

Determination of Carbaryl as an Amide Derivative by Electron-Capture Gas Chromatography

R. L. Tilden and C. H. Van Middlelem

A number of derivatives for *N*-methyl and *N,N*-dimethylcarbamate insecticides were synthesized and evaluated as to their potential electron capturing properties in a gas chromatograph. One of the most promising derivatives, 4-bromo-*N*-methylbenzamide, was used to quantitate, by electron capture gas chromatography, carbaryl residues extracted from field-treated spinach and chicory. Sulfuric acid hydrolysis was used to convert carbaryl simultaneously to a methylamine salt and the crop extractives to water-soluble products. Effective

separation of the methylamine from the water-soluble extractives was accomplished by making the aqueous phase strongly alkaline which also catalyzed the coupling of the free amine with the 4-bromobenzoyl chloride. To verify the gas chromatographic results, all field samples were also analyzed by the conventional colorimetric procedure for carbaryl. The lower limits of detection were 0.2 ppm for fortified crop extracts by employing the proposed gas chromatographic procedure. Pure standards could be detected at the 20-picogram level.

Because of the increasing commercial application of *N*-methyl and *N,N*-dimethylcarbamate insecticides, there is a definite need for developing rapid, sensitive gas chromatographic procedures for the detection of residues of these compounds on treated crops. Unfortunately, the parent molecules of these particular compounds cannot be satisfactorily chromatographed without derivation. Chemical alteration of the carbamate molecule is necessary to render it susceptible to electron capture detection.

In one of the first attempts to alter the molecular structure of a carbamate, Ralls and Cortes (1964) brominated 1-naphthyl-*N*-methylcarbamate, thereby producing a mixture of derivatives detectable by electron capture gas-liquid chromatography (EC-GLC). Unsatisfactory attempts to increase the sensitivity of carbaryl detection below 1.0 ppm were attributed to the relatively greater proportion of brominated products formed from green bean extract components, compared to the amount of brominated carbaryl. Direct bromination of the acetone-hexane extract of treated apples by Gutenmann and Lisk (1963) yielded an electron capture-sensitive derivative of diphenylamine. Subsequently, Gutenmann and Lisk (1965) converted carbaryl to a brominated 1-naphthyl acetate which was detected in the nanogram range by EC-GLC following hydrolysis, bromination, and acetylation of the parent compound. Hydrolysis and bromination of snap bean extracts by Van Middlelem *et al.* (1965) resulted in the EC-GLC detection of carbaryl residues at a sensitivity limit of 0.1 ppm.

A second approach to carbamate derivation has been through alkaline hydrolysis to phenolic degradation products (Figure 1). The phenols can in turn react in various ways to form the derivative of choice. Bowman and Beroza (1967) coupled the phenols of several carbamates with dimethyl chlorothiophosphate. The resulting derivative was chromatographed using a flame photometric detector. Another

procedure in which the phenols were used for derivative formation was developed by Butler and McDonough (1968) whereby the trichloroacetate derivative was produced by reacting trichloroacetyl chloride with phenolic products of selected carbamates using pyridine as the catalyst.

The amine is also available for derivation after hydrolysis of the carbamates as shown in Figure 1. Applications of amine derivation have been made by Crosby and Bowers (1968) and Holden *et al.* (1969). These procedures describe the conversion of amine to aniline compounds to facilitate detection by electron affinity. Other amine derivatives for EC detection

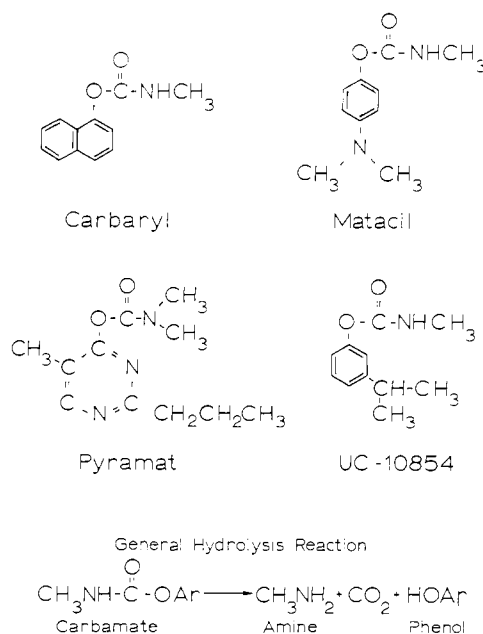


Figure 1. Several commercial carbamate pesticides susceptible to the general hydrolysis and coupling reaction

Pesticide Research Laboratory, Department of Food Science, University of Florida, Gainesville, Fla. 32601

were evaluated by Clarke *et al.* (1966). These investigators compared *N*-trifluoroacetyl, monochloroacetyl, pentafluoropropionyl, heptafluorobutryl, and acetyl derivatives of various amines and phenolic amines.

The object of the investigations presented here was to develop a practical means of analyzing residues of carbaryl by electron capture GLC. A unique derivative was developed initially which was applicable to the amine portion of the carbaryl molecule. Techniques were developed which allowed quantitative conversion of carbaryl to the derivative as well as elimination of background due to crop extractives. Finally, the method was tested by analyzing actual field-treated crops. Data obtained by this procedure were compared to results obtained from duplicate field samples by a conventional colorimetric method.

EXPERIMENTAL

Development of Electron-Capturing Derivatives. Considerable effort was made to synthesize a derivative whose molecular structure exhibited exceptional electron affinity. Four basic groups of compounds were synthesized from methyl- and dimethylamine, as illustrated in Figure 2. The first group synthesized were the nitrated anilines reported by Crosby and Bowers (1968) and Holden *et al.* (1969). These compounds resulted in much less EC detector response than the group of benzamides. Although the aliphatic amides were as sensitive as the benzamides, their preparation was more difficult. The fourth group synthesized were the sulfonamides which were relatively insensitive to electron capture and lacked sufficient volatility for effective chromatography at practical column temperatures. Therefore, the benzamide derivatives combined most of the required properties such as high sensitivity to EC detection, ease of preparation, plus thermal and chemical stability under appropriate column conditions. The 4-bromomethylbenzamide, although not the most sensitive of the benzamides tested, was the most satisfactory because the acid chloride from which it was derived contained the least impurities.

Three methods of synthesizing the benzamides were evaluated by reacting the amine with acetates, anhydrides, and acid chlorides. The latter procedure was the most rapid, and also resulted in the highest yields. After a suitable synthesis procedure had been determined, consideration was given to substitution of particular groupings on the ring and an evaluation of which positions were optimum for maximum sensitivity to electron capture.

The *N*-methylbenzamide without substitution was ineffective as an electron capturer. The effect of substitution was investigated by comparing the 4-nitro-*N*-methylbenzamide response to that of 4-bromo-*N*-methylbenzamide. Halogen substitution was determined to be the more sensitive to electron capture detection. Since Lovelock and Lipsky (1960) reported that the heavier halogens exhibited the highest electron capture potential, iodine substitution was investigated. Para-iodo-*N*-methylbenzamide was difficult to prepare because of the very low solubility of 4-iodobenzoyl chloride in benzene. Preparation of 4-chloro-*N*-methylbenzamide was also undesirable due to the high volatility of 4-chlorobenzoyl chloride.

It soon became apparent that it would be necessary to consider the physical consequences of substitution such as solubility and volatility, in addition to the influence of substitution on EC response. Although the 2,4-dichloro substitution produced approximately twice the EC response as compared to the 4-bromo, impurities in the 2,4-dichlorobenzoyl chloride

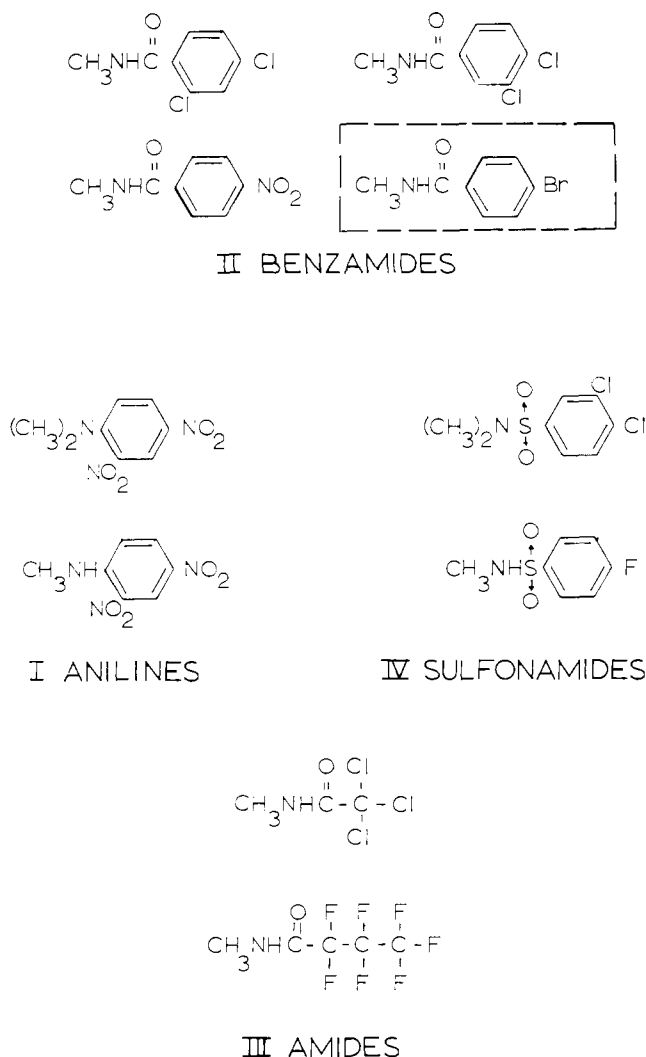


Figure 2. Compounds synthesized and evaluated for response to electron capture detection

resulted in such large interfering peaks that this derivative had to be eliminated from further consideration. Unlike the 2,4-dichloro homolog, 4-bromobenzoyl chloride was relatively pure in a crystalline state at room temperatures. Because of the essential requirement for high acid chloride purity, further investigation into other possible substitution patterns was discontinued. The EC sensitivity of the 4-bromo-*N*-methylbenzamide derivative was determined to be more than adequate to meet the analytical requirements for detection of microquantities of carbaryl.

Conversion of Carbaryl to a Benzamide. The conversion of carbaryl to 4-bromo-*N*-methylbenzamide, as conducted in this experiment, is illustrated in Figure 3. Acid hydrolysis is preferred to basic hydrolysis since its use results in quantitative retention of methylamine as a salt. The most suitable acid for this reaction was hot, concentrated sulfuric. The acid, containing methylamine salt, was diluted in water and then covered with a known volume of benzene containing an excess of the 4-bromobenzoyl chloride. Sufficient sodium hydroxide was added to neutralize the acid and alkalize the aqueous phase. This permitted the release of the methylamine from its water soluble salt, resulting in its being dissolved immediately in the benzene layer. In a modified Schotten-Baumann synthesis, the methylamine reacted with the acid chloride while being continuously agitated in the

Table I. Electron Capture Detector Peak Height Response to Low Levels of 4-Bromo-*N*-methylbenzamide

4-Bromo- <i>N</i> -methylbenzamide, (Nanograms)	Peak Height Response (mm.) (1×10^{-9} amp, full scale)
0.0	0
0.2	8
0.4	16
0.6	24
0.8	32
1.0	36
(3×10^{-9} amp, full scale)	
2.0	20
4.0	35
6.0	48
8.0	55
10.0	60

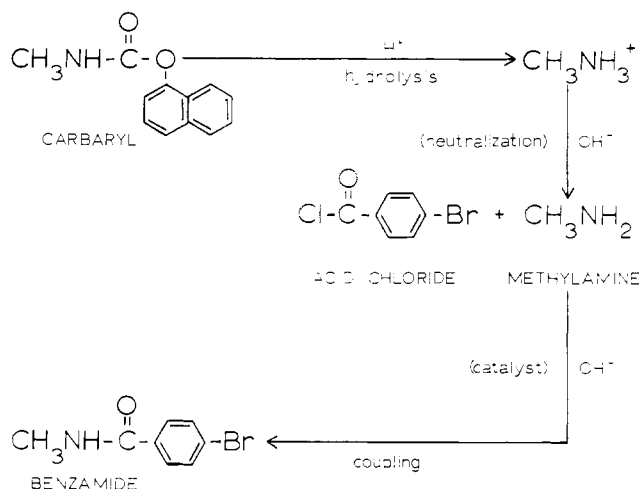


Figure 3. Acid hydrolysis of carbaryl to methylamine and subsequent coupling with acid chloride to derive 4-bromo-*N*-methylbenzamide

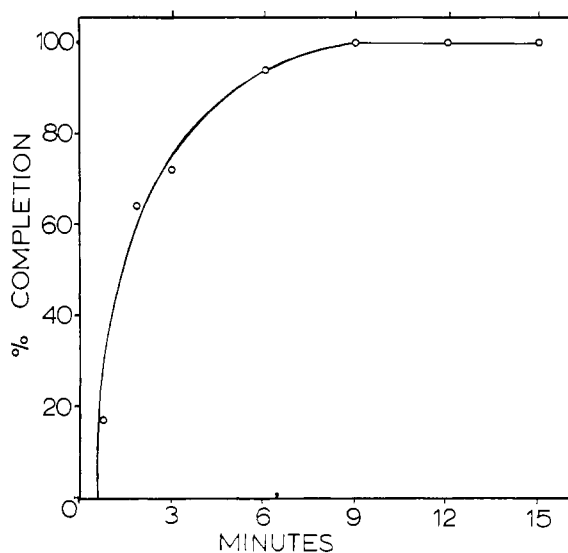


Figure 4. Percentage completion of methylamine coupling with 4-bromobenzoyl chloride to form benzamide derivative

water-benzene mixture. Excess sodium hydroxide acted as a catalyst for the coupling reaction. The overall hydrolysis and coupling was quantitative on the micromole scale necessary for pesticide residue analysis. Quantitative coupling was achieved in several minutes and the resulting benzene phase contained no plant extractives detectable by EC gas chromatog-

raphy. The benzene solution containing the derivative in a predetermined volume was sufficiently stable for satisfactory chromatography, even after several days of refrigeration.

No separate treatment for cleanup of the crop extracts was necessary since it was incorporated in the hydrolysis and coupling procedure. During hydrolysis of carbaryl, sulfuric acid simultaneously oxidized the plant extractives, converting them to water-soluble products and thereby effecting the first cleanup step. Final extract cleanup took place during the coupling reaction in which the methylamine was extracted into the benzene layer while the acid-treated plant extractives remained in the aqueous solution. Consequently, the benzene layer, containing the derivative, was relatively free of crop extractives which could be manifested as interfering peaks in gas chromatographic analysis.

Field Experiment. Commercial ground equipment was used to apply carbaryl to spinach and chicory in experimental field plots at Zellwood, Fla. Four applications of 1 and 2 pounds of active carbaryl per acre were applied to the leafy vegetables over a two-week period. Samples were selected for residue analysis one, three, and eight days following the last field application. Ten mature plants were selected at random for each field plot, placed in polyethylene bags and frozen immediately. After transportation to the laboratory, the plants were chopped in a Hobart foodcutter and returned to the freezer until extracted and analyzed.

Laboratory Procedure. APPARATUS. A Packard 7820 gas chromatograph with a Ni^{63} detector was modified by using a 10:1 voltage divider to reduce the detector voltage output to 5 volts D.C. A glass helix column, 4 mm. \times 6 feet was packed with 3% Carbowax 20M on 100/120 H.P. Chromosorb W. The temperatures employed were 235° C for the column and 250° C for the detector and injector. The nitrogen flow was 60 ml. per minute. One-microliter injections were delivered from a 10- μ l. syringe.

CHEMICALS. 4-Bromobenzoyl chloride solution was prepared by dissolving 1.0 gram of crystals (m.p. 39–40° C) in 1 liter of benzene. The sodium hydroxide solution contained 75.0 grams (ACS grade) in 500 ml. of distilled water. Standard carbaryl solution was 99.9% pure carbaryl dissolved in methylene chloride at a concentration of 10.0 μ g. per ml. All solvents were of redistilled quality.

SAMPLE PREPARATION. Fifty grams of crop sample were blended with 150 ml. of methylene chloride for 2 minutes. The extract was filtered through cheesecloth and another extraction using an additional 100 ml. of solvent was made on the same plant material. The second extract was added to the first and made to 250 ml. in glass-stoppered bottles. Fifty grams of granular, anhydrous sodium sulfate were added to remove water and clarify the solution. The extracts were refrigerated until analyzed.

CONTROL CROP CURVE. The control and unknown samples received identical treatment except for the addition of carbaryl. Twenty-five-milliliter aliquots of the extract were added to 125-ml. glass-stoppered Erlenmeyer flasks. Aliquots from untreated crop were fortified with 0, 30, 60, and 90 μ g. of carbaryl using the 10.0 μ g. per ml. standard solution. It was necessary to adjust the aliquots of the unknown samples accordingly when they did not contain between 20 and 80 μ g. of carbaryl.

ACID HYDROLYSIS AND DIGESTION. Evaporation was accomplished by placing the Erlenmeyer flask on a hot plate under a dry air stream. The samples were not allowed to boil and the last 2 to 5 ml. of solvent was evaporated in the Erlenmeyer flask without heating. All plant material was then

coated with 0.5 ml. of concentrated H₂SO₄. The flasks were then loosely stoppered and heated at 125° C for 2 hours. All samples were brought to room temperature before derivation.

DERIVATION. Twenty-five ml. of distilled water was added to each Erlenmeyer flask followed by 25.0 ml. of the acid chloride-benzene solution. Ten milliliters of the sodium hydroxide solution was added carefully to prevent splattering and to assure quantitative trapping of the released methylamine under the benzene layer. Several samples were checked with pH paper to verify the strong alkalinity of the aqueous phase. Thorough agitation of the stoppered flasks on a mechanical shaker resulted in complete coupling in ten minutes. After the solvent layers separated, 5 ml. of the clear benzene layer was transferred to 10-ml. capped vials and placed under refrigeration if not chromatographed the same day.

RESULTS AND DISCUSSION

Table I illustrates the detector response in the range of 0.2 to 10.0 nanograms of derivative. Purified 4-bromo-*N*-methylbenzamide synthesized in quantities of several grams, was used to prepare the standard solutions.

These standards were necessary in the experimental stage to determine the yields for coupling, hydrolysis, and recovery. The time and extent of coupling (Figure 4) was determined by using EC-GLC to monitor the reaction of microgram quantities of methylamine hydrochloride with an excess of acid chloride. The coupling sequence could then be used to determine the efficiency of hydrolysis. Carbaryl recoveries from crop extracts were determined to be over 90%.

The interfering and noninterfering peaks illustrated in Figure 5 are due to hydrolyzed impurities from the acid chloride reagent during the coupling reaction. This reagent-induced interfering peak (high blank) was of equal size in all samples, regardless of the amount of pesticide or crop extractives present. The interfering peak or high blank was automatically compensated for in the crop extract control curve. Extracts from as much as 50 grams of spinach or chicory resulted in no additional interfering peaks. Efforts to remove the interfering peak included "GC preparation" purification of the 2,4-dichlorobenzoyl chloride and atmospheric control to eliminate the possibility of methylamine contamination. In addition, an assortment of columns and temperature programming was used in an effort to displace the high reagent blank. The problem was also apparent with the 4-bromo compound but to a lesser extent.

To verify the analytical results obtained by the gas chromatographic procedure described, the carbaryl-treated spinach and chicory extracts were also analyzed by the colorimetric method of Johnson (1964). Evaluation of the data presented in Table II would indicate that there is generally satisfactory comparative data obtained by the two methods of analysis.

The described gas chromatographic procedure has several distinct advantages over the colorimetric method. Since all processes following extraction are carried out in one flask, the GLC procedure utilized considerably less glassware than is required in the other method used. The derivative in the GLC procedure is stable for days whereas the chromogenic agent in the colorimetric method is unstable. The colorimetric method is applicable only to carbaryl which forms 1-naphthol following alkaline hydrolysis. On the other hand, the gas chromatographic procedure should be applicable to most *N*-methyl and *N,N*-dimethylcarbamates. Preliminary work indicated that the proposed electron capture GLC procedure can be successfully applied to the analysis of residues of

Table II. Comparison of Carbaryl Residue Data Obtained by Two Analytical Procedures on Samples of Field-Treated Spinach and Chicory (expressed in ppm)

Int. Days ^a	Fld. Rep.	SPINACH			
		1.0 lb. Active/Acre		2.0 lb. Active/Acre	
		EC-GLC	Colorimetric	EC-GLC	Colorimetric
1	A	28	28	80	94
3		20	18	46	33
8		4.5	4.5	23	18
1	B	38	33	100	120
3		4.0	3.0	11	11
8		3.0	0.8	10	5.0
CHICORY					
1	A	29	25	130	130
3		27	24	76	76
8		7.2	7.5	29	37
1	B	66	47	80	83
3		35	41	37	50
8		7.0	4.9	7.0	11

^a Time interval since last of 4 field applications of carbaryl.

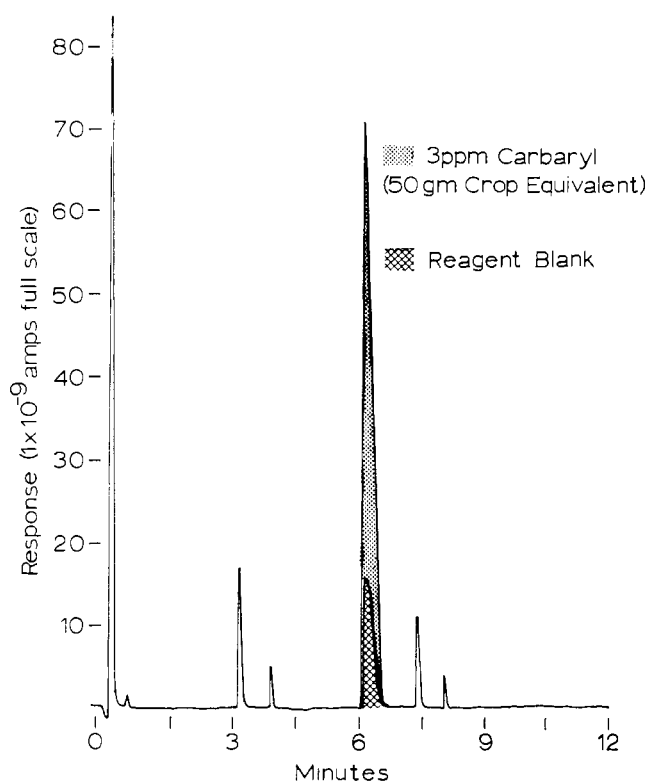


Figure 5. Carbaryl peak interference attributable to acid chloride impurities (no detectable interferences from crop extractives equivalent to 50 grams)

Matacil (4-dimethylamino-*m*-tolyl methylcarbamate). The primary derivative used in this study is relatively simple to synthesize and the usual time-consuming cleanup is eliminated by the hydrolysis procedure described. The limit of sensitivity of the EC-GLC procedure is approximately 0.2 ppm which compares favorably to the colorimetric limit of 0.1 ppm. Extreme sensitivity is not necessary for carbaryl analyses because of the relatively high tolerance established for this pesticide on agricultural commodities.

One of the disadvantages of the proposed gas chromato-

graphic procedure is the interfering high blank which is of no consequence when dealing with substrates containing carbaryl residues of 1.0 ppm or greater. Another possible disadvantage of the EC-GLC procedure may be the necessity for establishing a daily standard curve when analyzing a group of samples.

Following extraction, two technicians can analyze 15 to 20 samples per day by employing the electron capture gas chromatographic method presented. Further extension of this procedure may include many other categories of insecticides, herbicides, and fungicides subject to hydrolysis to simple amines as described by Crosby and Bowers (1968).

ACKNOWLEDGMENT

The authors are indebted to Tom H. Glenn and Terry J. Sprinkle for their technical assistance during the course of these investigations.

LITERATURE CITED

- Bowman, M. C., Beroza, M., *J. Assoc. Offic. Anal. Chemists*, **50**, 926 (1967).
Butler, L. I., McDonough, L. M., *J. Agr. Food Chem.*, **16**, 403 (1968).
Clarke, D. D., Wilk, S., Gitlow, S. E., *J. Gas Chromatog.*, **4**, 310 (1966).
Crosby, D. G., Bowers, J. B., *J. Agr. Food Chem.*, **16**, 839 (1968).
Gutenmann, W. H., Lisk, D. J., *J. Agr. Food Chem.*, **11**, 468 (1963).
Gutenmann, W. H., Lisk, D. J., *J. Agr. Food Chem.*, **13**, 48 (1965).
Holden, E. R., Jones, W. M., Beroza, M., *J. Agr. Food Chem.*, **17**, 56 (1969).
Johnson, D. P., *J. Assoc. Offic. Agr. Chemists*, **47**, 283 (1964).
Lovelock, J. E., Lipsky, S. R., *J. Amer. Chem. Soc.*, **82**, 431 (1960).
Ralls, J. W., Cortes, A., *J. Gas Chromatog.*, **2**, 132 (1964).
Van Middlem, C. H., Norwood, T. L., Waites, R. E., *J. Gas Chromatog.*, **3**, 310 (1965).

Received for review June 27, 1969. Accepted August 29, 1969. Work supported in part by USPHS research grant ES-00208 through the Division of Environmental Health Sciences, National Institutes of Health. Florida Agricultural Experiment Station, Journal Series No. 3295.