Analyses of various crops for heptachlor residues have shown a contamination of the heptachlor epoxide fraction with apparent heptachlor which interferes with the determination of heptachlor epoxide. Residues from heptachlor sprays on corn plants were placed on a Florisil column and eluted with pentane and 6% ether pentane. The pentane fraction contained all of the heptachlor. The first 45 ml. of the ether-pentane eluent contained gamma chlordan and the remaining ether-pentane eluent contained all of the heptachlor epoxide. The technique devised will eliminate interference of gamma chlordan in heptachlor epoxide determinations and will result in a more complete analysis of residues from heptachlor applications to crops.

200 ml. of pentane (heptachlor fraction). The columns were then eluted with 200 ml. of 6% ethyl ether in pentane, and fractions of the eluent were collected for evaluation. To each fraction was added 0.01 ml. of Nujol (medicinal grade heavy mineral oil), in pentane, and the volume reduced to 5 ml., using 3-ball Snyder condensers. The residue was transferred to graduated centrifuge tubes and reduced to dryness in a 40° C. bath, using a gentle stream of air to assist in evaporation.

One milliliter of modified Polen-Silverman (*4*) reagent and a small Carborundum chip were added, and the tubes were placed in an oil bath at 100° C. for 15 minutes and then removed and cooled to room temperature. The color developed by the reaction was evaluated qualitatively.

With both adsorbents the heptachlor fraction was recovered in the pentane, but the heptachlor epoxide and gamma chlordan remained on the column. The addition of ethyl ether to the pentane removed both gamma chlordan and heptachlor epoxide from the columns. In the Florex column no heptachlor epoxide was found in the first 25 ml. of the ether-pentane eluent; the next 75 ml. of this eluent contained a mixture of heptachlor epoxide and gamma chlordan. In the Florisil column, the first 25 ml. of ether-pentane eluent was free of heptachlor epoxide or gamma chlordan. The next 20 ml. of eluent gave a true violet color, indicating the presence of gamma chlordan and the absence of heptachlor epoxide. The remaining fractions (155 ml.) gave only the pure yellow associated with heptachlor epoxide. The Florex column did not separate gamma chlordan from heptachlor epoxide. The Florisil column released the gamma chlordan in the first 45 ml. of ether-pentane eluent, and heptachlor epoxide in the next 155 ml. of eluent.

Calibration of Column. For calibration of the Florisil column use an aliquot of plant extract from a sample of the untreated crop, and add 400 μg. of technical heptachlor and 30 μg. of heptachlor epoxide. Reduce the volume to 10 ml. and transfer to a 15-gram activated Florisil column previously wet with pentane. Rinse the sides of the column with about 2 ml. of pentane, and when the rinses have gone into the adsorbent, connect a reservoir containing 250 ml. of pentane and elute the column. Collect the eluent in a 500-ml. flask and reserve for heptachlor analysis. When all the pentane has reached the top of the Florisil, remove the flask and replace it with a graduated cylinder. Connect a reservoir containing 200 ml. of 6% ether-pentane to the column and continue the elution, collecting fractions to determine the rate of release and the separation of gamma chlordane and heptachlor epoxide. The ether-pentane solvent should be removed and the column activity determined by observing the colors produced in the various fractions by the Polen-Silverman reagent.

Results

Good column activity should produce the following results: all the heptachlor in the pentane fraction, no color in the first 25-ml. ether-pentane fraction, and violet, but no yellow, in the next two 10-ml. fractions. The last 10-ml. fraction should show only a faint violet or yellow, and the remainder of the ether-pentane eluent should contain practically all the epoxide added, when compared to a comparable quantity of epoxide run directly in a reaction tube.

With this procedure almost all the interfering gamma chlordan is recovered in the 45-ml. fraction, and a good measurement can be made of the epoxide present. Other degrees of activation or variability of the adsorbent may require different quantities of the ether-pentane eluent.

Literature Cited

(1) Meyer, C. F., Malina, M. A., Polen, P. B., J. AGR. FOOD CHEM. **8**, 183 (1960).
(2) Ordas, E. P., Smith, V. C., Meyer, C. F., *Ibid.*, **4**, 444 (1956).
(3) Polen, P. B., Velsicol Chemical Corp., private communication, 1960.
(4) Polen, P. B., Silverman, P., *Anal. Chem.* **24**, 733 (1952).

Received for review October 27, 1960. Accepted January 9, 1961.

ACARICIDE RESIDUES

A Modification of the Rosenthal Method for Rapid Determination of Kelthane Residues

DONALD A. GEORGE, JACK E. FAHEY,[1] and KENNETH C. WALKER

Entomology Research Division, U. S. Department of Agriculture, Yakima, Wash.

THREE METHODS are available for the determination of residues of Kelthane [4,4′-dichloro-α-(trichloromethyl)benzhydrol]. Rosenthal, Frisone, and Blinn (*6*) and Eiduson (*1*) described methods in which chloroform is liberated, swept from extraneous material in a special apparatus, and converted quantitatively to a red dye with an aqueous pyridine-alkali mixture. Gunther and Blinn (*5*) determined it directly by the absorption at 264 mμ or indirectly by absorption of the 2,4-dinitrophenylhydrazone in alcoholic alkali at 510 mμ. Rosenthal and coworkers established that, under mildly alkaline conditions, Kelthane will undergo a haloform type reaction to yield 4,4′-dichlorobenzophenone and chloroform. Griffon, Mossanen, and Legault-Demare (*4*) made a study of the Fujiwara (*2*) test for polyhalogen compounds with pyridine.

Analysis of a large number of samples

The 4,4 -dichloro-α-(trichloromethyl)-benzhydrol residue is extracted from plant material with *n*-hexane and the extract is cleaned up, evaporated to dryness, and reacted with aqueous pyridine-alkali. The reaction, carried out in a single test tube, produces chloroform and is sensitive to less than 10 μg. of Kelthane. No special equipment or reagents are required. The method has been used to determine Kelthane residues on or in apples and pears and their leaves, asparagus, bean hay, celery, cucumbers, lettuce, Lima beans, spinach, strawberries, string beans, and zucchini squash.

[1] Present address, 1118 Chestnut St., Vincennes, Ind.

Table I. Recovery of Kelthane Added to Various Crops[a]

Crop	Kelthane Added, P.P.M.	Recovery, %
Hyflo Super-Cel Cleanup		
Apples[b]	0.30	83.3
	0.57	89.4
	0.68	78.8
	0.72	81.9
	0.90	71.1
	Av.	80.9
Pears[b]	0.48	87.8
	1.30	69.2
	1.89	68.8
	Av.	75.3
Acid-Wash Cleanup		
Asparagus	4.00	85.0
		100.0
	Av.	92.5
Bean hay[b]	2.25	89.2
Celery	2.07	91.8
		113.0
	Av.	102.4
Cucumber	4.00	95.0
		105.0
	Av.	100.0
Lettuce	0.40	102.0
	2.80	94.2
	4.00	90.0
	Av.	95.4
Lima beans, beans and pods	3.30	117.7
Lima beans	3.30	69.1
		70.0
	Av.	69.6
Spinach	2.07	87.0
		99.0
	Av.	93.0
String beans	4.00	97.5
		102.0
	Av.	100.0
Zucchini squash	4.00	83.0
		102.5
	Av.	92.8
Attaclay and Acid-Wash Cleanup		
Strawberries	4.00	97.5
	2.00	94.0
		96.5
	Av.	96.0

[a] Total extraction, except where indicated.
[b] Surface extraction only.

Table II. Recovery of Kelthane Added to Leaves[a]

Leaves	Added, μg.	Recovery, %
Apple	10.0	88.0
	20.0	91.0
		74.0
	50.0	101.0
	Av.	88.5
Pear	20.0	94.5
		99.0
	Av.	96.8

[a] Surface extraction, Hyflo Super-Cel cleanup.

of various crops for pesticide residues requires analytical methods that are accurate, reproducible, and capable of routine application, and demand a minimum of equipment and reagents. The method presented is based on the reaction of Kelthane and alkali to produce chloroform and its reaction with pyridine. The liberation of chloroform and its colorimetric determination are carried out in a single test tube. The method is more rapid than those previously reported, requires no special equipment or reagents, and is adaptable to routine laboratory use. With careful manipulation the method is sensitive to 5 μg. of Kelthane and has been used for as much as 150 μg. Samples, apparatus, and equipment must be free of chlorinated solvents. The most commonly used solvents in this laboratory, chloroform, methylene chloride, and carbon tetrachloride, were found to interfere.

Experimental

Special Reagents. Kelthane Standard Solution. Dissolve 0.1000 gram of analytical grade Kelthane (Rohm & Haas Co., Philadelphia, Pa.) in 500 ml. of *n*-hexane; 1 ml. of this solution contains 200 μg. of Kelthane. Dilute an aliquot of this solution with *n*-hexane so that 1 ml. contains 10 μg. of Kelthane.

Cotton, acetone extracted and oven dried.

n-Hexane, redistilled Skellysolve B. (Any *n*-hexane with a low reagent blank can be used.)

Pyridine Solution. Purify the pyridine by refluxing over solid potassium hydroxide for 1 hour, cool, decant into a clean dry flask, and distill. Add 4% distilled water to the purified pyridine to obtain a 96% solution.

Potassium Hydroxide Solution. Thirty-three grams dissolved in 67 ml. of water.

Preparation of Standard Curve. Pipet aliquots of Kelthane standard solution containing 0 to 150 μg. into glass-stoppered test tubes. Evaporate the samples to dryness in a 40° to 60° C. water bath using a small stream of filtered air to speed evaporation. Add 5 ml. of the pyridine solution and 2 ml. of the potassium hydroxide solution, stopper the test tubes, mix the contents well, and immerse in a boiling water bath, or steam bath for 2 minutes. Swirl occasionally, while the samples are heating, and then remove the test tubes and cool in an ice bath for 1 minute. Decant through a loosely packed cotton plug in a small funnel, to remove any suspended particles, into 1-cm. cells. Experience has shown that a tightly packed cotton plug removed some of the colored product from the pyridine solution. Determine absorbance at 520 to 530 mμ, using distilled water or the pyridine solution as a reference. The color is stable for at least 30 minutes.

The standard curve obtained by this method for 0 to 150 μg. of Kelthane follows Beer's law. The average slope (*K* factor) of the curve was found to be 0.0066. The range of absorbance of standards determined on a Beckman Model B Spectrophotometer was: reagent blank 0.005 to 0.995 for 150 μg. of Kelthane.

Preparation of Samples. Surface residues on apples, pears, and similar crops were removed by tumbling the treated crops in an end-over-end tumbler, using redistilled *n*-hexane as the solvent. Those on apple and pear leaves were removed by placing a known area of leaf tissue in a glass-stoppered flask and shaking for 1 minute with redistilled *n*-hexane. Asparagus, bean hay, celery, cucumbers, lettuce, Lima beans, spinach, strawberries, string beans, and zucchini squash were processed in a food chopper into pieces less than 1/8-inch maximum diameter and extracted by adding *n*-hexane and isopropyl alcohol and tumbling. The isopropyl alcohol

was removed from the extract by washing with several portions of distilled water. All extracts were dried by filtration through anhydrous sodium sulfate.

Cleanup of Plant Extracts. Interfering materials from surface strippings of apples, pears, and their leaves were removed by shaking an aliquot of the extract for 2 minutes with Hyflo Super-Cel or Attaclay. The cleaned-up extract was filtered and the flask and filter paper were washed several times with *n*-hexane. After shaking, the extract may be centrifuged rather than filtered.

To remove interfering materials from extracts of chopped or ground crops, 100 ml. (or an aliquot diluted to 100 ml.) of the *n*-hexane solution was shaken for 1 minute with 10 ml. of 85% sulfuric acid. Acid was allowed to separate from *n*-hexane and was discarded. The sulfuric acid wash was repeated until all color was removed (usually two washes were sufficient). The *n*-hexane solution was then washed repeatedly (3 to 5 times) with 50 ml. of distilled water until the washings were neutral to methyl orange, then dried by shaking for 30 seconds with 20 ml. of saturated sodium chloride. The solution was dried further by filtration through a sodium sulfate pad, and flask and pad were washed with *n*-hexane. Excess solvent was evaporated off and volume adjusted to that of the original aliquot.

Analysis of Plant Extracts. Plant extracts were analyzed in the same manner as for the preparation of the standard curve using an aliquot of the cleanedup plant extract. Control or blank samples of the same food crop, not treated with Kelthane, were analyzed and found to contribute interference equivalent to from 0.0 p.p.m. of Kelthane (surface residues on apples) to 0.8 p.p.m. in plant extracts of asparagus.

Known amounts of Kelthane, in *n*-hexane, were added to the samples just prior to addition of the solvent for surface stripping or extraction and percentage recovery of the added Kelthane was determined. Values obtained are presented in Tables I and II.

Interferences. Kelthane, an acaricide, is generally used in combination with other pesticides. To check possible interferences, a number of insecticides were analyzed by this method. No interferences were found from 100 μg. of Chlorobenzilate (ethyl 4,4′-dichlorobenzilate), DDT, dieldrin, Dimite (4,4′-dichloro-α-methylbenzhydrol), endrin, Guthion [*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate], heptachlor epoxide, lindane, methoxychlor, Sevin (1-naphthyl methylcarbamate), and Tedion (2,4,4′,-5-tetrachlorodiphenyl sulfone). One hundred micrograms of chlordan gave an interference equivalent to 7.5 μg. of Kelthane. A similar amount of heptachlor gave an interference equivalent of 15 μg. of Kelthane and when these samples were heated for 5 minutes, instead of the suggested 2-minute period, the interference increased to 22.5 μg. Giang, Barthel, and Hall (*3*) reported interference for some insecticides that gave no interference when analyzed by this method. Giang and others mentioned that impurities in insecticides tested may have been responsible.

Recovery of Chloroform. Known amounts of chloroform, in a pyridine solution, added directly to the test tubes were analyzed by this method and results obtained were similar to those reported by Rosenthal and coworkers (*6*).

Discussion

Reaction products of Kelthane and chloroform gave identical transmittance wave-length curves between 350 and 600 mμ. Maxima on each curve occurred at 370 and 525 mμ. The curves were similar to those reported by Rosenthal and coworkers (*6*).

The simplicity of the method and the absence of special equipment or reagents make the method adaptable to the rapid routine examination of a large number of samples on a variety of crops.

Literature Cited

(1) Eiduson, H. P., *J. Assoc. Offic. Agr. Chemists* **42,** 561–62 (1959).
(2) Fujiwara, K., *Sitzber. Naturforsch. Ges. Rostock* **6,** 33–43 (1916).
(3) Giang, P. A., Barthel, W. F., Hall, S. A., J. AGR. FOOD CHEM. **2,** 1281 (1954).
(4) Griffon, H., Mossanen, N., Legault-Demare, J., *Ann. pharm. franc.* **7,** 578 (1949).
(5) Gunther, F. A., Blinn, R. C., J. AGR. FOOD CHEM. **5,** 517 (1957).
(6) Rosenthal, I., Frisone, G. J., Gunther, F. A., *Ibid.*, **5,** 514 (1957).

Received for review August 17, 1960. Accepted January 9, 1961. Division of Agricultural and Food Chemistry, 138th Meeting, ACS, New York, September 1960. Work done at a laboratory of Pesticide Chemicals Research Branch, Agricultural Research Service, Entomology Research Division, U. S. Department of Agriculture.

HERBICIDE DETERMINATION

A New Basic Procedure for Determining Phenoxy Acid Herbicides in Agricultural Products

ROLAND P. MARQUARDT and E. N. LUCE

The Dow Chemical Co., Midland, Mich.

THE NEW and increased uses of phenoxy acid herbicides in agricultural products made it desirable to seek a better analytical method than that of Marquardt and Luce (*3*), which is limited to the determination of phenoxyacetic acids and occasionally gives high blanks when interfering materials are difficult to remove. Besides being applicable to phenoxy acids in general and giving low blanks, a procedure that shortens analysis time and improves recoveries was sought. As a result of our investigations, a new analytical scheme was developed that involves cleavage with pyridine hydrochloride of the ether linkage common to phenoxy acids.

Pyridine hydrochloride is a very acidic onium salt, especially in the fused state (*1*). Prey (*4*) showed that the molten compound cleaves phenyl ethers. The authors found that under the same conditions, pyridine hydrochloride cleaves phenoxy acids, liberating the corresponding phenol derivatives.

In the following procedures illustrating a general scheme for determination of phenoxy acids in agricultural products, the phenol derivatives involved are determined photometrically by aminoantipyrine (*2*) methods as measures of the original compounds.

Determination of 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid) in Sugar Cane Juice

This method without modification is applicable to the determination of 0.05 to 2.0 p.p.m. of 2,4-dichlorophenoxyacetic acid in sugar cane juice. After removal and suitable cleanup, cleavage of the ether linkage in 2,4-dichlorophenoxyacetic acid with pyridine hydro-