the corresponding adipose tissue. It must also be mentioned that perchloroethylene (PCE), a common dry-cleaning and degreasing agent, is also a source of TCA[23–25] and thus of serum chloroform. In a very brief study of four workers of a dry-cleaning establishment, chloroform serum levels (presumably drom PCE) ranged from 300–6000 ppb depending on the amount of PCE exposure each worker experienced.

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