sugar cane, 800 ml. for cottonseed, and 850 ml. for other plant materials

C = Dexon concentration in µg. per ml. as read from the standard curve

A standard curve is obtained by adding known amounts of Dexon to 600 ml. of 1.0% sodium sulfite solution, processing as a sample beginning with the coupling reaction, and plotting optical density versus micrograms of Dexon present per milliliter. The curve obeys Beer's law. A concentration of 1 µg. per ml. in the final solution has an absorbance of 1.09.

P.P.M. Dexon in sample =

$$\frac{(10)\,(C)\,(100)\,(T)}{(W)\,(90)\,(A)}$$

This determination should also be carried out on untreated control samples. In most cases, the value obtained on these is negligible. In the event that significant values are obtained from control samples, they must be subtracted from the value observed for the treated samples in order to obtain the true values for the residues of Dexon.

Discussion

Recovery Experiments. Recovery experiments were conducted on each of the plant materials listed in Table II, in which known amounts of Dexon were added just prior to dialysis. While such experiments do not indicate the efficiency of the initial extraction system, they do show whether or not the material is lost in the steps subsequent to extraction.

The data presented in Table II indicate that satisfactory recovery of Dexon may be achieved by the procedure described. Where appropriate, the reported values are followed by the average deviation from the mean and, in brackets, by the number of independent determinations carried out.

Precision and Sensitivity. The data in Table II show the precision of results obtainable by this method. In the range up to 0.2 p.p.m., the average deviation from the mean is approximately 10% of the measured value. The precision is considered satisfactory for the low concentrations being measured. The instrumental limit of sensitivity is approximately 0.06 p.p.m. for cottonseed, 0.02 p.p.m. for sugar cane, and 0.015 p.p.m. for the other crops since these residue levels are necessary for an absorbance of 0.1. For all crops other than cottonseed, control values are well below these values so that the sensitivity of the method may be considered to be equivalent to the instrumental limit of sensitivity. Cottonseed control values ranged from <0.060 p.p.m. to 0.108 p.p.m.; therefore, the sensitivity for this crop is approximately 0.10 p.p.m.

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FUNGICIDE–MITICIDE RESIDUES

Extraction and Determination of Karathane Residues in Fruits

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A procedure is described for the extraction and determination of Karathane residues in apples, grapes, and strawberries. The residue is suitably extracted with mixed hexanes and purified either by column chromatography or by washing with sulfuric acid. Following solvent evaporation, the residue is dissolved in *N,N*-dimethylformamide for color development. The color which forms is measured at 444 mµ and is stable for at least 60 minutes. Recovery experiments and residue data are also presented.

KARATHANE (Rohm and Haas Co., trade name), 2-(1-methylheptyl)-4,6-dinitrophenyl crotonate, is used on a variety of crops for the control of powdery mildew diseases and certain species of mites. The method commonly used for the determination of Karathane residues (*3*) requires careful evaporation of the extracting solvent, steam distillation, extraction of the distillate, evaporation, and finally color development with a pyridine-water reagent. Recently, a much shorter procedure (*4*) has been described which utilizes ethanolic tetraethylammonium hydroxide as the color developing reagent.

Recent studies in this laboratory have revealed that Karathane forms an intense yellow color when dissolved in *N,N*-dimethylformamide without the addition of alkali required for color development with most mono- and dinitro compounds (*2*). A similar observation (*1*) has been made with Nitrosal, a feed medicament. Since apparently few nitro compounds form colors under these conditions, this color formation combined with a relatively simple cleanup procedure served as the basis for development of a new method for the determination of Karathane residues in fruits. In the present study, a short and sensitive procedure is described for the extraction and determination of Karathane residues in apples, grapes, and strawberries. In general, the residue is extracted with mixed hexanes and purified by column chromatography (or washed with sulfuric acid), the solvent is removed under reduced pressure, and the residual Karathane is dissolved in *N,N*-dimethylformamide for color development.

Reagents

N,N-dimethylformamide, spectro grade.

Florisil, 60 to 100 mesh, 1200° F. activation temperature. The florisil is not reactivated, being used as obtained from the Floridin Co., Tallahassee, Fla.

Mixed hexanes, b.p. 60° to 80° C.

2-(1-Methylheptyl)-4,6-dinitrophenyl crotonate (Karathane), Rohm and Haas Co., Philadelphia, Pa.

Procedure

Preparation of Standard Curve. Prepare a calibration curve by diluting aliquots of a stock solution (100 μg. of Karathane per ml. of mixed hexanes) with mixed hexanes to contain from 5 to 50 μg. of Karathane per ml. Transfer 1.0-ml. samples of the dilutions to each of several evaporation flasks, and evaporate the solvent under reduced pressure with a rotating flash evaporator. Once the solvent is removed, add 4.0 ml. of *N,N*-dimethylformamide to each of the flasks and swirl to ensure complete mixing of the contents. After 20 minutes, determine the absorption at 444 mμ against *N,N*-dimethylformamide.

Extraction Method. Macerate to fine pieces a representative sample of apples in a suitable food chopper. Weigh 500 grams of this material into a tared 1-gallon tin can equipped with a metal baffle for mixing purposes. Add 1 liter of mixed hexanes (b.p. 60° to 80° C.), seal tightly, and roll on a mechanical roller (35 r.p.m.) for 30 minutes. For recovery studies, fortify the samples with Karathane after the maceration step, but before the addition of extracting solvent. In the case of grapes or strawberries, the chopping step is omitted. The can should not be opened for at least 10 minutes after the rolling procedure to permit dispersion of the emulsion which forms. Decant the mixture into a beaker containing 200 grams of anhydrous sodium sulfate and mix thoroughly. Filter through fluted filter paper and store in a tightly capped bottle until analyzed.

Removal of Interfering Substances. Either one of the following two procedures can be used for the removal of interfering plant substances from the extracts.

Florisil Column Chromatography. Pass a 100-ml. aliquot of the sample (extractives from 50 grams fruit) through a 2.5- × 25-cm. column containing 20 grams of florisil (60 to 100 mesh) previously rinsed with 50 ml. of 6% ethyl ether in mixed hexanes. Wash the column successively with 50-, 100-, and 150-ml. portions of 6% ethyl ether in mixed hexanes. Discard the first 50-ml. wash, collecting the two subsequent washes in a 500-ml. evaporation flask. If a 200-ml. sample (extractives from 100 grams of fruit) is used, discard only the first 150-ml. of effluent from the column, collecting the remaining 50-ml. and all subsequent washings in a 500-ml. evaporation flask.

Sulfuric Acid Treatment. Transfer a 100- to 200-ml. aliquot of the sample (extractives from 50 to 100 grams of fruit) to a 500-ml. separatory funnel and add 20 ml. of concentrated sulfuric acid (c.p., sp. gr. 1.84). Stopper and shake gently for 3 minutes. Allow 5 minutes for the phases to separate, and then remove and discard the lower (acid) layer. The emulsified upper layer is retained in the funnel. Wash this upper phase successively with four 50-ml. portions of distilled water. Shake the funnel vigorously, and then discard the water which collects at the bottom after each addition. Pass the remaining mixture through a 2.5- × 25-cm. column containing approximately 100 grams of anhydrous sulfate. Collect the effluent in a 500-ml. evaporation flask. Rinse the separatory funnel twice with two 50-ml. aliquots of mixed hexanes, and add these washings to the column when the level of the fluid is approximately 1 inch above the sodium sulfate. Wash the column with an additional 50-ml. aliquot of mixed hexanes, and pool the effluent and washings in the evaporation flask.

Evaporation and Colorimetric Determination. Evaporate the solvent under reduced pressure with a rotating flash evaporator (Rinco Flash Evaporator, Rotating, Rinco Instrument Co., Greenville, Ill.), the evaporation flask being partially immersed in a 40° to 50° C. water bath. Remove the flask from the evaporator when the last traces of solvent have disappeared, and rinse down the sides with 4.0 ml. of *N,N*-dimethylformamide. Stopper and swirl the contents occasionally to ensure complete solution of all of the Karathane present. After 20 minutes, filter the solution through glass wool. This can be accomplished by passing the solution through a small funnel with glass wool packed in the stem. Transfer the solution to a cuvette (1-cm. light path), or filter directly into the cuvette and determine the absorbance of the resulting yellow color against *N,N*-dimethylformamide with a Beckman DU spectrophotometer at 444 mμ.

Results and Discussion

Solvent Extraction and Evaporation. Karathane is soluble in a variety of organic solvents, but mixed hexanes were chosen for the extraction procedure because this mixture extracts fewer interfering plant substances than most solvents. Also, the emulsion that forms when the sulfuric acid cleanup procedure is used breaks on standing more readily in mixed hexanes than with other solvents. Extraction of the fruits for 30 minutes by the tumbling procedure is a sufficient period to ensure complete removal of the residue; longer periods only increase the blank values.

Excellent recoveries of Karathane

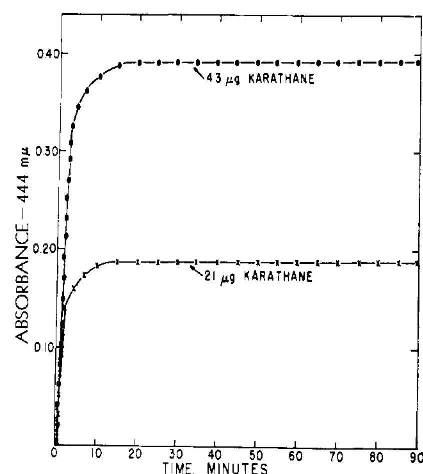

Figure 1. Karathane color development in *N,N*-dimethylformamide

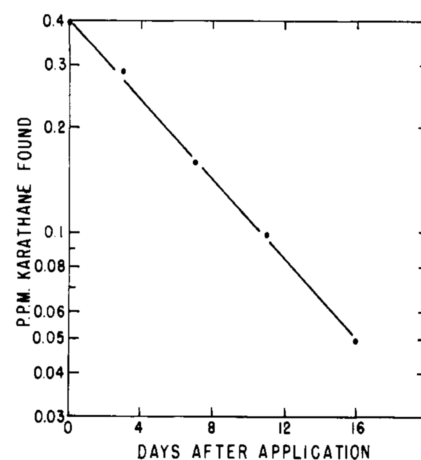

Figure 2. Decline of Karathane residues on strawberries

Table I. Recovery Values at Different Time Intervals after Removal of Solvent at 50° to 60° C.

Karathane Added, μg.	Absorbance, 444 mμ[a] Just to dryness	10 Minutes after dryness
10	0.088	0.086
20	0.173	0.174
30	0.268	0.265
40	0.360	0.362

[a] Reagent blank used as reference.

Table II. Recovery of Karathane Following Solvent Evaporation at Various Temperatures

Temperature, ° C.	Absorbance, 444 mμ
40	0.440[a]
57	0.458
77	0.449

[a] 50 μg. of Karathane used.

Table III. Recovery of Karathane from 50 Grams of Fruit

Karathane Added, μg.	Florisil Column Chromatography: Karathane found, μg.	Florisil Column Chromatography: Recovery, %	Sulfuric Acid Treatment: Karathane found, μg.	Sulfuric Acid Treatment: Recovery, %
		APPLE		
50	41.8	83.6	44.8	89.6
30	26.8	89.3	28.5	95.0
10	9.1	91.0	8.6	86.0
		GRAPE		
50	42.8	85.6	44.6	89.2
30	27.2	90.7	26.3	87.7
10	9.1	91.0	9.6	96.0
		STRAWBERRY		
50	41.4	82.8	44.7	89.4
30	27.1	90.3	26.9	89.7
10	9.3	93.0	8.8	88.0
	Av.	88.6		90.0

were obtained when the solvent was removed under reduced pressure at 50° to 60° C. with the flash evaporator. The flasks can remain on the evaporator for at least 10 minutes after the last visible traces of solvent have been removed with no significant loss of the residue (Table I). Increased water bath temperatures have little effect on recoveries (Table II).

Color Development and Interferences. Following the addition of *N,N*-dimethylformamide to Karathane residues, a yellow color develops which has a peak absorption at 444 mμ. Maximum color development is obtained in 20 minutes and the color which is formed remains stable for at least 1 hour (Figure 1). Standard curves prepared by the procedure obey Beer's law up to at least 50 μg. of Karathane in 4 ml. of *N,N*-dimethylformamide. Under these conditions, standard solutions containing 5, 10, 20, and 50 μg. of Karathane have absorbances of 0.045, 0.090, 0.180, and 0.440, respectively, when measured in cuvettes (1-cm. light path) with a Beckman DU spectrophotometer.

Compounds such as parathion, 2,6-dichloro-4-nitroaniline, 1,3,5-trichloro-2,4-dinitrobenzene, and 1,3-difluoro-4,6-dinitrobenzene do not form measurable colors under conditions of maximum color development for Karathane. In addition, very little interference is obtained from unfortified check samples. Blank samples prepared from 100 grams of unfortified fruit have very low absorbances, ranging from 0.035 to 0.050.

Recovery and Sensitivity. With the florisil column, the average recovery of Karathane from treated fruits is 88.6%, and the recovery is 90% when the sulfuric acid procedure is used (Table III). Moreover, as low as 0.05 p.p.m. of Karathane on strawberries can be detected with an average recovery of 86% (std. dev. ±5.4). Thus, it is apparent that either cleanup procedure can be used with very satisfactory results.

Field-Treated Samples. In order to test the reliability of the procedure, field experiments were conducted. A test plot of strawberries was sprayed 21 days before harvest (the recommended time of application) with a commercial preparation of Karathane (6 ounces of Karathane per acre), and representative samples of the treated fruit were analyzed for residues. In Figure 2, the degradation curve shows a logarithmic decline, and only 0.05 p.p.m. of Karathane remains after 16 days following application.

Acknowledgment

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NEMATOCIDE RESIDUES

Determination of 1,2-Dibromo-3-chloropropane and Tetrachlorothiophene in Crops

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ANALYSIS of nematocides is often complicated by their low boiling points or high vapor pressures. Recovery of these compounds from crop material is often accompanied by high losses which occur during concentration of the extracts prior to final analysis. The need for specific analytical methods to detect the presence of traces of the original compound is apparent. Previous attempts to develop such methods have often lacked sensitivity.

Specific residue methods for the determination of 1,2-dibromo-3-chloropropane (various trade names) have not been reported. Analytical data based on total bromine content of agricultural crops have been used for an estimation of residues of this nematocide (*3*). Archer *et al.* (*1*) showed that several nematocides could be readily detected using gas chromatography. They showed that dibromochloropropane could be detected in 6 minutes using an 8-foot, 1/4-inch o.d., 20% silicone grease column at 132° C. with a thermal conductivity detector. The study was a comparison of two types of columns with two types of detectors. A similar technique has been found applicable to