tographic cleanup methods. The authors' years of experience with the apparatus (*8*) indicate that up to six to eight samples of 0.5 ml. each of concentrated citrus extractives can be cleaned up before the stripping tube is overloaded. From the injection of the sample to the final recovery of the solvent-free concentrated extractive, which is directly usable for gas chromatographic analysis, not more than 30 minutes of elapsed time are required. Since it is hoped that this procedure will also be found advantageous for the cleanup of volatile pesticide-containing extracts prior to paper or thin-layer and gas chromatographic analysis, construction details of the apparatus are given.

Acknowledgment

The authors gratefully acknowledge the opportunity and encouragement given in this experimental study by W. E. Baier, Director of Research and Development Division, Sunkist Growers, Inc.

Literature Cited

(1) Baetz, R. A., *J. Assoc. Offic. Agr. Chemists* **47,** 322 (1964).
(2) Coulson, D. M., Devries, J. E., Walther, B. A., Stanford Research Institute, *Rept.* **12,** 4 (1960).
(3) Eidelman, M., *J. Assoc. Offic. Agr. Chemists* **45,** 672 (1962).
(4) Eiduson, H. P., *Ibid.*, **44,** 183 (1961).
(5) Farrow, R. P., Elkins, E. R., Jr., Beacham, L. M., III, *Ibid.*, **48,** 738 (1965).
(6) Frear, D. E. H., "Chemistry of the Pesticides," 3rd ed., Chap. II, III, IV, Van Nostrand, New York, 1955.
(7) Gunther, F. A., Blinn, R. C., Ott, D. E., Abstracts, 139th Meeting, American Chemical Society, p. 26A' 1961.
(8) Kim, J. JS, Wilson, C. W., *Sunkist Growers Res. Develop. Rept.* **4** (17), 46 (1965).
(9) McKinley, W. P., Mahon, J. H., *J. Assoc. Offic. Agr. Chemists* **42,** 725 (1959).
(10) McKinley, W. P., Savary, G., Webster, C., J. Agr. Food Chem. **10,** 226 (1962).
(11) Mills, P. A., *J. Assoc. Offic. Agr. Chemists* **42,** 734 (1959).
(12) Moats, W. A., *Ibid.*, **45,** 355 (1962).
(13) *Ibid.*, **46,** 172 (1963).
(14) *Ibid.*, **47,** 587 (1964).
(15) Ott, D. E., Gunther, F. A., J. Agr. Food Chem. **12,** 239 (1964).
(16) Storherr, R. W., Watt, R. R., *J. Assoc. Offic. Agr. Chemists* **48,** 1154 (1965).

Received for review April 25, 1966. Accepted August 28, 1966.

RESIDUE DETERMINATION

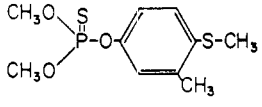
Determination of Fenthion Residues in Plant and Animal Tissues by Electron-Capture Gas Chromatography

R. J. ANDERSON, J. S. THORNTON, C. A. ANDERSON, and D. B. KATAGUE

Chemagro Corp., Kansas City, Mo.

A specific residue method is described for the determination of fenthion and its five metabolites in plant and animal tissues. The method is based on oxidation of the various compounds to the oxygen analog sulfone which in turn is hydrolyzed to the corresponding phenol. The phenol is brominated and acetylated prior to detection measurement by electron-capture gas chromatography. The method is sensitive to 0.1 p.p.m. of the compound.

Fenthion—(*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate—is the common name for the organic phosphorus insecticide and parasiticide sold under the registered trademarks Baytex, Entex, and Tiguvon. The structural formula is as follows:



The metabolism of fenthion in plants has been studied by Niessen, Tietz, and Frehse (*5*) who found that the compound was converted to its sulfoxide and sulfone as well as the oxygen analog sulfoxide and sulfone. The oxygen analog was not observed. However, Rawson and Arthur (*6*) reported detection of small quantities of the oxygen analog in cotton plants.

Brady and Arthur (*2*), in their study of the metabolism of fenthion in rats, found that the compound was rapidly oxidized to the sulfoxide and sulfone, and to the oxygen analog and its sulfoxide and sulfone.

Knowles and Arthur (*4*) found that dermal application of P^{32}-labeled fenthion to dairy cows resulted in small amounts of radioactivity in the milk. Fractionation of the acetonitrile-soluble portion of this activity on Celite partition columns gave several distinct peaks, one of which corresponded to fenthion, another to the sulfoxide and oxygen analog, and a third to the oxygen analog sulfoxide and oxygen analog sulfone. Acetonitrile-soluble activity in tissues was negligible. Intramuscular injection resulted in the same number of acetonitrile-soluble activity peaks as obtained for the dermal application. The method developed, therefore, was designed to measure not only fenthion but also its five metabolites.

The primary concern in the development of the method was adequate sensitivity and specificity for all of the compounds. Preliminary experiments involved the use of a Dohrmann microcoulometric gas chromatograph equipped with a sulfur-sensitive titration cell. Although acceptable results were obtained for fenthion, attempts to chromatograph some of the metabolites were unsuccessful.

A suitable gas chromatographic procedure based on the phenol of the oxygen analog sulfone was finally developed. Acetylation of the phenol gave a moderately sensitive response in a gas chromatograph equipped with an electron-capture detector. As expected, sensitivity was increased further by brominating the phenol prior to acetylation. This acetylated phenol sulfone as well as the halogenated derivative undoubtedly could have been determined using a Dohrmann chromatograph, but this was not attempted because electron capture offered greater sensitivity. Using electron capture, the desired sensitivity of 0.1 p.p.m. and specificity were easily achieved.

Analytical Method

Apparatus and Reagents. An F & M Model 700 gas chromatograph equipped with a pulsed-type electron capture detector set at a pulse interval of 15 μseconds was used. The electrometer range and attenuation set-

tings were 100 and 2, respectively. The recorder chart speed was 1/4 inch per minute. Operating temperatures for the injection port, column oven, and detector cell were 220°, 180°, and 200° C., respectively. The carrier gas was 5% methane in argon set at 70 ml. per minute with a 5-ml.-per-minute bleed on the purge line. The column was 4-mm. i.d. borosilicate glass packed with 16 inches of 7% D.C. 200 silicone fluid on 30- to 60-mesh, acid-washed, dimethyldichlorosilane treated Chromosorb G.

All separatory funnels were equipped with Ultramax stopcocks.

m-Chloroperbenzoic acid is available from F.M.C. Corp., Villa Park, Ill.

Fenthion analytical standard is available from Chemagro Corp., Kansas City, Mo. All solvents except analytical reagents were redistilled from an all-glass apparatus.

A Swissco rotary vacuum evaporator (Swissco Instruments, Greenville, Ill.) or equivalent all-glass system should be used for all evaporations in the procedure.

Sample Preparation. Grind the entire frozen sample in a Hobart food chopper in the presence of an equal weight of dry ice, and place the sample in frozen storage overnight to allow the dry ice to sublime. This procedure is used for all samples.

Extraction of Fat Samples. Weigh 25 grams of the chopped frozen sample into a 1-quart Waring Blendor jar. Add 200 ml. of Skellysolve B and blend at high speed for 3 minutes. Filter with suction through an 11-cm. Whatman No. 42 filter paper covered with a 3- to 4-mm. layer of Hyflo Super-Cel. Transfer the filtrate to a 500-ml. separatory funnel. Return the filter cake and filter paper to the blender jar, add 200 ml. of acetonitrile, and blend at high speed for 2 minutes. Filter as above, omitting the layer of Super-Cel. Transfer the filtrate to the same separatory funnel and shake the funnel for 30 seconds. Allow the layers to separate and draw off the acetonitrile phase into a second 500-ml. separatory funnel containing 100 ml. of Skellysolve B. Shake the second funnel for 30 seconds, allow the layers to separate, and draw off the acetonitrile layer into a 1000-ml. round-bottomed flask. Repeat the extraction of both Skellysolve B phases with two 200-ml. portions of acetonitrile. Evaporate the combined acetonitrile extracts to dryness using an all-glass rotary vacuum evaporator at 40° C. (Continue at Oxidation.)

Extraction of Animal Tissue Samples, Other Than Fat. Weigh 50 grams of the chopped frozen sample into a 1-quart Waring Blendor jar. Add 200 ml. of acetone and blend for 3 minutes at high speed. Filter with suction through an 11-cm. Whatman No. 42 filter paper. Transfer the filtrate to a 1000-ml. separatory funnel. Return the filter cake and filter paper to the blender jar, add 200 ml. of chloroform, and blend for 3 minutes at high speed. Filter with suction through an 11-cm. Whatman No. 42 filter paper covered with a 3- to 4-mm. layer of Hyflo Super-Cel. Rinse the blender jar with 100 ml. of chloroform and add the rinsings to the filter cake. Transfer the filtrate to the same separatory funnel and shake the funnel for 30 seconds. Allow the layers to separate and draw off the lower phase through a 32-cm. Whatman No. 12 fluted filter paper into a 1000-ml. round-bottomed flask. Evaporate the sample to dryness using a rotary vacuum evaporator at 40° C.

Transfer the sample to a 250-ml. separatory funnel with 100 ml. of Skellysolve B. Rinse the flask with 100 ml. of acetonitrile and add the rinse to the separatory funnel. Shake the funnel for 30 seconds, allow the layers to separate, and draw off the acetonitrile layer into a second 250-ml. separatory funnel containing 50 ml. of Skellysolve B. Shake the second funnel for 30 seconds, allow the layers to separate, and draw off the acetonitrile layer into a 500-ml. round-bottomed flask. Repeat the extraction of both Skellysolve B phases with two 100-ml. portions of acetonitrile. Evaporate the combined acetonitrile extracts to dryness on a rotary evaporator. (Continue at Oxidation.)

Extraction of Green Forage Crops. Weigh 50 grams of the chopped frozen sample into a 1-quart Waring Blendor jar. Add 200 ml. of acetone and 15 grams of Hyflo Super-Cel and blend for 3 minutes at high speed. Filter with suction through Whatman No. 42 filter paper. Rinse the blender with 50 ml. of acetone and use this to rinse the filter cake. Rinse the filter cake with 100 ml. of chloroform. Transfer the filtrate to a 1000-ml. separatory funnel. Rinse the filter flask with 150 ml. of chloroform and add this to the separatory funnel containing the filtrate. Shake the separatory funnel for 30 seconds and allow the layers to separate. Draw off the lower phase through a 32-cm. Whatman No. 12 fluted filter paper into a 1000-ml. round-bottomed flask. Evaporate just to dryness on a rotary evaporator. Dissolve the residue in 50 ml. of chloroform.

Prepare a chromatographic column as follows: Tamp a plug of glass wool into the bottom of a 20 × 400 mm. glass chromatographic tube equipped with a 300-ml. integral reservoir and Ultramax stopcock. Slurry and add 20 grams of alumina—acid washed, chromatographic grade, Merck—in chloroform solution. Drain the chloroform down to the level of the alumina. Transfer the sample quantitatively to the chromatographic column and allow it to percolate through the column at the rate of about 2 to 4 drops per second. Elute the column with an additional 150 ml. of chloroform. Evaporate the eluate just to dryness on a rotary vacuum evaporator at 40° C.

Oxidation. (Start a 5-μg. fenthion standard at this point.) Dissolve the residue from the extraction steps in 10 ml. of a 10% w./v. solution of *m*-chloroperbenzoic acid in isopropyl ether. Allow the sample to stand at room temperature for 30 minutes with occasional swirling. (Note: Do not allow the oxidation to proceed beyond 45 minutes.) Add 10 ml. of isopropyl ether and transfer the sample to a 125-ml. centrifuge-type separatory funnel. Complete the transfer with 80 ml. of 2*N* hydrochloric acid. Shake the funnel for 30 seconds, allow the layers to separate, and draw off the aqueous phase into a second 125-ml. centrifuge-type separatory funnel containing 20 ml. of isopropyl ether. Shake this separatory funnel for 30 seconds, then centrifuge for 5 minutes at 800 r.p.m. Drain the aqueous phase into a 500-ml. separatory funnel. Add 80 ml. of 2*N* hydrochloric acid to the first separatory funnel and repeat the entire extraction procedure. Combine the aqueous phases. Add 200 ml. of chloroform to the 500-ml. separatory funnel and shake for 30 seconds. Allow the layers to separate and draw off the chloroform phase into a second 500-ml. separatory funnel. Repeat the extraction with 100 ml. of chloroform and combine the extracts. Add 100 ml. of 0.5*N* NaOH to the combined chloroform extracts and shake the separatory funnel for 30 seconds. Allow the phases to separate and drain the lower, chloroform phase through a 32-cm. Whatman No. 12 fluted filter paper, into a 500-ml. round-bottomed flask. Rinse the filter paper with about 20 ml. of fresh chloroform. Evaporate the sample to dryness using a rotary evaporator at 40° C. Remove any traces of chloroform with an air jet at room temperature.

Hydrolysis. Dissolve the residue in 15 ml. of 1*N* NaOH, and place the sample flask in a 40° C. water bath for 45 minutes, swirling occasionally. Transfer the sample to a 250-ml. separatory funnel containing 12 grams of sodium chloride. Rinse the flask with 20 ml. of 2.5*N* sulfuric acid and add this to the separatory funnel. Shake to dissolve the salt. Add 100 ml. of chloroform to the separatory funnel and shake the funnel for 30 seconds. Allow the layers to separate and drain the lower phase into a second 250-ml. separatory funnel. Repeat the extraction with an additional 100 ml. of chloroform. Dry the combined chloroform extracts by adding 20 grams of powdered anhydrous sodium sulfate. Decant the chloroform solution through an 11-cm. Whatman No. 541 filter paper into a 250-ml. round-bottomed flask. Rinse the separatory funnel with 20 ml. of chloroform and add the rinse to the flask. Evaporate the sample to about 8 ml. using a rotary evaporator and transfer to a 15-ml. centrifuge tube. Rinse the flask with about 4 ml. of acetone and add this to the tube. Evaporate the sample to dryness under an air jet at 40° C.

Bromination. Add 0.6 ml. of 6% v./v. liquid bromine in glacial acetic acid, rinsing down the walls of the tube in the process. Stopper the tube and place in a 40° C. water bath for 60 minutes at a depth which just exceeds the level of the reagent. Evaporate the sample to dryness under a gentle stream of air in a 40° C. water bath. Dissolve the residue in 5 ml. of isopropyl ether and transfer the sample to a 60-ml. separatory funnel. Rinse the tube with 5 ml. of isopropyl ether followed by two 10-ml. rinses with 4% w./v. aqueous sodium bicarbonate, adding all the rinses

to the separatory funnel. Shake the separatory funnel for 30 seconds, allow the layers to separate and drain the aqueous phase into a 250-ml. separatory funnel containing 8 grams of sodium chloride. Add 5 ml. of 2.5*N* sulfuric acid and agitate the funnel to expel the carbon dioxide. Shake the funnel to dissolve the salt. Add 150 ml. of chloroform and shake the funnel for 30 seconds. Allow the layers to separate and drain the chloroform phase into a second 250-ml. separatory funnel. Dry the chloroform by adding 15 grams of powdered anhydrous sodium sulfate. Decant the chloroform through an 11-cm. Whatman No. 541 filter paper into a 250-ml. round-bottomed flask. Rinse the separatory funnel with 20 ml. of chloroform and add the rinse to the flask. Evaporate the sample to dryness using a rotary evaporator. Remove the last traces of chloroform with a gentle air stream at room temperature.

Acetylation. Dissolve the residue in 4 ml. of benzene. Transfer 1 ml. of the benzene solution to a 15-ml. glass-stoppered centrifuge tube. Add 50 μliters of a 2.5% v./v. solution of concentrated sulfuric acid in 99 to 100% acetic anhydride and mix the sample thoroughly. Stopper the tube and place in a 40° C. water bath for 30 minutes. Cool to room temperature and add 8 ml. of 10% w./v. sodium bicarbonate. Shake the tube until the evolution of carbon dioxide is no longer evident. Using a microliter syringe, inject 4 μliters (8 μliters for fat samples) of the benzene solution into the gas chromatograph. The retention time is about 8 minutes. An appropriate standard is injected also before and after each set of samples as a comparison for quantitation and for retention time verification.

Calculations. Since fat samples are only one half as large as the other tissue samples, twice as much of the final benzene solution is injected in order to get the same response. Using the aliquots and sample sizes recommended in the method, calculations are made in the following manner.

$$\text{P.P.M.} = \frac{(A_1)\ (0.1)}{(A_2)}$$

where A_1 = peak area of the sample
A_2 = average peak area of the standards

If different attenuation settings are used for the samples and standards or if dilutions are necessary to keep on the linear portion of the curve—see discussion—appropriate corrections must be made.

Discussion

To utilize the gas chromatographic procedure described, a method for the quantitative oxidation of fenthion and its metabolites to the oxygen analog sulfone was developed. Initial efforts involved the use of a sodium tungstate-catalyzed hydrogen peroxide oxidation carried out in an acetone-water mixture (*7*). Oxidation in this system was complete in 3 hours at room temperature. However, the amount of sodium bisulfite needed to destroy the excess peroxide created difficulties in sample processing and resulted in an undesirable white residue of sodium sulfite in the final extract.

A second and more desirable procedure involved oxidation with *m*-chloroperbenzoic acid (*7*). Isopropyl ether was used as a solvent instead of chloroform because of the greater ease of extraction after oxidation. In this case, oxidation was complete in 30 minutes at room temperature. Although extended periods of oxidation—over 1 hour—could cause some degradation of the oxygen analog sulfone, this is not serious because the product can be quickly isolated from the oxidation medium by acid extraction. The comparatively high concentration of acid used is necessary to prevent emulsions.

Oxidation is advantageous inasmuch as the measurement of fenthion and its metabolites as a single peak is allowed, thereby simplifying chromatographic analysis. Moreover, processing of the sample subsequent to oxidation is facilitated because only one compound has to be dealt with. Furthermore, the oxygen analog sulfone is easier to hydrolyze and has greater water solubility than the compounds from which it is derived. An increase in the water solubility is particularly important because the use of water-solvent partitions which give excellent cleanup is permitted. Without such cleanup, interferences resulting from bromination of sample extractives mask the fenthion peak.

Chromatography on alumina is necessary in the preparation of forage crops. Without this step, certain of the extractives interfere with the complete oxidation to the sulfone.

Bromination of the sulfone phenol using 6% v./v. liquid bromine in glacial acetic acid at 40° C. is complete in less than 1 hour. Addition of iodine as a catalyst and increasing the temperature —to 60° C.—had little effect on the reaction time. Following bromination, most of the excess bromine reagent is removed by evaporation. However, an aqueous extraction step is included to remove the last traces of bromine trapped in the residue which, if not removed, would inhibit the acetylation reaction.

Acetylation of the brominated phenol is necessary to increase its volatility and to prevent irreversible adsorption on the column packing. Acetylation is by a modification of the procedure of Hoff and Feit (*3*) for the esterification of primary alcohols.

In the chromatographic analysis, an inert solid support, dimethyl dichlorosilane-treated Chromosorb G is used to minimize peak tailing. This is coated with a nonpolar substrate, D.C. 200 silicone fluid. This combination gives a larger, more symmetrical peak than was observed using columns prepared with Chromosorb W.

Recovery experiments were conducted on all of the tissues and crops listed in Table I by adding known amounts of the compound at the blending step and processing the samples by the appropriate procedure. The results are listed in Table I. Recovery experiments included only fenthion, fenthion oxygen analog sulfoxide, and fenthion oxygen analog sulfone. Recovery of these compounds, representing the extremes in

Table I. Recovery of Fenthion and Its Oxygen Analog Sulfoxide and Sulfone from Animal Tissues and Forage Crops

Sample	Compound Added	Added, P.P.M.	Mean Recovery, %[a]
Brain	Fenthion	0.1	102 ± 9 (3)
	Oxygen analog sulfoxide	0.1	93 ± 3 (3)
	Oxygen analog sulfone	0.1	91 ± 2 (3)
Fat	Fenthion	0.1	82 ± 8 (3)
	Oxygen analog sulfoxide	0.1	99 ± 16 (3)
	Oxygen analog sulfone	0.1	108 ± 12 (3)
Heart	Fenthion	0.1	82 ± 3 (3)
	Oxygen analog sulfoxide	0.1	91 ± 5 (3)
	Oxygen analog sulfone	0.1	103 ± 6 (3)
Kidney	Fenthion	0.1	83 ± 13 (3)
	Oxygen analog sulfoxide	0.1	84 ± 2 (3)
	Oxygen analog sulfone	0.1	78 ± 12 (3)
Liver	Fenthion	0.1	78 ± 3 (3)
	Oxygen analog sulfoxide	0.1	73 ± 10 (3)
	Oxygen analog sulfone	0.1	85 ± 8 (3)
Steak	Fenthion	0.1	77 ± 8 (3)
	Oxygen analog sulfoxide	0.1	71 ± 4 (3)
	Oxygen analog sulfone	0.1	79 ± 5 (3)
Alfalfa	Fenthion	0.1	102 (1)
	Oxygen analog sulfoxide	0.1	103 (1)
	Oxygen analog sulfone	0.1	107 (1)
Pasture grass	Fenthion	0.1	77 (1)
	Oxygen analog sulfoxide	0.1	87 (1)
	Oxygen analog sulfone	0.1	108 (1)

[a] Values are followed by the average deviation from the mean and, in parentheses, by the number of determinations.

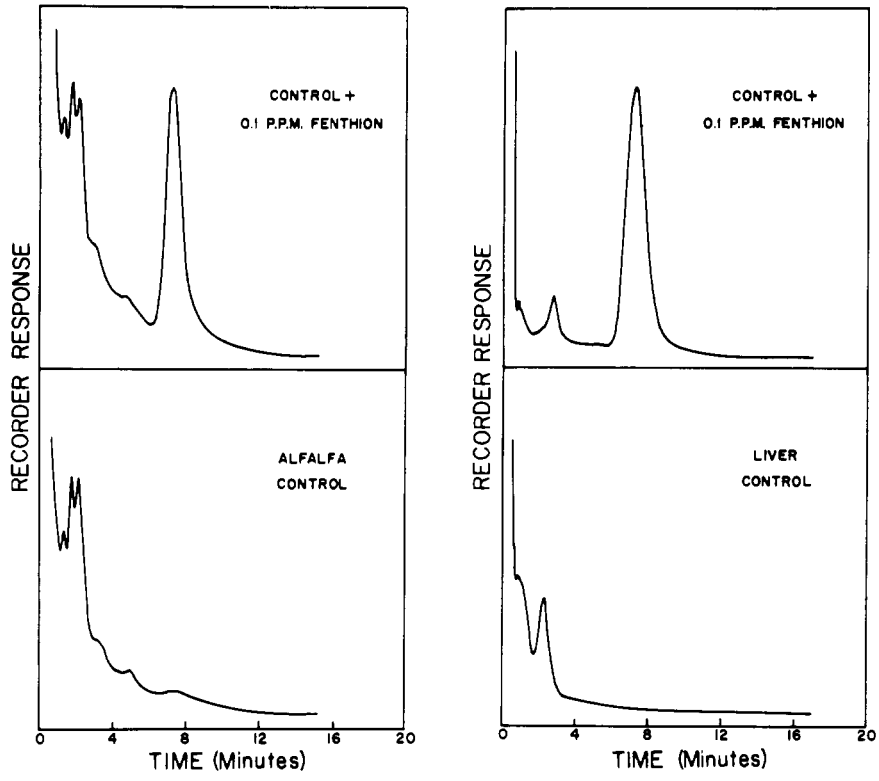


Figure 1. Chromatograms for the recovery of fenthion from alfalfa and liver

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 experiments. 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 reinjected 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 stepwise procedure and the number of reactions the compound undergoes, the response for standards run at different times may vary as much as ±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

RACHEL BIELORAI and EUGENIA ALUMOT

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

Most of the work on the determination of the fumigants in feeds by gas chromatography was done by studying the gas phase (*2, 11*). 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, reextracting the acetone extract with hexane, and using aliquots of the latter extract for gas chromatography. Their conclusion was that the method would be