An Improved Colorimetric Method for Determining Endosulfan (Thiodan) Residues in Vegetables and Beef Fat

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Residues of endosulfan (Thiodan) were determined in chard, collards, lettuce, sugar beets, table beet tops, turnip greens, and beef fat. The samples were extracted with *n*-hexane or *n*-pentane, the extracts cleaned up, evaporated to dryness, and reacted with methanolic NaOH and aqueous pyridine. The reaction, carried out in a single test tube, requires no special apparatus, is adaptable to routine laboratory work, and is applicable to 5 to 100 μg. of endosulfan (Thiodan). Chlorinated solvents, such as chloroform, carbon tetrachloride, or dichloromethane, cause no interference, and special purification of the pyridine reagent is not required. Captan, chlordan, heptachlor, and ovex are the only pesticides out of 45 tested that interfere with the method.

ANALYTICAL METHODS for the determination of endosulfan (Thiodan) (6,7,8,9,10,10 - hexachloro - 1,5,5*a*,6,9,-9*a* - hexahydro - 6,9 - methano - 2,4,3-benzodioxathiepin-3-oxide) have been reviewed by Butler, Fahey, and Maitlen (*1*). The method used in the work reported here is a modification of that proposed by those authors. A lower reagent blank is produced in the modified method, and filtration of the solution at the color development stage is not required. The method is not affected by chlorinated solvents, and no special purification of the pyridine reagent is necessary. Captan, chlordan, heptachlor, and ovex caused some interference, but 41 other pesticides did not. The method will determine both isomers of endosulfan, is applicable to amounts of 5 to 100 μg. of the technical material, and the absorbance follows Beer's Law throughout this range.

Reagents

Endosulfan Standard Solution Dissolve 0.1063 gram (94% technical endosulfan (Niagara Chemical Division, FMC Corp.) in 1 liter of *n*-hexane that has been distilled over metallic sodium. One milliliter of this solution contains 100 μg. of technical endosulfan. Dilute an aliquot of this solution with distilled *n*-hexane so that 1 ml. contains 10 μg.

Pyridine Solution. Add 4 ml. of distilled water to 96 ml. of ACS-grade pyridine.

Methanolic Sodium Hydroxide (0.025*N*). Dissolve 4 grams of ACS-grade NaOH in ACS-grade methanol, cool to room temperature, and make to 100 ml. with the methanol. Dilute 10 ml. of this solution to 400 ml. with methanol. The reagent is stable for several weeks.

Pyridine–Methanolic Sodium Hydroxide Reagent. Add 10 ml. of the 0.025*N* methanolic NaOH to 50 ml. of the pyridine solution. This reagent is stable for only about 6 hours and should be made just before using.

n-Hexane. Distilled over metallic sodium.

Adsorbent Mixture. Nuchar C-190-N (carbon, decolorizing) and Magnesol (magnesium oxide), mixed at 1:1 ratio by tumbling in an end-over-end fashion for 1/2 hour.

Cotton. Acetone extracted and oven dried.

Apparatus

Oil bath (100° C. ± 2° C.).
Spectrophotometer.
Water bath (about 50° C.).

Preparation of Standard Curve

Pipet aliquots of the endosulfan standard solution containing 0 to 100 μg. into glass-stoppered test tubes. Dilute to a volume of 10 ml. with distilled *n*-hexane. Evaporate to dryness in the warm-water bath using a gentle stream of air. Remove samples from the bath, add 5 ml. of the methanolic NaOH–pyridine reagent, stopper the tubes, and place in the oil bath for 4 minutes. Remove from the bath and place in ice water for 1 minute. (Care should be taken at this point to loosen the stoppers since cooling may cause them to freeze in the tubes.) Decant the solution into 1-cm. cells and determine the absorbance within 10 minutes at 520 mμ, using distilled water or a reagent blank as a reference.

Discussion of Method

The color was checked at regular time intervals for a period of 1/2 hour after

Table I. Recovery of Endosulfan (Thiodan) Added to Control Samples

Sample[a]	Added (μg.)	Recovery (%)
Chard	200	90
	800	89
	6000	87
Collards	200	91
	300	89
	500	86
	1000	90
	1500	84
	3000	73
	4000	80
Sugar beets	9	78
	9	88
	12.5	92
	15	102
	15	120
	15	98
	15	88
	15	100
	15	82
	20	75
	30	88
	30	128
	30	78
	30	76
	60	86
Turnip greens	300	86
	500	84
	500	85
	500	83
	3000	88
Leaf lettuce	200	88
	500	83
	5000	82
Table beet tops	100	102
	200	87
	300	87
	500	87
	3000	83
Beef fat[b]	50	76
	50	78

[a] 100-gram sample. [b] 10-gram sample.

Table II. Values for Control Samples

Sample[a]	Number of Samples Analyzed	Weight of Aliquot Analyzed, Grams	Parts per Million Calculated as Endosulfan	
			Low	High
Chard	6	1 to 5	0	0
Collards	7	1 to 5	0	0.04
Sugar beets	12	100	0	0.01
Turnip greens	9	5 to 10	0	0.08
Leaf lettuce	7	100	0	0.06
Table beet tops	6	100	0	0.02
Beef fat	6	10	0.2	0.2

[a] 100-gram samples extracted and aliquots of the extract taken for analysis.

Table III. Endosulfan (Thiodan) Residues in Treated Crops, Expressed in Parts Per Million

Crop	Residue[a] Days after Last Application					
	0	7	10	14	150	180
Chard	57.1	6.8	...	2.3	...	...
Collards	38.7	10.1	5.3	2.9	...	...
Lettuce	68.5	8.0	...	1.6	...	...
Table beet tops	34.4	4.7	3.2	1.2	...	...
Turnip greens	37.5	...	5.4	5.1	...	...
Sugar beets	...	...	...	...	0	0

[a] Corrected for control sample values.

development, and no change was noted during the first 10 to 15 minutes. The intensity decreased slowly after 15 minutes. The slope of the curve for technical endosulfan is 0.0095 with a reagent blank of 0.004 absorbance. The slope for the low-melting-point isomer is 0.0092, and 0.0097 for the high melting-point isomer.

The normality of the methanolic NaOH–pyridine reagent was varied from 0.025*N* to 1.0*N*, and the heating time, at 100° C. (±2° C.), from 3 to 6 minutes. The maximum color development was found when the normality was maintained at 0.025 and the samples were heated for 4 minutes.

Pyridine used for the determination of endosulfan by the method of Butler *et al.* (*1*) was purified by the method cited by George *et al.* (*2*). A comparison of two lots of ACS-grade pyridine with the specially purified product showed that purification was unnecessary for the modified procedure.

Standard curves run on consecutive days indicated that the methanolic NaOH–pyridine is not stable and that the reagent should be made just before using. The data indicated that the reagent does not change during the first 6 hours after preparation.

Interferences

The interference of chlorinated solvents, such as chloroform, carbon tetrachloride, and dichloromethane, was measured by adding 0.1 to 0.2 ml. of the solvent directly to the test tube prior to heating in the oil bath. No interference from these three solvents was found.

Forty-one insecticides, two herbicides, and two fungicides were checked for interference in the analytical method by using 50 μg. or more of each material. Chlordan, heptachlor, captan, and ovex (966 μg.) were found to interfere. The interferences, calculated as endosulfan, were 43.4, 3.0, 1.2, and 21.2 μg., respectively. The following pesticides did not cause interference:

Aldrin, Amitrol, Aramite, chlorobenzilate, Delnav, demeton, DDT, Diazinon, naled (Dibrom), dicapthon, dieldrin, Dilan, dimethoate, Dimite, Di-Syston, endrin, EPN, ethion, Genite 923, Guthion, heptachlor epoxide, Karathane, Kelthane, lindane, malathion, methoxychlor, methyl parathion, parathion, Perthane, phorate, phorate oxygen analog, Phosdrin, Phostex, TDE, Tedion, toxaphene, carbophenothion (Trithion), 2,4-D, schradan, Sevin, and Sulphenone.

Endosulfan interfered in the determination of Kelthane in the method of George *et al.* (*2*). Kelthane does not interfere in the analysis of endosulfan by the proposed method. A mixture of Kelthane and endosulfan may be analyzed by first determining both compounds by the Kelthane method, then determining endosulfan by the modified method. The Kelthane content can then be calculated by difference.

Analysis of Samples

Chard, Collards, Lettuce, Table Beet Tops, and Turnip Greens. Samples were collected and quick frozen. The frozen samples were ground in a hand-operated food chopper, and a weighed aliquot was extracted by tumbling for 30 minutes with a mixture of distilled *n*-hexane–isopropanol (2:1). The isopropanol was removed by repeated washings with distilled water and the washed extract dried with anhydrous sodium sulfate.

Sugar Beets. Mature sugar beets were washed free of soil, run through a gang-saw arrangement, and weighed amounts of the sugar beet sawdust collected and frozen. The samples were allowed to thaw before being extracted in the same manner as the above plant materials.

An appropriate aliquot of the extract was made up to 30 to 50 ml. and shaken for 3 minutes on a wrist-action shaker with 1 gram of the adsorbent mixture. The extract was filtered through a plug of cotton overlaid with a layer of anhydrous sodium sulfate, and the flask with filter washed four times with 15-ml. portions of ethyl ether–distilled *n*-hexane (5:95). The filtrate was carefully evaporated to dryness.

Beef Fat. The samples were ground, mixed with anhydrous sodium sulfate, and extracted with *n*-pentane. An aliquot equivalent to 10 grams of fat was diluted to 50 ml. and extracted with 50 ml. of acetonitrile by shaking in a separatory funnel. Two more extractions were made using 25-ml. portions of acetonitrile. The extracts were combined, 150 ml. of distilled water was added, and the aqueous solution was extracted with *n*-pentane. The *n*-pentane solution was dried over anhydrous sodium sulfate, filtered, and the volume reduced to about 5 ml. on a steam bath, using an Erlenmeyer flask fitted with a three-section Snyder column.

A chromatographic column was prepared as described by Murphy and Barthel (*3*). The column was prewashed with 200 ml. of *n*-pentane containing 5% ethyl ether and the sample introduced with *n*-pentane. The endosulfan was then eluted with 200 ml. of *n*-pentane containing 5% ether. The activity of the adsorbents may vary, and the column should be calibrated whenever a new lot of Florisil or carbon is used. After chromatographing, the solvent was carefully evaporated.

The residues from the cleaned-up extracts were treated with 5 ml. of the methanolic NaOH–pyridine reagent, and the final color was developed and read as in the preparation of the standard curve.

Recovery of Endosulfan

Known amounts of endosulfan were added to control samples prior to the addition of the solvent for the extraction, and the percentage recovered was determined. Values obtained are presented in Table I.

Control samples of the various materials were analyzed to determine the interference due to plant materials in the extracts. The sample sizes for the chard, collards, lettuce, and turnip greens were purposely made small in anticipation of the high endosulfan residues on the treated crops (Table II).

Endosulfan Residues on Treated Crops. Chard, lettuce, and table beets received two spray applications of endosulfan at the rate of 1 pound per acre. Collards and turnip greens were sprayed four times with 0.75 pound of technical endosulfan per acre. One plot of sugar beets was treated about 6 months before harvest with a granular formulation at the rate of 1 pound of technical endosulfan per acre. A second plot was treated in the same manner, then treated a second time 1 month later. The residues found are given in Table III.

Literature Cited

(1) Butler, L. I., Fahey, J. E., Maitlen, J. C., J. AGR. FOOD CHEM. **10,** 479 (1962).
(2) George, D. A., Fahey, J. E., Walker, K. C., *Ibid.*, **9,** 264 (1961).
(3) Murphy, R. T., Barthel, W. F., *Ibid.*, **8,** 442 (1960).

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INSECTICIDE RESIDUES

Determination of Residual 4-Dimethylamino-3,5-xylyl Methylcarbamate and 4-Dimethylamino-3,5-xylenol by Use of Luteoarsenotungstic Acid

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Procedures for the residue determinations of 4-dimethylamino-3,5-xylyl methylcarbamate ("carbamate") and 4-dimethylamino-3,5-xylenol ("xylenol") in peaches and undelinted cottonseed are given. The "xylenol" is determined colorimetrically with a highly specific reagent, luteoarsenotungstic acid; the "xylenol" produced by the saponification of 4-dimethylamino-3,5-xylyl methylcarbamate is determined as a measure of the "carbamate."

ZECTRAN (registered trademark of The Dow Chemical Co.) insecticide, containing the active ingredient 4-dimethylamino-3,5-xylyl methylcarbamate, has proved useful on deciduous fruits and other edible products. It has also been used in the treatment of cottonseed and ornamental plants. Thus analytical methods for residual amounts of this chemical in a variety of media were needed.

The methods given account for the analysis of residual quantities of the active ingredient as well as the hydrolytic product, 4-dimethylamino-3,5-xylenol, in two typical crops, peaches and cottonseed. The usual procedure for this type of carbamate ester is to determine colorimetrically the phenol derivative produced by its saponification. Determination of the 4-dimethylamino-3,5-xylenol before and after saponification would thus distinguish between the two compounds.

However, most of the usual colorimetric metods for phenols do not work with 4-dimethylamino-3,5-xylenol. Procedures using nitrous acid (*4*), diazotized *p*-nitroaniline (*6*), 2,6-dibromoquinonechloroimide (*2*), or 4-aminoantipyrine (*3*) fail to give a color reaction or lack sensitivity.

Phosphotungstomolybdic acid, as in the phenol reagent of Folin and Ciocoltin (*1*), gives a sensitive color test with 4-dimethylamino-3,5-xylenol; however, it likewise gives a color with the carbamate ester. Furthermore, since this reagent is very sensitive to reducing agents, excessive cleanup of the pesticide from agricultural products is necessary to obtain acceptable blanks.

Luteoarsenotungstic acid [$H_6(18WO_3 . As_2O_8)$] (*5*) gives a blue color with 4-dimethylamino-3,5-xylenol but does not give any color with 4-dimethylamino-3,5-xylyl methylcarbamate. In addition, the luteoarsenotungstic acid has a high degree of specificity for this phenol because *o*-aminophenol and *p*-aminophenol and their derivatives are the only monophenols that give a positive test with this reagent, although polyphenols will give a blue color. Since the reagent is insensitive to mild reducing agents, cleanup of the carbamate pesticide and its hydrolytic product from agricultural products in order to obtain low blanks is relatively easy.

By using the ideal properties of luteoarsenotungstic acid as a colorimetric reagent, the following procedures for the residue determinations of 4-dimethylamino-3,5-xylenol and 4-dimethylamino-3,5-xylyl methylcarbamate were developed.

Colorimetric Procedures

Reagents. Sulfuric acid, approximately 6*N* stock solution. Cautiously add 168 ml. of concentrated sulfuric acid (95.5%) to about 750 ml. of water. Cool the aqueous solution to room temperature and dilute it to 1 liter. Dilute 1 volume of 6*N* sulfuric acid to 3 volumes with water to obtain approximately 2*N* sulfuric acid.

Potassium hydroxide, approximately 2.5*N*. Dissolve 165 grams of 85% potassium hydroxide pellets in about 750 ml. of water. Cool the solution, dilute to 1000 ml., and keep in a polyethylene bottle.

Luteoarsenotungstic acid reagent (*5*). Add 200 ml. of water to 50 grams of arsenic pentoxide and 20 grams of sodium tungstate, $Na_2WO_4.2H_2O$, in a 500-ml., round-bottomed flask. Reflux the mixture for 1½ hours. Pour the solution into a 400-ml. beaker; add 50 grams of lithium sulfate, $Li_2SO_4.H_2O$, and 5 or 6 drops of bromine. Boil the solution for 8 to 10 minutes to remove the excess bromine. Cool the solution, dilute to 250 ml. with water, and filter. Keep the reagent in a glass-stoppered amber bottle. The reagent is stable for at least 2 months and probably indefinitely.

Sodium carbonate, approximately 15% solution. Dissolve 150 grams of ACS-grade sodium carbonate in about 750 ml. of water and dilute the solution to 1000 ml.

4-Dimethylamino-3,5-xylenol, m.p. 93.5–95° C.

4-Dimethylamino-3,5-xylyl methylcarbamate, m.p. 85° C.

The last two chemicals may be obtained from Bioproducts Department, The Dow Chemical Co.

Apparatus. Coleman spectropho-