Chemical Determination of Perthane Residues on Agricultural Crops

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An analytical method has been developed for the determination of Perthane residues on agricultural crops. The method involves the extraction of Perthane residues with chloroform, dehydrochlorination, and reaction of the dehydrochlorination products with concentrated sulfuric acid at room temperature. The characteristic peach color obtained has a strong absorption maximum at 493 mμ. Perthane residues as low as 0.1 p.p.m. can be determined on asparagus and corn.

PERTHANE, 1,1 - dichloro - 2,2 - bis(*p*-ethylphenyl)-ethane, (Q-137 Experimental Insecticide, Rohm & Haas Co., Philadelphia, Pa.) is a relatively new insecticide of low mammalian toxicity, generally less insecticidal than DDT or TDE, but specific against certain insects (*3*). To correlate control of the asparagus beetle, *Crioceris asparagi* (L.), with insecticide deposits on the plants, analytical data on the Perthane residues were required. The ultraviolet absorption method of Stanley and Jackson (*5*) was not considered suitable for extracts of agricultural crops. Reactions involving Perthane which would result in a colored product suitable for colorimetric determination were investigated. Nitration with fuming nitric–concentrated sulfuric acid mixture and with fuming nitric acid alone, and reaction of the nitrated product with sodium methylate, similar to the Schechter-Haller method (*4*) for DDT, were unsuccessful.

Nitration, reduction of the nitro group, diazotization, and coupling with *N*-(1-naphthyl)ethylenediamine dihydrochloride gave a rosy-pink color with absorption maximum at 545 mμ, but the color was only about one third as intense as that produced by an equal amount of DDT carried through the same procedure. This method was very lengthy and involved, and was not pursued further.

When Perthane was dehydrochlorinated and the dehydrochlorination products were treated with strong sulfuric acid at room temperature, a characteristic peach color was obtained, which had its absorption maximum at 493 mμ. This series of reactions is similar to that used by Fairing and Warrington (*2*) for the analysis of methoxychlor [1,1,1-trichloro - 2,2 - bis(*p* - methoxyphenyl)-ethane], but with that material a wine-red color is produced with absorption maximum at 555 mμ. While the optimum color density is produced with methoxychlor by 85% sulfuric acid, Perthane gives the most intense color with the concentrated acid (96%).

Reagents

Purified Perthane, melting point 56–57° C., recrystallized from ethyl alcohol.

Chloroform.

Anhydrous sodium sulfate.

Alcoholic potassium hydroxide, 1% w./v., made fresh daily.

Petroleum ether, redistilled, boiling point 35° to 60° C.

Decolorizing mixture (*1*).

Sulfuric acid, concentrated (96%).

Paraffin wax, 1% w./v., in chloroform.

Procedure

The material to be analysed is tumbled in glass jars for 5 minutes at about 50 r.p.m. with chloroform in the proportion of 2 grams of material to 1 ml. of chloroform. The chloroform extract is dried with anhydrous sodium sulfate and filtered. No cleanup is necessary at this stage. An aliquot of filtrate is evaporated to dryness in a large borosilicate glass test tube, 28 × 150 mm. (with standard taper No. 24 outer joint), using a gentle current of filtered air. Fifteen milliliters of 1% alcoholic potassium hydroxide and a boiling chip are added, and the test tube is placed in a boiling-water bath until only about 0.5 ml. of solution remains. This step takes about 20 to 25 minutes. The test tube is cooled, and 25 ml. of petroleum ether are added by volumetric pipet. The tube is stoppered and shaken for 3 minutes to extract the dehydrochlorination product. Anhydrous sodium sulfate (3 grams) and 1 gram of decolorizing mixture are added, and the tube is shaken for 2 minutes.

After the mixture settles, a 15-ml. aliquot is removed by means of a volumetric pipet, the tip of which has been covered with a wad of absorbent cotton. The 15-ml. petroleum ether aliquot is transferred to a dry test tube, and the solvent is completely removed by a current of filtered air. It is convenient to keep the tubes in a water bath at 35° to 40° C.; undue cooling and condensing of water vapor are prevented. Concentrated sulfuric acid (5 ml.) is added, the residue is stirred with a glass stirring rod, and the color is allowed to develop for 15 minutes. The absorbance of the colored solution is measured in a Beckman Model DU spectrophotometer at 493 mμ.

Discussion

The effect on the final color, due to varying the sulfuric acid concentration is shown in Table I. The slow fading of the color, with time, is shown in Table II.

Purified Perthane gives almost twice the color density by this method as does the technical product, but both have an absorption maximum at 493 mμ. The purified and technical Perthane standards both give clear, colored final solutions in the concentrated sulfuric acid. When the method is used for the analysis of Perthane residues on asparagus, waxy turbidities are sometimes obtained along with the final color. Column chromatography of the chloroform plant extracts, and also of the petroleum ether extracts of the dehydrochlorination products, did not entirely eliminate this turbidity and resulted in extreme loss of final color.

When this turbidity is present, the colored sulfuric acid should be shaken

Table I. Effect of Concentration of Sulfuric Acid on Absorbance

H_2SO_4, %	Absorbance at 493 mμ.
96	0.730
93	0.690
91	0.505
88	0.405

Table II. Stability of Color with Time

Time, Minutes	Absorbance at 493 mμ
5	1.115
10	1.116
15	1.114
20	1.113
25	1.107
30	1.105
60	1.088

with an equal volume of benzene. The benzene extracts the waxes, leaving, on separation, a lower layer of clear, colored sulfuric acid which may be transferred by pipet to a cuvette for absorbance reading. The turbid sulfuric acid may be filtered through a sintered-glass microfilter, but this procedure can be troublesome if the waxes clog the filter plate, and is not as convenient as extraction with benzene.

Variable brown colors in the petroleum ether extracts are sometimes obtained even from untreated samples. The 2-minute decolorizing step has eliminated these off-colors from all samples tested.

The petroleum ether extract of the dehydrochlorination products could be filtered in the normal way, but precautions would have to be taken to prevent variable concentration, by evaporation, of the solvent. The method described, for the withdrawal of the petroleum ether aliquot, filters it through the cotton at the pipet tip. A minimum of glassware is used.

As little as 5 γ of Perthane can be determined when added to a 25-ml. aliquot of chloroform extract of asparagus. With the proportions of solvent to sample used in the procedure, this would correspond to 0.1 p.p.m. of residue.

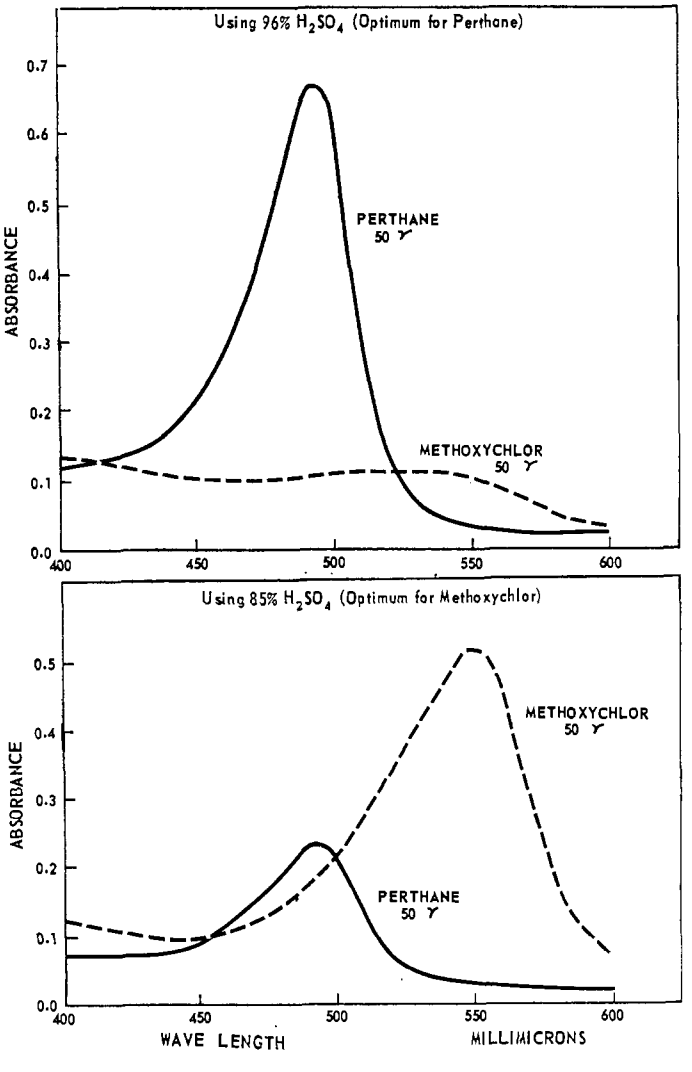

Figure 1. Absorption curves of products of dehydrochlorinated Perthane and methoxychlor treated with sulfuric acid

Recoveries

When known amounts of Perthane were added to chloroform extracts of plant material and carried through the analytical procedure, recoveries greater than 100% were obtained, relative to the same amounts of Perthane in chloroform solution. The waxy materials extracted from the asparagus and corn plants protect the Perthane, either during evaporation or dehydrochlorination, giving larger recoveries than when these materials are absent. Standard curves should be derived by adding varying amounts of Perthane to chloroform extracts of untreated samples of the plant material being examined. If untreated samples are not obtainable, the addition of 1 ml. of 1% paraffin wax in chloroform to the standard solution will give the same protective effect as the extractives from 50 grams of asparagus.

Specificity

Fairing and Warrington, in determining methoxychlor by dehydrochlorination and reaction with sulfuric acid, stated that "no other organic insecticide now in use produces any color under similar conditions." The peach color produced by Perthane is sufficiently different from the red color produced by methoxychlor for easy identification, even at the lowest concentrations.

By developing the color with 96% sulfuric acid and measuring the absorbance at 493 mμ the method is made specific for Perthane, as shown in Figure 1. Perthane will not interfere with the analysis of methoxychlor where the color is developed with 85% sulfuric acid and the absorbance measured at 550 mμ. The two insecticides would probably not be present on an agricultural crop at the same time.

Literature Cited

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