being further metabolized to smaller fragments.

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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 ±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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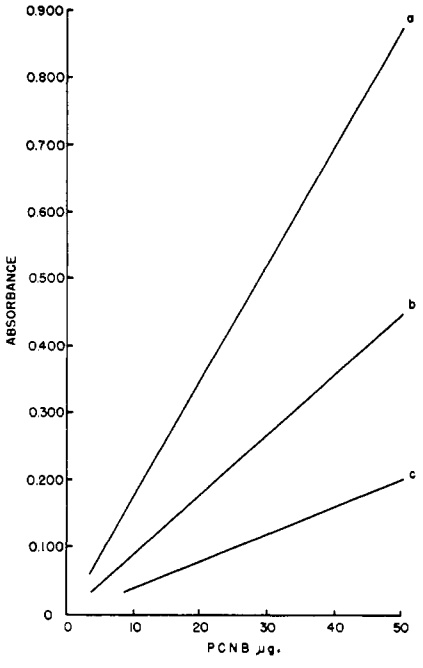

Figure 1. Standard Calibration Curve for PCNB

(a) 5.00-cm. light path via present method; (b) 2.54-cm. light path via present method; (c) 2.54-cm. light path via method (4)

centrated sulfuric acid, wash once with distilled water, once with 2% sodium bicarbonate solution, and with distilled water until such washes are neutral to pH indicator paper. Dry the petroleum ether with anhydrous granular sodium sulfate and filter. Distill the filtrate through a 400-mm. column of 2-mm. glass beads, discarding the initial 100-ml. forecut and 100-ml. tail product from each 4-liter batch. Store the distillate in glass bottles.

PENTACHLORONITROBENZENE (reference grade). Dissolve 100 grams of technical grade PCNB in 500 ml. of benzene; wash with concentrated sulfuric acid, distilled water, 2% aqueous sodium bicarbonate, and water until neutral. Dry with sodium sulfate and filter. Evaporate the benzene in a vacuum oven at 50° C. Dissolve the product in hot USP ethanol; filter through a heated funnel. Remove the last traces of ethanol in a vacuum oven and store the pentachloronitrobenzene in a dark glass bottle.

MAGNESIUM OXIDE. The originally recommended (*1*) Westvaco No. 2641 has been discontinued. If this adsorbant is to be used, Sea Sorb 43 (Fisher Scientific Company) is a suitable substitute.

FLORISIL (pretreated, activated). Pour 120 grams of Florisil (Floridin Co., Tallahassee, Fla.) 60–100 mesh (110° or 260° grade) into a 1-liter, borosilicate glass beaker containing 700 ml. of 1:1 hydrochloric acid. Stir continuously for exactly 1 minute. Pour the slurry immediately into a 90-mm., fritted disk glass funnel set in a suction flask with vacuum applied. Wash with distilled water, but do not agitate with a rod, until the filtrate is neutral. Dry overnight at 120° C. Activate the dry material at 650° C. for 2 hours in a preheated furnace. Transfer the activated product immediately to glass-stoppered bottles and keep stored at 120° C. until used.

COPPER COILS. Prepare coils of No. 16 B and S copper wire by wrapping it around a 5-mm. glass rod to give 12 loops to the coil. Clean immediately before use in concentrated nitric acid and rinse successively with water, acetone, and purified petroleum ether.

ETHANOLIC POTASSIUM HYDROXIDE, 0.5 *N*. Allow to stand overnight; filter and store in the dark. Discard when yellow.

COLOR REAGENT. Dissolve 0.350 gram of 1-naphthylamine in 88 ml. of glacial acetic acid and 200 ml. of deionized water. Add 7.500 grams of procaine hydrochloride. Dilute to 1000 ml. with deionized water. Store in a dark, glass-stoppered bottle.

TREATED CELITE. Slurry Celite 545 with 1 to 1 hydrochloric acid, filter, and wash with deionized water until neutral. Dry at 110° C.

Preparation of Standard Calibration Curve. Prepare standard test solutions of reference grade pentachloronitrobenzene in purified petroleum ether to deliver 10 μg. of PCNB per 1.00 ml. at 20° C.

Transfer 0, 1, 2, 3, 4, and 5 ml. of the test solution to 150-ml. acetylization flasks. Adjust the volume of each to 5 ml. with purified petroleum ether. Add 1.00 ml. of 0.5*N* ethanolic potassium hydroxide and 1.0 ml. of ACS grade acetone to each. Heat exactly 7 minutes at 80° C. in a water bath, with the bottoms of the flasks immersed only 1/4 to 1/2 inch in the water.

Cool in a cold water bath. Add 20.0 ml. of color reagent and 25 ml. of purified petroleum ether. Stopper and shake mechanically for 15 minutes. Transfer to 60-ml. separatory funnels. Filter each aqueous layer under vacuum, portionwise, through 1 × 1 cm. treated Celite (*1*) in a medium porosity filter stick, collecting the filtrates in test tubes set inside a suction flask or similar vacuum assembly.

Measure absorbance, *A*, of the solutions in 5-cm. cells in a suitable spectrophotometer at 525 mμ. Use water as a reference or compare standards with the reagent blank. Plot *A*, corrected by the reagent blank, against μg. of PCNB. The color produced conforms to Beer's law within the range of 0 to 50 μg. (Figure 1, curve *a*). The average *A* for 32 determinations at the 50-μg. level was 0.881 ± 0.031.

Such a calibration curve should be used as reference only for the standard solutions of PCNB analyzed simultaneously with reagent blanks, crop or soil blanks, recovery standards, and treated samples. Calculation of apparent μg. of PCNB in the extracts should be performed by the following equations:

$$\text{Apparent } \mu\text{g. PCNB} = \frac{A \text{ of crop or soil sample} - A \text{ of crop or soil blank}}{F} \quad (1)$$

where F =

$$\frac{A \text{ of PCNB standard} - A \text{ of reagent blank}}{\mu\text{g. PCNB in standard}} \quad (2)$$

Procedure. SOIL. Collect sufficient replicate core or plug samples of soil (*3*), air-dry in an uncontaminated area, mix, and divide to obtain representative subsamples for moisture and PCNB analyses.

Determine the moisture content of a 5.0-gram subsample dried at 110° C. for 5 hours, or to a constant weight.

Transfer 100 grams of air-dried subsample of soil to a 500-ml., glass-stoppered Erlenmeyer flask. Add 200 ml. of purified petroleum ether, stopper, and shake for 1 hour. Allow solids to settle. Decant the supernatant solvent through prewashed glass wool, collecting 100 ml. of the filtrate in a volumetric flask at 20° C.

Perform preliminary analyses of similar aliquots of the extracts of treated soil samples, control soil blanks, and recovery standards—i.e., control soil spiked with known amounts of PCNB.

If the soil recovery standard fails to give the required color developed for such a quantity of PCNB, it is possible that sulfur was present in the soil and that a cleanup is necessary.

If the soil blank contains materials giving absorbance values equivalent to large quantities of apparent PCNB, a cleanup through pretreated, activated Florisil, described below, may be effective in removing such an interference. If this type of interference persists, it is more likely to be indicative of PCNB contamination.

If the concentration of net PCNB in the aliquot of the treated soil is beyond the desired 3- to 50-μg. range, select a more appropriate aliquot for analysis and apply the cleanup procedures indicated by the proposed preliminary tests, if such are necessary.

Data for recovery of PCNB from local soil are presented in Table I. The local soil blanks are indicative of a background equivalent to less than 0.06 p.p.m. apparent PCNB in a 50-gram sample. The background interference rises proportionately as the size of the equivalent weight of soil in the aliquot of the extract decreases. This is inherent in this method, since the lowest reliable amount of PCNB measurable is 3 μg. An average 91.7% recovery was obtained within the 1 to 10 p.p.m. range.

Table I. Local Soil Blanks and PCNB Recoveries

Blanks		Recovery Standards		
Equivalent grams soil in aliquot	Apparent p.p.m. found	PCNB Added µg.	P.P.M.	Recovered, %
0.5	<6	5	10	88.0
1.0	<3	50	50	94.8
	<3	25	25	94.0
5.0	<0.6	25	5	96.4
	<0.6	5	1	82.0
10.0	<0.3	50	5	96.4
25.0	<0.12	25	1	72.8
50.0	<0.06	50	1	98.4
				Av. 91.7

Table II. Residual Terraclor in Greenhouse Soil

Controls					Treated[a]			
Equivalent grams soil in aliquot	Blanks, apparent p.p.m. found	Recovery Standards P.P.M. added per 10 grams	µG. in aliquot	Recovery, %	Equivalent grams soil in aliquot	Net P.P.M. PCNB Found A	B	C
0.2	60	2500	50	101.7	0.02	1276	2988	
0.2	57	2500	50	103.7	0.02	1281	2563	
0.2	60	2500	50	105.0	0.02	1252	2904	
0.2	59	2500	50	106.1	0.02		2700	
0.02	193	250	5	63.2	0.02		2700	
					0.004			5635
					0.004			5370
					0.004			5601
Av.	86			96.0		1270	2671	5535

[a] A = 2000 p.p.m. applied; B = 4000 p.p.m. applied; C = 20,000 p.p.m. applied.

In Table II, typical results are presented for sets of soil from control and Terraclor-treated beds. The objectives of the test were studies of treatment of beds for growing ornamental plants and sequent loss of Terraclor through irrigation and ordinary weathering factors. The soil was treated at 2,000; 4,000; and 20,000 p.p.m. active PCNB and irrigated 48 times at a rate of 1 quart of water per square foot. A total of 88 gallons of water was used in this process. The soil samples were analyzed by the procedure described above. The control samples were used as blanks and were spiked with PCNB to obtain recovery data. Due to the high concentrations of PCNB found in the treated soil, comparative recovery standards and blanks of approximately the same equivalent weight of soil were analyzed. The blanks appear to be high in background of apparent PCNB. This is partially due to the calculation of small quantities of apparent PCNB in the relatively small weights of soil. The average recovery agrees favorably with the one in Table I. The results presented in Table II under *Treated* are indicative of the apparent loss based only on the theoretical amounts applied.

Florisil Cleanup. Prepare a 30 × 400 mm. column to contain 100 mm. of pretreated, activated Florisil, topped by 20 mm. of anhydrous granular sodium sulfate. Prewash with 75 ml. of purified petroleum ether, allowing about 2 mm. of solvent to remain above the sulfate layer.

Deliver an appropriate aliquot of the extract to the column. If the aliquot exceeds 25 ml., concentrate to this volume before introducing it into the column. Allow it to pass into the sulfate layer and collect the filtrate in a clean, 150-ml. acetylization flask. Elute with 150 ml. of 6% diethyl ether–purified petroleum ether mobile solvent. Concentrate the filtrate from the Florisil column to 5 ml. and proceed with the analysis for PCNB.

Elemental sulfur may be eluted from the Florisil column if such is present in the soil extract, and the following technique is recommended for its removal.

Sulfur Cleanup. Deliver an appropriate aliquot of soil extract to a 150-ml., tapered acetylization flask and concentrate, or dilute with petroleum ether, to about 25 ml. Insert a coil of freshly cleaned copper wire and reflux in a warm water bath. Remove the copper wire if it becomes discolored by deposits of cupric sulfide, and rinse quantitatively with petroleum ether. Repeat refluxing with clean copper wire until discoloration does not occur. Remove the copper wire, rinse, concentrate the extract to 5 ml., and proceed with the analysis for PCNB.

Discussion

Comparisons of polarographic (*2,4*) and gas chromatographic (*4*) procedures to the spectrophotometric (*1,4*) method of analysis for PCNB have indicated that the latter method is preferred for its specificity and more consistent recoveries of PCNB from raw agricultural commodities. Modifications to the extraction and cleanup processes have been proposed by Bache and Lisk (*2*) and by Klein and Gajan (*4*). The variations to the spectrophotometric method, offered in the latter paper, appeared to give significantly higher reagent blanks than obtained by the original procedure (*1*).

The specificity of this method more closely approaches the ideal since the only known interference, appearing as PCNB in extracts of raw agricultural commodities, would be tetrachloronitrobenzene (TCNB). The latter compound would normally not be used in combination with PCNB for protection of a crop. Those pesticides known to give interference to the polarographic and gas chromatographic methods are, however, quite frequently applied in normal pesticide control applications and would offer significant problems to the isolation of PCNB.

The sensitivity of the spectrophotometric method for pentachloronitrobenzene has been significantly increased by the use of purified reagents and solvents. The procedure of Klein and Gajan (*4*) was reported to produce a reagent blank equivalent to an apparent 8-µg. quantity of PCNB, whereas the typical blank obtained under the circumstances herein presented should not exceed an apparent 3.6±0.8 µg. of PCNB. Furthermore, the slope of the standard calibration curve produced by their procedure is far lower than obtainable by this method. (Figure 1, curve *c*). The causes for such inconsistencies may well be attributed to the reagents and the instrument (and cells) used for measurement of absorbance values. Purified reagents and solvents must be used. Increased sensitivity may be obtained if the 5.00-cm. cells are used in a Beckman Model B or DU Spectrophotometer or equivalent instrument. The contrast between 5.00-cm. and 2.54-cm. light path absorbance values is illustrated in Figure 1, curves *a* and *b*, respectively.

The modifications to preparation of reagents, solvents, and adsorbants, as well as the specifications presented in this paper, are considered essential to obtaining increased sensitivity and reproducibility by this method.

Distilled water has frequently been found to contain sufficient oxidizing agents to destroy the microquantities of nitrite to be determined.

Impurities and color imparted by 1-naphthylamine which has become dark with age have been responsible for high reagent blanks.

Reagent grade petroleum ether sold

under various trade names and even those reportedly redistilled fractions with more narrow boiling ranges, as well as a product distilled from sulfuric acid, have often failed to meet the prescribed specifications. The presence of impurities in this or any other solvent used in proportionately large quantities for extraction of the raw agricultural commodity or soil and subsequently concentrated and submitted to the analytical procedure must always be checked for potential interferences. Most frequently, impurities have caused low values where known quantities of PCNB have been added.

The recommendations for purification of PCNB are particularly applicable when such a high quality reference material is needed in development of an improved end determination or a procedure for determination of PCNB residues in a new crop or resolution of a problem suspected to be due to an interference. Under routine conditions, the purity of PCNB in technical grade Terraclor—e.g., 99% active PCNB—may be adequate as a standard of comparison.

The procedure (*1*) for preparation of the standard calibration curve, used as the general analytical method for all extracts in that publication, has been modified. Sulfuric acid treatment of some crop extracts resulted in entrainment of some acid in the final solutions to be analyzed. It is advisable to check and adjust the final azo dye solutions from such acid-treated extracts so that they fall within the optimum pH 2.0–2.5 range. Standard PCNB solutions and other extracts may be analyzed by the modified procedure presented.

The recommendation to use the standard calibration curve only as a reference for a standard PCNB solution analyzed simultaneously with crop or soil extracts is offered. This permits detection of deviation from standard curve and makes certain that such a deviation is inherent in the reagents used in the end determination. If such should occur within the expected limits, but to the outer area of such limits, then calculation of apparent μg. of PCNB in the extracts by Equation 1 cancels such an error attributable to the reagents.

An extension to the range of this method may be obtained, but a slight deviation from Beer's law is evident at the higher concentrations. In the event that analysis of an extract develops a color beyond the expected limits, its estimated absorbance may be obtained by use of a 1-cm. cell or the multiple selector switch on a Beckman Model B spectrophotometer. However, it is advisable to prepare and analyze a new PCNB standard solution of concentration equivalent to that estimated in the extract and to obtain a new F by Equation 2 before calculating the apparent μg. of PCNB. Even though the new F agrees with the reliable F obtained within the normal concentrations, it is recommended that a smaller sample be extracted or an aliquot of the extract be analyzed which would contain PCNB within the 0- to 50-μg. range.

Upon developing the purified, activated Florisil, it was demonstrated that this adsorbant could be substituted for the more complex mixture No. 1 (*1*) to remove pigments from extracts of those crops described in Procedure A (*1*). It was noted that uniformity of the product prepared in this manner and stability of its activated properties when stored in stoppered bottles at 120° C. appear to give an adsorbant of extreme usefulness to this procedure. The work of Mills (*5*) and Moddes (*6*), as well as others who have recommended Florisil, is acknowledged as instrumental to the above modification. It has been effectively applied to several different lots of Florisil and has repeatedly improved the product for the cleanup of PCNB extracts. Prolonged agitation of Florisil in dilute aqueous hydrochloric acid or agitation while washing with water may produce a gel which impedes filtration. When such occurred, the batch was discarded and the fritted glass funnel was cleaned with 2% aqueous sodium hydroxide and water.

In the course of investigations leading to the development of extraction procedures for certain crops being experimentally treated with Terraclor, denatured (Formula 2B) ethanol was selected as the solvent. Petroleum ether, hexane, benzene, and methylene chloride invariably remained relatively immiscible with the crop. The tissue of the crop formed a globular, gelatinous mass, into which the above-mentioned solvents could not penetrate. A quantity of anhydrous granular sodium sulfate twice the weight of the crop, added with the alcohol and blended immediately, served to break tissues and cells, as well as to act as a dehydrating agent. Thus, the substrate was essentially an aqueous alcohol mixture of adequately high proportions, 75% or better, of alcohol to assure solubility and extraction of PCNB.

Isolation of the alcohol from the substrate by filtration afforded an opportunity for partitioning PCNB into purified petroleum ether by means of two 400-ml. extractions of the alcohol filtrate diluted to 360 ml. to obtain a 50% aqueous mixture. Emulsion problems and subsequent loss of PCNB occurred in mixtures containing greater quantities of water. The proposed conditions for partitioning PCNB into petroleum ether also resulted in the separation and removal of red and yellow pigments in the aqueous alcohol phase.

The technique of refluxing the extracts with clean copper wire to remove elemental sulfur was more efficient than the multiple operations required in bromination procedures (*7*).

The decolorized extracts of some crops obtained from the Florisil column may also contain some oils or waxes which can be removed by a sulfonation process similar to the one described for cottonseed (*1*). A single 50-ml. wash of the decolorized extract with concentrated sulfuric acid, followed by water, 2% aqueous sodium bicarbonate, and water, until neutral, normally removed such materials. Extracts receiving acid treatment should be analyzed for PCNB by the procedure in the earlier paper (*1*) to assure proper pH concentrations in the final azo dye solution.

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E. C. Gasiokiewicz, University of Massachusetts Agricultural Experimental Station, Waltham, Mass., conducted the greenhouse test of Terraclor in soil and provided the samples for analyses reported in Table II.

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