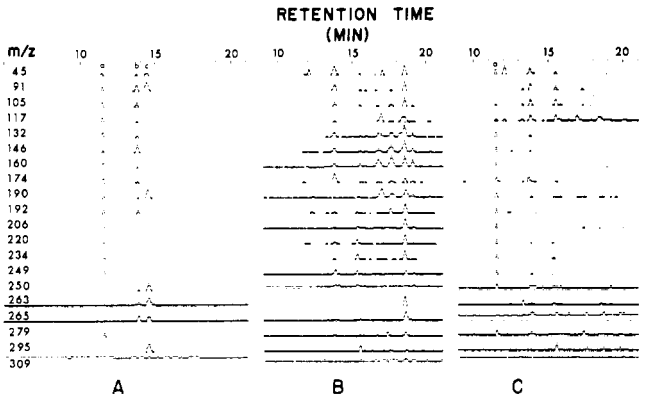

Figure 3. Multiple-ion monitoring chromatogram on SE-54 capillary column of (A) metalaxyl (a), benzyl alcohol (b), and ring 3-OH (c) metabolites, (B) check potato tuber extract, and (C) a field-treated potato tuber extract.

tention times (Table IV). On the SE-30 column, mixtures of cyprofuram and oxadixyl were only partially resolved. The acidic compounds (metalaxyl acid and benzyl acid metabolites) showed a low response and some peak tailing whereas after methylation, the expected response and symmetry were obtained. Depending on the injector temperature and the cleanliness of the capillary inlet system, metalaxyl benzyl alcohol may elute as the rearrangement lactone or as two peaks indicating some thermal decomposition, and ofurace often has a propensity to dechlorinate and elute as ofurace alcohol.

A nitrogen–phosphorus detector can be used to determine these compounds with good sensitivity and specificity to most crop extracts, even without cleanup. The mass selective detector in the multiple-ion monitoring mode coupled with the separation power of the capillary column adds a high degree of specificity to the determination. The common ions associated with the acylalanines and their metabolites allow simultaneous determination of these compounds in an extract (Figure 3). Cooke et al. (1982) used packed column GC and multiple-ion monitoring to confirm the presence of ofurace in potato foliage after soil treatment. Incorporation of partitioning or fractionation steps in the analytical methodology will also add specificity and confirmation to the analysis.

Registry No. CGA-48988, 57837-19-1; CGA-38140, 57646-30-7; SAN 371, 77732-09-3; RE-20615, 58810-48-3; RE-26745, 67932-71-2; RE-26940, 70622-00-3; CGA-29212, 52888-51-4; CGA-100255, 96258-85-4; CGA-100255 methyl ether, 96258-86-5; CGA-100255 ethyl ether, 96258-87-6; CGA-94689, 85933-49-9; CGA-94689 rearrangement lactone product, 96258-88-7; CGA-94689 methyl ether, 96258-89-8; CGA-94689 ethyl ether, 96258-90-1; CGA-108905, 96258-91-2; CGA-108905 methyl ester, 96258-92-3; CGA-108905 ethyl ester, 96258-93-4; benalaxyl, 71626-11-4; cyprofuram, 69581-33-5; metalaxyl acid, 87764-37-2; metalaxyl acid ethyl ester, 57837-86-2; ofurace alcohol, 66637-59-0.

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Received for review November 21, 1984. Accepted March 15, 1985.

Preparation and Use of Mixed Fumigant Standards for Multiresidue Level Determination by Gas Chromatography

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Two multicomponent stock concentrations of the following standard-grade fumigants are made in purified 2,2,4-trimethylpentane: methyl bromide, dichloromethane, carbon disulfide, chloroform, 1,2-dichloroethane, carbon tetrachloride, trichloroethylene, chloropicrin, 1,2-dibromoethane, and tetrachloroethylene. One concentration is used with electron-capture detection, the other with Hall detection. Analyst exposure to these toxic fumigant substances is minimized by fortifying samples and making working concentrations through single-step dilutions. Recovery data are accurate and complete. Single injections of the working concentrations permit rapid ppb level screening determination of residual fumigants in grain and grain-based products.

INTRODUCTION

Most of the following fumigant methods were developed to determine 1–4 specific residues at levels above 1 ppm: leaching (Heuser and Scudamore, 1969; Berck, 1974; Fairall and Scudamore, 1980; Clower 1980), extraction and partition (Newsome and Panopio, 1982; Daft, 1983a), sweep and codistillation (Malone, 1969; Rains and Holder, 1981; Hughes et al., 1983; Iwata et al., 1983), headspace (Entz and Hollingfield, 1982; Page and Charbonneau, 1984; Bowers, 1984), and purge-closed loop (Wang and Lenahan, 1984). Recently, however, because of the attention given to the 1984 ban of the fumigant 1,2-dibromomethane (EDB) and because several residues from among about 15 commonly used fumigants can be detected in one sample

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Table I. Dilution Scheme for Making Solutions of Mixed Fumigant Standards (Electron-Capture and Hall Detection)

compd	d^{20} (lit.)	ECD amnt to 100 mL	ECD stock concn, mg/mL	HECD amnt to 100 mL	HECD stock concn, mg/mL	working concn of ECD and HECD, ng/mL
CH_3Br	1.730[a]	210 μL[b]	1.21	84 μL[b]	0.48	121.0, 48.0
CH_2Cl_2	1.326	200 μL	2.65	7 μL	0.093	265.0, 9.3
CS_2	1.263	250 μL	3.16	none		316.0
$CHCl_3$	1.484	10 μL	0.148	8 μL	0.119	14.8, 11.9
EDC	1.257	1 mL	12.57	10 μL	0.126	1257.0, 12.6
CCl_4	1.594	20 μL[c]	0.032	120 μL[c]	0.191	3.2, 19.1
TCE	1.465	11 μL	0.161	11 μL	0.161	16.1, 16.1
CP	1.656	10 μL	0.166	15 μL	0.248	16.6, 24.8
EDB	2.179	20 μL	0.436	40 μL	0.872	43.6, 87.2
PCE	1.623	5 μL	0.081	15 μL	0.243	8.1, 24.3

[a] d^0. [b] Prediluted 3X, 0 °C. [c] Prediluted 10X, 20 °C.

by these methods, analytical focus has trended toward multiple-residue screens at levels below 1 ppm.

The gas chromatographic (GC) determination of multifumigant residues in grain and grain-based products at these lower detection levels requires working standards that are safe, dependable, and easy to use. Although most single-component standards can be purchased prediluted, the diluting medium itself may not be suitable for a particular fumigant method. Therefore, when making suitable original standards in the laboratory, safe practices must be employed since fumigant compounds are volatile and toxic (Federal Register, 1984). Extra care is also needed when making dilutions of a compound such as methyl bromide which is comparatively more volatile (bp 3.56 °C) than most of other fumigants and is more susceptible to loss in solution.

Moreover, all primary standards should be free of error-causing impurities. Pesticide-grade tetrachloroethylene, for example, cannot be used in mixed standards at these detection levels because it sometimes contains traces of chloroform or carbon tetrachloride. Yet, a safe straightforward procedure for making low concentrations of mixed reference solutions is possible.

This report describes a procedure for making multiple-component fumigant standards volumetrically by using microliter syringes (Sawyer, 1980; Clower, 1981). The application of these standards to residue determination is then discussed.

MATERIALS AND METHODS

Standards. GC background impurities were removed from the 2,2,4-trimethylpentane (isooctane) stock/working solvent by one of two methods. Method one: 4 L of isooctane were passed through to successive 55-mm i.d. columns of activated alumina (Iwata et al., 1983) at a flow rate of 10–15 mL/min. The upper column contained 100 g of Alumina I, basic or neutral (Heikes, 1984); the lower one contained 100 g of Alumina I, neutral. This method was repeated as necessary to remove background contaminants. Method two: 4 L of isooctane were refuxed over 200 g of sodium metal in a 5-L flask (with cold water condenser) for about 20 h; about 3 L was then distilled from the sodium (Puma 1984). Both the relux and the distillation operations were carried out in a hood under a 200 mL/min constant gas purge of breathing quality air. (Danger: sodium is extremely reactive with moisture. The labeling and identity of the isooctane should be double checked beforehand. Also, a scaled-down distillation apparatus, e.g., a 1-L flask capacity, limits the amount of sodium in use at one time.)

The following standard-grade compounds (all in liquid form) were purchased from Chem Service, West Chester, PA: (1) methyl bromide (CH_3Br), (2) dichloromethane (methylene chloride, CH_2Cl_2), (3) carbon disulfide (CS_2), (4) chloroform ($CHCl_3$), (5) carbon tetrachloride (CCl_4), (6) 1,2-dichloroethane (ethylene dichloride, EDC), (7) trichloroethylene (TCE), (8) 1,2-dibromoethane (ethylene dibromide, EDB), and (9) tetrachloroethylene (perchloroethylene, PCE). Chloropicrin (CP) was obtained from Eastman Kodak, Rochester, NY. In making stock solutions of these standards, strict safety precautions were employed (Federal Register, 1984; Guidelines Establishing Test Procedures for the Analysis of Pollutants, 1984; Safety in Academic Chemistry Laboratories, 1979; Working with Carcinogens, 1977; OSHA Safety and Health Standards, General Industry, 1976). Rubber gloves were worn by the analyst (butyl, neoprene, or latex—but not PVC) (Prokopetz and Walters, 1985). A gas respirator (NIOSH/MHSA approved) was available to the analyst. All dilutions were done in a well-ventilated fume hood.

Before diluting the methyl bromide, all glassware, syringes, and solvents were first cooled to about −4 °C in a freezer. Liquid CH_3Br (about 1 mL) was shipped in a sealed glass ampule which was cooled to about 0 °C (or just below) in the freezer. The seal was broken—in the hood—and the CH_3Br was poured into a cold 5-mL graduated clyinder (graduated to 0.1 mL or less) and measured. Cold isooctane was added to a 1:2 dilution with careful mixing. When a cold 100-μL Dynatech locking gas-tight syringe was used, specific amounts of this dilution (equivalent to 70 and 28 μL of liquid CH_3Br, respectively) were slowly injected into separate 30-mL portions of cold isooctane in 50-mL Actinic Erlenmeyer flasks (see Table I). These steps were done as quickly as possible to maintain a fairly constant CH_3Br temperature of 0 °C. (Note: the locking syringe was bulky and could not be inserted into the necks of volumetric flasks.)

Next, when well-rinsed 10- to 100-μL GC syringes were used, specific amounts of the remaining components at 20 °C were injected into the same 30-mL solutions according to the dilution scheme in Table I. The isooctane solutions were then transferred to 100-mL Actinic volumetric flasks and taken to volume with the same solvent at room temperature. Working solutions were made by diluting each stock solution 10^4X, then transferring to amber septum-sealed containers.

Gas Chromatograph. The gas chromatograph used was a dual column Tracor 560 equipped with nickel-63 electron-capture (ECD) and Hall-electroconductivity chloride-mode (HECD-Cl) detectors and 1.8 m or 3.6 m × 4 mm i.d. glass columns packed with 20% OV-101. Additional columns used with ECD were (1) 20% OV-225/20% OV-17 (2 + 1 mixed bed), (2) 10% SP-1000, and (3) 20% OV-17; all packings were 80–100 mesh. GC operating conditions were as follows: injector ports, 130–150 °C; columns, 80–90 °C isothermal; ECD, 350 °C; carrier

gas, 5% CH_4/Ar; HECD, reactor base 250 °C; reactor 900 °C; conductivity solvent flow (*n*-propanol), 0.4 mL/min; H_2 reaction gas flow, 60 mL/min; automated vent time, 0 min; carrier gas, He. Carrier gas flows were 30–90 mL/min. Recorder attenuation was 10X for ECD, and 2X (range 10) for HECD; chart speed was 10–12.7 mm/min. Sensitivities were about 50% full scale deflection for 0.2 ng of chloroform (t_R = $3^1/_2$ min), both detectors.

Methods. The fumgant methods used were modified procedures of the AOAC (Official Methods of Analysis, 1980). Whole-kernel grain (50 g) was leached 48–72 h in 100 mL of 5:1 acetone–water with occasional swirling. One milliliter of the resultant leachate was transferred to a 15-mL screw-cap culture tube with 10 mL of 8% NaCl solution (w/v) and 1 mL of purified isooctane. After securing the cap, the tube was shaken vigorously 1 min. Six to ten microliters of the upper isooctane layer (3–5 mg sample) were syringe drawn for residue determination by GC (Pederson and Cornwell, 1984).

Grain-based products (and citrus fruits) were analyzed by a modified procedure still being tested and improved. The product (10 g) was put into a 125-mL Actinic Erlenmeyer flask containing 50 mL of 25% acetone (v/v):10% NaCl (w/v):10% phosphoric acid v/v):0.2% glycerin (v/v) solution and 10 mL of 0.1 benzene in isooctane (v/v). The flask was shaken (or its contents homogenized 5 s if not broken up by shaking) and allowed to stand overnight. Water (50 mL) was added. The flask was then shaken vigorously 1 min. After the layers separated (sometimes with centrifugation at 1500 rpm for 10 min), 6–10 μL of the isooctane layer were drawn for residue determination.

In both methods, fortification was made by injecting into the sample solution an amount of mixed stock solution that would equal the working concentration after partitioning; e.g., whole-kernel grain whose leachate was usually partitioned at a 1:1 ratio was fortified by injecting 10 μL of each stock solution into separate samples, a 10^4X dilution of each stock.

RESULTS AND DISCUSSION

Standard Preparation. Solvents other than isooctane, e.g., acetone or methanol, both soluble in aqueous and many organic solutions, probably could have been used to make the mixed stock solutions. But our supplies have not been impurity free, and convenient purification methods were not available. Isooctane, on the other hand, could be purified, sodium distillation being more effective than alumina chromatography but also more dangerous. Moreover, single-component working solutions of the 10 purchased compounds—made from stocks in purified isooctane—showed no background impurities at the stated instrument sensitivities, indicating the standards themselves were impurity free.

Also, the peak height pattern of 10-component solutions remained identical between separately made stock and working solutions. Seven-component stock solutions, tested earlier, made in isooctane and stored in a freezer lasted about one year without noticeable deterioration of GC response, i.e., about 10%, except for chloropicrin which deteriorated fastest (Castro and Belser, 1981). CH_3Br, CH_2Cl_2, and CS_2 in mixed solution have not been tested for that length of time. However, data from a six-month period indicated CS_2 is stable in cold stock solution, but CH_3Br and CH_2Cl_2 in the same solution each deteriorate at a rate of about 10% a month. Acidifying the stock solutions with two drops of H_3PO_4 enhanced the stability of chloropicrin, CH_3Br, and CH_2Cl_2 slightly.

Yet, acidifying the working solutions did not enhance the stability of CH_3Br and CH_2Cl_2. These solutions at room temperature in Actinic glassware lasted 1–2 weeks without noticeable change, after which CH_3Br and CH_2Cl_2 were gradually lost. Also, single-component working solutions of CH_3Br in clear glassware lost about 30% of their original GC response over a three-day period. Conversely, single-component working solutions of CH_2Cl_2 were stable for days. These data suggest solutions of CH_3Br and CH_2Cl_2 need to be made separately and more often than the other eight components for best quantitatation.

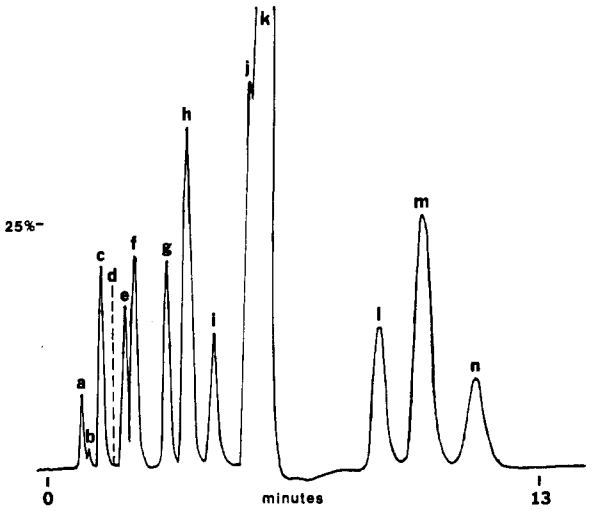

Figure 1. Chromatogram of 10-component fumigant standard in isooctane: (a) air, (b) unknown, (c) 1.0 ng of CH_3Br, (d) t_R acetone, (e) 2.1 ng of CH_2Cl_2, (f) 10 ng of CS_2, (g) 0.12 ng of $CHCl_3$, also t_R hexane, (h) 10 ng of EDC, (i) 0.03 ng of CCl_4, (j) 0.13 ng of TCE, (k) solvent peak (off scale), (l) 0.2 ng of CP, (m) 0.35 ng of EDB, and (n) 0.05 ng of PCE; 20%; OV-101, 80 °C, electron-capture detection.

An advantage, however, in having 10-component stock solutions is that both the sample-fortifying and the working solutions can be made simultaneously from the same sources, minimizing analyst exposure to opened stock solutions. Also, concurrent application of fortifying and working solutions to sample analysis lends accuracy and completeness to the recovery data which ranged from 20% to 120% for all components.

Because of its volatility, pure methyl bromide is difficult to transfer accurately. Prediluted CH_3Br was purchased (0.2 mg/mL), but the batch we received contained a background peak with ECD. Consequently, in making the original stock solutions, the task of transferring volumetric amounts of pure liquid CH_3Br was facilitated by first putting it into solution with cold isooctane. The resultant working standard compared favorably (±10%) to another one made gravimetrically, also cold.

Determination. A problem in the GC determinative step of this analysis is the coelution of working solvents and incurred residues. With ECD, for example, isooctane coelutes with trichloroethylene (see Figure 1). On the same column, a hexane working solvent coelutes with chloroform, a frequently incurred residue. Which working solvent is best?

Both hexane and isooctane are used in methods by codistillation (Rains and Holder, 1981; Iwata et al., 1983; Hughes et al., 1983) and by liquid–liquid partitioning (Newsome and Panopio, 1977; Daft, 1983a). In the methods of this study, isooctane is used because it is almost completley immiscible with aqueous solution, and it provides relatively good analyte stability. Also, isooctane is not as volatile (bp 99 °C) at ambient temperatures as most other working solvents, and it is relatively odor free. Furthermore, with HECD, the isooctane solvent peak

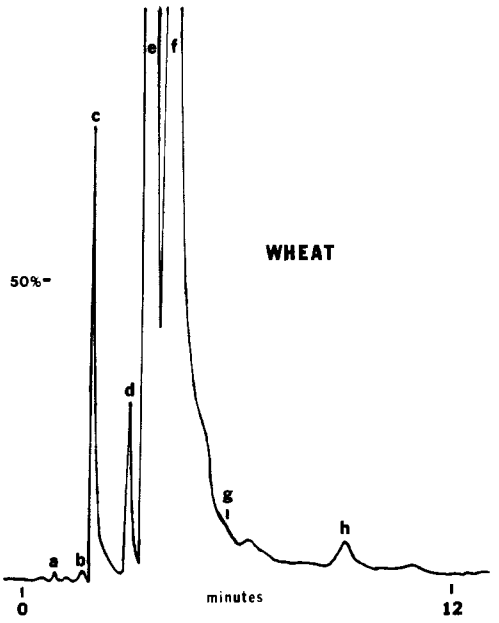

Figure 2. Chromatogram of wheat sample (3 mg): (a) air, (b) acetone, (c) 110 ppb of CH_2Cl_2 reagent-background peak, (d) 90 ppb of $CHCl_3$, (e) 1500 ppb of EDC, (f) 800 ppb of CCl_4, (g) t_R isooctane, and (h) 120 ppb of EDB; 20% OV-101, 90 °C, Hall detection.

disappears after a few injections of standard or sample (see Figure 2), completely disclosing trichloroethylene residues during GC screens.

Nevertheless, fumigant determination is done primarily with ECD by employing two (or more) GC columns which alternately shift the elution patterns of isooctane and fumigants differently, thus disclosing the retention regions of all potential residues and providing a suitable means for confirming the identity of incurred residues (Daft, 1983b). Determination by such dual-column screens is faster when single injections of mixed standards can be made. The same is probably true with other methods. In conclusion, whichever method or whichever working solvent is chosen for fumigant determination, the analysis is safer and much more efficient by using carefully made mixed standards.

ACKNOWLEDGMENT

I thank B. Puma for helpful instruction and advice on purifying isooctane and members of the Total Diet section for supporting this work.

Registry No. EDC, 107-06-2; TCE, 79-01-6; PC, 76-06-2; EDB, 106-93-4; PCE, 127-18-4; CH_3Br, 74-83-9; CH_2Cl_2, 75-09-2; $CHCl_3$, 67-66-3; CS_2, 75-15-0; CCl_4, 56-23-5.

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Received for review November 19, 1984. Accepted April, 4, 1985.