Determination of Residues of Methyl- and Dimethylcarbamate Insecticides by Gas Chromatography of Their 2,4-Dinitroaniline Derivatives

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A procedure was developed for determining residues of methyl- or dimethylcarbamates in plant materials. The dichloromethane extract of the crop is subjected to a coagulation step, carbamates are hydrolyzed with alkali, and the liberated amines are reacted with 1-fluoro-2,4-dinitrobenzene to form dinitroaniline derivatives that are determined by electron-capture gas chromatography. Derivative formation is essentially quantitative. Background interference varied with different crops, but was usually less than the equivalent of 0.05 p.p.m. of carbamate. Recoveries from fortified samples of several crops were 90 to 100%.

The introduction of carbamate insecticides has brought with it a need for sensitive analytical methodology to determine their residues in food crops. The direct application of gas chromatography is generally unsatisfactory with monomethylcarbamates because on-column decomposition occurs (Ebing, 1965) and because detector response to carbamates is weak unless the carbamate happens to contain a group—e.g., halogen—that is sensed by one of the highly specific detectors. The possibility that these difficulties could be overcome by converting carbamates to stable derivatives giving good response was investigated.

Inasmuch as the carbamate insecticides contain either a methyl- or a dimethylamine group that may be liberated by hydrolysis, quantification of the liberated amines by their reaction with 1-fluoro-2,4-dinitrobenzene and electron-capture gas chromatography of the resulting dinitroanilines appeared to be a promising approach. Day *et al.* (1966), who proposed this method for determining the pure C_1–C_4 amines, showed the determination to be a very sensitive one. However, when this procedure was applied to the determination of insecticidal carbamates in crops, excessive interference was encountered. The interference was eliminated by employing a modification of the acetone–phosphoric acid–ammonium chloride coagulation cleanup of Johnson (1964) and by some revision of the reaction conditions.

EXPERIMENTAL

Reagents and Solvents. Aqueous solutions containing 1.0*N* sulfuric acid, 0.5*N* potassium hydroxide, and 5% borax were prepared. The two latter solutions were purified by adding 2 grams of acid-washed charcoal (Norit SG Extra, American Norit, Jacksonville, Fla.) to a liter of the solution, shaking intermittently for 5 minutes, and filtering through Celite 545 (Johns-Manville, New York).

The carbamate insecticides were the best quality obtainable from their manufacturers. In most cases, they were analytical grade materials. The pesticides used in the study are identified in Table I.

Coagulating solution: Twenty grams of ammonium chloride and 40 ml. of 85% phosphoric acid were diluted to 400 ml. with distilled water; after treatment with charcoal as above, 20 ml. of this stock solution were further diluted to 800 ml. with distilled water.

Reagent grade acetone (distilled in glass) was used without further purification. Reagent grade dichloromethane, benzene, and pentane were purified by mixing one gallon with 40 grams of silicic acid, shaking well, and filtering through a fritted-glass funnel.

The coupling agent, 1-fluoro-2,4-dinitrobenzene (J. T. Baker, Phillipsburg, N. J.), was purified by distillation at 128° C. and 1 mm. pressure.

Procedure. Blend 50 grams of chopped plant material with 200 grams of anhydrous sodium sulfate and 200 ml.

Table I. Pesticides Cited

Generic Name or Other Designation	Chemical Name
Azodrin	3-Hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate
Banol	6-Chloro-3,4-xylyl methylcarbamate
Baygon (Bay 39007)	*o*-Isopropoxyphenyl methylcarbamate
Bidrin	3-Hydroxy-*N*,*N*-dimethyl-*cis*-crotonamide dimethyl phosphate
Carbaryl	1-Naphthyl methylcarbamate
Carbofuran (Niagara NIA-10242)	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
Dimethoate	*O*,*O*-Dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate
Dimetilan	1-Dimethylcarbamoyl-5-methyl-3-pyrazolyl dimethylcarbamate
Diphenamid	*N*,*N*-Dimethyl-2,2-diphenylacetamide
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
Matacil	4-(Dimethylamino)-*m*-tolyl methylcarbamate
Mobam	Benzo[*b*]thien-4-yl methylcarbamate
Norea	3-(Hexahydro-4,7-methanoindan-5-yl)-1,1-dimethylurea
Pyrolan	3-Methyl-1-phenylpyrazol-5-yl dimethylcarbamate
Union Carbide UC-21149	2-Methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl) oxime
Zectran	4-(Dimethylamino)-3,5-xylyl methylcarbamate

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of dichloromethane. Reduce the sample size if the total content of carbamate exceeds 100 micrograms. Filter through a 1/4-inch layer of Celite in a Büchner funnel, breaking up the solid matter with a spatula to prevent caking during filtration. Rinse with 250 ml. of dichloromethane and take to thorough dryness in a rotary evaporator. Add 5 ml. of acetone, and warm the flask with swirling for about 30 seconds to dissolve the residue. Cool the solution and add 75 ml. of coagulating solution and a scoop of about 2 grams of Celite. Swirl and filter through a 1-inch layer of Celite. Repeat the coagulation step and filtration; then rinse the flask and filter with 25 ml. of coagulating solution. For pesticides that contain an aminophenyl group—e.g., Zectran and Matacil—add 10 ml. of 5% borax and an equal volume of 0.5*N* potassium hydroxide to the filtrate. Extract the filtrate five times with 25-ml. portions of dichloromethane, filter the dichloromethane layers through anhydrous sodium sulfate into a 500-ml., round-bottomed flask, and evaporate to thorough dryness. Rinse down the pesticides with 2 ml. of pentane and 10 ml. of 0.5*N* potassium hydroxide. Place the lower part of the flask in a 40–2° C. water bath for 30 minutes, then cool and add 10 ml. of 1*N* sulfuric acid. Transfer the liquid to a separatory funnel with about 25 ml. of water and extract four times with 15-ml. portions of dichloromethane. Transfer the aqueous phase into a clean 500-ml., round-bottomed flask (final volume can be any amount from 50 to 200 ml.). Add 10 ml. of 0.5*N* potassium hydroxide. Spot test with pH paper and adjust the pH of the solution to 7, if necessary. Add 10 ml. of 5% borax and 2 ml. of a freshly made benzene solution containing 1 drop of 1-fluoro-2,4-dinitrobenzene per ml., and heat on a steam bath for 30 minutes. Cool the solution to room temperature rapidly; immediately add 25 ml. of benzene, stopper the flask, and agitate it vigorously on a mechanical shaker for 2 minutes. Pour the mixture into a separatory funnel, drain off the aqueous phase, and wash the benzene solution twice with distilled water. Drain the benzene into a large test tube, stopper, and store in a refrigerator while awaiting analysis by gas chromatography.

Prepare standard solutions by carrying 12.5 μg. of the pesticide through the analysis, beginning with the addition of 2 ml. of pentane and 10 ml. of the 0.5*N* potassium hydroxide; dilute the final solution twofold and fourfold to obtain the lesser concentrations needed to establish a working curve of concentration *vs.* peak height.

Gas chromatography: A Packard Model 802 gas chromatograph equipped with an electron-capture detector and with a 4-foot × 1/4-inch o.d. glass column containing 2% XE-60 on 50/60 mesh Anakrom ABS (Analabs, Hamden, Conn.) at 190° C. was used. The flow rate of the nitrogen carrier gas through the column when new was 180 ml. per minute. The rate of nitrogen flow had to be reduced gradually to about 100 ml. per minute during the four-to-six-months life of the column to maintain retention times constant. The instrument setting was 3×10^{-10} amp. ful scale, and the detector potential was 75 volts.

RESULTS AND DISCUSSION

Efforts to utilize the methodology of Day *et al.* to react amines with 1-fluoro-2,4-dinitrobenzene were generally unsatisfactory for the low levels of pesticides found in crops. Impurities in the reagents caused excessive interference, and results were not reproducible. Although some improvement was registered when the reagents were purified, it was not possible to eliminate the interference from the dioxane used by Day *et al.* as a solvent for the coupling reagent. Dioxane also made the control of pH difficult. These difficulties were overcome by using benzene as the reaction solvent. A further improvement was effected by avoiding the use of strong base after the coupling reaction because it tended to destroy the *N,N*-dimethyl-2,4-dinitroaniline derivative; the destruction was complete if an organic solvent was not present.

The new procedure, which provides good precision, requires a fixed pH for the coupling reaction. The solution was accurately neutralized and then strongly buffered to meet this requirement. The effect of adding acid or base on the reaction yields (determined by comparison with standards of the pure dinitroanilines) is depicted in Figure 1. The solution was neutral at 0 ml. As 0.5*N* potassium hydroxide was added, the yield of the dimethyl product was unaffected, but yield of the methyl derivative started to drop after the addition of 2.5 ml. The addition of 0.5*N* hydrochloric acid caused the yields of both products to decrease gradually. However, errors in neutralization were easily limited to ±0.5 ml. of the acid or base solution, and within these limits, the conversion of dimethyl- and methylamine to their dinitroaniline derivatives was 100 and 96%, respectively, with less than 1% variation. In dilute benzene solutions, the dimethyl compound was subject to photodecomposition, which necessitated daily preparation of standards. However, solutions of both dinitroanilines, including those of the standards, were stable for several weeks when they were stored in a refrigerator.

The standard curves obtained with a dimethyl- and a methylcarbamate—Pyrolan and carbofuran, respectively—are shown in Figure 2. The results were linear except for a slight curvature with amounts of the methylcarbamate less than 1.0 ng.

A typical chromatogram, obtained by analyzing a fortified spinach sample, is shown in Figure 3. Peaks of the pesticide derivatives (broken lines) are superimposed on the chromatogram of the blank spinach extract (solid line). The solvent peak was broadened somewhat by unreacted coupling agent, and the ill-defined peaks between 15 and

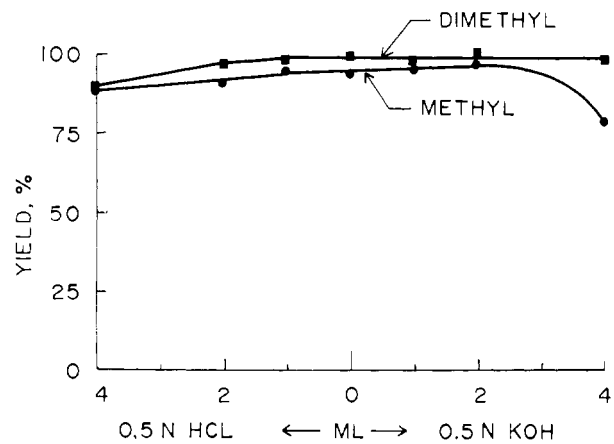

Figure 1. Effect of adding acid or base on yield of *N*-methyl- and *N,N*-dimethyl-2,4-dinitroaniline from 2.5 μg. of the amines. 0 ml. = neutrality

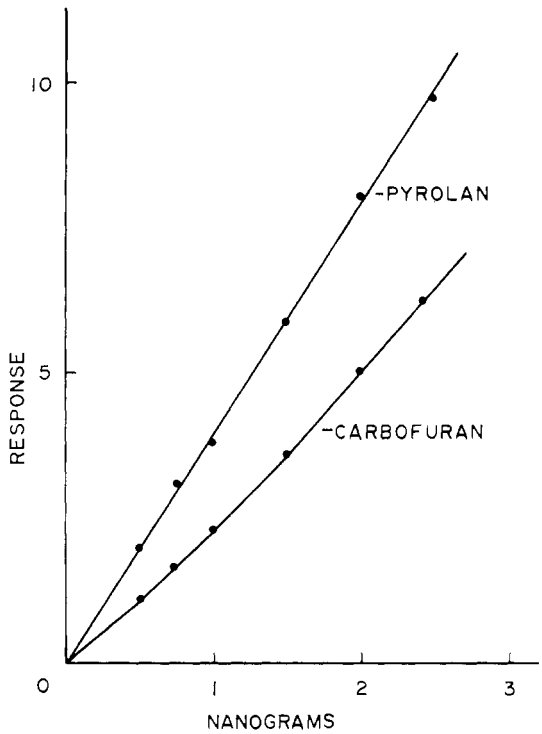

Figure 2. Standard curves of a methyl- (carbofuran) and a dimethylcarbamate (Pyrolan)

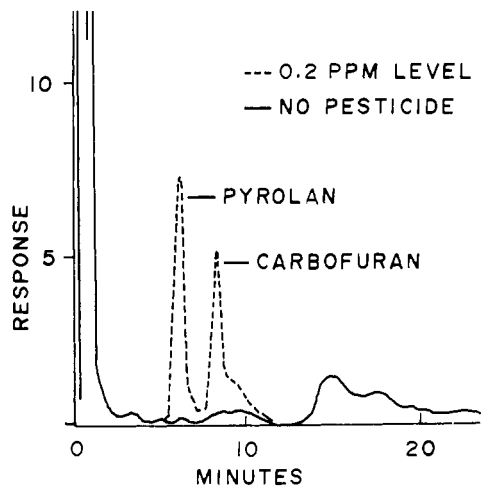

Figure 3. Typical chromatograms obtained when Pyrolan and carbofuran were added to spinach

Peaks of derivatives from the fortified sample (broken lines) are superimposed on the chromatogram of the unfortified sample (solid line)

20 minutes are believed to be, at least partially, products of excess reagent. These peaks, which are always present in variable amounts, do not affect the results.

Chromatograms of blank determinations for several crops are shown in Figure 4, with the peak positions of the two derivatives indicated by arrows. Fluctuations in the baselines were negligible in the analysis of extracts of spinach, cucumbers, and tomatoes. Extracts of apples showed several small peaks, none of which interfered. Lettuce and string beans produced peaks that did interfere

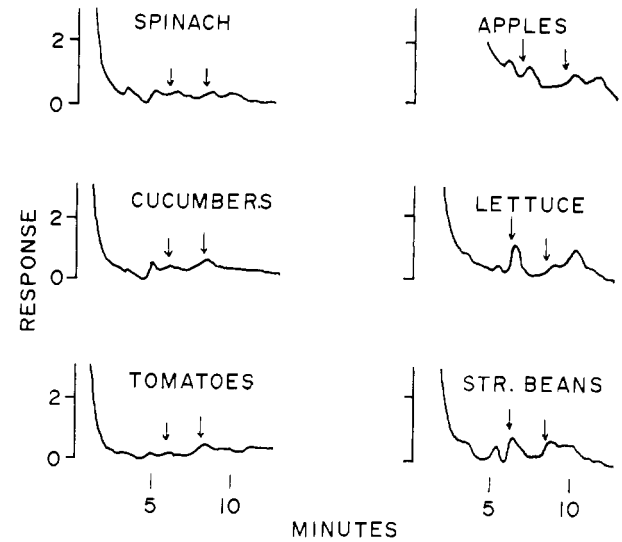

Figure 4. Chromatograms obtained from six unfortified crops

Arrows show the points at which the peaks of the dinitroaniline derivatives from the dimethyl- and the methylcarbamates appear

with the derivative peaks, but they did not exceed the equivalent of 0.05 p.p.m. of carbamate. Results may be corrected for this interference. Although seven crops thus proved to be sufficiently free of interference, other crops will have to be checked in this regard.

The volume of coagulating solution used affects the amount of interference from crops. With the prescribed addition of 75 ml., the dimethylcarbamate blank was 0.01 p.p.m. or less for all crops, and with 25 ml. the methylcarbamate blank was 0.02 or less for all crops. Deviations from these volumes increased the background. Thus, if there is interest in only one of the two types of carbamates, the volume of coagulating solution can be adjusted to achieve minimum interference.

Effects of Noncarbamate Nitrogen Groups. The aromatic dimethylamino groups of Zectran and Matacil prevent complete extraction of the compounds from acid solutions. This problem is avoided by adjusting the pH of the filtrate from the coagulation step (by the addition of borax and potassium hydroxide) prior to the extraction with dichloromethane. The dimethylamino groups of these carbamates also tend to split off during the hydrolysis step to produce a peak of the dimethyl derivative in addition to that of the methyl derivative from the carbamate group, which is the one used for quantitation.

The presence of free methyl- or dimethylamine in the original sample did not interfere. However, pesticides with an amide or urea group that released methyl- or dimethylamine during hydrolysis did interfere. Some common pesticides that have such groups were tested to determine the extent to which they would hydrolyze to amines. Dimethoate released methylamine equivalent to 9% of its amide group; no amine was released by Azodrin. The dimethylamine released from diuron and norea (ureas) and from Bidrin and diphenamid (amides) was equal to about 4% of the theoretical amount present. Thus, the conditions of hydrolysis were sufficiently mild to hold these potential interferences to a minimum. It would appear that

these compounds will not cause serious interference unless excessive amounts are present.

Recovery Data. A methylcarbamate, carbaryl, and a dimethylcarbamate, dimetilan, were added to seven crops at the 0.2-p.p.m. level. Their recoveries (Table II) ranged from 90 to 99%, the average being 93% for each pesticide. Although other crops were not tested, the results indicate that the method is likely to be applicable to a large number of other fruit and vegetable crops.

The recoveries of 10 carbamates from spinach fortified at the 0.2-p.p.m. level are given in Table III; they ranged from 85 to 102%. At levels of 0.1 and 0.05 p.p.m., recoveries ranged from 78 to 105%. As expected, fluctuations in recoveries were greater with lower levels of the pesticides.

Additional Comments. A number of procedures for forming derivatives of insecticidal carbamates to facilitate the analysis of carbamate residues have been reported. The group introduced registers with high sensitivity on one of the relatively specific detectors (electron-capture or flame-photometric). The derivative is usually formed from the phenolic rather than the amine portion of the carbamate. [Crosby and Bowers (1968) suggested derivatization of amine fragments, but no details of their method were given.] Although the analysis of the phenolic derivative provides greater specificity, such methods are only applicable to certain carbamate residues—e.g., Argauer, 1968; Bache *et al.*, 1968; Bowman and Beroza, 1967a, b; Butler and McDonough, 1968; Cavagnol and Betker, 1967; Gutenmann and Lisk, 1965; Maitlen *et al.*, 1968; Ralls and Cortes, 1964; Van Middelem *et al.*, 1965. In contrast, the analysis based on the methyl- or dimethylamine portion allows one to check for all insecticidal carbamates that may be present, rather than to resort to individual analysis for each of the carbamates. Accordingly, the procedure would be of greatest use in determining total carbamate content of food crops when there is no prior knowledge as to whether any carbamates are present. Should carbamates be found in appreciable amounts, they may be identified by rapid procedures, such as the thin layer chromatography methods developed by Finocchiaro and Benson (1965, 1967) and by Engst and Spranger (1964). Other procedures of interest are cited by Williams and Cook (1967) in their comprehensive review of pesticide residues.

The type of multiple analysis described should be of increasing value in safeguarding foods as the number of commercial carbamate insecticides grows. The procedure may also be useful in monitoring crops for carbamate persistence under field conditions.

Table II. Recovery of Two Insecticides from Seven Vegetable Crops Fortified at the 0.2-P.P.M. Level

Crop	Recovery, % Carbaryl	Dimetilan
Apples	98	93
Broccoli	92	95
Cucumbers	92	90
Lettuce	94	99
Spinach	95	91
String beans	90	91
Tomatoes	93	92
Means	93.4	93.0

Table III. Recoveries of 10 Insecticides from Spinach

Insecticide	Added, P.P.M.	Recovered, %
Methylcarbamates		
Banol	0.2	96
Baygon	0.2	90
Carbaryl	0.2	95
Carbofuran	0.05	105
	0.1	87
	0.2	93
	0.2	89
	0.2	96
	2.0	95
Matacil	0.2	85[a]
Mobam	0.2	99
UC-21149	0.2	102
Zectran	0.2	93[a]
Dimethylcarbamates		
Dimetilan	0.2	96
Pyrolan	0.05	78
	0.1	81
	0.2	90
	0.2	89
	0.2	95
	0.2	95
	0.2	95
	2.0	90
Mean		92.4

[a] Filtrate from coagulation step made alkaline before extraction of insecticide with dichloromethane.

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