Co-Ral, although systematically ineffective against newly hatched screwworm larvae and stable flies when applied dermally, appeared to be an effective residual insecticide.

Literature Cited

(1) Brundrett, H. J., McGregor, W. S., Bushland, R. C., *Agr. Chem.* **12** (No. 6), 36, 123 (1957).
(2) Comar, C. L., "Radioisotopes in Biology and Agriculture," p. 132, McGraw-Hill, New York, 1955.
(3) Hanes, C. S., Isherwood, F. A., *Nature* **164,** 1107 (1949).
(4) Krueger, H. R., Casida, J. E., Niedermeir, R. P., J. AGR. FOOD CHEM. **7,** 182 (1959).
(5) Robbins, W. E., Hopkins, T. L., Eddy, G. W., U. S. Dept. Agriculture, Corvallis, Ore., personal communication, May 1957.
(6) Robbins, W. E., Hopkins, T. L., Eddy, G. W., J. AGR. FOOD CHEM. **5,** 509 (1957).
(7) Roth, A. R., Eddy, G. W., *J. Econ. Entomol.* **48,** 201 (1955).
(8) Smith, C. L., Richards, R., *Ibid.*, **47,** 712 (1954).

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INSECTICIDE RESIDUES

Microdetermination of TDE in Spray Residues

ISADORE ROSENTHAL, C. F. GORDON, and E. L. STANLEY

Rohm and Haas Co., Philadelphia, Pa.

TDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane, has been used to protect crops from damage by insects. A method is described for its determination as a residue in plant extracts. After partial purification by solvent partitioning and treatment with adsorbent, the residue is dehydrohalogenated to 1-chloro-2,2-bis(p-chlorophenyl)ethylene, in a rapid, selective manner using sodium ethylate in dimethylformamide. Treatment of this alkene with sulfuric acid yields a colored carbonium ion complex with a maximum absorption at 502 mμ. Extraction and cleaning procedures are described, with a discussion of the method.

THE INSECTICIDE 1,1-dichloro-2,2-bis (*p* - chlorophenyl)ethane (TDE, DDD, or Rhothane) is used as an economic poison to protect a variety of crops against insects. The study, as presented here, represents endeavors to develop an analytical method to determine microamounts of spray residues on raw agricultural products.

TDE spray residues are difficult to determine in the presence of certain other chlorinated pesticides, as the residue methods now employed—Schechter-Haller (*9*) and total organic chlorine (*4*)—do not have a high degree of specificity for TDE.

Earlier work in this laboratory had indicated the possibility of a method applicable to dehydrohalogenated TDE using sulfuric acid. Researchers in this field had already pointed out the usefulness of such a procedure with DDT (*1, 3*), methoxychlor (*2*), and Perthane (*8*). Adaptation of existing procedures to TDE failed, because the conventional cleanup sequences gave poor recovery of TDE, and the dehydrohalogenation step could not be carried out reproducibly by boiling in alcoholic potassium hydroxide.

Reagents

All materials are reagent grade, unless specified otherwise.

Alumina, Merck reagent grade.

n-Hexane, technical grade, 95 mole % minimum. This is used for extraction and stripping of fruits and vegetables.

n-Hexane, purified, technical grade, 95 mole % minimum, passed through activated alumina. Using a column 4 cm. in diameter, 1 pound of alumina will clean up 2 gallons of solvent.

Acetonitrile, purified, technical grade, distilled or reagent grade, passed through activated alumina (see *n*-hexane, purified).

Equilibrated solvents. Saturate purified *n*-hexane and purified acetonitrile with each other.

Rhothane purified, technical material, recrystallized from methanol twice. Melting point 110–10.5° C.

Sodium ethylate, 0.1*N*. Store and disperse from an automatic buret protected from the atmosphere by silica gel and Ascarite.

Absorbent mixture.

77 parts sodium sulfate (anhydrous)
5 parts Attasol
5 parts Filter Cel
2 parts charcoal, activated (Nuchar)

Mix well and dry for 24 hours at 110° C. Keep tightly stoppered until used.

Extraction

Because of the multiplicity of problems presented by the variety of samples it is difficult to outline definite extraction procedures. The main purpose is to achieve complete removal of TDE from the sample. Ordinarily, hexane is used and the solvent to sample ratio can be varied according to needs.

Depending on the nature of the analysis, the sample can be macerated before extraction and then a methanol-hexane extraction may be necessary. Extracts are dried over anhydrous sodium sulfate prior to storage. Such extracts can be stored for several months without loss of TDE.

Separation

A 5- to 15-ml. aliquot of the extract, or an amount containing 10 to 50 γ (optimum 30) of TDE, is placed in a 60-ml. cylindrical separatory funnel. Enough *n*-hexane (saturated with acetonitrile) is added to make a total volume of 25 ml., followed by 25 ml. of acetonitrile (saturated with *n*-hexane). The phases are then shaken for 2 minutes for thorough equilibration. After the phases have separated, the acetonitrile layer (lower) is withdrawn into a tube of Type 2 (Figure 1). Four grams of absorbent mixture are added to the tube, which is shaken for 2 minutes. The solution is filtered with suction through a fritted-glass filter funnel (Type 3, Figure 1, funnel only with suction attachment) into a dehydrochlorination tube of Type 1 (Figure 1).

Dehydrochlorination

The acetonitrile solution is evaporated almost to dryness in a water bath at 50° to 55° C., under a gentle stream of nitrogen, with the last bit of solvent being removed at room temperature under nitrogen. Three milliliters of dimethylformamide are added to dissolve the residue, followed by 1 ml. of 0.1*N* sodium ethylate. The mixture is swirled for 1

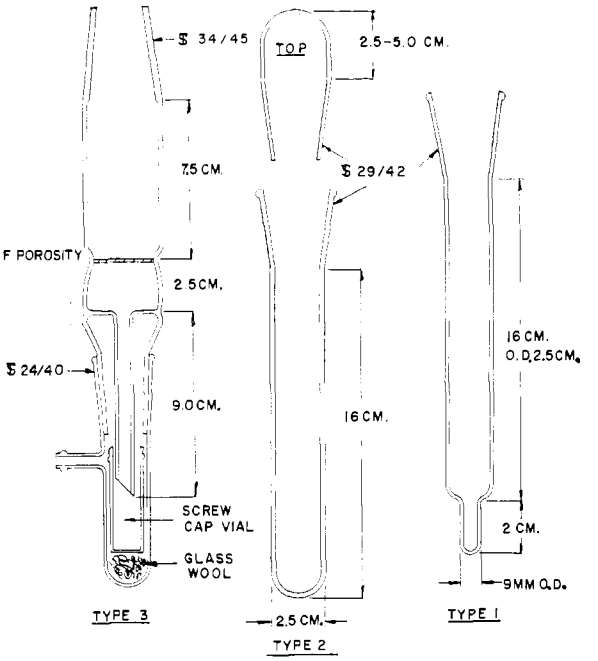

Figure 1. Special apparatus used in the procedure

minute. At exactly 1 minute after the addition of the sodium ethylate, 25 ml. of distilled water are added and the tube is swirled until its content appears clear.

Thirty milliliters of *n*-hexane are added to the tube containing the dehydrochlorinated TDE, and stoppered tightly, and shaken for 2 minutes. When the phases have separated, approximately 25 ml. of the lower water layer are withdrawn. Another 25 ml. of distilled water are added and the shaking is repeated for 2 minutes. When the phases have separated, enough of the lower layer is removed so that the interphase is in the nipple of the tube.

Color Development and Absorption Measurement

Twenty-five milliliters of the *n*-hexane layer are withdrawn and transferred to a tube of Type 2. The *n*-hexane is evaporated, on a water bath at room temperature, under a gentle stream of nitrogen. Overdrying the residue, leads to considerable losses at this point. Five milliliters of concentrated sulfuric acid are added, while smothering the tube in a blanket of nitrogen. The tube is capped tightly and swirled gently after tilting and rolling the acid on the sides of the tube to pick up all residue. After 15 minutes the absorbance is determined in a 1-cm. cell at 502 mμ using concentrated sulfuric acid as a reference. This colored solution is known to be stable for at least 24 hours.

In the analysis of some crops, waxy residues may carry through which are insoluble in the concentrated acid used for color development. To remove these suspended waxes from the acid solution, the acid is filtered through a coarse, fritted funnel. The filtrate is caught in a 10-ml. screw-cap vial (Figure 1, Type 3) to minimize loss and facilitate handling.

Calibration

The same procedure is followed using aliquots of a standard solution containing 5, 10, 20, 30, and 50 γ of TDE. The absorbance values follow Beer's law through this range and a typical curve will have a slope of 0.1 absorbance unit per 6.6 γ. A calibration curve is prepared for each substrate by carrying through the same procedure on an untreated control sample and on other untreated controls mixed with known amounts of TDE in the laboratory. A blank with added TDE should be run with each set of samples.

Discussion

TDE is completely dehydrochlorinated under the conditions described. Unlike methoxychlor, DDT, or Perthane, a rigorous caustic treatment must be avoided, as this leads to poor recoveries. The use of dimethyl formamide to promote dehydrohalogenation is not only reproducible, but is also faster than the conventional approach of boiling in alcoholic potassium hydroxide. It is possible that nonaqueous dehydrohalogenation techniques under different conditions may be useful in giving rapid, reproducible dehydrohalogenation from DDT, Perthane, and methoxychlor.

The recovery achieved is dependent upon the method of handling when evaporating solvent during the procedure. Care must be exercised to prevent the residue from overdrying and the last few milliliters must be removed at room temperature, while rotating the tube by hand. If a dry tube with residue in it is exposed to nitrogen and heat, large losses will occur. This has been shown to be the case for related compounds (*5*, *6*). In concentrating the residue the temperature must not exceed 55° C. and in removing solvent from the dehydrochlorinated product the water bath temperature should not be above room temperature (21° to 27° C.).

Nitrogen is used to blanket the tube during the addition of the sulfuric acid at the color development step, in order to produce a stable color. Should the acid be added in the presence of air, the color initially produced will fade rapidly in an irregular manner.

The cleanup procedure used with TDE differs from that for Perthane (*2*), 1,1-dichloro - 2,2 - bis(ethylphenyl)ethane, in that the absorbent is used before dehydrohalogenation instead of after. In addition, the use of an acetonitrile hexane extraction is recommended. The adsorbent is used before dehydrohalogenation, because dehydrochlorinated TDE is more strongly adsorbed than the parent compound. This is expected, because it is known that unsaturation, especially conjugated unsaturation, generally gives rise to stronger adsorption. The loss for TDE is about 54% on absorption after dehydrohalogenation (*vs.* 8.5% for Perthane). Using the adsorbent before this step cuts the TDE loss to 22%. The extraction is added as a general step, although in some cases the blank is satisfactory without it. However, it serves two useful functions. In the case of exceptionally waxy samples at low residue levels, the adsorbent does not seem to have enough capacity, but the extraction step takes care of this. If the adsorption step is used after dehydrohalogenation, colored materials generated by the action of the base on vegetable materials are removed. With TDE the formation of these materials must be prevented, because there is no cleanup after dehydrohalogenation. Therefore, the solution going into the dimethylformamide must be as clean as possible. Adsorption losses are smaller from acetonitrile than from hexane.

Interferences

The method has been applied successfully to spinach, cucumbers, cherries, apples, lettuce, broccoli, kale, and turnip greens. Low control values are achieved with this procedure. A reagent blank has an absorbance of 0.010. Untreated controls also give low values such as 0.58 p.p.m. for 10 grams of lettuce, 0.52 for spinach, and 0.82 for apples.

A full study has not been completed as to the extent to which most other insecticides interfere. Some of those which have been investigated and shown not to interfere are DDT, Perthane, heptachlor, and methoxychlor. As shown with Perthane (*7*) the use of 96% sulfuric acid eliminates the interferences of methoxychlor. The absorbance of DDT at 502 mμ, under the conditions used, is very low. Even at relatively

high concentrations (500 γ of DDT), no significant interference is found.

Sensitivity

The method is capable of detecting as low as 1.0 γ or approximately 0.07 p.p.m. in a 15-gram sample.

Recovery and Reproducibility

TDE is lost at the various steps in the procedure: extraction, 15%; adsorption, 22%; and dehydrohalogenation and water washes, 8%. The over-all recovery is 62 to 65%, not 55%, as losses cannot be added, but must be applied consecutively. This represents the losses based on the absolute amount of TDE initially present. The relative recovery values range from 95 to 102%, depending on the substrate.

Replicate determinations over a period of time gave:

Added	No. of Runs	Recovery, %	Std. Dev., %
10	5	97.7	±7.0
20	5	98.5	3.2
30	4	99.9	1.8
40	5	100.1	5.7
50	4	101.6	5.1

Literature Cited

(1) Bradbury, F. R., Higgons, D. J., Stoneman, J. P., *J. Soc. Chem. Ind. (London)* **66,** 65–8 (1947).
(2) Doble, J., Thornburg, W., "Methoxychlor, A Summary of Analytical Methods," E. I. du Pont de Nemours & Co., Wilmington, Del., 1951.
(3) Fairing, J. D., Warrington, H. P., *Advances in Chem. Ser.*, No. **1,** 260–5 (1950).
(4) Gunther, F. A., Blinn, R. F., "Analysis of Insecticides and Acaracides," pp. 481–4, 598, Interscience, New York, 1955.
(5) Johnson, D. P., *J. Assoc. Offic. Agr. Chemists* **39,** 1490–7 (1956).
(6) Jones, L. R., Riddick, J. A., *Anal. Chem.* **24,** 569–71 (1952).
(7) Kunze, F. M., *J. Assoc. Offic. Agr. Chemists* **37,** 578–81 (1954).
(8) Miles, J. R. W., J. Agr. Food Chem. **5,** 349–50 (1957).
(9) Schechter, M. S., Soloway, S. B., Hayes, R. A., Haller, H. L., *Ind. Eng. Chem., Anal. Ed.* **17,** 704–9 (1945).

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INSECTICIDE RESIDUES

Colorimetric Estimation of Malathion Residues in Cottonseed

M. V. NORRIS and E. J. KUCHAR[1]

Central Research Division, American Cyanamid Co., Stamford, Conn.

A colorimetric method reported previously for the estimation of spray residues of malathion on other plant and animal products has been adapted successfully to the determination of these residues on cottonseed. It is suitable for determining concentrations down to 0.1 p.p.m. in 200 grams of sample.

The basic colorimetric method reported for the determination of malathion [*S*-[1,2-bis(ethoxycarbonyl)-ethyl]*O*,*O*-dimethyl phosphorodithioate] residues in plant and animal tissue (*2, 3*) has been adapted successfully to cottonseed from which edible oil is to be extracted. The residual malathion is removed from the pulverized cottonseed by Soxhlet extraction with hexane. It is extracted from hexane into acetonitrile, which is treated with acid-washed alumina to remove interfering colored components. The malathion is extracted from an aqueous acetonitrile solution into carbon tetrachloride and analyzed by a procedure similar to the above-mentioned method.

The applicability of the method has been tested by analyzing cottonseed samples fortified with known amounts of the insecticide (Table I).

Procedure

Reagents. Acetonitrile, commercial grade. Neutralize with glacial acetic acid, if alkaline.

Alumina, acid-washed powder. Heat 1 pound of aluminum hydroxide powder (Baker and Adamson No. 1233) in a large porcelain dish for 8 hours at 400° C. Cool, then slurry with 750 ml. of 1*N* aqueous hydrochloric acid and stir for 15 minutes. Decant the supernatant liquid and repeat the acid treatment twice. Filter off the solid alumina using suction, wash with water until free from acid, and then wash three times with 150-ml. portions of methanol. Dry in a large, open dish, overnight, in an oven at 100° C. Store in stoppered glass container.

Prepare other reagent as in the colorimetric method (*2*).

Apparatus. Alumina column. Place a cotton plug at the bottom of an ordinary 50-ml. buret cut off at the 30-ml. mark, apply a slight vacuum at the bottom, and pour in dry, acid-washed, alumina powder to give approximately a 7-inch column. Place a cotton plug on top and pass 50 ml. of acetonitrile through the column at the rate of about 5 ml. per minute. Withdraw the acetonitrile until the level reaches about 2 cm. above the alumina and the column is ready for use.

Soxhlet extraction apparatus, large size with 7 × 2⅜ inch paper thimbles.

Calibration Curve. Prepare the calibration curve as described (*2*) for meat, fat, etc., but use aliquots of 0, 0.5, 1, 3, 8, 10, and 15 ml. of the standard solution.

Extraction and Determination of Residues. Pulverize a 200-gram sample of ginned cottonseed in a suitable apparatus. (Blending dry in a Waring Blendor is suitable for pulverizing 20-gram portions at a time.) Extract the finely divided seed for 6 hours, using a Soxhlet apparatus with about 350 ml. of *n*-hexane (commercial) and a solvent distillation rate of about 45 ml. per minute. Filter the hexane extract through a fluted paper; place it on a steam bath, and concentrate to 300 ml. with the aid of a jet of air. Cool and transfer the solution to a 500-ml. separatory funnel with the aid of 50 ml. of *n*-hexane. Add 50 ml. of acetonitrile, shake vigorously for 1 minute, then filter the lower acetonitrile phase through a fluted filter paper into a 400-ml. beaker. Repeat the extraction of the upper layer three more times with 50-ml. portions of acetonitrile and filter each portion into the beaker. Pass the combined aceto-

Table I. Recovery of Malathion from Cottonseed

Sample, Grams	Malathion, P.P.M. Added	Found[a]	Recovery, %
100	0	0.015	
200	0	0.015	
	0	0.025	
	0	0.010	
	0.08	0.05	63
	0.08	0.04	50
	0.15	0.10	67
	0.15	0.12	80
	0.15	0.12	80
	0.30	0.22	73
	0.30	0.21	70
	0.30	0.20	67
	0.73	0.64	88
	0.73	0.60	82
	0.97	0.86	89
	1.14	0.97	85
	2.27	2.03	89
100	4.53	4.23	93

[a] Results on spiked samples are corrected for "apparent" malathion found in untreated control samples.

[1] Present address, Olin Mathieson Chemical Corp., New Haven, Conn.