It has been used to extract and clean up other animal product samples containing fat prior to analysis for the five insecticides. Work on the use of this method for cleanup of animal blood, eggs, chicken fat, tallow, lard, and ground meat will be reported at a later date.

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INSECTICIDE RESIDUES

Dilan Residue Determination by Microcoulometric Gas Chromatography

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A cleanup procedure using either activated carbon or nonactivated Florisil was successfully applied to extracts of pears for the residue analysis of Dilan. Preliminary survey analysis of Dilan was accomplished by gas chromatography with programmed temperature and a thermal conductivity detector. Residue analyses sensitive to 1 to 2 μg. of Dilan were made by microcoulometric gas chromatography. The analysis of commercial Dilan formulations by programmed temperature gas chromatography is feasible.

DILAN is a mixture of Bulan [2-nitro-1,1 - bis(*p* - chlorophenyl)butane], Prolan [2-nitro-1,1-bis(*p*-chlorophenyl)-propane], and related compounds. The commercial mixture is composed of 53.3, 26.7, and 20.0%, respectively, of the above components. The related compounds are principally the *o-p'* isomers similar to those associated with DDT (*7*).

Dilan is reported to have insecticidal properties and has been used for the control of pear psylla in California in conjunction with studies on the pear decline disease. The psylla was implicated as a possible agent in the transmission of this disease. As a result of treatments to control this pest, residue data were required before California recommendation could be made for the use of this pesticide. Cooperative work between university entomologists and this laboratory are required to develop the necessary control measures and recommendations for the use of the pesticide based on insecticide performance, safety, and residue levels.

Two methods of analysis for Dilan have been reported. Mitchell (*8*) described a paper chromatographic procedure for the separation of the components of Dilan, and the subsequent location and measurement of the spots on the paper. Jones and Riddick (*6*) described a colorimetric procedure involving the conversion of the aliphatic nitro groups to the aci-form. The converted product is complexed with ferric chloride in an acid medium to form a colored product. In the latter method, quantities less than 50 μg. were not reliable and quantities less than 10 μg. were not detectable. The best results were obtained with 100- to 500-μg. quantities of Dilan which thus would involve sample quantities as large as 500 grams to achieve the desired sensitivity. Blank problems were reported, which may have been partially caused by the absence of any cleanup measures.

A cleanup procedure and method of analysis utilizing a gas chromatograph with a microcoulometric detector has been developed for the analysis of Dilan in pears. Residues as low as 1 to 2 μg. can be detected.

Experimental

Preliminary investigations on Dilan were made with a programmed temperature gas chromatograph using a thermal conductivity detector. A commercial mixture was resolved into two distinct peaks (Figure 1), indicating a 75:25 component ratio. A sample of purified Dilan containing a mixture of *p,p'* isomers of Prolan and Bulan showed a similar chromatogram. Also, a formulated product made from a technical

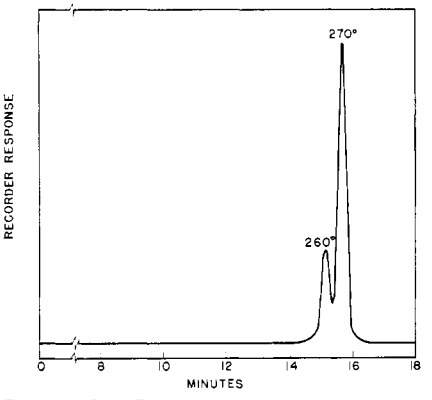

Figure 1. Programmed temperature gas chromatogram

25 μg. Dilan in 25 μl. injected on an F and M Model 500 gas chromatograph, thermal conductivity detector; initial temperature 100° C., program rate 11° per min.; 2-foot stainless steel column containing 20% General Electric SE-30 silicone rubber gum on 40- to 50-mesh Chromosorb P, Prolan response at 260° C. and Bulan response at 270° C.

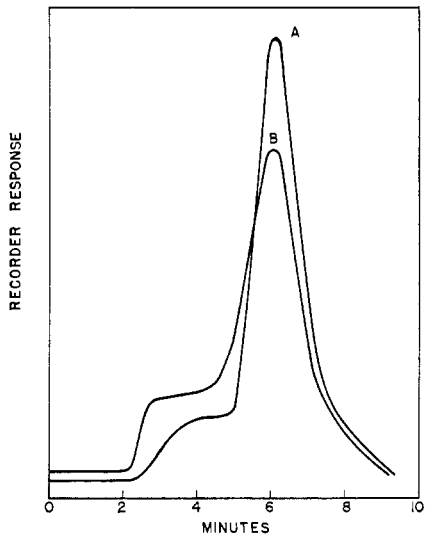

Figure 2. Chromatogram of (A) 7.5 μg. of Dilan standard in 7.5 μl. solvent, and (B) Dilan residue from 10 grams of pears (50-μl. injection)

Dohrmann Model 100 gas chromatograph, microcoulometer detector, 6-foot quartz column 6 mm. O.D., 4 mm. I.D., 20% Dow-11 silicone grease "purified" (*1*), on 40- to 50-mesh Chromosorb P; injection port 270°C., column 260° C., furnace 825° C., resistance 128 ohms, nitrogen carrier gas 100 cc. per min., auxiliary nitrogen 40 cc. per min., oxygen 100 cc. per min., recorder 1 mv. full scale, chart speed 2 min. per inch

Table I. Dilan Residue Data[a] on Pears

Sample weight, 10 grams; Dilan added, 10.2 μg.

Code	Days from Treatment	Carbon Cleanup Found, μg.	Carbon Cleanup Recovery, %	Florisil Cleanup Found, μg.	Florisil Cleanup Recovery, %	Florisil Cleanup, P.P.M.
Control	0	9.3	91	10.4	102	
B		16.1		17.2		1.72
C		9.8		10.9		1.07
Control	7	9.2	90	10.3	101	
B		7.4		8.5		0.83
C		8.2		9.3		0.91
Control	14	7.5	74	8.8	86	
B		7.7		8.9		0.87
C		7.5		8.8		0.86
Control	21	8.9	87	10.0	98	
B		5.9		7.0		0.69
C		6.5		7.6		0.74

[a] All data corrected for apparent background halogen response of check samples.

grade material produced both chromatographic peaks of the same relative height. Burke and Johnson (*4*) are cited by Cassil (*5*) for their work on retention times of various halogenated pesticide standards which include Bulan, Dilan, and Prolan. They reported two peaks for each of the three compounds. The Dohrmann gas chromatograph with the microcoulometer detector was employed for the analysis of residues. A procedure was developed for the analysis of Dilan in pears in a range which would detect residues as low as 1 to 2 μg. (Figure 2).

Method

A quantity of frozen pears was reduced to a pulp with a Hobart food chopper. A 500-gram aliquot was transferred to a 1-gallon can containing two internal baffles, 1000 ml. of redistilled benzene was added, and the can was sealed with a friction lid and then tumbled for 1 hour. The benzene extract was decanted from the pulp, filtered through fluted paper containing sodium sulfate, and stored in bottles at room temperature for subsequent residue analysis. A fortified sample was prepared by adding to the check sample a quantity of Dilan equivalent to a 1.0 p.p.m. residue.

For analysis of crop material treated with Dilan only, an activated carbon cleanup procedure for the removal of pigments and some waxes (*3*) was sufficient. A 100-ml. aliquot of plant extract equivalent to 50 grams of sample was agitated with 2 grams of Nuchar C-190N for 1 hour and then filtered through a double thickness of fluted filter paper. The filtrate volume was recorded for future calculations and then concentrated to 250 μl. for gas chromatographic analysis.

The concentration step involved evaporation of the extract to near dryness using a rotary flash vacuum evaporator. The evaporation flask did not exceed 50° C., and the cold trap was held at dry ice bath temperature. The residue was transferred with benzene to a sedimentation tube with 5- or 10-μl. divisions in the calibrated portion. Further concentration was made with a gentle stream of air directed at the surface of the liquid. The final solution was stirred with an off-center stopper on the shaft of a small electric motor.

During this study, it was suspected that certain samples also contained DDT, which overlapped and confused the chromatographic data of the Dilan residues. Therefore, a procedure was devised for the separation of the components of a DDT-Dilan mixture and is recommended for cleanup only when samples contain the mixture. An aliquot of 100 ml. of extract is concentrated to dryness on a rotary flash evaporator. The residue is dissolved in a small volume (5 to 10 ml.) of petroleum ether and transferred quantitatively to a chromatographic column (1 × 35 cm.) containing 30 cm. of unactivated Florisil. Two-hundred milliliters of petroleum ether is allowed to pass through the column to remove the DDT. The Dilan is then eluted from the column with 30 to 40 ml. of benzene. The eluent is concentrated to 250 μl. for gas chromatographic analysis.

The concentrated extract from either of the cleanup procedures represents all or most of the 50 grams of plant material. An aliquot of the 250-μl. concentrate, equivalent to 10 to 20 grams of pears, is injected into the Dohrmann gas chromatographic system for analysis. Figure 2 illustrates the response of 7.5 μg. of a Dilan standard compared to the Dilan residue observed in 10 grams of pear sample at a sensitivity of 128 ohms using a 1-mv. recorder. Quantitation is based on the halogen content of the compound, which is directly related to the area under the curve. Therefore, a sample properly cleaned up will give a measurable response with 1 to 2 μg. of Dilan at a sensitivity of 256 ohms. Standard curves will give a measure of column efficiency which may be used as a basis for determining the method efficiency and residue levels.

Results and Discussion

The information obtained from the thermal conductivity instrument showed that gas chromatographic analysis of Dilan was feasible. The authors intended to use the Dohrmann gas chromatograph with the microcoulometer detector for the determination of residues of Dilan. Operating conditions of the thermal conductivity instrument using linear temperature programming helped to determine the best operating parameters for the Dohrmann instrument.

The problem of obtaining 100% recovery of DDT and DDT-related compounds from a gas chromatograph with a microcoulometric detector has been the subject of previous papers (*1*, *2*). Dilan may be classed in this same category inasmuch as recovery, based on halogen content, is never complete. However, since only ring halogens are involved, a much higher recovery is noted as compared to DDT. This agrees with the premise that halogen loss is primarily due to aliphatic halogen in DDT-type compounds.

Table I shows recovery data of the laboratory-fortified check samples together with residue data on pears sampled periodically after treatment of the fruit. Each recorded value is the result of at least duplicate analyses. Experience has taught us not to expect better than

±3% accuracy on duplicate injections of the same solution. This, too, will vary with instruments, operators, and operating conditions. Each laboratory will have to choose its own set of operating parameters. The method sensitivity using the activated carbon cleanup procedure was 0.2 p.p.m., while the Florisil cleanup allowed a sensitivity of 0.1 p.p.m. The difference in sensitivity resulted from a background of apparent halogen response which was partially removed by the Florisil treatment. The removal of an interfering DDT-type compound by selective elution from a Florisil column was necessary because both compounds (DDT and Dilan) had overlapping retention times. The Florisil procedure was effective and allowed good recovery of the Dilan.

A procedure, including cleanup, for the analysis of Dilan residues in pears was achieved. Evaluation of entomological data will undoubtedly be reported elsewhere.

Acknowledgment

The expert technical assistance of Nancy Nash is to be noted in preparing the samples and obtaining the residue data.

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PESTICIDE RESIDUES

Colorimetric Determination of 6-Methyl-2,3-quinoxalinedithiol Cyclic Carbonate (Morestan) Residues in Apples and Pears

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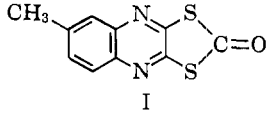
An analytical method for microdetermination of spray residues of Morestan is described. The procedure involves a hydrolysis with concentrated ammonium hydroxide to give 6-methyl-2,3-quinoxalinedithiol. Subsequent treatment with ammoniacal nickel reagent gives a red-colored chelate which is measured at 540 mμ. The method has been used for determination of Morestan residues in apples and pears.

MORESTAN (trademark, Farbenfabriken Bayer, A.G.) is 6 methyl-2,3-quinoxalinedithiol cyclic carbonate. The compound, formerly referred to as Bayer 36205, has been shown to be very effective in controlling several mite species on a wide range of crops. Control of both resistant and nonresistant strains has been reported for periods of up to 4 weeks. Morestan is also effective against pear psylla, white flies, and aphids, and has given excellent control of powdery mildew on a variety of crops.

The structure of Morestan is as follows:

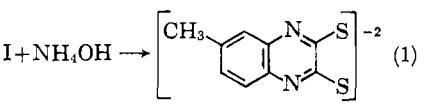


I

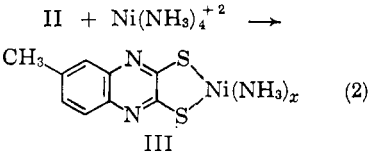
Little information appears in the literature regarding the chemical and physical properties of the compound. It is closely related to the insecticide Eradex, which is 2,3-quinoxalinedithiol cyclic trithiocarbonate.

Previous work in the authors' laboratory has shown that Eradex, upon treatment with ammonium hydroxide, is hydrolyzed to give 2,3-quinoxalinedithiol. The latter compound has been proposed by Morrison and Furst for the colorimetric determination of nickel (*7*). Morestan under the same conditions gives 6 methyl-2,3-quinoxalinedithiol which, as expected, also reacts with ammoniacal nickel to give a colored complex.

A partial equation for the reaction is as follows:



II



III

Structure III is analogous to the one postulated by Morrison and Furst (*7*) for the complex derived from 2,3-quinoxalinedithiol. Maximum absorbance of III is at 540 mμ. The color is stable and follows Beer's law in concentrations from 0 to 15 μg. per ml.

Morestan is relatively nonpolar, and can be quantitatively extracted from a 2:1 acetone:water solution into an equal volume of Skellysolve B. It is not strongly held by adsorbants, such as alumina, and can be easily eluted using nonpolar solvents. The compound is rapidly hydrolyzed in concentrated ammonia. A 10-minute hydrolysis and a 20-minute color development following addition of nickel reagent gave completely reproducible results and was adopted for routine use.

Samples are analyzed by blending with acetone, filtering, and extracting the filtrate with Skellysolve B. Morestan partitions into the Skellysolve B, and the bulk of the crop extractives remains in the aqueous acetone phase. This extraction provides considerable, though not sufficient, cleanup. Chromatography on alumina removes enough of the remaining interferences to permit

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