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# Determination of Volatile Purgeable Halogenated Hydrocarbons in Human Adipose Tissue and Blood Serum

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Organohalogens have been detected in virtually all chlorinated drinking waters (ROOK 1974, BELLAR et al. 1974, SYMONS et al. 1975, THOMASON et al. 1978). During a nationwide study (SYMONS et al. 1975), concentration levels in the finished drinking water of 79 cities of the United States were established for six volatile purgeable halogenated hydrocarbons (VPHH's): chloroform ($CHCl_3$), bromodichloromethane (BDCM), dibromochloromethane (DBCM), bromoform ($CHBr_3$), carbon tetrachloride ($CCl_4$) and 1,2-dichloroethane (DCE). The health implications of these findings stimulated the development of a project to determine if any of these six substances or trichloroethylene (TCE) could be detected in relatively small samples of human adipose tissue (250 mg) or blood serum (0.5 ml) obtained from residents of Dade County, Florida, an area in which chloroform levels in excess of 300 µg/l (ppb) have been reported. Accordingly, the purge/trap/desorb method of BELLAR and LICHTENBERG (1974) was modified to accomplish these objectives. The procedure reported here requires no extraction or clean-up step and is relatively inexpensive to perform. Each analysis is completed in about 30 minutes.

## MATERIALS AND METHODS

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 6-ft x 0.25-in 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 $140^0$, and a transfer line temperature of $210^0$. The Hall detector furnace was maintained at $900^0$ with a hydrogen flow-rate of 40 ml/min and a solvent (1:1 n-propanol: distilled water) flow of 0.4 ml/min.

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.

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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. Trichloroethylene, 1,2-dichloroethane and bromoform were from Aldrich Chemical Co.; bromodichloromethane and dibromochloromethane, from Columbia Organic Chemical Co. Dow Corning antifoam emulsion B was from Fisher Scientific Co. and the n-octane on 100-120 mesh Porasil C chromatographic packing was purchased from Supelco, Inc.

Preparation of Standards. Two ml each of carbon tetrachloride, dibromochloromethane and bromoform and 1 ml each of chloroform, trichloroethylene, bromodichloromethane and 1,2-dichloroethane are diluted with hexane to a final volume of 100 ml (solution 1). The concentration of each component is calculated by using the respective specific gravities. One ml of solution 1 is quantitatively diluted to 100 ml with hexane (solution 2), and a convenient working standard is prepared by diluting 0.1 ml of solution 2 to 25 ml with hexane. Use of 5 µl of this solution leads to acceptable peak heights when the Hall detector attenuation is 10 x 8.

A standard curve may be obtained by using three hexane dilutions of solution 2: the working standard, 0.1 ml diluted to 50 ml (for 10 x 4 attenuation) and 0.2 ml diluted to 25 ml (for 10 x 16 attenuation). (Although solutions 1 and 2 are stable at room temperature, fresh working standards must be made daily.)

Procedure for Blood Serum. One ml of 1% aqueous antifoam is added to a 5-ml purging device and the sample concentrator is operated in the Trap Bake Mode for 20 min while the trap temperature is $200^0$. (This procedure purges the system of all potentially interfering volatile compounds; however, a blank run may be made at this time to be certain that the system is uncontaminated.) The trap is then cooled to the ambient temperature. By means of a gas tight syringe, 0.5 ml of serum is introduced into the purging device and a purge flow-rate of 10 ml/min is started. The lower portion of the purging device is immersed in a $115^0$ stirred glycerol bath for 30 min.* To prevent steam contamination of the Tenax/silica gel trap, a small glass vapor trap or interceptor is placed between the purging device and the adsorbent trap. After the purge/trap period is complete, the adsorbed compounds are desorbed and transferred to the analytical column ($60^0$) by heating the trap at $150^0$ for 6 min. The GC column is then temperature programmed $7^0$/min to $140^0$.

*Both serum and fat appear to have an inherent binding capacity for chloroform which can be overcome by purging at elevated temperatures.

Procedure for Adipose Tissue. Between 200 and 500 mg of frozen adipose tissue is cut into thin strips and pushed to the bottom of a pre-purged, 5-ml Tekmar purging device. The purge flow-rate is adjusted to 10 ml/min, and the lower portion of the device is immersed in a 115$^0$ stirred glycerol bath for 20 min. After the volatile components have been purged from the liquefied fat, the analytical procedure given above for serum analysis is followed. Hexane is added to the purging device containing the purged adipose tissue, and the residue is quantitatively extracted to separate the fat component from residual connective tissue. Values are reported in ng VPHH/g of hexane extractable fat.

GC/MS Component Confirmation. The identities of the components quantified by the LSC/GC method are confirmed by using an LSC/GC/MS analytical system. Any confirmation is based on both relative retention values (GC data) and mass fragmentation data (m/e values and isotopic ratios).

## RESULTS AND DISCUSSION

Chloroform is the major volatile purgeable halogenated hydrocarbon identified during the analysis of human adipose tissue and blood serum when this methodology is used.

Reproducibility. One serum sample was analyzed ten times over a two-day period. The chloroform concentration ranged from 23 to 36 µg/l with a mean value of 27 µg/l and a standard deviation of 4.

One fat sample was analyzed ten times over a three-day period. The chloroform values ranged from 104 to 140 ng per gram of hexane extractable fat with a mean value of 122 ng/g and a standard deviation of 6.

Recovery. Because of the volatility of the compounds in this study, recovery studies were performed within the purging device. Serum was pre-purged, then analyzed to insure that no halogenated compounds were present. For each replication, a 5-µl aliquot of the working standard was added to the serum in the purging device. The components of the standard were allowed to mix with the serum for several minutes; then the serum was treated like an unknown sample. Table 1 indicates how much of each VPHH was added, the average per cent recovered and the recovery range for ten replications.

TABLE 1
Recovery of VPHH's from Human Blood Serum

| | Amount Added (µg/l) | Average Recovery (%) | Recovery Range (%) |
|---|---|---|---|
| $CCl_4$ | 1.3 | 112 | 108-124 |
| $CHCl_3$ | 0.6 | 100 | 100 |
| TCE | 0.6 | 98 | 83-100 |
| BDCM | 0.8 | 92 | 88-100 |
| DCE | 1.0 | 100 | 93-110 |
| DBCM | 2.1 | 87 | 78-100 |
| $CHBr_3$ | 2.3 | 90 | 79-100 |

A heated sample of human adipose tissue was purged for 30 minutes to remove all volatile purgeable halogenated compounds. For each replication, a 5-µl aliquot of the working standard was added to the fat in the purging device and allowed to mix for several minutes. Then the fat was treated as an unknown sample. Table 2 indicates how much of each VPHH was added, the average per cent recovered and the recovery range for ten replications.

TABLE 2
Recovery of VPHH's from Human Adipose Tissue

| | Amount Added (µg/l) | Average Recovery (%) | Recovery Range (%) |
|---|---|---|---|
| $CCl_4$ | 1.3 | 96 | 90-100 |
| $CHCl_3$ | 0.6 | 92 | 88-100 |
| TCE | 0.6 | 101 | 100-110 |
| BDCM | 0.8 | 109 | 100-125 |
| DCE | 1.0 | 98 | 93-100 |
| DBCM | 2.1 | 105 | 90-118 |
| $CHBr_3$ | 2.3 | 110 | 83-137 |

Blood Serum Analyses. Ten serum samples were collected from healthy human subjects and analyzed within 24 hours of collection. The chloroform values ranged from 13 to 49 µg/l as indicated in Table 3.

Sample S-1 was analyzed several times over a period of two months; each result was within 2 µg/l of the initial value.

Adipose Tissue Analyses. Ten fat samples, taken from near the anterior abdominal wall at autopsy, were analyzed. Chloroform concentrations ranged from 20 to 460 ng per gram of hexane extractable fat as indicated in Table 4.

TABLE 3
Human Blood Serum
Chloroform Levels

| Sample | µg/l | Sample | µg/l |
|---|---|---|---|
| S-1 | 13 | S-6 | 25 |
| S-2 | 13 | S-7 | 26 |
| S-3 | 49 | S-8 | 30 |
| S-4 | 13 | S-9 | 30 |
| S-5 | 45 | S-10 | 13 |

TABLE 4
Human Adipose Tissue
Chloroform Levels

| Sample | ng/g* | Sample | ng/g* |
|---|---|---|---|
| F-1 | 80 | F-6 | 20 |
| F-2 | 230 | F-7 | 28 |
| F-3 | 460 | F-8 | 140 |
| F-4 | 65 | F-9 | 95 |
| F-5 | 240 | F-10 | 240 |

*hexane extractable fat

The identities of the reference compounds and the chloroform in human serum and fat were confirmed by LSC/GC/MS methods. When coupled with the appropriate GC retention data, the cluster having m/e = 83, 85 and 87 corresponding to the respective positively charged fragments $CHCl_2^{35}$, $CHCl^{35}Cl^{37}$ and $CHCl_2^{37}$ was particularly useful to confirm the presence of chloroform in the biological samples.

The exact source of the chloroform detected in human fat and serum by this procedure is presently unknown. Possible sources include municipal drinking water and chronic exposure to trichloroethylene (TCE) and/or tetrachloroethylene (perchloroethylene, PCE).

Municipal drinking water contains both residual chlorine and chloroform generated during the chlorination process from reactions between humic substances in raw water and either dissolved chlorine or hypochlorous acid (MORRIS 1978). It has also been proposed that "intermediate bonding states" between halogen and various organic molecules can exist (GLAZE et al. 1977; NICHOLSON et al. 1977). If this latter situation occurs to any great extent, ingestion of municipal drinking water could conceivably lead to in vivo generation of chloroform from the precursors. Moreover, the residual chlorine in finished drinking water could also form chloroform precursors after the water has been consumed. A clear explanation of the origin of chloroform in human tissue is thus not currently possible.

A satisfactory explanation is even more difficult if exposure to TCE and/or PCE is considered. During an earlier study and from periodic water analysis reports, neither of these compounds has been detected in amounts comparable to the chloroform levels usually found in Miami drinking water. However, both TCE and PCE are metabolic precursors of chloroform (BUTLER 1949, IKEDA 1977), and FISHBEIN (1976) has reviewed two additional routes of exposure, air and food (McCONNELL et al. 1975, CAMISA 1975). McCONNELL et al. (1975) reported tissue ranges of TCE and PCE for humans between less than 0.5 µg/kg and 29 µg/kg wet tissue. These values are far below the chloroform levels reported here for adipose tissue. Although it is impossible to rule out the possibility that some of our experimental subjects had been exposed to these two organochlorides, it is reasonable to assume that they were not. None of

the subjects were involved in either dry cleaning or degreasing occupations nor did any of them work near these types of establishments. If a portion of the serum chloroform reported in this investigation is traceable to metabolized TCE or PCE, the intact chlorinated ethylene(s) will be present in corresponding samples of adipose tissue from the individuals. Such a study is in progress.

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