Determination of Residues of Banol and Other Carbamate Pesticides after Hydrolysis and Chloroacetylation

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An analytical method was developed to determine the residue of Banol (6-chloro-3,4-xylyl methylcarbamate) and 6-chloro-3,4-xylenol in Bermuda grass. After a liquid chromatographic cleanup and separation of 6-chloro-3,4-xylenol from Banol by extraction from methylene chloride with aqueous sodium hydroxide, the Banol was hydrolyzed in aqueous sodium hydroxide, the solution was shaken with chloroacetic anhydride in benzene, and an aliquot of the dried benzene phase was injected into a gas chromatograph. Recoveries of Banol in Bermuda grass at 3 and 0.1 p.p.m. were 86 and 95%, respectively. For Banol, the minimum detectability was 0.04 p.p.m. for a 25-gram aliquot. The aliquot size was increased to 100 grams to improve detectability at this lower level. The use of this method to determine the residues of Banol in milk, apples, cucumbers, and tomatoes was examined, and the percentage recoveries of Banol, 6-chloro-3,4-xylenol, carbaryl, and 1-naphthol were investigated. The relative responses of electron-capture gas chromatography of Banol, carbaryl, and eight other methylcarbamates after hydrolysis and chloroacetylation were compared to heptachlor epoxide.

The purpose of this investigation was to develop a sensitive GLC electron-capture method that could quantitatively determine both Banol (6-chloro-3,4-xylyl methylcarbamate) and its hydrolysis product (6-chloro-3,4-xylenol) in each other's presence in range grass with a minimum number of operational manipulations. Several methods have been developed to determine similar compounds by derivatization and subsequent gas chromatography. Bowman and Beroza (1967) reported formation of *O,O*-dimethyl phosphorothioate derivatives with detection by flame photometry. Aue and Ertingshausen (1967) reported formation of diethyl phosphate derivatives and determination with alkali flame ionization. Gutenmann and Lisk (1965a) hydrolyzed, brominated, and acetylated carbaryl to prepare a sensitive derivative suitable for electron-capture detection. Gutenmann and Lisk (1965a) and Stanley (1966) used a methylation method that required the careful preparation of an ether-diazomethane reagent. Butler and McDonough (1968) used a trichloroacetylation procedure for the determination of three carbamate pesticides, but did not include a separation from possible phenol interferences. Landowne and Lipsky (1963) measured the response of several mono-, di-, and trihaloacetates of cholesterol and found the chloroacetate derivative to be the most sensitive.

The chloroacetylation method of Argauer (1968) appeared especially suitable for this investigation, since the reagent would react directly with either the phenol obtained from an alkaline extraction of a methylene chloride plant extract, or with the final alkaline solution obtained after hydrolysis of the carbamate, without additional workup after reaction.

To determine if the procedure developed for the determination of Banol in Bermuda grass could be applied to other carbamate pesticides, the relative sensitivities of the chloroacetate derivatives of 10 hydrolyzed carbamate pesticides were compared. In addition the percentage recoveries of Banol from several agricultural products were compared with those obtained for carbaryl by this procedure.

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EXPERIMENTAL

Materials and Apparatus. Florisil, as received from the Floridin Co., lost 4.4% of its weight on heating to 500° C. An additional 20% water was added to the as-received Florisil for column chromatography. Chloroacetic anhydride (Eastman White Label) was recrystallized from benzene. A conventional gas chromatograph (Varian Aerograph Model 200) was equipped with a 5-foot × $^1/_8$-inch i.d. stainless steel column containing 2% (w./w.) General Electric XE-60 silicone polymer coated on 80- to 100-mesh acid-washed DMCS-treated Chromosorb W (Applied Science Laboratories, Inc., State College, Pa.), and maintained at 165° C. The tritium detector was held at 205° C., the injection heater at 210° C., and the nitrogen flow at 30 ml. per minute at the column outlet.

Extraction and Column Chromatography. BERMUDA GRASS, APPLES, CUCUMBERS, AND TOMATOES. A 50-gram portion of the shredded sample of Bermuda grass was blended with 200 ml. of methylene chloride in a Lourdes Model MM-1 Multimixer for 5 minutes. For the apples, cucumbers, and tomatoes, 100-gram subsamples were used. The emulsions that formed when apples and tomatoes were used broke if they were allowed to stand 10 minutes before filtering. The contents of the blender were carefully filtered to keep evaporation of the solvent to a minimum. A 50-ml. aliquot of the methylene chloride extract was percolated through 15 grams of Florisil in a Shell-type 20-mm. o.d. glass column previously washed with 50 ml. of water-saturated solution of methylene chloride. The column was eluted with an additional 75 ml. of water-saturated methylene chloride. The eluate (about 125 ml.) was collected in a 250-ml. separatory funnel.

MILK. Fifty grams of milk were blended for 5 minutes with 150 ml. of acetone and filtered through Eaton-Dikeman fluted filter paper, grade 512, into a 500-ml. separatory funnel. The filtrate was extracted first with 100 ml. and then with 50 ml. of methylene chloride. The combined methylene chloride–

Table I. Banol and 6-Chloro-3,4-xylenol (P.P.M.) Found by Duplicate Analyses of Treated Bermuda Grass

Replication	Days after Treatment					
	0	1	2	4	7	14[a]
1	28, 29 (<0.3)[b]	14, 17 (<0.1)	7.5, 8.2 (<0.1)	2.3, 1.6 (<0.1)	0.68, 0.54 (<0.1)	0.08, 0.10 (0.07, 0.06)
2	30, 35 (<0.3)	17, 21 (<0.1)	5.9, 6.9 (<0.1)	1.6, 1.4 (<0.1)	0.66, 0.72 (<0.1)	0.03, 0.05 (0.06, 0.08)
3	24, 29 (<0.3)	12, 12 (<0.1)	8.2, 7.2 (<0.1)	1.0, 1.1 (<0.1)	0.22, 0.34 (<0.1)	0.04, 0.05 (0.06, 0.06)
4	18, 33 (<0.3)	10, 11 (<0.1)	4.2, 5.1 (<0.1)	0.8, 1.4 (<0.1)	0.42, 0.48 (<0.1)	0.05, 0.07 (0.04, 0.08)
Check	<0.1, (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.03 (<0.02)

[a] Aliquot sizes increased to equivalent of 50 or 100 gram plant, to obtain satisfactory recorder response.
[b] Parentheses indicate 6-chloro-3,4-xylenol.

acetone extracts were percolated through anhydrous sodium sulfate and evaporated to near-dryness under a water-aspirator vacuum. The residue was dissolved in 50 ml. of methylene chloride for percolation through Florisil.

Separation of Carbamates from Their Phenols. Twenty-five milliliters of 0.25*N* NaOH were added to the methylene chloride eluate (125 ml.), and the separatory funnel was shaken for 3 minutes. The sodium hydroxide phase was transferred to a 125-ml. Erlenmeyer flask for analysis of phenols.

The methylene chloride phase was evaporated to near dryness under a water-aspirator vacuum at room temperature or lower in a 250-ml. Erlenmeyer flask. The residue was dissolved by adding 50 ml. of petroleum ether (b.p. 30° to 60° C.) and then 25 ml. of 0.25*N* NaOH. Hydrolysis of the carbamates present was allowed to proceed for 18 hours in the dark, though no study was made of the effect of light. The contents of the flask used for the evaporation and hydrolysis were transferred to a 125-ml. separatory funnel, where the sodium hydroxide layer was separated and washed with an additional 25 ml. of petroleum ether. The sodium hydroxide layer (or an aliquot made up to a 25-ml. volume with 0.25*N* NaOH) was transferred to a 125-ml. Erlenmeyer flask.

Chloroacetylation and Gas Chromatographic Analysis. Ten milliliters of reagent (1 gram of chloroacetic anhydride per 200 ml. of benzene) were added to the 125-ml. Erlenmeyer flask containing the sodium hydroxide solution. The flask was shaken mechanically for 3 minutes. Part of the benzene layer was transferred to a vial containing anhydrous sodium sulfate. A 5-μl. aliquot of the benzene phase was taken from the vial and injected into the gas chromatograph, and the response was compared with that obtained with 5-μl. injections of the standards.

Standards. Solutions of phenols and carbamates were prepared in concentrations of 500, 250, and 125 μg. per ml. in benzene and stored in polyethylene-capped amber-colored glass bottles (effect of ultraviolet light, Eberle and Gunther, 1965). Aliquots representing 5, 2.5, 1.25, and 0.62 μg. of the phenols and carbamates were added to 25 ml. of 0.25*N* NaOH in a 125-ml. Erlenmeyer flask. For the carbamates, hydrolysis was allowed to proceed for 18 hours as before. These solutions were then carried through the steps for chloroacetylation and gas chromatographic analysis along with the samples.

RESULTS AND DISCUSSION

Gunther *et al.* (1962), Johnson *et al.* (1963), and Whitehurst *et al.* (1963) separated phenols from carbamate pesticides in chloroform or methylene chloride by brief extraction with aqueous alkali. Methods of hydrolyzing the carbamate that remained in the organic phase included evaporation of methylene chloride in the presence of 0.25*N* NaOH and then solvent extraction of unwanted precipitates and contact hydrolysis of the carbamate dissolved in several immiscible solvents with 0.25*N* NaOH over an 18-hour period. However, failure with other solvents either to redissolve interferences after hydrolysis or to hydrolyze the carbamate in the organic phase resulted in the use of petroleum ether (b.p. 30° to 60° C.) as the only satisfactory solvent for this procedure. The lengthy hydrolysis period is not as objectionable as it first appears. During a work day, 10 or more samples can be extracted and chromatographed, and the phenols separated for analysis the same day. While the phenol fractions are being analyzed, the carbamate fractions are undergoing hydrolysis for completion of analysis the next morning. When hydrolysis of the carbamate fraction was speeded up by refluxing, a significant degree of saponification of certain plant materials took place that caused an emulsion to occur when the reagent in benzene was added.

Column chromatography was used to eliminate an interference caused by precipitation of certain plant extractives when sodium hydroxide solution was added to the petroleum ether and to remove colored material that would be injected into the gas chromatograph with the benzene. The addition of large amounts of water to the Florisil was essential. Relative recoveries of 95 to 100% were obtained for carbamate and phenol standards percolated through the column, as described when compared with identical standards not percolated through the column. Though a yellow discoloration occurred in the elute (strongly red for ripe tomatoes) due to a decrease in the adsorptive properties of the water-loaded Florisil, the color remained with the methylene chloride layer in the phenol-carbamate separation step and with the petroleum ether layer in the hydrolysis step.

Table I shows the residues found on Bermuda grass treated with Banol at a rate of 1.12 kg. of active ingredient per hectare. The variability of these results on duplicates, not aliquots, ranges as high as $\pm 20\%$ in some instances and probably is due to sampling. Since the residues obtained at 14 days after spraying were low, larger aliquots (50 or 100 grams) were used to obtain sufficient response on the recorder. The recoveries of Banol in Bermuda grass averaged 83% at a level of 30 p.p.m., 86% at 3 p.p.m., and 95% at 0.1 p.p.m. The recoveries of 6-chloro-3,4-xylenol in Bermuda grass averaged 70% at 5 p.p.m., and 60% at 0.1 p.p.m. Also, when this method, primarily designed for the determination of Banol in Bermuda grass, was applied to apples, tomatoes, and cucumbers, the recoveries averaged 91% at a level of 0.2 p.p.m. and 74% at a level of 0.1 p.p.m. To establish these recoveries, microgram amounts of standards were added to the crops prior to the extraction step of the procedure. These fortified crops were then carried through the gas chromatographic step of the procedure and compared with standards that were added to 60-ml. Erlenmeyer flasks, hydrolyzed, and chloroacetylated as described in the procedure. The concentrations of the standards used was such that for a 100% recovery, the response obtained for the sample aliquot would be identical with that for the flask that contained the largest

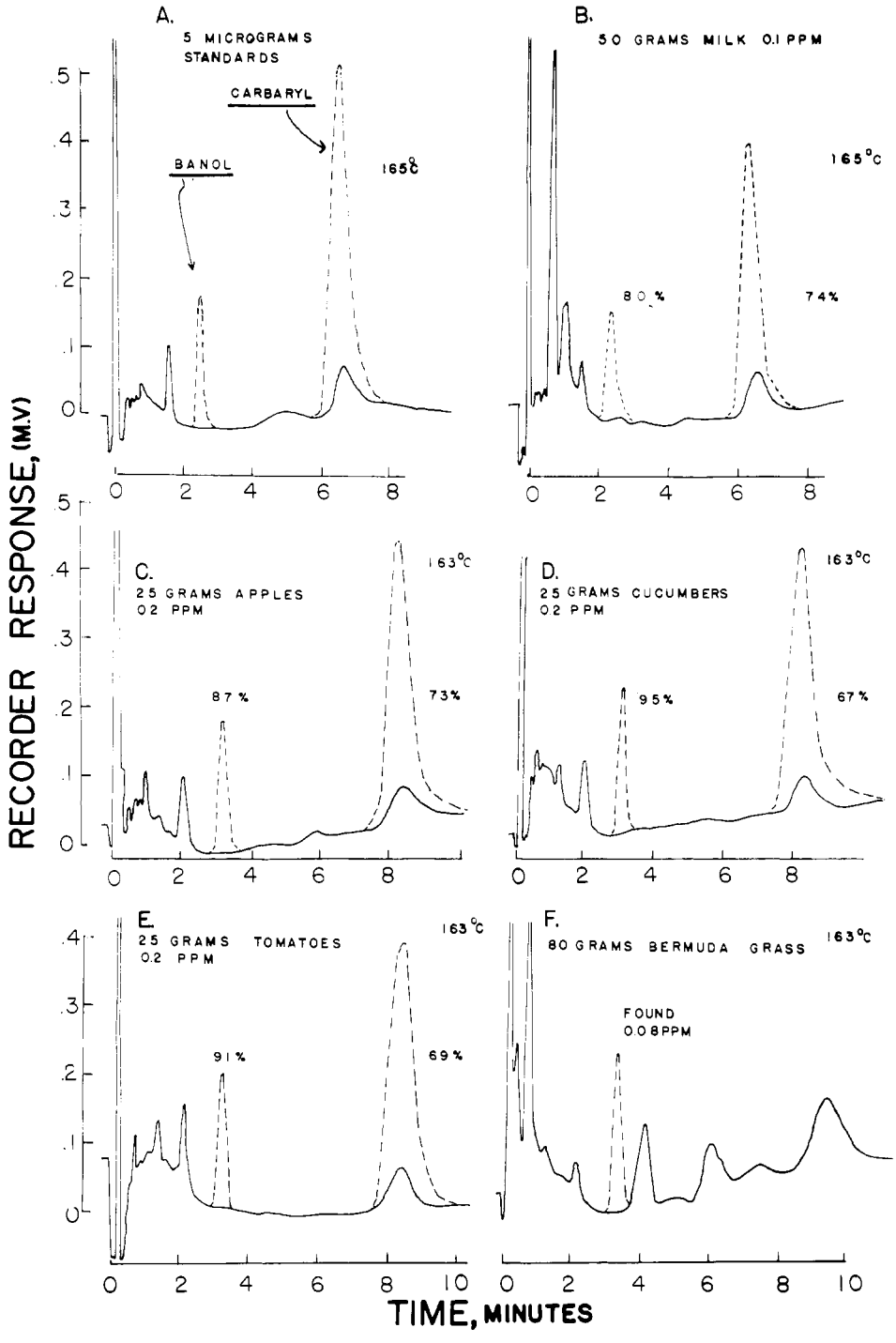

Figure 1. Chromatograms after hydrolysis and chloroacetylation

5-μl. aliquot of 10-ml. benzene phase injected
A. 5 μg. of Banol and carbaryl standards in 25 ml. NaOH solution
B,C,D,E. Recoveries in milk, apples, cucumbers, and tomatoes
F. Banol in Bermuda grass; solid line represents reagent and crop blanks

amount of standard. Hence, a comparison with the less concentrated standards was readily available to estimate recoveries of less than 100%.

Actual chromatograms showing sample recoveries of Banol and carbaryl are given in Figure 1; chromatograms showing recoveries of the corresponding hydrolysis products, 6-chloro-3,4-xylenol and 1-naphthol, are given in Figure 2. An interference under the carbaryl and 1-naphthol peaks equivalent to about 0.05 mv. (from Figure 3, a 5-μl. injection of the 2-μg. Banol standard would produce the same recorder deflection) was attributed to the chloroacetic anhydride after benzene, sodium hydroxide, and water were eliminated as sources of this impurity. Recoveries of carbaryl in Bermuda grass averaged 80% at a level of 0.2 p.p.m. for two determinations. Typical recoveries of carbaryl in apples, tomatoes, and cucumbers at levels of 0.1 and 0.2 p.p.m. ranged between 67 and 79%. In milk, the average recovery of Banol at 0.1 p.p.m. was 82% and that of carbaryl was 76% for two determinations. In general, the recovery of 6-chloro-3,4-xylenol averaged 76% and that of 1-naphthol averaged 69%. In all studies of recovery, the crop was fortified at the appropriate level before extraction with solvent. Peak heights were com-

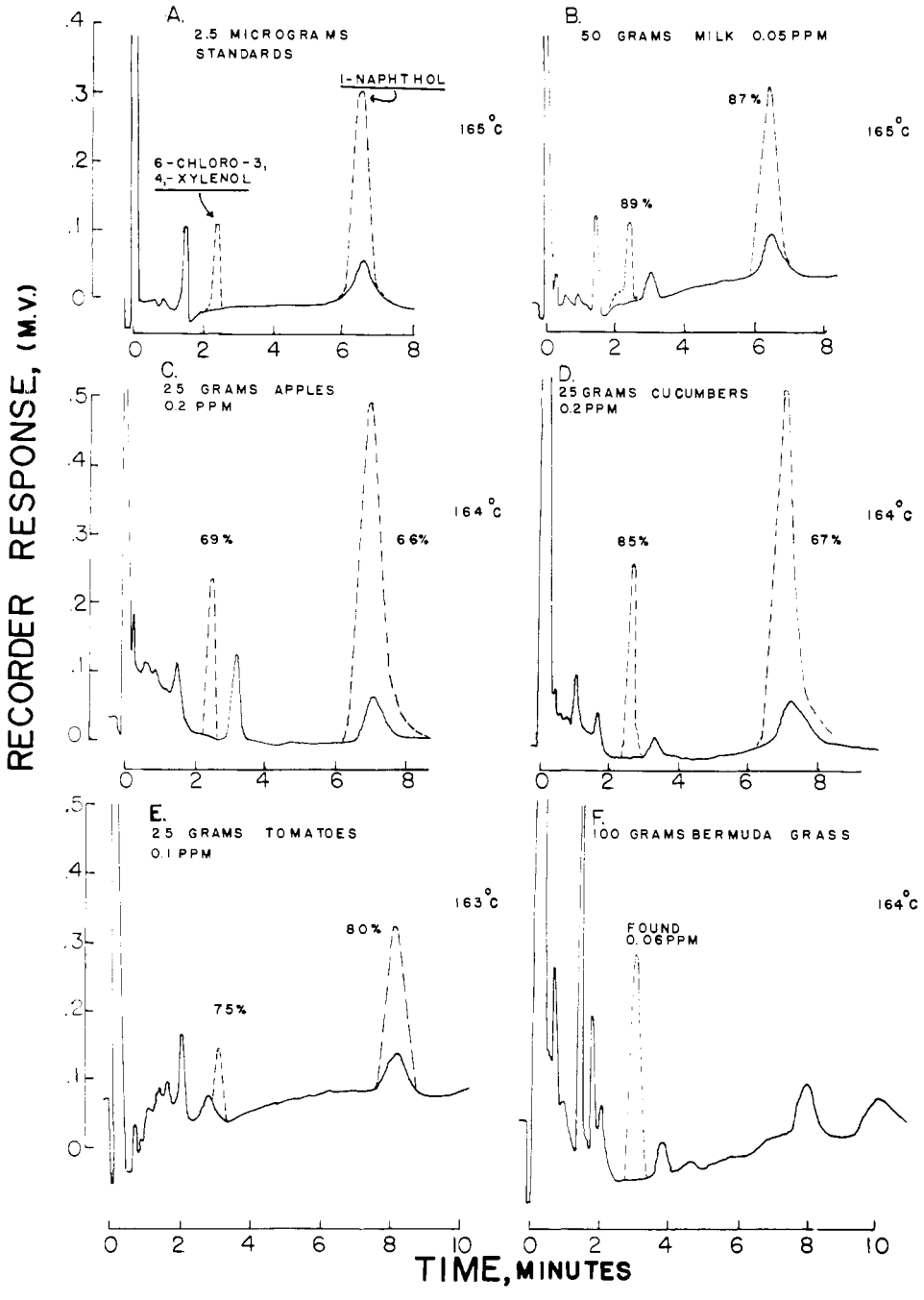

Figure 2. Chromatograms after chloroacetylation

5-μl. aliquot of a 10-ml. benzene phase injected
A. 2.5 μg. of 6-chloro-3,4-xylenol and 1-naphthol standards in 25 ml. NaOH solution
B,C,D,E. Recoveries in milk, apples, cucumbers, and tomatoes
F. 6-Chloro-3,4-xylenol in Bermuda grass; solid line represents reagent and crop blanks

pared against prepared standards and chromatographed the same day. The apparent variation in retention times was caused by changes in column oven temperature and rate of gas flow.

Banol, carbaryl, and eight other carbamates at five concentration levels (1.0, 2.5, 5.0, 10, and 20 μg.) were hydrolyzed in 25 ml. of 1% NaOH, then chloroacetylated, and the response of the derivatives to electron capture was determined. The results plotted in Figure 3 give the relative retention times and the linear range of recorder response for these derivatives compared with a heptachlor epoxide (1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindan) standard. Since heptachlor epoxide is a widely used pesticide standard in most residue laboratories and offers excellent response to electron-capture detection, it was chosen as a reference to indicate the minimum response that should be expected for the chloroacetate derivatives. Also, the relative response is compared in Table II by using the product of retention time and recorder response at maximum peak height at similar levels of concentration. The response factors for the chloroacetates, beside reflecting the electron-withdrawing strength of the substituents, include an additional factor. The chloroacetates were prepared by reaction of a benzene reagent solution with an aqueous solution containing the hydrolyzed carbamate. The rate of chloroacetate formation probably is not the same for all the carbamates considered.

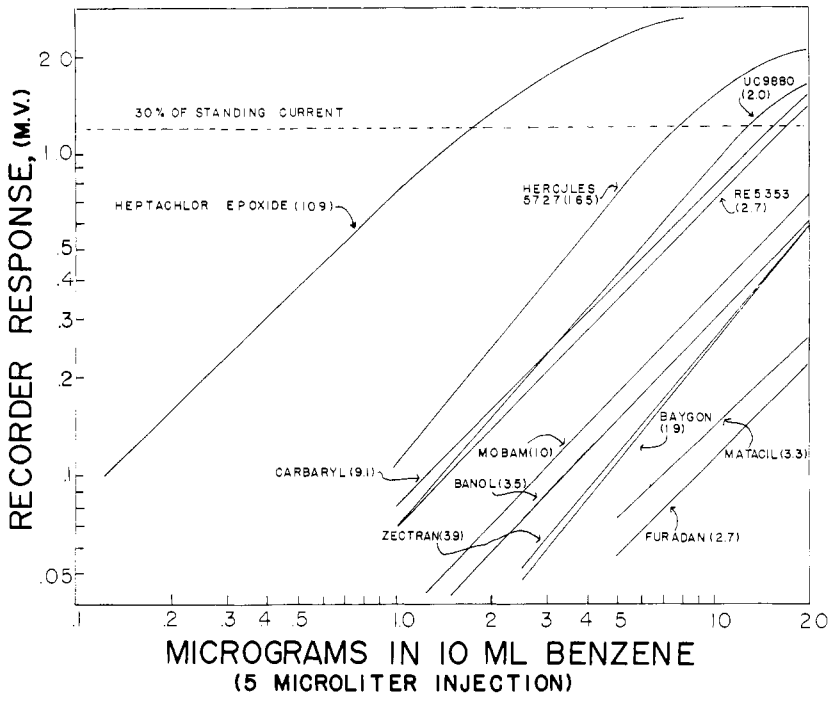

Figure 3. Peak heights for chloroacetate derivatives of 10 hydrolyzed carbamate pesticides compared with heptachlor epoxide standard

Values in parentheses indicate retention time in minutes from benzene front. Since 1 mv. represents full scale recorder deflection, electrometer attenuation was increased to plot values above 0.8 mv.

Table II. Relative Response of Chloroacetate Derivatives of Several Hydrolyzed Carbamates with Heptachlor Epoxide to Electron-Capture Detection

Pesticide	Methylcarbamate	Relative[a] Response Factor
Niagara NIA-10242 (Furadan)	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl	48
Bay 39007 (Baygon)	*o*-Isopropoxyphenyl	67
Matacil	4-(Dimethylamino)-*m*-tolyl	71
Zectran	4-(Dimethylamino)-2,5-xylyl	130
Banol	6-Chloro-3,4-xylyl	165
Union Carbide UC-9880	*m*-Cym-5-yl	285
Chevron RE 5353	*m*-(1-Methylbutyl)phenyl	300
Hercules 5727	*m*-Isopropylphenyl	395
Mobam	Benzo[*b*]thien-4-yl	600
Carbaryl	1-Naphthyl	1200
Heptachlor epoxide	(Comparison standard in benzene)	13000

[a] GC electrometer: range input impedance EC 10, attenuation × 1. Distance in cm. from benzene solvent × peak height × 0.1 from chromatograms of 5-μg. carbamate standards taken through steps for standards and chloroacetylation and gas chromatography.

Where the rate of formation is slow, compared with the loss of reagent by hydrolysis, the amount of that specific hydrolyzed carbamate derivatized will be small, as may be reflected by the response factors obtained.

The minimum reproducible detectability for other carbamates can be estimated by comparing the peak heights of the reagent and control samples shown in Figure 1 with the retention times and recorder response shown in Figure 3—for example, Union Carbide UC-9880 (retention time 2.0) has a relative recorder response about three times greater than Banol. However, a peak interference equivalent to 0.1 mv. of recorder response for 25 grams of apples would mean that the lower limit of practical detection could not be extended below concentrations less than 2.5 μg. in 25 grams (0.1 p.p.m.).

The usefulness of the chloroacetylation procedure has been demonstrated by analyzing two carbamates found in several agricultural products. Though the recoveries for Banol are satisfactory, the results obtained for carbaryl suggest that possible modification should be considered before the procedure is extended to other carbamates in general.

As is usually the case, the practical lower limit of sensitivity depends on the amount of plant substances present that may interfere with the final determination. In several instances where a large amount of extracted plant substances appeared present, the Florisil in the chromatographic step was increased to 50 grams with an additional 25 ml. of methylene chloride used for elution, without affecting the recovery of that step.

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