Method for the Determination of Residues of Carbamate Insecticides by Electron-Capture Gas Chromatography

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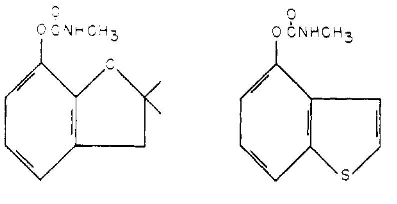
After hydrolysis to the phenols, residues of carbaryl, Mobil MC-A-600 (benzo[*b*]thien-4-yl methylcarbamate), and Niagara NIA-10242 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) were heated with pyridine and trichloroacetyl chloride to produce trichloroacetates which were measured by electron-capture gas chromatography. Hydrolysis and trichloroacetylation were performed without any intermediate washing step. The time for analysis was about 1 hour after cleanup. Cleanup procedures consisted of a coagulation step, followed by liquid chromatography on Florisil or liquid chromatography only on a combination adsorbent of alumina, Florisil, and Norit A. The method has been used to determine residues in potatoes, sugar beets, apples, and range grass. The lower limits of detection of the pure standards in nanograms were NIA-10242, 0.01; carbaryl, 0.02; MC-A-600, 0.02 (signal to noise ratio of 3 to 1). In crops, the lower limits of sensitivity were generally 0.01 to 0.10 p.p.m.

New carbamate insecticides are increasingly being applied to soil and foliage. To analyze the resulting residues, a general and sensitive analytical method was needed that preferably could be used to analyze for one carbamate in the presence of others.

Most carbamate insecticides are methylcarbamates of various phenols. Many of the methods of determining residues that have been reported rely upon reactions of the phenol from the hydrolyzed carbamate. Johnson (1964) reported a colorimetric method for carbaryl based on coupling 1-naphthol to *p*-nitrobenzenediazonium fluoroborate. Gutenmann and Lisk (1965) developed a gas chromatographic method for carbaryl, based on hydrolysis, bromination, and acetylation, that was sensitive to 0.05 p.p.m. Although this method was satisfactory for carbaryl, the authors could not extend it to Mobil MC-A-600 (benzo[*b*]thien-4-yl methylcarbamate) or to Niagara NIA-10242 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) (Figure 1). Van Middelem *et al.* (1965) demonstrated a method for carbaryl sensitive to 1 p.p.m. which was based upon the same principle as that of Gutenmann and Lisk (1965), except that they did not acetylate the brominated 1-naphthol. They also showed that their method could be used for a qualitative survey for Bayer 37344 [4-(methylthio)-3,5-xylyl methylcarbamate] and for Union Carbide UC-10854 (*m*-isopropylphenyl methylcarbamate) when the residue levels were above 2 p.p.m.

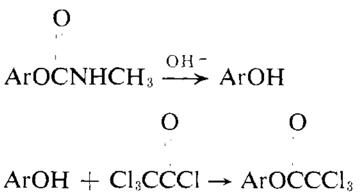


NIA 10242 MC-A-600

Figure 1. Two experimental carbamate insecticides

Bowman and Beroza (1967a) reported a gas chromatographic method for NIA-10242 that was based on hydrolysis and conversion of the resulting phenol to a dimethyl thiophosphate. The derivative was detected with a flame photometric detector, at a sensitivity of 0.02 to 0.04 p.p.m. Bowman and Beroza (1967b) also reported a method for MC-A-600 which used GLC and the flame photometric detector and was sensitive to 0.01 to 0.03 p.p.m. Finocchiaro and Benson (1967) have summarized other methods of carbamate analysis.

Our specific purpose was to develop a sensitive GLC electron-capture method that could be applied quantitatively to NIA-10242 and MC-A-600, as well as to carbaryl. Hydrolysis of the carbamate and trichloroacetylation of the resulting phenol (see equation) provided derivatives which were successfully determined quantitatively in crops of sugar beets, potatoes, apples, and range grass at a sensitivity of 0.01 to 0.10 p.p.m.



$$\mathrm{Ar}\overset{\mathrm{O}}{\overset{\|}{\mathrm{OC}}}\mathrm{NHCH_3} \xrightarrow{\mathrm{OH^-}} \mathrm{ArOH}$$

$$\mathrm{ArOH} + \mathrm{Cl_3C}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}\mathrm{Cl} \rightarrow \mathrm{ArO}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}\mathrm{CCl_3}$$

REAGENTS AND MATERIALS

Apparatus. The gas chromatograph (Research Specialties with Sr_{90} electron-capture detector) was operated under the following conditions: 183 × 0.4 cm. I.D. glass column packed with 10% DC 200 on 60- to 80-mesh Gas-Chrom Q (Applied Science Lab., State College, Pa.); nitrogen carrier gas with a flow rate of 75 to 100 ml. per minute; column temperature, 190° C.; injector temperature, 210° C., detector temperature, 230° C.

Reagents. COLUMBIA ACTIVATED CHARCOAL (National Carbon Co., Fostoria, Ohio).

FLORISIL (60- to 100-mesh) had a weight loss of 2.3% after 20 hours at 110° C. (Floridin Co., New York, N.Y.).

NORIT A had a weight loss of 1.9% after 20 hours at 110° C. (Fisher Scientific Co., Fair Lawn, N.J.).

ALUMINUM OXIDE (Baker's Analyzed Reagent, not chromatographic quality) had a weight loss of 1.0% after 20

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hours at 110° C. (J. T. Baker Chemical Co., Phillipsburg, N.J.).

HEXANE (Scientific Supplies, Seattle, Wash.). Shake 2 liters of hexane with 60 grams of a 1 to 2 mixture of Florisil–Columbia activated charcoal, and allow the mixture to stand overnight. Filter and distill the hexane.

PYRIDINE SOLUTION, reagent grade pyridine (J. T. Baker, Phillipsburg, N.J.). Pass 50 ml. of pyridine through a 10-mm. I.D. liquid chromatography column, packed from bottom to top with glass wool, a thin layer of anhydrous sodium sulfate, 8 grams of Florisil, and about 0.5 inch of anhydrous sodium sulfate. Discard the first eluate that is used to wet the column. Dilute 0.10 ml. of the chromatographed pyridine to 100 ml. with methylene chloride, and store the stock solution in a dark bottle.

MINERAL OIL SOLUTION. Dilute 1 gram of Shell Ondina Oil (Shell Oil Co.) to 100 ml. with methylene chloride.

COAGULATING SOLUTION. To 1.5 grams of ammonium chloride, add 3 ml. of 85% phosphoric acid, and make to 1 liter with distilled water.

TRICHLOROACETYL CHLORIDE SOLUTION was prepared by the method of Mory *et al.* (1958). Weigh 163 grams (1 mole) of trichloroacetic acid (Eastman, White Label) into a 250-ml. round-bottomed flask equipped with a well for a thermometer. Add 7.8 ml. (0.1 mole) of *N,N*-dimethylformamide (Eastman, White Label) and 80 ml. (1.1 moles) of thionyl chloride (Matheson Co.), and immediately connect to a condenser capped with a drying tube of calcium chloride. Heat at 65° to 85° C. for about 2.5 hours. The temperature will rise to 100° C. when the reaction is complete. Distill the reaction mixture under vacuum through an 18-inch Vigreux column, and collect the fraction with b.p. 31° C./32 mm. (yield 70%). In a 10-ml. volumetric flask, dilute 0.1 ml. of the distilled trichloroacetyl chloride to 10 ml. with methylene chloride.

1-NAPHTHYLTRICHLOROACETATE. About 1 gram of the title compound was prepared for use as an analytical standard for carbaryl. It had m.p. 86° to 87° C.

Analysis. Calcd. for $C_{12}H_7O_2Cl_3$: C, 49.78; H, 2.44; Cl, 36.73. Found: C, 49.90; H, 2.56; Cl, 36.52.

PROCEDURE FOR STANDARDS

Place 0.1 to 50 μg. of the insecticide dissolved in chloroform in a test tube, add 0.1 ml. of mineral oil solution (keeper), and evaporate the solution to dryness with a gentle stream of air in a 40° C. water bath. Do all subsequent evaporations in this manner. Add 0.2 ml. of 0.1*N* sodium hydroxide in methanol, rotate the test tube to wet its sides with the mixture, and shake for 5 minutes on a shaker.

Evaporate the methanol, add 1 ml. of the pyridine solution, and heat the tube in an oil bath at 100° to 105° C. until the methylene chloride has evaporated (about 1 minute). Remove the test tube, and cool it in ice water; add 1 ml. of the trichloroacetyl chloride solution, shake briefly, and again heat in the oil bath until the methylene chloride has evaporated. Remove the test tube from the oil bath, add 10 ml. of hexane, shake briefly, add 5 ml. of water, and transfer the contents of the tube to a 60-ml. separatory funnel. Rinse the tube with 5 ml. of hexane. Shake the separatory funnel for 30 seconds, draw off the water phase, add 5 ml. of a saturated solution of sodium bicarbonate, and shake for 15 seconds. Draw off the sodium bicarbonate phase, and wash once with 5 ml. of water. Draw off the water layer, transfer the hexane layer to a 50-ml. Erlenmeyer flask, and add anhydrous sodium sulfate; allow the hexane solution to stand for 15 minutes before filtering it into a volumetric flask through folded filter paper containing anhydrous sodium sulfate. Wash the flask and filter paper with enough hexane to make the hexane solution up to 50 ml. Inject 1 to 5 μl. of the hexane solution into the gas chromatograph.

SAMPLE PREPARATION

Apples, Potatoes, and Sugar Beets. Blend 100 grams of the crop for 5 minutes with 200 ml. of chloroform for apples and potatoes or 250 ml. for sugar beets. Pour the mixture into a 400-ml. beaker, add about 100 grams of anhydrous sodium sulfate while stirring vigorously, and allow the mixture to stand for at least 10 minutes. Filter the mixture through a cotton plug covered with anhydrous sodium sulfate into glass bottles, and refrigerate.

Range Grass. Place 100 grams of range grass in a jar with 800 ml. of chloroform, and allow the mixture to steep overnight. Tumble the jar for 1 hour, and then proceed as for apples.

For the determination of percentage recovery of the carbamates, samples of apples, potatoes, sugar beets, or range grass were fortified prior to this step.

CLEANUP PROCEDURES

Apples or Sugar Beets (for Carbaryl or MC-A-600). Evaporate a portion of the extract (representing 10 to 100 grams of crop sample) to dryness, add 4 ml. of acetone, heat for 30 seconds on a steam bath, and add 50 ml. of coagulating solution. After 15 minutes, filter the mixture through a cotton plug into a 250-ml. separatory funnel, and wash the flask and cotton plug twice with 10-ml. portions of coagulating solution. Extract the solution with a 50- and a 25-ml. portion of chloroform. Dry the chloroform extract with anhydrous sodium sulfate, filter it and three 10-ml. rinses of chloroform into a flask, and evaporate the solution to 10 ml. Prepare a liquid chromatography column (20-mm. I.D.) as follows: pack it (from bottom to top) with glass wool, anhydrous sodium sulfate, 45 grams of Florisil, and anhydrous sodium sulfate. Prewash the column with 100 ml. of chloroform, and dry it with suction. Add the 10-ml. chloroform solution and three 10-ml. rinses of the flask to the Florisil column. As the last of the liquid sinks into the column, change receiving flasks, and elute the insecticide with 150 ml. of chloroform. Add 0.1 ml. of the mineral oil solution (keeper) to the eluate, evaporate it to a small volume, transfer it to a test tube, and evaporate it to dryness. Proceed as with the standards.

Potatoes (for Carbaryl, MC-A-600, or NIA-10242). Use the same procedure as for apples and sugar beets except use 15 grams of Florisil for liquid chromatography, and elute the insecticide with 50 ml. of chloroform.

Range Grass (for MC-A-600). Use a portion of range grass extract, representing 1 to 20 grams of sample, and proceed as for apples and sugar beets.

Table I. Gas Chromatographic Data for Trichloroacetates[a] Derived from Carbamates

Insecticide	Retention Time Relative to Aldrin[b]	Level of Detection, Ng.
Bay 44646 (Matacil)	0.31	0.01
Bay 39007 (Baygon)	0.33	0.01
NIA-10242	0.45	0.01
Carbaryl	0.85	0.02
MC-A-600	0.86	0.02
Bay 37344 (Mesurol)	1.08	0.02

[a] The gas chromatographic conditions were the same as those given in the Reagents and Materials section except that the column temperature was 180° C. The lower temperature allowed a more accurate determination of the retention times of Bay 44646 and Bay 39007.

[b] The retention time of aldrin was 9.8 minutes.

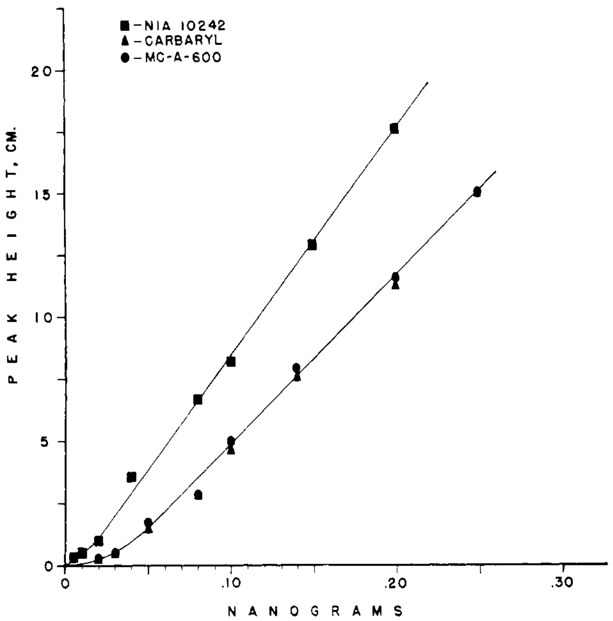

Figure 2. Standard curves for the trichloroacetates of the phenols from carbaryl, MC-A-600, and NIA-10242

Sugar Beets, Apples, Potatoes, or Range Grass (for NIA-10242). Evaporate a portion of the extract, representing 1 to 30 grams of sample, to a volume of 10 ml. With suction, pack a chromatograph column (15 cm. × 2-cm. I.D.) from bottom to top with glass wool, 1 gram of anhydrous sodium sulfate, 10 grams of Florisil, 3 grams of aluminum oxide (Baker's Analyzed), 1 gram of Norit A, and 1 gram of anhydrous sodium sulfate. Add 100 ml. of chloroform to the column. As the last of the chloroform sinks into the adsorbent, change the receiving flask, and add the sample extract. Rinse the sample container three times with 10 ml. of chloroform, and add each rinse to the chromatograph column. Elute with 25 ml. of chloroform. During chromatography of the sugar beet extract, stir the Norit A with a spatula to prevent it from caking. Add 0.1 ml. of mineral oil solution (keeper) to the eluate, and proceed as with the standards.

The Florisil, which was used in these two cleanup procedures, was used as received from the manufacturer. Since lots of adsorbent occasionally vary, the chromatographic column used for cleanup should be checked with a standard carbamate solution and, if necessary, adjustments in elution volumes should be made.

RESULTS AND DISCUSSION

Since the hydrolytic and gas chromatographic stabilities of the desired derivatives were unknown, the trichloroacetates of the phenols from several carbamate insecticides were prepared on a synthetic scale. Their retention times relative to aldrin and their limits of detection are summarized in Table I. The infrared spectra of these compounds showed characteristically low carbonyl absorptions at 5.61 to 5.66 microns and absence of absorption in the OH region.

The purified trichloroacetate from carbaryl was used as a standard to determine the percentage of reaction during development of the analytical procedure. Once conditions were established for 100% reaction, the same conditions were used for the phenols of MC-A-600 and NIA-10242.

During development of the procedure, trichloroacetyl chloride was initially purified by a simple distillation, but the trichloroacetates prepared from this reagent contained long retention time GLC peaks, which made the analysis long. Distillation of the acid chloride through a Vigreux column effectively removed these peaks. Because acid chlorides are easily hydrolyzed, a substantial excess of acid chloride is used.

The sodium hydroxide that was used for hydrolysis of the carbamates was initially removed by neutralization and extraction of the phenol with ether. However, the time for analysis was significantly reduced by not removing the base and by preparing the trichloroacetate in situ.

A hexane solution of the trichloroacetate is stable for about 15 minutes, but when the hexane solution was washed with water (the recommended procedure), the trichloroacetate was stable for several hours. (After a week, about 10% was lost.) When benzene extraction of the trichloroacetates was tried, several interfering GLC peaks were obtained.

Standard curves are shown in Figure 2. NIA-10242 is more sensitive partly because it is more strongly electron capturing; also it is normally eluted faster than carbaryl or MC-A-600.

The cleanup procedures are convenient and consist either of a coagulation step followed by liquid chromatography on Florisil, or just liquid chromatography on a combination column of alumina, Florisil, and Norit A. In the latter procedure, Baker's Analyzed alumina, which was not designated chromatographic quality, was used. When the Baker's Analyzed chromatographic quality was used, the carbamates could not be recovered. The chromatographic quality alumina is more strongly adsorbing. It may have decomposed the carbamates, since they could not be removed even with polar solvents. Similarly, the charcoal should be Norit A. When Nuchar C-190 was used, the recoveries were low.

Figures 3 to 6 illustrate typical GLC chromatograms of

Table II. Percentage Recovery of Carbaryl, MC-A-600, and NIA-10242 from Crops Fortified with Known Amounts of the Insecticides Prior to Extraction and Cleanup

Crop	Added, P.P.M.	Recovery, %
	CARBARYL	
Apples	0.0	0.0
	0.05	102.5
	0.10	76.4
Potatoes	0.0	0.0
	0.01	90.7
	0.01	88.1
	0.05	109.1
	0.10	97.2
	0.40	83.0
Sugar beets	0.0	0.0
	0.10	82.1
	MC-A-600	
Apples	0.0	0.0
	0.10	90.2
Potatoes	0.0	0.0
	0.01	70.0
	0.05	99.7
	0.10	99.0
	0.10	89.5
	0.10	117.3
Range grass	0.0	0.0
	0.10	94.8
	0.40	84.3
	0.40	90.0
	0.80	96.8
	8.0	86.1
	30.0	120.0
Sugar beets	0.0	0.0
	0.05	76.2
	0.10	80.3
	NIA-10242	
Apples	0.0	0.0
	0.02	110.0
Potatoes	0.0	0.0
	0.005	80.0
	0.01	88.2
	0.02	95.0
	0.04	86.9
	0.05	89.3
	0.10	80.3
	0.40	100.0
	4.0	80.6
Range grass	0.0	0.0
	0.01	104.1
Sugar beets	0.0	0.0
	0.04	93.8
	0.05	87.3
	0.10	95.5
	0.50	90.5

residues in crops and the corresponding control samples. In Figures 3 and 6, the base line is not well defined. This leads to an uncertainty in peak height of ±2.5% and ±1%, respectively. In this method, the limit of sensitivity is determined not by the strength of the gas chromatographic peak of the trichloroacetate (if it were, a sensitivity of 0.1 p.p.b. could be realized), but by the ratio of this peak height to that of the plant materials that were not removed by the cleanup procedure. As a result, the limit of sensitivity is defined as the point at which the interference from

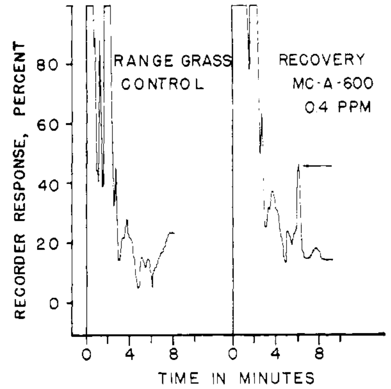

Figure 3. Chromatograms of cleaned-up range grass with and without MC-A-600 (fortified prior to extraction)

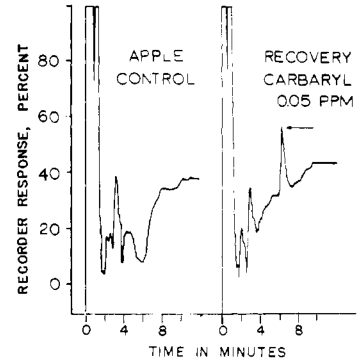

Figure 4. Chromatograms of cleaned-up apples with and without carbaryl (fortified prior to extraction)

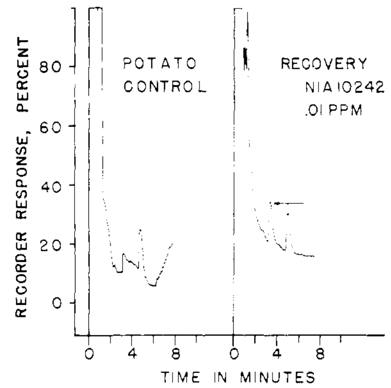

Figure 5. Chromatograms of cleaned-up potatoes with and without NIA-10242 (fortified prior to extraction)

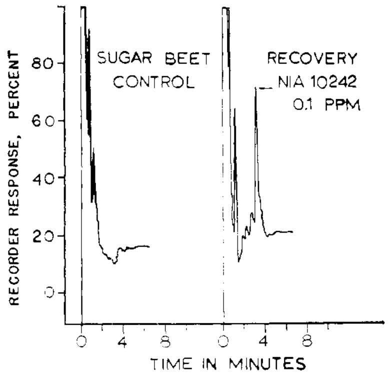

Figure 6. Chromatograms of cleaned-up sugar beets with and without NIA-10242 (fortified prior to extraction)

the peaks of plant materials becomes objectionable. As the residue levels increase above the limit of sensitivity, the base line becomes increasingly well defined.

Recoveries are summarized in Table II. The lower limits of sensitivity given are not necessarily the best obtainable. For example, determination of MC-A-600 in range grass below 0.10 p.p.m. was not attempted.

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