II (desethylphosphamidon), 0.20; metabolite V, 0.59; and metabolite VII, 0.49. The R_f values can be subject to considerable variation depending on the type of plant, the paper itself, the temperature, and other factors, and have to be compared with standards run on the same chromatogram. Furthermore, it has proved extremely difficult to detect the smears of metabolite VII, and that of metabolite V was found in the same region of the paper as the plant pigments, thereby being masked by them. Also, metabolites V and VII are not phosphorus compounds and therefore cannot be determined by the phosphomolybdenum blue method. This method then is sensitive to only phosphamidon and its metabolite II, both qualitatively by paper chromatography and quantitatively by the phosphomolybdenum blue reaction. The intensity of the blue smears caused by metabolite II is approximately 25% that of the phosphamidon itself. The limit of detection for phosphamidon and metabolite II (desethylphosphamidon) with the blue tetrazolium reagent is about 0.5 and 1 μg., respectively, per spot.

Although this procedure is very time consuming in the amount of time required to carry one individual sample through the procedure, it is a very rapid procedure when the total number of analyses that one person is able to complete in a given amount of time is considered. It has been possible to complete as many as 30 samples per day. The sensitivity of the method is 0.2 to 0.4 p.p.m. with the sample weights and aliquots as described, based on the limits of detection of the blue smears on the papers. A higher sensitivity can be realized by using larger aliquots in the initial stages.

Because this method depends on the phosphomolybdenum blue reaction, other phosphorus-containing compounds will interfere if they are soluble in methylene chloride and methanol and occur in the same R_f values on the paper chromatograms.

Acknowledgment

The assistance of Katherine A. Nelson and J. S. Burke in these studies is gratefully acknowledged. This study was supported in part by grants from the Ortho Division of the California Chemical Co. and Regional Research Project NE-36 on Pesticide Residues.

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NEMATOCIDE RESIDUES

The Spectrophotofluorometric Determination of *O,O*-Diethyl *O*-2-Pyrazinyl Phosphorothioate (Zinophos) and Its Oxygen Analog in Soil and Plant Tissues

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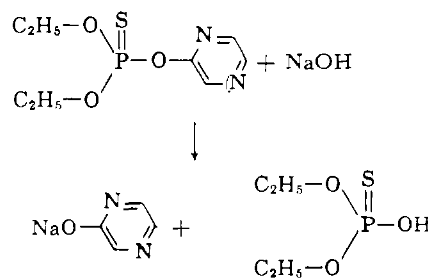
Residues of O,O-diethyl O-2-pyrazinyl phosphorothioate (Zinophos) and its oxygen analog have been measured by spectrophotofluorometry in several crops and in soil. The method depends upon hydrolysis of the phosphate ester to produce the sodium salt of 2-pyrazinol which fluoresces strongly in the region 375 mμ when activated by light of 315 mμ. The method is reliable at Zinophos levels as low as 5 μg. (0.05 p.p.m.). Beans, potatoes, table beets, corn, and peppermint grown in treated soils contain less than 0.05 p.p.m. The method has also been tested on asparagus, cauliflower, cherries, strawberries, carrots, and soil. Of 10 insecticides tested, only Guthion interferes to any appreciable extent.

THE NEMATOCIDE ZINOPHOS, *O,O*-diethyl *O*-2-pyrazinyl phosphorothioate, (formerly known as EN 18133) has shown considerable promise for the control of nematodes, symphylids, wireworms, and other soil-borne pests. Development of this pesticide for commercial use depends in part on the availability of a specific and sensitive analytical method. Such a method using spectrophotofluorometry for the quantitative measurement of 2-pyrazinol, the major hydrolysis product of Zinophos, is described in this report.

When refluxed in the presence of mild alkali, Zinophos reacts according to the following equation:



In the presence of strong alkali (pH 10 or above) and under the influence of incident light of wave length 315 mμ, 2-pyrazinol fluoresces strongly in the region 375 mμ. This fluorescence is sufficient to allow detection of as little as 5 μg. of Zinophos per 100 grams of plant tissue. Interferences from crops tested to date have been minor when the proper cleanup methods were employed. Most of the insecticides likely to occur in mixtures with Zinophos have shown little or no interference with the determination.

The technique of spectrophotofluorometry has found limited usage in pesticide residue determinations. Hornstein (*5*) surveyed the fluorescent properties of a number of pesticides and found that Guthion, Potasan, Warfarin, and some synergists and plant growth regulators exhibited fluorescence, but that many of the common insecticides showed

no activity. Anderson *et al.* (1) have described a fluorometric method for Co-Ral, Giang (4) for *O,O*-diethyl *O*-naphthalimido phosphorothioate, and Freed and Hughes (3) for 1,1′-ethylene-2,2′-bipyridylium dibromide.

Reagents

Alumina, Merck.

Attaclay adsorbent, mix two parts of Attaclay (Minerals and Chemicals Corporation of America, Philadelphia 5, Pa.) with one part of Celite 545 (Johns-Manville Products Corp., Celite Division, San Francisco, Calif.).

Charcoal, C 190N, activated, acid washed (Kensington Scientific Corp., Berkeley, Calif.).

Ion exchange resin, Dowex 21K, 50–100 mesh, chloride form (J. T. Baker Chemical Co., Phillipsburg, N. J.).

O,O-Diethyl *O*-2-pyrazinyl phosphate (American Cyanamid Co., Princeton, N. J.).

Sodium pyrazinolate, analytical grade (American Cyanamid Co., Princeton, N. J.).

Zinophos, analytical grade (American Cyanamid Co., Princeton, N. J.).

Apparatus

Chromatography column, 20 mm. i. d., approximately 40 cm. long with 200-ml. capacity bulb at the top, a medium porosity, sintered-glass plate, and a side arm for vacuum at the bottom.

Ion exchange column, 10 mm. i. d., approximately 30 cm. long with tubing constricted for rubber connection at one end.

Kuderna-Danish evaporative concentrator.

Spectrophotofluorometer, Aminco-Bowman, or equivalent.

Procedure

Extraction. Macerate a representative sample of the plant tissue to be analyzed in a food chopper in the presence of anhydrous sodium sulfate. Crops grown in Zinophos-treated soil should be thoroughly washed before extraction. The amount of sodium sulfate to be used depends on the sample, but 1 gram per gram of tissue is usually sufficient. Transfer this mixture to a blender and homogenize in the presence of chloroform or benzene for 5 minutes. One to 2 ml. of solvent per gram of tissue usually provides good solvent mobility. Allow the slurry to stand for 1 hour to allow hydration of the sodium sulfate, and then tumble the mixture end over end for 1 hour. Remove the extract by decantation and filtration through anhydrous sodium sulfate and centrifuge to break emulsions.

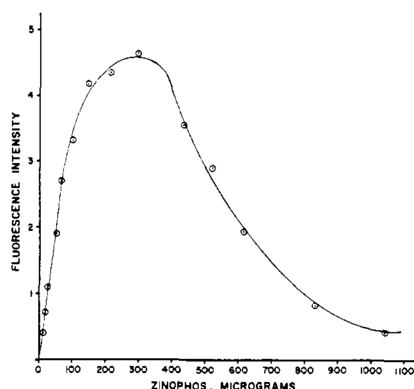


Figure 1. The relationship between fluorescence and Zinophos level

Cleanup. Prepare the chromatography column by placing a small pad of glass wool on the plate in the bottom of the column and by adding 10 grams of the Attaclay adsorbent. Apply a vacuum of approximately 200 mm. Hg to the side arm and level off the surface of the adsorbent with a glass rod. Top the adsorbent with a 2.5-cm. layer of anhydrous sodium sulfate. Prewash the column with 100 ml. of benzene adjusting the liquid flow to about one drop per second by regulation of the vacuum applied to the column.

Concentrate an aliquot of the extract, equivalent to 100 grams of sample, to 10 ml. on a steam bath. Change the receiving flask and quantitatively add the sample to the column. Draw down the sample, rinsing with benzene. Add 200 ml. of benzene and collect about 250 ml. of eluate. Remove the flask and quantitatively transfer the benzene solution to a Kuderna-Danish evaporative concentrator.

For some crops (e.g., tobacco), an adsorbent mixture of charcoal-alumina provides additional cleanup. Prepare this column by placing a pad of glass wool at the bottom of the column and by adding alternately 0.5-gram portions of alumina and charcoal to a height of about 10 cm. Place another pad of glass wool at the top of the adsorbent and wash the column with 100 ml. of benzene. Quantitatively transfer the concentrated eluate from the Attaclay column to this column and elute with two 30-ml. portions of benzene.

Hydrolysis. Attach a Snyder column to the evaporative concentrator and evaporate the solvent on a steam bath to about 5 ml. Remove the tube and evaporate the remaining solution to about 0.5 ml. under an air jet at room temperature. Add 1.0 ml. of ethanol and again evaporate the solution to 0.5 ml. Repeat this operation twice, then add sufficient ethanol to make the volume 4 ml. Add 4 ml. of 0.2*N* sodium hydroxide, attach the tube to a water-cooled condenser, and reflux in a boiling water bath for 1 hour.

After cooling the hydrolysate, acidify with 6*N* hydrochloric acid, transfer to a 125-ml. separatory funnel, and extract with two 10-ml. portions of chloroform. Combine the chloroform extracts and wash with 10 ml. of water.

Table I. Zinophos Residues in Beans Grown in Treated Soil

Zinophos Treatment, Lb. Active/Acre	Days after Treatment[a]	Zinophos Residues, P.P.M.[b]	
		Foliage	Pods
Emul. concn., 10 lb.	20	<0.05	
	34	<0.05	
	58		<0.05
	80 (Harvest)		<0.05
Emul. concn., 5 lb.	20	<0.05	
	34	<0.05	
	58		<0.05
	80 (Harvest)		<0.05
5% Gran., 10 lb.	12	1.60	
	24	0.16	
	40	<0.05	
5% Gran., 5 lb.	12	1.32	
	24	0.18	
	40	<0.05	
	56 (Harvest)		<0.05

[a] Treated 1 day prior to planting.
[b] Values corrected for crop blank.

Table II. Recovery of Zinophos from Fortified Crop Extracts

Crop Extract[a]	Crop Blank, Fluorescence Intensity	Zinophos, P.P.M. Added	Zinophos, P.P.M. Found	Recovery, %
Beans, foliage	0.035	0.049	0.044	90
Beans, pods	0.160	0.089	0.066	74
Peppermint hay	0.069	0.128	0.099	77
Peppermint oil	0.171	3.48	1.18	34
Orange	0.000	0.098	0.000	0
Cherry	0.062	0.098	0.085	87
Cauliflower	0.124	0.098	0.085	87
Carrots	0.076	0.098	0.075	77
Strawberries	0.125	0.098	0.086	88
Asparagus	0.172	0.098	0.077	79
Potatoes, foliage	0.020	0.043	0.039	91
Potatoes, tubers	0.020	0.087	0.085	98
Corn, foliage	0.016	0.17	0.13	76
Corn, kernels	0.000	0.17	0.13	76
Table beets	0.000	0.087	0.082	94
Soil	0.000	1.18	0.73	62
	0.022	4.0	2.42	61

[a] Represents 100 grams except peppermint oil (10 grams) and soil (20 grams).

Table III. Recovery of the Oxygen Analog of Zinophos from Fortified Crop Extracts

Crop Extract[a]	Crop Blank, Fluorescence Intensity	Oxygen Analog of Zinophos, P.P.M. Added	Found	Recovery, %
Beans, foliage	0.019	0.84	0.72	86
Beans, pods	0.013	0.84	0.69	82
Corn, foliage	0.016	0.84	0.59	70
Corn, kernels	0.012	0.84	0.76	91
Potatoes, foliage	0.016	0.84	0.73	87

[a] Represents 50-gram sample.

Table IV. Measurement of Zinophos in the Presence of Other Pesticides

Pesticide	Amount Present, µG.	Zinophos, µG. Added	Found	Recovery, %
Aldrin	1000	...	0.0	...
	1000	40.5	41.1	102
Dieldrin	1000	...	0.0	...
	1000	40.5	38.8	96
DDT	500	...	0.1	...
	500	40.5	37.5	93
Toxaphene	500	...	0.1	...
	500	40.5	38.0	94
Heptachlor	500	...	0.0	...
	500	40.5	42.0	104
Tedion	500	...	0.0	...
	500	40.5	37.0	91
Sevin	500	...	0.5	...
	500	40.5	40.0	99
Diazinon	500	...	1.6	...
	500	40.5	41.5	102
Guthion	100	...	ca. 300	...
	100	40.5	>300	...
Parathion	500	...	0.0	...
	500	40.5	29.0	72

Table V. Precision of Zinophos Method

Analyst	Date of Analysis	Fluorescence Intensity of Zinophos Standards[a] 5 µg.	10 µg.	20 µg.	30 µg.	50 µg.
A	6/24/59	0.177	0.376	0.781	1.02	1.76
	6/25/59	0.191	0.392	0.770	1.14	1.96
B	1/28/60	0.188	0.381	0.772	1.08	1.74
	2/17/60	0.186	0.379	0.782	1.11	1.82
	4/5/60	0.193	0.392	0.783	1.16	1.99
	5/8/61	0.207	0.398	0.794	1.19	2.01
C	5/31/60	0.195	0.399	0.771	1.20	1.92
	6/1/60	0.181	0.391	0.786	1.18	1.98
D	5/12/61	0.184	0.389	0.774	1.09	1.72
	7/20/61	0.204	0.404	0.786	1.20	2.02
E	10/6/61	0.195	0.392	0.782	1.15	1.98
Av.		0.191	0.390	0.780	1.14	1.90
Std. Dev.		0.0092	0.0087	0.0075	0.018	0.037

[a] Fluorescence intensity values corrected for reagent blank (av., 0.053; std. dev., 0.0099).

Discard the chloroform extracts and combine the water wash with the original aqueous hydrolysate. Transfer to a 50-ml. beaker and make strongly basic (pH 10 or higher) with 50% sodium hydroxide. Concentrate the aqueous phase to about 5 ml. on a steam bath using an air jet. If the solution at this point is turbid, clarify by centrifugation and transfer the supernatant to a 10-ml. volumetric flask and dilute to the mark. Measure the fluorescence at 375 mµ with an activating wave length of 315 mµ. To be sure that the fluorescence is not being self-quenched by excess 2-pyrazinol, the fluorescence measurement must be repeated after diluting the mixture 1 to 1 with water (Figure 1).

Test for Quenching Agents. Since naturally occurring materials passing through the cleanup procedure may absorb fluorescence (quench) of the wavelength being used in the 2-pyrazinol determinations, a test for quenching must be performed on each type of sample being analyzed. After determining the fluorescence intensity of the sample, add a known amount of 2-pyrazinol (10 to 20 µg.) to the sample and repeat the measurement. If quenching does not occur, the fluorescence intensity due to the additional 2-pyrazinol will be additive. If the proper response is not observed, the extent of quenching can be estimated from the response of the added 2-pyrazinol and a correction made for the 2-pyrazinol content of the original sample. Quenching should be avoided for the most accurate measurements, and if it occurs to an appreciable extent, the cleanup technique should be modified accordingly.

Analysis of Soil. Extract a representative sample of soil with chloroform at the rate of 1 ml. per gram by shaking on a mechanical shaker for 1 hour. Remove the solvent by filtering with suction. Concentrate an aliquot of the soil extract containing 0 to 50 µg. of Zinophos to a small volume in a Kuderna-Danish concentrator, and remove the last traces of chloroform by the alcohol-sweeping technique already described. Hydrolyze the sample as before and perform an additional cleanup by passing the hydrolysate through an ion exchange resin. Dowex 21-K is prepared by washing with water until the washings become colorless. Then place a pad of glass wool in the bottom of a 10-mm. chromatography tube and add the resin to the column in a thin slurry to a height of 5 cm. Wash the resin with additional water and then with 100 ml. of 6N hydrochloric acid. Regulate the flow of the liquid by means of a screw clamp and tubing at the bottom of the column. Pass the acid through the column in about 30 minutes, then pass water through the column until the pH is 4 or higher. Adjust the pH of the hydrolysate to 10 to 12 and add it to the column through Whatman No. 42 filter paper. Rinse the hydrolysis tube through the filter paper using 12 ml. of 25% (v./v.) methanol and 20 ml. of water. Regulate the elution rate to about one drop per second discarding the first 30 ml. of the eluate. Then add 50 ml. of 0.2N HCl to the column and collect 50 ml. of eluate. Make the eluate strongly basic (pH 10 or higher) with concentrated NaOH, and concentrate to about 8 ml. Transfer to a 10-ml. volumetric flask, dilute to the mark, and determine the fluorescence intensity.

Determination of the Oxygen Analog of Zinophos. The extraction procedure already described will also extract the oxygen analog of Zinophos from the plant tissue, but the oxygen analog is not eluted from the Attaclay column with benzene. If the determination of this metabolite is desired, it can be eluted from the Attaclay column after the benzene elution of Zinophos. Change the receivers after all benzene has been drawn into the adsorbent and elute the column with 100 ml. of technical chloroform or redistilled chloroform containing 1% ethanol. Use the procedure for Zinophos to concentrate and hydrolyze the chloroform eluate. Acidify the hydrolysate after cooling with 6N hydrochloric acid and transfer to a 125-ml. separatory funnel. Extract with three 10-ml. portions of chloroform followed by one extraction with 10 ml. of ethyl acetate. Backwash the combined chloroform layer with 10 ml. of water and combine this with the aqueous phase. Discard the organic layers and concentrate the aqueous layer to about 8 ml. after making it strongly basic. Then follow the procedure for the determination of Zinophos, including the test for quenching.

Calibration Curve. Dissolve 23.8 mg. of sodium pyrazinolate in distilled water and dilute to 500 ml. Store in a refrigerator and prepare fresh every 2 weeks. One milliliter of this solution is equivalent to 100 µg. of Zinophos. Prepare standards over the range 0

Table VI. Extent of Hydrolysis of Zinophos under Various Conditions

Concentration of Base, N	Reflux Time, Min.	Hydrolysis,[a] %
0.1	60	100
0.1	30	100
0.05	60	100
0.05	30	100
0.025	60	100
0.01	60	52
0.005	60	7

[a] Based on comparison with equivalent 2-pyrazinol.

to 70 μg. in 10-ml. volumetric flasks, dilute to approximately 9 ml. with water, add one drop of 50% NaOH, and dilute to mark. Then measure the fluorescence intensity and prepare a calibration curve.

Discussion

At its present stage of development, Zinophos has been used principally in preplanting applications against soil-borne pests. It appears to persist in the soil about 12 to 14 weeks under early summer conditions of moderate rainfall. Residue studies on crops grown in soil treated with 2.5 to 10 pounds of active Zinophos per acre have indicated little tendency for Zinophos or its oxygen analog to accumulate in the tissues of mature plants. During the first few days of growth, beans accumulate detectable residues, but these disappear as the plant matures. Typical residue data are shown in Table I. Residue studies on other crops grown in soil treated with 2.5 to 10 pounds of active Zinophos per acre resulted in Zinophos residues less than 0.05 p.p.m. Crops included potatoes 130 days after treatment at 3 pounds per acre, corn 102 days after treatment at 10 pounds per acre, and table beets, 104 days after treatment at 10 pounds per acre.

The method has also been tested on extracts of cherries, cauliflower, carrots, asparagus, strawberries, and oranges. Oranges and peppermint oil proved to resist accurate analysis, and results with soil were only fair. The results of the various recovery studies are tabulated in Tables II and III.

The reasons for the limited success of tne method in measuring soil residues in Zinophos are not clear. Recoveries of 60% have been attained quite consistently on freshly fortified soils. This may be due to the partial adsorption of Zinophos by the soil or to the presence of interferences which are not removed by the various cleanup methods. Tests for quenching have indicated that this is not the cause of the low recoveries.

Table VII. Recovery of Zinophos-C^{14} During Analysis

Steps in Analysis	Recovery, %[a] This laboratory[b]	This laboratory[b]	Bowman et al. (2)[c]
Extraction	98	97	87
Adsorption chromatography	91	103	102
Hydrolysis	97	99	96
Chloroform extraction	98	...[d]	96
Ion exchange chromatography	...[d]	86	...[d]
Over-all recovery	85	85	87

[a] Determined before and after each step using liquid scintillation counter.
[b] Zinophos-C^{14} added to bean slurry.
[c] Zinophos-C^{14} added to banana slurry.
[d] Not determined.

The possibility that soil hydrolysates contain excessive amounts of salts which might interfere with the ion exchange chromatography has been investigated without leading to improvement in the method. The analysis of soils freshly fortified with 2-pyrazinol results in poor recoveries (ranging no higher than 25 or 30%).

With adequate cleanup, the method is sensitive to 5 μg. of Zinophos in 100 grams of crop tissue. Two kinds of interferences occur—high blank due to fluorescent materials in the plants and quenching by naturally occurring substances. The latter interference is the probable cause of the low recoveries from extracts of oranges shown in Table II.

Tests of the method against other pesticides which might be encountered with Zinophos (Table IV) show that Guthion interferes by fluorescing in the same region, while parathion seems to produce some quenching.

The precision of the method in the hands of various analysts is indicated by the results in Table V. The extent of hydrolysis can be seen by a study of Table VI which shows data obtained during the development of the hydrolysis procedure.

The reasons for the self-quenching test described earlier are clear from a study of the standard curve (Figure 1). Between 0 and 70 μg., fluorescence is proportional to the quantity of Zinophos. Beyond this point, self-quenching becomes a factor, and fluorescence actually decreases when Zinophos level exceeds 300 μg. in 10 ml. During analysis, it is important to determine whether the Zinophos content of the sample lies on the ascending or the descending portion of this curve. This can be determined as described earlier by a dilution of the sample after the fluorescence intensity measurement, followed by a second measurement of intensity.

In the development of analytical methods, it is often difficult to determine the efficiency of recovery in sequential steps in a tentative method using conventional means of detection. Radioisotopes, however, offer a reliable and sensitive means for checking any step in the method without interference from crop materials or reagents. Carbon-14 ring-labeled Zinophos was used to check the individual steps in the method. Results in Table VII indicate that small losses occur at each step during the determination. These studies show that the extraction procedures used result in nearly quantitative removal of Zinophos from beans or bananas.

To find a suitable solvent for extraction of Zinophos residues, water solutions of Zinophos were extracted with various solvents. Hexane removed 97% Zinophos from water and benzene or chloroform removed 99%. The latter solvents were chosen because of their greater suitability for the cleanup method.

The elution of the oxygen analog of Zinophos from the Attaclay column is best accomplished with technical chloroform. Chloroform purified by distillation is unsatisfactory, as is redistilled chloroform containing traces of water or formic acid. Redistilled chloroform containing 1% ethanol, methanol, or acetic acid provides a suitable elution solvent, although such a mixture removes more undesirable plant constituents from the column than does technical chloroform. If the former elution solvent is used, it may be necessary to increase the number of chloroform or ethyl acetate extractions after the hydrolysis step. The elution of the Attaclay column with benzene followed by chloroform effectively separates Zinophos and its oxygen analog. Analyses of plant extracts fortified with the two compounds have shown good recoveries of both materials, 93% for Zinophos and 80% for the oxygen analog.

A desirable feature of the method would be its application to the determination of free 2-pyrazinol in crop or soil samples. At least two properties of this compound have thus far prevented the achievement of this goal, however. Its high water solubility prevents the use of nonaqueous solvents in its removal from plant tissue, and its tendency to be adsorbed or otherwise bound by plant and soil tissue also interferes with extraction. At the present stage of development, therefore, the method must be considered as adequate only for intact Zinophos or its oxygen analog. This may not be a serious handicap in the development of Zinophos residue information because of the fact that 2-pyrazinol in the soil may be lost rapidly by leaching. Furthermore, metabolic studies have shown (*6*) that 2-pyrazinol in plant tissue has only a limited existence,

being further metabolized to smaller fragments.

Acknowledgment

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FUNGICIDE RESIDUES

Modifications to the Spectrophotometric Analysis of PCNB (Terraclor) in Soil and Crops

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Purified reagents have been found essential to obtain the increased sensitivity of the spectrophotometric method for PCNB. A modified end determination requires less time and is applicable to extracts which have not been treated with sulfuric acid. The pretreatment, activation, and storage of Florisil described has been effective in obtaining a more uniform and stable absorbant. Removal of elemental sulfur from extracts of raw agricultural commodities has been accomplished by refluxing with clean copper wire. Direct extraction of Terraclor with ethanol from some food products has afforded the opportunity to partition PCNB into petroleum ether, while red and yellow pigments are discarded in the aqueous alcohol phase. Soil extracts can be directly analyzed for Terraclor by this method.

THE SPECTROPHOTOMETRIC METHOD (*1*) of analysis for pentachloronitrobenzene [Terraclor (PCNB), registered tradename for pentachloronitrobenzene by Olin Mathieson Chemical Corp.] residues has been in continuous use since it was developed in 1954. Due to the extensive experimental testing and broad use of this fungicide, the application of this method has afforded opportunities to appraise, improve, and subject it to rigorous conditions.

The comments of Klein and Gajan (*4*) indicate a preference for this spectrophotometric method because of its near specificity. Although tetrachloronitrobenzene can give an additive interference, it is not common practice to use it in combination with PCNB.

More detailed specifications and proposed methods of purification of reagents are presented. The use of only 1.0 ml. of alcoholic potassium hydroxide for hydrolysis eliminates the adjustment of pH of the azo dye solution. Thus, time is saved, transfers are eliminated, and dilution of the solution is avoided. All contribute to greater sensitivity. If extracts must be treated with sulfuric acid to remove oils, the larger volume of alcoholic potassium hydroxide and subsequent adjustment of pH (*1*) might be required.

Extraction of soil and subsequent analysis of an appropriate aliquot by this method have been accomplished without interference. The cleanup techniques now available may be applied to extracts of soil and raw agricultural products to remove interfering materials most commonly encountered.

Refluxing extracts with clean coils of copper wire has been effective in eliminating elemental sulfur which normally would prevent development of color for PCNB.

A more uniform and a more stable Florisil has been obtained in these laboratories when the prescribed cleanup, activation, and storage methods have been followed. It has been used as a substitute for the more complex mixture No. 1 (*1*) in removal of pigments from extracts of the crops described.

An alternate approach for extraction of PCNB is based upon development investigations. Certain crops were not readily miscible with nonpolar solvents, and recoveries were erratic. Blending with ethanol and sodium sulfate and subsequent partitioning of the diluted aqueous alcohol with petroleum ether produced more consistent recoveries and an effective partitioning of red and yellow pigments in the discarded aqueous phase.

Experimental

Reagents. WATER. Deionized or equivalent.

1-NAPHTHYLAMINE. Recrystallized from hot cyclohexane, then from warm purified petroleum ether.

PURIFIED PETROLEUM ETHER (b.p., 30°–60° C.). To meet specification: "Upon concentration of 800 ml. of reagent grade petroleum ether to 5 ml., sufficient interference shall not be obtained to produce a reagent blank equivalent to more than 3.6 ± 0.8 μg. apparent PCNB. Nor shall such a concentrate, to which a 5-ml. aliquot of a standard purified petroleum ether solution containing 50 μg. of PCNB has been added, and subsequently concentrated to 5 ml., deviate more than $\pm 10\%$ in absorbance value, compared to a similar standard not containing the concentrates of 800 ml. of petroleum ether."

A purification procedure is presented to be used if the recommended method in the previous publication (*1*) does not produce a solvent which meets the above specification. Prewash the petroleum ether with three successive lots of con-

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