Residues of Volatile Halocarbons in Foods Using Headspace Gas Chromatography

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As part of a pilot project to measure the exposure of population groups to contaminants in air, food, and water, volatile halocarbon (VHC) residues were determined in foods by using gas chromatographic headspace analysis. Thirty-nine different food items were purchased at retail markets in each of three geographical areas in accordance with the Food and Drug Administration's Total Diet Market Basket program. The foods were prepared as for consumption and divided into four composites representing dairy products; meat, fish, and poultry; oils, fats, and shortening; and beverages. Twenty composites (five sets of the four listed) were analyzed; eight contained VHC residues (chloroform, bromodichloromethane, 1,1,1-trichloroethane, and trichloroethylene), generally at ppb (nanograms per gram) concentrations. The individual foods in the eight positive composites were also analyzed to determine which foods contained VHCs. Evaluation of the approach indicates that it is suitable for screening a wide variety of foods for VHC residues.

Volatile halocarbons (VHCs) such as chloroform, carbon tetrachloride, 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene are used primarily as industrial solvents and chemical intermediates. Tens to hundreds of millions of pounds each of these compounds are produced annually (International Trade Commission, 1979). In addition, trihalomethanes (THMs) may be produced through the chlorination of water. VHCs are of interest because their large production volumes may lead to their presence as contaminants of foods and because some are carcinogenic in animals. Some VHC residues in foods have been reported (McConnell et al., 1975; Page and Kennedy, 1975; Page and Charbonneau, 1977; Gilbert et al., 1978; Entz and Hollifield, 1982).

The Food and Drug Administration's (FDA) Bureau of Foods was asked by the Environmental Protection Agency (EPA) to participate in a pilot study as part of a program to measure human exposure to various toxic pollutants through the pathways of air, water, and food. The pilot study was undertaken in conjunction with Research Triangle Institute, the chief EPA contractor for the project, to refine and test procedures for the analysis of foods for VHCs. The techniques used in this study were based on previous work on headspace gas chromatography (GC) (Entz and Hollifield, 1982). Eight compounds were selected for evaluation of the analytical procedure: chloroform ($CHCl_3$), bromodichloromethane ($CHCl_2Br$), carbon tetrachloride (CCl_4), 1,2-dichloroethane (EDC), 1,2-dibromoethane (EDB), 1,1,1-trichloroethane (MC), trichloroethylene (TCE), and tetrachloroethylene (PCE).

Foods were purchased at retail markets in accordance with FDA's Total Diet Study Adult Market Basket (Duggan and McFarland, 1967; Duggan and Cook, 1971). Elizabeth, NJ, Chapel Hill, NC, and Washington, DC, were the sampling sites.

The Adult Market Basket, representing the diet of a teenage male, is divided into 12 food groups. Under this approach, individual foods are prepared table ready (as generally consumed in the home), and foods from each group are blended together in the proper proportions to form a "composite". If unusual or excessive residues are found in the composites, the individual foods are also analyzed. Four of the twelve composites were sampled: composite I = dairy products; composite II = meat, fish, and poultry; composite X = oils, fats, and shortening; composite XII = beverages. These groups were chosen because they contain the food types thought to be most likely to contain VHC residues.

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EXPERIMENTAL SECTION

Food Preparation. Foods were purchased and transported to this FDA laboratory for preparation and analysis. Perishable foods were shipped and stored frozen but were allowed to thaw before preparation. Solids, if not finely divided, were chopped, grated, or ground. Preparation of the foods involved cooking the meat, fish, and poultry (composite II) and brewing tea and coffee (composite XII). No other preparation was needed for composite I (dairy) or composite X (oils and fats). Foods were composited by blending weighed amounts of each individual food (termed subsample) to attain a homogeneous mixture. Samples of both the individual foods and the blended composite were retained for analysis. Aqueous samples were refrigerated; all other samples were frozen.

Apparatus and Reagents. The apparatus and reagents have been described (Entz and Hollifield, 1982). An automated headspace analyzer, a Perkin-Elmer Model F-42 gas chromatograph, equipped with an electron capture detector, and a Spectra Physics Model 1 integrator were used for the initial analyses. Manual injection of headspace onto a second GC column in a Varian 3700 gas chromatograph was used for tentative confirmation of identity. Gas chromatographic–mass spectrometric (GC–MS) confirmation was performed by using a Finnigan 4000 quadrupole mass spectrometer in the electron impact mode with manual injection of headspace.

Gas Chromatography. The conditions for GC and the three columns used have been described (Entz and Hollifield, 1982). Column 3, a 50-m glass capillary coated with OV-101, was used for the initial identification and quantitation of residues, column 1, a 3.7-m 15% OV-17 packed column, was used for tentative confirmation by GC, and column 2, a 3-m 20% SP2100/0.1% Carbowax 1500 packed column, was used for GC–MS confirmation.

Procedure. The general procedure was similar to that outlined earlier (Entz and Hollifield, 1982). A portion of the sample was sealed in a 24-mL glass vial by using a Teflon-lined rubber septum. For some samples, 15 mL of 20 N H_2SO_4 was added to the vial before sealing to digest the food. The vials were heated 1 h in a 90 °C water bath, and an aliquot of the headspace was injected into the gas chromatograph. One-gram samples of composites I and

II and individual meat, fish, and poultry items were digested with 20 N H_2SO_4 before analysis. Two-gram samples of composites X and XII were analyzed neat, as were the individual foods making up composites I, X, and XII. Residues were quantitated by using the method of standard additions; in some cases the quantitation was checked by using external standards. An internal standard was added to each vial to check for replicate injection volumes and to check for leaks from the sealed vials.

Fortification. A double quantity of the necessary foods was purchased in Washington, DC. Half was analyzed and the remainder was used to prepare fortified composites. Four foods (milk, ground beef, mayonnaise, and lemon/lime soft drink) were individually fortified with $CHCl_3$, MC, CCl_4, TCE, EDB, and PCE and then combined with appropriate other foods that were free of VHC residues to produce fortified samples of the four composites.

RESULTS AND DISCUSSION

A series of experiments were carried out to assess the capabilities of the headspace technique and the procedures for compositing the foods. The recovery of analyte through the preparation and compositing steps was examined by analyzing both fortified foods and food composites. Storage stability and the precision of analyses were evaluated through replicate analyses of the fortified composites over a 4–6-month period. The partitioning of VHCs from each composite was studied through standard addition experiments.

EDC was not used during the method evaluation because of the poor electron capture response for this compound. In addition, $CHCl_2Br$ was not studied in the fortification experiments because it coeluted with TCE on the capillary GC column used in this phase of the study. It was assumed to have characteristics similar to those of $CHCl_3$.

The losses of analytes due to preparation and handling of individual foods were determined by comparing the analytical results immediately after fortification with those obtained after preparation. The overall recovery through both the handling and compositing steps was determined by comparing the fortification level in the individual food to the concentration of analytes determined in the composite, after accounting for the dilution due to other foods included in the composite.

Single fortification experiments were performed for each of the four composites. Recoveries of the analytes ranged from 61 to 84% (average 74%) for composite I and 58–128% (average 84%) for composite X. The cooking of the subsamples in composite II (meat, fish, and poultry) removed most of the VHCs. Only 22–58% of the VHCs were recovered from the cooked ground beef. Low recoveries of analytes, ranging from 10 to 82% (average 51%) were obtained for composite XII (beverages). The recovery of VHCs from the fortified lemon/lime soft drink was much higher, 69–141% (average 97%), indicating that the major loss of analytes occurred during the compositing.

Replicate analyses of fortified composites I and X, performed over a 5-month period, had relative standard deviations (RSDs) ranging from 8 to 27% (six replicates). No significant changes in the analyte levels were observed for these composites during storage.

Three replicate analyses of composite XII were performed at about 2-week intervals. Each analysis was performed on a portion of the composite from an individually sealed gas-tight container. RSDs of 5–13% were obtained. Subsequent reanalysis of the contents of the containers indicated a 30–70% loss of analyte. Losses were not observed for replicates of these same samples when resealed without overlying headspace and stored for 3 months. The loss observed for the beverage composites was attributed to the partitioning of VHCs into the overlying headspace and subsequent loss upon opening the container.

Table I. Food Composite Partitioning Data—Standard Additions[a]

compound	composite I (dairy)	composite II (meats)	composite X (oils, fats)	composite XII (beverages)
$CHCl_3$	40.5[b] (13)	29 (12)	9.1 (8)	59 (4)
MC	111 (41)	75 (20)	29 (27)	130 (8)
CCl_4	1023[b] (22)	532 (40)	512 (78)[c]	515 (18)[c]
TCE	52[b] (18)	29 (19)	7.0 (10)	70 (3)
$CHCl_2Br$	199 (4)	114[d] (25)	30[d] (5)	35[d] (16)
EDB	11[b] (22)	6.8 (44)	2.8 (15)	47 (6)
PCE	234[b] (27)	110 (22)	19.4 (18)	432 (3)

[a] Mean of slopes from best fit lines for standard additions experiments in integrator counts per nanogram added (sensitivity). Data from two to five composites were used for calculations. Relative standard deviation (percent) is in parentheses. [b] One data point was excluded from the calculation as outlier (greater than 3σ from the mean). [c] Coeluting components were present in foods. [d] Two data points were used for calculation.

Table II. Estimates of Quantitation Limits for VHCs in Food Composites[a]

compound	composite I (dairy)	composite II (meats)	composite X (oils, fats)	composite XII (beverages)
$CHCl_3$	12.5[b]	18	28	4.2
MC	4.2[b]	7.0	8.8	1.9
CCl_4	0.5[b]	0.87	3.0	0.5
TCE	10[b]	18	36	3.5
$CHCl_2Br$	2.3	4.5	8.3	0.5
EDB	48.5[b]	84	92	5.8
PCE	2.3	4.6	13	0.5

[a] Quantitation limits (mean) in ppb (nanograms per gram) calculated from the slope of the best fit line; concentration of analyte necessary to give 500 integrator counts. [b] One data point was excluded from the calculation as outlier (greater than 3σ from the mean).

The repeatability of the analyses of the composites is of the same order as that observed earlier for individual foods, with RSDs less than 20% (Entz and Hollifield, 1982). The storage stability of these composites appears adequate. Repeated sampling of aqueous samples may produce losses.

Quantitation by both standard additions and external standards was carried out during the replicate experiments. No difference was observed between the two methods within the precision listed above for the replicates.

Because the composites were prepared by using foods from different retailers, producers, and processors, as well as from different lots, the composition could vary, especially in the fat content of the dairy and meat composites. Variation in the composition could also be introduced during preparation of the foods. For determination of the reproducibility of the composition of the composites, five sets of the four composites prepared from foods from different sources were studied. The variation in analyte partitioning from sample to headspace was used as a measure of the reproducibility of the composition of the composites. Composites were studied through a series of standard additions over a 10–20-fold concentration range. The variation in the slope of the line relating the quantity of added analyte to the peak area in a chromatogram re-

Table III. Analysis of Composites for VHCs

sample site, no.	composite I (dairy)	composite II (meats)	composite X (oils, fats)	composite XII (beverages)
Washington, 1	nq[a]	nq	19 ppb of MC[b]	nq
Chapel Hill, 1	nq	nq	920 ppb of TCE[b]	32 ppb of $CHCl_3$[b] 0.3 ppb of $CHCl_2Br$
Elizabeth, 1	17 ppb of $CHCl_3$ 1.5 ppb of MC 1.2 ppb of $CHCl_2Br$	nq	tr[c] of $CHCl_3$(<12 ppb) tr[c] of MC(<5 ppb) 56 ppb of TCE[b]	12 ppb of $CHCl_3$ 0.6 ppb of $CHCl_2Br$
Chapel Hill, 2	nq	nq	nq	12 ppb of $CHCl_3$
Elizabeth, 2	nq	nq	nq	6 ppb of $CHCl_3$

[a] No compound was observed above the quantitation limit. [b] GC-MS confirmation was obtained for the subsample from this composite. [c] Trace; not quantitated.

flects the variation in the detector response and/or the variation in the partitioning of the analyte from the food matrix into the headspace. These data are listed in Table I. The agreement among the slopes for various samples of composite XII indicates that the detector response was nearly constant over the course of the study. The RSD of the slopes ranged from 3 to 18% for the compounds tested. The differences in the variations of the slopes for other composites were attributed to variation in partitioning rather than variations in detector response.

The homogeneity of the composite appeared to be the most important factor in determining the variation in partitioning. Composite XII, a nearly completely aqueous matrix, had little variation in the slope. Likewise, composite X, nearly all fatty foods, showed less variation than the others; the RSD of the slopes ranged from 5 to 27%. Composites that contained both fatty and nonfatty foods showed greater variation. Composite I (dairy products) and composite II (meat, fish, and poultry) exhibited the greatest variation. The RSD of the slopes ranged from 4 to 41% and 12 to 44%, respectively. Previous FDA experience with composite I has indicated that it is difficult to maintain a homogeneous mixture of the fatty components (butter and cheese) in the matrix. For the more homogeneous food composites, the variation in the slopes was on the same order as the variation in quantitation of VHCs in replicate samples. This indicates that it is possible to reproduce these composites with similar partitioning characteristics for volatile compounds, which indicates that the composites' composition is reproducible. For the other composites the larger variation in the slopes may be due to either a lack of homogeneity of the composite, a variation in the composite's composition, or both.

The quantitation limit was defined as the concentration of analyte added to the sample necessary to give a 500-count integrator peak area (Table II). This quantity was estimated from the linear least-squares best fit of the standard additions data. Only the slope term was used to calculate the quantitation limit. This calculation does not take into account interferences present in the chromatogram or lines that do not intersect the origin.

To determine if laboratory contamination occurred, we routinely exposed corn oil to the ambient atmosphere in a Petri dish during preparation and compositing of the samples. Corn oil should absorb and retain lipophilic volatile compounds to a similar or greater extent than the foods being composited. No VHCs were observed above the quantitation limit (about the same as that for composite X, Table II) in the corn oil blanks related to samples containing VHC residues.

The results of analyses of the food composites (Table III) and selected subsamples (Table IV) showed that VHCs were found in 8 of the 20 composites. In general, low levels of THMs were found in composite XII and in one sample of composite I. The presence of THMs in composite XII (beverages) reflects the THMs found in the bottled soft drinks and may also reflect the use of tap water from the sampling site in the brewing of tea and coffee. Tap water from the sampling site was not analyzed as a separate subsample. No residues of either EDC or EDB were observed in the foods analyzed.

Table IV. Analysis of Subsamples[a] for VHCs

sample site, no.	food	composite	VHC found ppb (ng/g)
Washington, 1	margarine	X	MC,[b] 45
Chapel Hill, 1	margarine	X	TCE,[b] 3600
	cola soft drink	XII	$CHCl_3$,[b] 178 $CHCl_2Br$, 3.4
	noncola soft drink	XII	$CHCl_3$, 32
Elizabeth, 1[c]	milk	I	$CHCl_3$, 17
	ice cream	I	$CHCl_3$, 23 MC, 2
	processed American cheese	I	$CHCl_3$, 17 MC, 9
	natural cheese (nonprocessed)	I	$CHCl_3$, 15 MC, 7
	butter	I	$CHCl_3$, 56 MC, 16 $CHCl_2Br$, 7
	mayonnaise	X	$CHCl_3$, 34
	margarine	X	TCE,[b] 440 MC, 40
	cola soft drink	XII	$CHCl_3$, 22 $CHCl_2Br$, 3.8
Chapel Hill, 2	cola soft drink	XII	$CHCl_3$, 9
	noncola soft drink	XII	$CHCl_3$, 14.5
Elizabeth, 2	cola soft drink	XII	$CHCl_3$, 36 $CHCl_2Br$, 2.3

[a] Subsamples were found to contribute to VHCs in the composite. [b] Confirmed by GC-MS. [c] All individual foods from this set of composites were analyzed. Foods containing residues are listed.

Analysis of individual foods (Table IV) indicated that the chief source of VHCs in composite X was the margarine. Other foods besides the soft drinks and margarine also contained VHC residues. Dairy products from one sample had residues of MC at the low ppb level as well as THMs ranging from 7 to 56 ppb.

Foods producing an electron capture response at the retention time of TCE were analyzed on a second column to separate the coeluting TCE and $CHCl_2Br$. All other findings were based on single column retention times. Interferences from electron capturing compounds were observed near the retention times of CCl_4 and TCE in several foods from composites I and X (e.g., milk, butter, mayonnaise, and margarine). MS confirmation of residue identity was obtained for three margarine subsamples whose analyte concentrations were sufficient for identification, one containing MC and the other two TCE.

$CHCl_3$ was also confirmed in one soft drink subsample. Good agreement was obtained between full-scan electron impact mass spectra for analytes and standards. Because concentrations of approximately 50 ng/g were necessary for GC–MS confirmation, other residues found in the subsamples or composites could not be confirmed.

CONCLUSIONS

The compositing technique studied allows screening (quick semiquantitative analysis) for VHCs in a wide variety of foods. For composites I and X, the compositing technique provides quantitative information. However, the losses of analytes during preparation or compositing of foods for composites II and XII permit only qualitative information to be obtained; individual foods must be analyzed if quantitative data are desired. Careful attention to sample preparation is necessary to ensure similar partitioning characteristics for VHC determination in the heterogeneous food composites.

VHCs were detected at ppb levels in a variety of the foods collected; the compounds found, as well as their levels, differed from sample to sample. Because of the limited sampling, no conclusions regarding the general magnitude of VHC residues in foods should be drawn.

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LITERATURE CITED

Duggan, R. E.; Cook, H. T. *Pestic. Monit. J.* **1971**, *5*, 37.
Duggan, R. E.; McFarland, F. J. *Pestic. Monit. J.* **1967**, *1*, 1.
Entz, R.; Hollifield, H. *J. Agric. Food Chem.* **1982**, *30*, 84.
Gilbert J.; Startin, J. R.; Wallwortz, M. A. *J. Chromatogr.* **1978**, *160*, 127.
International Trade Commission "Synthetic Organic Chemicals, United States Production and Sales 1978"; ITC: Washington, DC, 1979; U.S. ITC Publication 1001.
McConnell, G.; Ferguson, D. M.; Pearson, C. R. *Endeavour* **1975**, *34*, 13.
Page, B. D.; Charbonneau, C. F. *J. Assoc. Off. Anal. Chem.* **1977**, *60*, 710.
Page, B. D.; Kennedy, B. P. C. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 1062.

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Determination of Volatile Aldehydes in Meat as 2,4-Dinitrophenylhydrazones Using Reversed-Phase High-Performance Liquid Chromatography

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Volatile aldehydes formed by oxidative degradation of unsaturated fatty acids were isolated from meat and determined as 2,4-dinitrophenylhydrazones (DNPHs). Meat samples were homogenized in ethanol and the volatile aldehydes distilled under vacuum into a trap filled with 2,4-dinitrophenylhydrazine. The derivatization took place in a two-phase reaction with acidic ethanol solution and hexane. The recovery of aldehydes with more than five carbon atoms is better than 80% at trace concentrations (micrograms per gram). Determination is carried out by reversed-phase HPLC on C_{18}-RP columns with the eluent acetonitrile–water–tetrahydrofuran (75:24:1) at 50 °C using photometric detection at 360 nm. The HPLC method was optimized for the determination of volatile aldehydes formed during oxidative degradation of the unsaturated fatty acids occurring in food. The detection limit at 360 nm was found to be 5 pmol. The utility of the method for the detection of beginning oxidative rancidity was demonstrated with a storage experiment of frozen pork liver.

Low concentrations of aldehydes are found in most foods. Aldehydes are characterized by very low olfactory threshold concentrations and for this reason constitute important components of the natural flavor of a food. In fat-containing foods, these components of the flavor are formed during maturation by enzymatic and nonenzymatic reactions, preferentially by oxidation of unsaturated fatty acids (Ohloff, 1973; Tressl et al., 1975). In the course of storage, these reactions may result in a elevated formation of aldehydes, which then would represent typical components of the off-flavor. A particularly well-known off-flavor is rancidity, which is mainly due to the formation of carbonyl compounds as a consequence of the autoxidation of fatty acids (Grosch, 1975).

Two different methods are employed in the analytical detection of aldehydes in low, however, relevant concentrations in foods. In the first one, the carbonyl compounds are concentrated together with all volatile ingredients of the food sample with subsequent gas chromatographic separation and determination. The other method consists in a reaction with 2,4-dinitrophenylhydrazine (2,4-DNP) and chromatographic determination of the hydrazones. Owing to the high extinction coefficient of the 2,4-dinitrophenylhydrazones (2,4-DNPHs), photometric detection permits an identification of aldehydes in the nanogram range. Additionally, 2,4-DNPHs are well accessible to methods of separation by thin-layer and liquid chromatography.

Direct derivative formation in an acid aqueous solution has been used first for preparative reactions with oxo compounds. At the low concentrations as common in the analytical field, however, a yield of the corresponding 2,4-DNPHs of only 70% was found (Selim, 1977). Fur-

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