

Figure 1. Chromatograms for the recovery of fenthion from alfalfa and liver

chemical and physical characteristics, indicates that the other metabolites can be recovered as well. All recoveries were run at the 0.1-p.p.m. level.

In calculating recovery of the metabolites of fenthion correction factors must be applied to the peak areas obtained to compensate for differences in molecular weight since recoveries are based on a fenthion standard. The following factors are applied directly to the areas obtained before calculating per cent recovery.

Fenthion	1.00
Fenthion sulfoxide	1.06
Fenthion sulfone	1.12
Oxygen analog	0.94
Oxygen analog sulfoxide	1.00
Oxygen analog sulfone	1.06

The method is capable of measuring 0.1-p.p.m. residues with good precision, and there is little interference from control peaks for the crops and tissues examined. Figure 1 shows typical chromatograms from the recovery experi-

ments. The inherent sensitivity of the method is considerably better than 0.1 p.p.m. For example, milk samples have been analyzed at the 0.01-p.p.m. level.

The chromatographic response is linear up to 75 nanograms—1.5 p.p.m.—of fenthion. Therefore, samples should be diluted and re-injected if they contain more than 50 nanograms per injection. This will ensure that the response falls along the linear portion of the curve.

Owing to the complexity of the step-wise procedure and the number of reactions the compound undergoes, the response for standards run at different times may vary as much as $\pm 20\%$; therefore, a standard must be included with each set of samples.

Literature Cited

- (1) Blinn, R. C., *J. Assoc. Offic. Agr. Chemists* **47**, 641-5 (1964).
- (2) Brady, U. E., Jr., Arthur, B. W., *J. Econ. Entomol.* **54**, 1232 (1961).
- (3) Hoff, J. E., Feit, E. D., *Anal. Chem.* **36**, 1002 (1964).
- (4) Knowles, C. O., Arthur, B. W., Department of Zoology-Entomology, Auburn University, Auburn, Ala., private communication, September 1961 and December 1961.
- (5) Niessen, H., Tietz, H., Frehse, H., *J. Chromatog.* **9**, 111 (1962).
- (6) Rawson, J. W., Arthur, B. W., "Field and Laboratory Experiments on Cotton Insect Control and Metabolism, Stability and Selectivity of Insecticides," p. 52. Auburn University, Auburn, Ala., 1958.
- (7) Schultz, H. S., Freyermuth, H. B., Bue, S. R., *J. Org. Chem.* **28**, 1140 (1963).

Received for review April 22, 1966. Accepted August 17, 1966.

RESIDUE DETERMINATION

Determination of Residues of a Fumigant Mixture in Cereal Grain by Electron-Capture Gas Chromatography

MOST of the work on the determination of the fumigants in feeds by gas chromatography was done by studying the gas phase (2, 17). In the present work, an attempt was made to develop a gas chromatographic method for the determination of residual fumigants in cereal grain. This method enables one to determine directly the rate of desorption up to the point when the grain contains only traces of the fumigant.

A method for determination of fumigants in cereal products has to be based on two main steps:

Extracting the fumigant residues from the grain with a suitable solvent and removing interfering substances from the extract before injection into the gas chromatograph.

Finding a suitable column-packing material for separating the different components and the solvent, and devising

a sufficiently sensitive detection method.

The cleanup procedure after the extraction is difficult for fumigants because of their high volatility. Goodwin, Goulden, and Reynolds (5) tried to overcome this difficulty by macerating and extracting the feedstuff with acetone, re-extracting the acetone extract with hexane, and using aliquots of the latter extract for gas chromatography. Their conclusion was that the method would be

RACHEL BIELORAI and
EUGENIA ALUMOT

Division of Animal Nutrition,
Volcani Institute of Agricultural
Research, Rehovot, Israel

A simple gas chromatographic method for the determination of residues of a fumigant mixture of chloroform, carbon tetrachloride, trichloroethylene, and carbon disulfide, applied in a liquid admixture to cereal grain, is described. Fumigated samples were submitted to steam distillation in the presence of toluene and an aliquot of the toluene layer was injected into the gas chromatograph. Nanogram quantities of mixture components were determined using an electron-capture detector.

suitable for vegetable crops, but not for grain, which yields a high chromatographic background. Recently, Thornton and Anderson (10) followed the suggestion of Jones and Riddick (7) of selectively extracting the insecticides by acetonitrile, thus eliminating fats and waxes and other interfering substances. The acetonitrile extract was then submitted to gas chromatography without further purification.

In previous work in this laboratory (4) ethylene dibromide (EDB) was determined by gas chromatography after distillation with benzene from the fumigated materials (8), thus avoiding the cleanup procedure. A similar extraction method, with some modification, was tried for a mixture of carbon disulfide, chloroform, carbon tetrachloride, and trichloroethylene (26.0, 37.0, 5.0, and 32.0% by volume, respectively), which is used commercially for grain fumigation in this country under the trade name Calandrex (prepared by Abic, Ltd., Tel-Aviv).

The chromatography was conducted with the aid of an electron-capture detector (9). This is the most sensitive device for the detection of electron-absorbing compounds such as the components of the fumigant mixture. Its selectivity permits the detection of parts per billion levels of the above compounds in materials which are not electron-absorbing, such as most of the more commonly used nonhalogenated solvents.

A short report of this work was presented at the XXXVth Meeting of the Israel Chemical Society (3).

Experimental

Extraction. The fumigant residues were distilled off from the fumigated material by the method described previously (1), except that toluene was used instead of benzene, since benzene appears with carbon tetrachloride on the chromatogram. Each batch of toluene was tested before use for the presence of electron-absorbing contaminations.

Because of the greater volatility of the above-mentioned fumigants, the recovery after steam distillation was not as complete as for ethylene dibromide and depended on the boiling points of the compounds. To reduce losses during distillation, the apparatus used previously (8) was modified as shown in Figure 1.

In the modified apparatus, the toluene collection tube is water-cooled and the

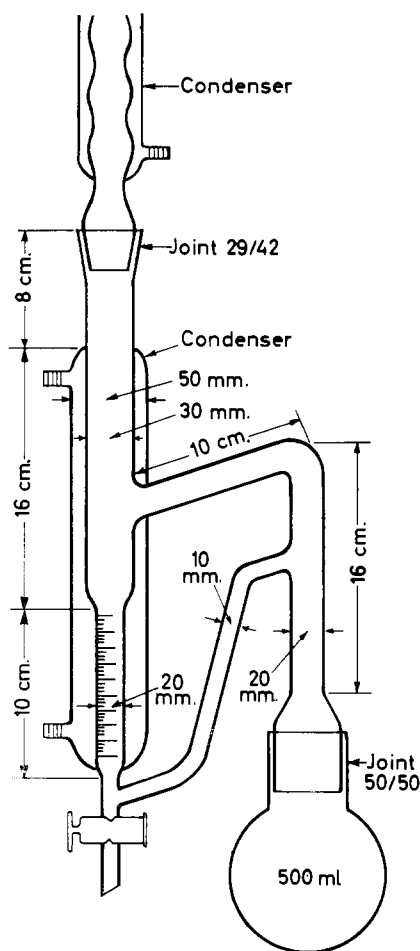


Figure 1. Modified apparatus for distillation and extraction of fumigant mixture

distillation arm leading to the collection tube is inclined downwards, to prevent the return of the condensate into the flask during distillation and rinsing.

Distillation Procedure. Weighed amounts of cereal grain (10 to 50 grams) were suspended in 250 ml. of water in a 500-ml. flask, placed in a heating mantle. Five milliliters of toluene were added to the suspension. After the condenser had been set up and the collection tube and the connecting arm filled with water, the suspension was mixed by shaking and was brought to a boil. The continuous distillation procedure was carried out for 15 minutes. No antifoam was used, since it interfered with the chromatographic analysis. After the whole apparatus had been cooled, the toluene layer was separated from the water and withdrawn into a small beaker containing anhydrous sodium sulfate.

The dry toluene solution was transferred to a 25-ml. volumetric flask. The apparatus was rinsed several times with small amounts of toluene. The rinsings were dried in the same beaker and added to the volumetric flask. The solution was then brought to volume. The entire operation should be carried out as quickly as possible.

To check the completeness of the distillation-extraction procedure, another 5 ml. of toluene were added to the flask, and the distilling procedure was repeated. Generally only traces of fumigants were detected in the second extract. Aliquots of the dry toluene solution were used directly for gas chromatography.

To determine the per cent recovery of each of the mixture components, 10 to 20 grams of untreated sample were distilled with 5 ml. of a standard fumigant solution in toluene (about 110 μg . of the mixture per ml.).

The distillate was brought to 25 ml. as described above and aliquots of 1 μl . were injected into the gas chromatograph. After several distillations of the standard mixtures, the average recovery (in per cent of undistilled standard \pm standard deviation) was: trichloroethylene, 96 ± 1 ; carbon tetrachloride, 94 ± 1 ; and chloroform, 82 ± 2 . In view of the constant recovery, a correction factor was applied for each of the above-mentioned compounds. Carbon disulfide, the most volatile component of the mixture, could be determined only approximately by this method, giving a recovery of about 40 to 50%.

Gas Chromatography. APPARATUS. Perkin Elmer, Model 801, with electron-capture detector. Column, 6 feet, all-glass, $\frac{1}{4}$ -inch o.d. packed with 10% w./w. silicone oil DC-710 on 80- to 100-mesh Chromosorb W HMDS. Carrier gas, nitrogen, 99.9% pure. Conditions of analysis: nitrogen flow rate, 20 ml. per minute; column temperature 60°C .; injector temperature 150°C .; detector temperature 120°C .; attenuation, $\times 500$. Potential across the detector, 45 volts. Recorder, 5-mv. Leeds & Northrup Co., Model Speedomax G; recorder chart speed $\frac{1}{2}$ inch per minute.

Under these conditions the gas chromatographic analysis including the toluene peak, which appears last, took 8 minutes.

Analysis of Fumigated Cereal Grain. Before the fumigation, the grain samples were distilled with toluene as described above. The toluene extracts were injected into the gas chromatograph, to check the absence of any volatile com-

Table I. Effect of Sample Weight on Recovery of Fumigant Residues in Sorghum, after Airing

Days of Airing	Sample Weight, G.	Quantities of Fumigants, P.P.M.		
		Chloroform	Carbon tetrachloride	Trichloroethylene
0	10	226.0	13.9	94.5
	20	240.0	12.4	87.5
1	10	104.0	9.4	43.3
	20	102.0	7.7	37.2
4	20	73.0	2.7	16.8
	30	69.0	2.7	16.6
16	50	22.5	...	9.4
	100	23.1	...	9.5

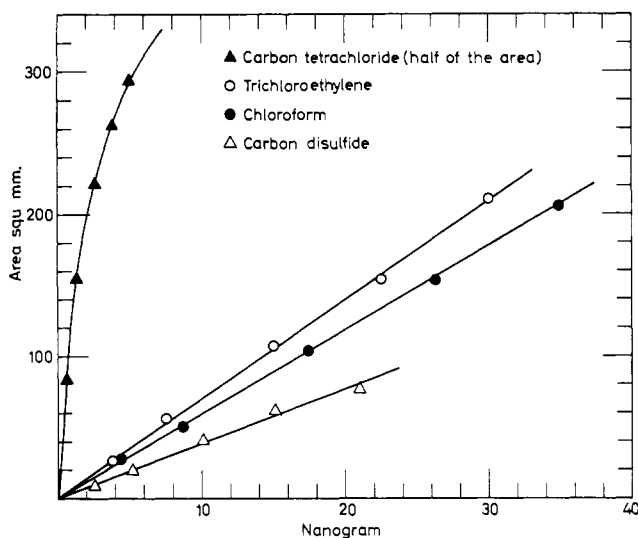


Figure 2. Calibration curve of fumigant mixture components

pound with the same retention times as the fumigants used. No such peaks were obtained in untreated grain, but unknown, interfering peaks occurred in grain that had been fumigated in the past.

Wheat, barley, corn, and sorghum were fumigated as described previously (7), for different periods of time and with different amounts of the fumigant mixture. At the end of the fumigation the samples were aired by placing them on perforated metal trays. Weighed samples of 10 to 50 grams were withdrawn for analysis at the beginning and at different stages of airing.

The distillation was carried out with 5 ml. of toluene, as described above, and aliquots of the toluene solution, after suitable dilution, were injected into the gas chromatograph. Standard solutions of the fumigant mixture in toluene were injected periodically as a check of the constancy of the instrument response. The amounts of the different components were calculated, taking into account the percentage recovery of each component after distillation.

The effect of sample weight on the recovery of fumigants was checked by simultaneously extracting two different amounts of the same fumigated sorghum at various times during the airing period. At higher concentrations of the residual fumigants, 10 and 20 grams of grain were analyzed, and at lower concentrations 50 and 100 grams were assayed at

a time. The results, given in Table I, show that the method gives reproducible results.

Results and Discussion

Calibration curves for each of the mixture components were established, since the response of the detector depends not only on the quantity of the compound but also on its electron-absorbing affinity. Figure 2 presents the calibration curves of the compounds determined.

The most active electron absorber, carbon tetrachloride, gave a linear response only in the lower range of the curve (up to 2 nanograms). If a larger amount is to be analyzed, the sample must be diluted, since attenuation of the signal does not help in reaching the linear region of the calibration curve. A typical chromatogram of a standard sample is presented in Figure 3.

The peaks of chloroform and carbon tetrachloride partially overlap (Figure 3), but the areas could be calculated without difficulty. The amounts of the mixture components analyzed are (in nanograms): carbon disulfide, 5.25; chloroform, 8.72; carbon tetrachloride, 1.28; and trichloroethylene, 7.50.

Figure 3 shows that carbon disulfide is as highly electron absorbing as halogen-

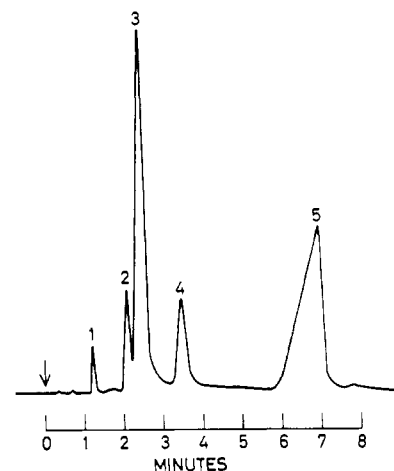


Figure 3. Chromatogram of fumigant mixture on silicone oil DC-710 column

10% w./w. on 80- to 100-mesh Chromosorb W HMDS. Arrow indicates starting point

1. Carbon disulfide
2. Chloroform
3. Carbon tetrachloride
4. Trichloroethylene
5. Toluene

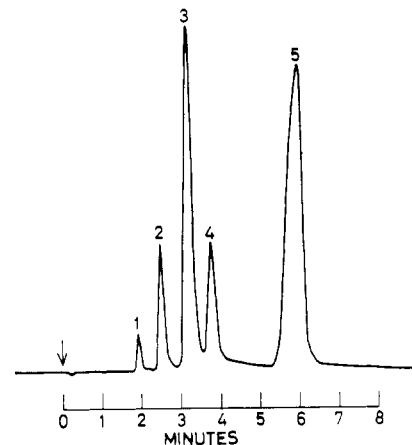


Figure 4. Chromatogram of fumigant mixture on SE-30 silicone gum rubber column

10% w./w. on 60- to 80-mesh Chromosorb W HMDS. Arrow indicates starting point

1. Carbon disulfide
2. Chloroform
3. Carbon tetrachloride
4. Trichloroethylene
5. Toluene

ated hydrocarbons. Grant and Vaughan (6) also found that carbon disulfide can be detected by the electron-capture detector. The authors could detect 0.05 μg . Under our conditions of analysis, much smaller amounts can be detected with the detector on its maximum sensitivity.

The high sensitivity of the detector toward carbon tetrachloride makes the separation of compounds close to carbon tetrachloride very difficult, especially when carbon tetrachloride is present in great amounts.

To obtain better separation of chloro-

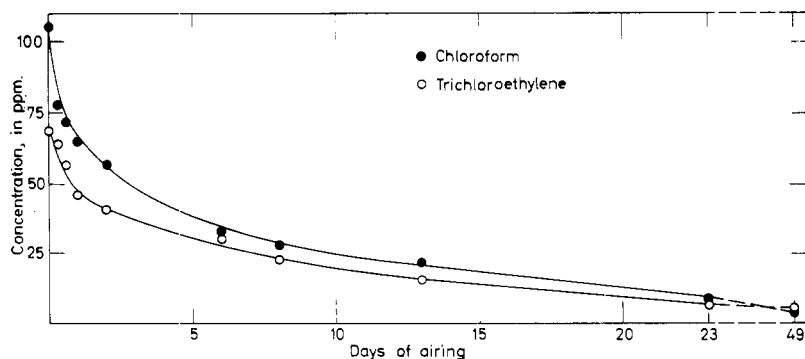


Figure 5. Residues of fumigants during airing of corn

Table II. Residues of Fumigants in Wheat and Barley Treated with Fumigant Mixture (40 Grams per Cu. Meter) for 72 Hours

Days of Airing	Quantities, P.P.M.					
	Chloroform		Carbon Tetrachloride		Trichloroethylene	
	Wheat	Barley	Wheat	Barley	Wheat	Barley
0	21.0	27.9	1.53	2.2	12.7	21.9
1	17.6		1.47		12.0	
2	16.8	11.1	1.4	1.2	11.2	9.4
5	8.9		1.38		7.7	
7	8.1	7.8	1.3	0.8	7.3	4.9
17	4.6	5.3	0.7	0.6	3.7	2.3

form and carbon tetrachloride, a 6-foot stainless steel column, 1/4-inch o.d., packed with 10% w./w. SE 30 silicone gum rubber on 60 to 80 Chromosorb W HMDS was used. The separation of the components on this column is illustrated in Figure 4, which shows that the column gave an improved separation of the mixture components. A glass or steel column could be used interchangeably.

The results obtained with corn, fumigated with 240 grams per cu. meter of the mixture for 72 hours, are illustrated in Figure 5. The fumigation was performed in two containers and duplicate analyses were made from each container separately. The results were very close, the maximum difference being $\pm 5\%$ from the average.

Airing curves for only trichloroethylene and chloroform are given in Figure 5, since carbon tetrachloride was present in very small amounts, from 7 p.p.m. at the beginning to 1 p.p.m. at the end of the airing period.

After 45 days of airing, the corn still contained about 4 p.p.m. of chloroform and 5 p.p.m. of trichloroethylene.

Results of determination of the fumigant residues in wheat and barley are given in Table II. The fumigation was carried out with 40 grams per cu. meter for 72 hours.

The quantities of residual fumigants present in grain fumigated with small amounts of the mixture are negligible after 2 days of airing (Table II).

The sensitivity of the gas chromatographic method described is in the nano-

gram range; this corresponds to parts per million concentrations of residual fumigants in the grain. However, the full sensitivity of the detector was not utilized, and much smaller quantities can be detected.

The method presented is more sensitive than the known chemical methods, especially for mixtures of halogenated hydrocarbons, where chemical determination of constituents is difficult and time-consuming.

Acknowledgment

The authors thank P. Budowski and M. Katz for their valuable advice. The technical assistance of Chava Genige and Pia Holstein is gratefully acknowledged.

Literature Cited

- (1) Alumot, E., Calderon, M., *J. Sci. Food Agr.* **16**, 464 (1965).
- (2) Berck, B., *J. AGR. FOOD CHEM.* **13**, 373 (1965).
- (3) Bielorai, R., Alumot, E., *Israel J. Chem.* **3**, 93 (1966).
- (4) Bielorai, R., Alumot, E., *J. Sci. Food Agr.* **16**, 594 (1965).
- (5) Goodwin, E. S., Goulden, R., Reynolds, J. G., *Analyst* **86**, 697 (1961).
- (6) Grant, D. W., Vaugham, G. A., *Nature* **208**, 75 (1965).
- (7) Jones, L. R., Riddick, J. A., *Anal. Chem.* **24**, 569 (1956).
- (8) Kenneth, B. H., Huelin, F. E., *J. AGR. FOOD CHEM.* **5**, 201 (1957).
- (9) Lovelock, J. E., Lipsky, S. R., *J. Am. Chem. Soc.* **82**, 431 (1960).
- (10) Thornton, J. S., Anderson, C. A., *J. AGR. FOOD CHEM.* **13**, 509 (1965).
- (11) Vincent, L. E., Lindgren, D. L., *J. Econ. Entomol.* **55**, 264 (1962).

Received for review June 8, 1966. Accepted August 22, 1966. Work financed by Grant No. FG-Is-117, U. S. Department of Agriculture. Contribution from The National and University Institute of Agriculture, Rehovot, Israel. 1966 Series, No. 1020-E.

ANALYTICAL METHOD

Gas Chromatographic Determination of Compound 4072 and Shell SD-8447 by Electron-Capture and Flame-Photometric Detection

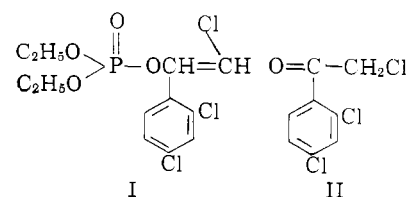
A GAS chromatographic method has been developed for Compound 4072 [2-chloro-1-(2,4-dichlorophenyl)-vinyl diethyl phosphate], an insecticide as effective as DDT against insects that attack corn (8). Data are also presented on the hydrolysis product of the insecticide Shell Compound SD-8447

[2-chloro-1-(2,4,5-trichlorophenyl)-vinyl diethyl phosphate].

A recent article (6) indicated that Compound 4072 (I) could not be determined by gas chromatography (GLC) directly; it was therefore hydrolyzed with dilute sulfuric acid to give 2,2',4'-trichloroacetophenone (II), which was determined gas chromatographically.

MORTON BEROZA and
M. C. BOWMAN

Entomology Research Division,
Agricultural Research Service,
U. S. Department of Agriculture,
Beltsville, Md., and Tifton, Ga.



Since the authors had already used GLC to determine directly the closely