Table I. Recovery of Fenthion from Florisil Column

Fraction	Weight, %	Fraction	Weight, %
1	79.7	6	0.9
2	7.8	7	0.8
3	3.5	8	0.6
4	2.1	9	0.5
5	1.8	10	0.2

Total = 97.9%

Table II. Reproducibility Study Given in Per Cent

Day 1	Day 2	Day 3	Day 4
91.1	89.8	89.9	89.1
90.7	90.0	89.3	91.3

Average = 90.0
Standard deviation = 0.8
95% confidence limits = ±1.9

fenthion. The over-all recovery of fenthion was determined by chromatographing replicate 500-mg. samples of standard fenthion as described. The four 500-mg. samples yielded: 95.7, 94.6, 94.7, and 95.8% for an average of 95.2%. Each batch of Florisil is standardized and a recovery factor (usually 1.05) applied to all subsequent determinations. Thus, essentially 100% of the fenthion added was recovered.

A recovery of 94.7% was obtained when 50 mg. of standard fenthion was chromatographed using 1 gram of Florisil in a 6-mm. column. This shows that the reduction in the column size and sample size gave the same recovery. Thus, if limited sample is available, smaller columns of proportionate size and capacity can be used.

Replicate determinations on one sample by two people on 4 days are shown in Table II. Nonreproducible results may be caused by incomplete adsorption of the polar impurities owing to improper packing and/or a high flow rate. Florisil should be protected from moisture at all times in order to maintain reproducibility.

(I) is the only nonpolar compound which interferes at 252 mμ. However, it can be determined independently by GLC or TLC then applied as a correction to the fenthion value.

Acknowledgment

The author is indebted to members of the Method Development Group for technical assistance. He is grateful to the Process Development Group for samples of pure compounds and many fruitful discussions.

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ANALYTICAL PROCEDURE

Colorimetric Determination of Dimethoate Residues in Plants and Milk

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An improved colorimetric method for the quantitative estimation of dimethoate (Cygon, Rogor) residues in plants and milk is based on treatment of dimethoate residues with methanolic sodium hydroxide and 1-chloro-2,4-dinitrobenzene to form a colored product, which is measured spectrophotometrically. The calibration curve conforms to Beer's law at the peak wavelength of 505 mμ. No interference was encountered from 38 other pesticides or from the organic solvents used in the method. Residue data were obtained on lima beans, green beans, cabbage, range forage, turnip greens, and tomatoes.

PUBLICATIONS devoted to the quantitative determination of residues of dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate, Cygon, Rogor] have been discussed by Giang and Schechter (*1*). These authors have developed and discussed in detail an analytical procedure for residues of this compound based on the reaction between thioglycolic acid and phospho-18-tungstic acid.

In the colorimetric method described herein, dimethoate is treated with methanolic sodium hydroxide and 1-chloro-2,4-dinitrobenzene to form a colored product. The color development procedure is a modification of the amine test reported by Snell and Snell (*4*), based upon the work of McIntire, Clements, and Sproull (*3*). Kolbezen, Eckert, and Bretschneider (*2*) reported a modification to eliminate the high background absorbance due to the formation of 2,4-dinitrophenetole. The procedure appears to be less subject to interference from different contaminants that may be present in sample extracts than the method of Giang and Schechter.

The oxygen analog of dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothioate] also reacts to form the color by the present method, but it tends to get lost in the cleanup procedure, so that both dimethoate and its oxygen analog cannot be determined in the same crop sample. Walker and Beroza (*7*) report separation of the two by using thin-layer chromatography, and suggest that it could be used as the differentiation procedure for cleanup of residue samples. Van Middelem and Waites (*6*) compared gas chromatography results with the colorimetric method.

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However, they reported that the oxygen analog could not be satisfactorily separated from the parent material or quantitatively detected under the gas chromatographic conditions specified. Steller and Curry (5) described a total phosphorus method incorporating a thin-layer chromatographic procedure for the determination of both dimethoate and the oxygen analog.

Reagents

CHLORODINITROBENZENE SOLUTION, 2%. Recrystallize 1-chloro-2,4-dinitrobenzene by dissolving it in a minimum volume of 95% ethyl alcohol, filter the solution, and precipitate the dissolved material by the addition of distilled water. Filter the precipitate, wash with water, and dry overnight in a vacuum desiccator. Prepare the solution by dissolving 2.0 grams of the recrystallized material in 100 ml. of absolute ethyl alcohol. Mix the reagent solution daily or just before use and store in a refrigerator. (1-Chloro-2,4-dinitrobenzene is toxic by contact and also can cause acute allergic reactions in susceptible individuals; use with care.)

METHANOLIC SODIUM HYDROXIDE, 0.5*N*. Dissolve reagent grade sodium hydroxide in methanol (reagent grade, redistilled).

LANOLIN SOLUTION, 0.5%. Dissolve anhydrous lanolin in chloroform (reagent grade, redistilled).

COAGULATING SOLUTION. Dissolve 0.5 gram of ammonium chloride in 400 ml. of water containing 1.0 ml. of phosphoric acid.

NUCHAR C-190-N (Industrial Chemical Sales Division, West Virginia Pulp and Paper Co., Covington, Va.) or equivalent.

COTTON. Extract cotton in a Soxhlet extractor with acetone; dry in air and then in a 100° C. oven.

DIMETHOATE STANDARD SOLUTION. Dissolve 10 mg. of dimethoate, 99% purity (American Cyanamid Co., P. O. Box 400, Princeton, N. J.), in 100 ml. of redistilled methylene chloride. Dilute an aliquot of this solution with methylene chloride, so that 1 ml. contains 10 μg. of dimethoate.

Preparation of Standard Curve

Pipet aliquots, containing 0 to 100 μg. of the standard dimethoate solution, into a series of glass-stoppered test tubes. Add 1 drop of lanolin solution to each tube to prevent loss during the evaporation. Carefully evaporate the solvent in a 70° C. water bath in the hood, and remove the last traces of solvent with slight vacuum at room temperature. Then add 1.0 ml. of methanolic sodium hydroxide to each sample and heat in a 60° C. water bath for 10 minutes. Cool the solution at once in a cold water bath. Add 0.1 ml. of the 1-chloro-2,4-dinitrobenzene reagent to each of the tubes; stopper and shake the tubes on a mechanical shaker for 10 minutes. Add 2.0 ml. of absolute ethyl alcohol and swirl. Fill matched Corex D glass cuvettes (10-mm. light path) with the colored solutions and measure the absorbance in a spectrophotometer (Beckman Model B or equivalent) at 505 mμ against the blank solution which has been carried through the same procedure. Prepare the standard curve by plotting the absorbance readings against micrograms of dimethoate.

The curve follows Beer's law at 505 mμ, in the range of 5 to 100 μg. of dimethoate, and it has a slope of 0.0118 absorbance unit per microgram (or 0.1 absorbance unit per 8.5 μg.). The reagent blank solution (using absolute ethyl alcohol as reference) gives an average absorbance of 0.040 ± 0.003.

Analysis of Plant Samples

Cut the sample into small pieces, macerate with a measured volume of methylene chloride (reagent grade, redistilled) (about 2 ml. per gram of plant material is usually sufficient) in a blender for about 3 minutes, and strain the extract (if necessary) through cheesecloth. If the methylene chloride and aqueous layers do not separate readily, add granular sodium chloride to the blender or transfer the extract into a centrifuge bottle and centrifuge for 10 minutes. Filter the methylene chloride layer through a Gooch crucible holder containing cotton and anhydrous sodium sulfate. If the extract contains large amounts of plant pigments, shake with 5 grams of Nuchar and aluminum oxide mixture (1 to 1 ratio) and filter again. Measure the volume recovered and evaporate the solvent carefully with a rotatory evaporator on a warm water bath, or on a steam bath with the use of a Snyder column. Remove the last traces of solvent with slight vacuum at room temperature. Evaporate only until the odor of methylene chloride has been removed.

Heat the waxy residue with 50 ml. of the coagulating solution on the steam bath, with occasional swirling until the wax is melted, then chill thoroughly in an ice bath. Filter the solution through a short chromatographic column containing 6 grams of a mixture made by mixing 4 parts of Hyflo Super-cel and 6 parts of Attapulgus clay (Attapulgus Clay Co., Philadelphia, Pa.) into a separatory funnel. When the level of the coagulating solution reaches the top of the column, wash the column with two 25-ml. aliquots of water, and filter into the same separatory funnel.

Extract the aqueous layer with 25 ml. of methylene chloride by vigorously shaking for at least 1 minute. Filter the methylene chloride layer through anhydrous sodium sulfate into a flask. Extract the aqueous layer in the funnel again with two successive 15-ml. portions of methylene chloride and filter through the same sodium sulfate filter.

Add 2 drops of lanolin to the methylene chloride extract and evaporate the solvent with a rotatory evaporator on a warm water bath or on a steam bath with a Snyder column until about 5 ml. is left. Quantitatively transfer the extract into a test tube with two successive 3-ml. methylene chloride rinses. Evaporate the solution carefully almost to dryness in a hot water bath; remove the last traces of solvent with slight vacuum at room temperature.

Add 1.0 ml. of methanolic sodium hydroxide solution to the residue in the tube, and finish the analysis as described for preparation of the standard curve.

Analysis of Milk Samples

Cool 50 ml. of the milk sample in a large separatory funnel to 10° C., and then extract by shaking with 2 equal volumes of cold methylene chloride. Add a few milliliters of water to hasten the separation into two distinct layers; if necessary, centrifuge the methylene chloride layer. With the aid of a rotatory evaporator or of a three-bulb Snyder column on a steam bath, concentrate the combined extract to approximately 5 ml.

Using 25 ml. of acetonitrile (reagent grade, redistilled), transfer the concentrated extract into a 125-ml. separatory funnel. Rinse the flask with two 5-ml. portions of acetonitrile, and add the rinses to the funnel. Wash the combined acetonitrile extract in the funnel with three 50-ml. portions of pentane (reagent grade, redistilled) by vigorous shaking. Drain the acetonitrile extract into an Erlenmeyer flask and concentrate the extract down to approximately 5 ml. at 60° C. with a rotatory evaporator. Quantitatively transfer the extract into a test tube with two successive 3-ml. methylene chloride rinses. Add a drop of the lanolin solution and evaporate the extract in a warm water bath in the hood. Remove the last traces of solvent carefully with a little vacuum at room temperature. From this point on, complete the analysis as described for preparation of the standard curve.

Tests Made with Method

Recovery from Plant Materials. A weighed amount of the plant sample

Table I. Recovery of Dimethoate Added to Plants and Milk

Sample	No. of Analyses	Av. Size of Sample, Grams	Dimethoate Added, μg. Low	Dimethoate Added, μg. High	Av. Recovery, %
Dry lima beans	4	25	8	20	80.5 ± 3.5
Green beans	14	60	9	60	74.2 ± 19.5
Cabbage	17	30	4	90	80.8 ± 22.0
Range forage	18	4	6.5	100	78.0 ± 19.0
Turnip greens	4	25	25	50	78.2 ± 12.5
Tomatoes	4	40	12	40	88.0 ± 15.0
Milk	7	100	5	66	75.3 ± 11.5

was cut up and placed in a blender, and an aliquot of a standard methylene chloride solution of dimethoate was added. The sample was blended with methylene chloride and analyzed by the method described. Results obtained with dry lima beans, green beans, cabbage, range forage, turnip greens, and tomatoes are shown in Table I.

Recovery from Whole Milk. Aliquots of a standard methylene chloride solution of dimethoate were added to a number of weighed whole milk samples. Each was extracted with methylene chloride and analyzed by the method. The results are also included in Table I.

Typical blank results obtained from the analyses of a number of plant and milk control samples (samples analyzed without the addition of the dimethoate standard solution) by the described method are shown in Table II.

Dimethoate was sprayed on field crops of lima beans, green beans, cabbage, range forage, turnip greens, and tomatoes. Samples were taken from the field at intervals after the spray and kept frozen until the time of analysis. The data and results of the study are shown in Table III.

Discussion

In extracting fibrous plant samples, such as range forage or bean plants, straining the methylene chloride extract through a few layers of cheesecloth was found necessary. With gentle pressing or squeezing, more extract can be expressed from the plant pulp.

For plant extracts with high pigmentation, treatment with a larger amount of Nuchar and aluminum oxide mixture is helpful; for certain samples several treatments may be required. The filtrate from the extraction should be colorless in order to avoid any high absorbance reading from the control sample of the plant material.

The authors found that dimethoate extract may be stored in methylene chloride for a few days in a refrigerator or in a cold room, but once the sample is hydrolyzed with methanolic sodium hydroxide, the analysis should be carried through the remainder of the method.

Thirty-four insecticides, three herbicides, and one fungicide, as well as the organic solvents used in the method, were tested for possible interfering effects on the proposed colorimetric method and it was found that none of the compounds causes any interference. The following pesticides did not interfere in the analysis: Aramite, aldrin, amitrole, benzene hexachloride, chlordan, chlorobenzilate, DDT, dioxathion, demeton, diazinon, dicapthon, dieldrin, Dilan, Dimite, disulfoton (Di-Syston), endrin, ethion, azinphosmethyl (Guthion), heptachlor, heptachlor epoxide, dinocap,

Table II. Interference Values of Control Samples

Sample	No. of Analyses	Av. Size of Sample, Grams		Apparent Residue, P.P.M.[a]	
		Low	High	Low	High
Dry lima beans	13	32.5	32.5	<0.1	0.31
Green beans	12	5.0	65.0	<0.1	0.07
Cabbage	17	25.0	60.0	<0.1	0.17
Range forage	13	2.1	33.3	<0.1	0.30
Turnip greens	4	5.0	10.0	0.3	0.52
Tomatoes	8	50.0	50.0	<0.1	0.09
Milk	6	50.0	50.0	<0.1	0.05

[a] Apparent dimethoate residue values are calculated.

Table III. Residue of Dimethoate in Field-Sprayed Plants

Plant	Dimethoate per Acre, Lb.	Sampling, Days after Application	Sample Analyzed, Grams	Residue, P.P.M.[a,b]
Lima beans	0.5	34	22.5	<0.1
	1.0	34	32.5	<0.1
Green beans	0.7	0	5.0	3.7
		7	10.0	1.1
		14	50.0	0.3
		21	50.0	0.1
		28	50.0	<0.1
	1.4	0	5.0	8.2
		7	10.0	2.1
		14	50.0	0.7
		21	50.0	0.1
		28	50.0	<0.1
	0.75	37	50.0	<0.1
Cabbage	0.5	0	50.0	0.8
		3	50.0	0.2
		7	50.0	<0.1
		14	50.0	<0.1
Range forage	0.11	0	2.0	3.2
	0.22	0	2.0	14.2
		1	2.0	3.9
		7	5.0	1.4
	1.0[c]	0	4.0	3.5
		1	3.5	1.8
		3	7.4	1.1
		7	21.8	0.4
		14	31.7	0.3
		21	28.7	0.4
	3	0	4.0	15.6
		3	3.8	2.8
		7	3.6	2.2
		14	17.0	1.7
		21	33.0	2.0
	1	0	4.0	6.2
		3	3.6	2.0
		7	7.1	0.7
		14	23.0	0.2
		21	50.0	<0.1
	3	0	4.2	23.0
		3	4.0	8.4
		7	16.5	1.7
Range forage	3	14	32.0	1.1
		21	33.8	1.0
	2.0	0	2.4	23.7
		1	2.4	18.8
		2	4.0	2.5
		4	7.5	0.8
	4.0	0	2.3	67.4
		1	2.3	50.4
		2	2.3	30.6
		4	2.5	15.5
		8	4.5	1.8
Turnip greens	0.5	0	2.0	35.6
		10	10.0	1.3
		14	10.0	0.8
Tomatoes	0.75	40	50.0	<0.1

[a] Corrected for control sample readings.
[b] Average of two or three determinations.
[c] Green grass.

dicofol (Kelthane), malathion, maleic hydrazide, methoxychlor, methyl parathion, naled, ovex, parathion, phorate, phosphamidon, schradan, carbaryl, tetradifon, endosulfan, toxaphene, carbophenothion, and 2,4-D.

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PLANT REGULATOR DETERMINATION

Determination of 2,4-Dichlorophenoxyacetic Acid and 2-(2,4,5-Trichlorophenoxy)propionic Acid in Citrus by Electron Capture Gas Chromatography

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Very dilute sprays (20 p.p.m.) of 2,4-dichlorophenoxyacetic acid and 2-(2,4,5-trichlorophenoxy)propionic acid will prevent preharvest fruit drop in citrus. A gas chromatographic electron capture method is described in which various forms of 2,4-D and 2,4,5-TP are isolated as the free acid and converted to 2-butoxyethyl esters for analysis. These esters are readily resolved from interfering citrus materials. Data show that 0.125 μg. can be determined in 500 grams of citrus peel (0.00025 p.p.m.) with 89 to 93% recovery and good reproducibility. The method demonstrates an important means of studying the metabolism of very low concentrations of these growth regulators in citrus.

WARM, dry, fall and winter weather, in Florida, can bring about preharvest fruit drop resulting in losses of 50 to 75% of the midseason Pineapple orange crop. Dilute sprays (20 p.p.m.) of 2,4 - dichlorophenoxyacetic acid (2,4-D) and 2-(2,4,5-trichlorophenoxy)-propionic acid (2,4,5-TP) inhibit preharvest abscission of oranges.

Isopropyl 2,4-D has been investigated in California and Florida for control of fruit drop (*2, 5, 7*), and 2,4-D is now registered for this use.

In Florida, 2,4,5-TP as the propylene glycol butyl ether ester (Kuron, Dow Chemical Co.) (*3*) and as the triethanolamine salt (*6*) has been demonstrated to be effective for fruit drop control of citrus. 2,4,5-TP is of importance because it is as effective as 2,4-D and causes less foliage damage to the spring flush of growth; however, 2,4,5-TP is not registered. Therefore, a sensitive and accurate method was needed for determining the relative residues of 2,4-D and 2,4,5-TP in citrus occurring under Florida climatic conditions.

Erickson and Hield (*1*) reported 2,4-D residues of 0.1 p.p.m. of free acid in whole oranges within one day after spraying. This approached the lower limit of measurement by their microcoulometric gas chromatographic method.

Since the electron capture detector is reported to be 1000 times more sensitive to many halogenated compounds, use of this type of detection should provide more definitive information concerning growth regulator residue concentrations below 0.1 p.p.m.

Cleanup procedures for citrus present special problems when the electron capture detector is used because of the wide variety of chemical entities in the oils and waxlike materials. If not removed, these rapidly foul the electron capture detector, greatly lowering sensitivity.

Many substances remaining in citrus even after cleanup have retention times very similar to those of 2,4-D and 2,4,5-TP methyl esters most commonly used in gas chromatography (*1, 4*). Presence of these interfering substances makes accurate quantitative measurements difficult or impossible because they produce peaks which are ill-defined, frequently unresolved from the solvent peak (Figure 1). Another ester was sought that would have a more desirable retention time. Yip (*8*) studied six commercial herbicide esters using microcoulometric gas chromatography and reported that the butoxyethyl ester of 2,4-D had the longest retention time. Therefore, this ester was selected for investigation.

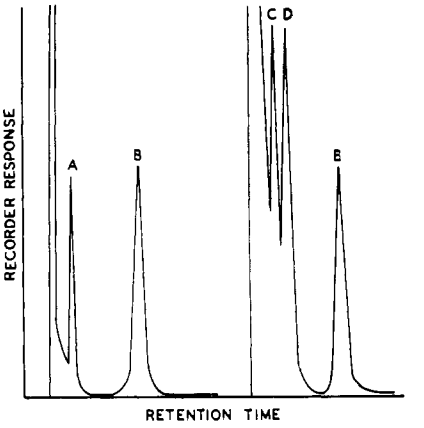

Figure 1. Relative retention times of 2,4-D methyl ester (Peak A) and 2,4-D butoxyethyl ester (Peak B)

In the tracing on right, butoxyethyl ester peak (B) is shown in the presence of citrus extractives. C and D are peaks from citrus. The appearance of peak B following the solvent peak represents a time lapse of 3 minutes

Experimental

2-Butoxyethanol—e.g., butyl Cellosolve—in the presence of dry HCl rapidly esterified the growth regulator acids. Butoxyethyl ester peaks appear after interfering citrus peaks on the chromatograph recording (Figure 1). Furthermore, 2-butoxyethanol has excellent solvent properties for citrus extractives and excess quantities of the reagent can readily be removed from the reaction mixture.