The product was recrystallized twice from 10 ml. of isopropyl alcohol to give a yield of 2.58 grams of pure dieldrin or 62.6% based on aldrin. The infrared spectrum was identical with that of an authentic sample of pure dieldrin and the melting point, 181° C., was the same as that of the authentic sample. The over-all yield of dieldrin based on barium carbonate was 22%. The specific activity was 1.32 mc. per mmole or 3.5 mc. per gram.

Because 79 mc. of C^{14} in 200 moles of barium carbonate were used, dieldrin with a specific activity of 4.2 mc. per gram would be expected. However, the 19% dilution of radioactive trichloroethylene with inert trichloroethylene in the preparation of octachlorocyclopentene results in a net expected activity of 3.4 mc. per gram. This is in remarkably good agreement with values obtained and indicates that no exchange of C^{14} occurred in any of the reactions involved.

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Literature Cited

(1) Burton, W. B., Potter, J. C., Silverman, M., Division of Agricultural and Food Chemistry, 132nd Meeting, ACS, New York, September 1957.
(2) Cramer, R. D., Kistiakowski, G. B., *J. Biol. Chem.* **137,** 549 (1941).
(3) Krall, R. E., Ph.D. thesis, University of Colorado, pp. 132–3, 1956.
(4) Prins, H. J., *Rec. trav. chim.* **69,** 1003–6 (1950).

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FUNGICIDE DETERMINATION

Rapid Polarographic Determination of Pentachloronitrobenzene on Forage and Comparison with Spectrophotometric Method

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A method is described for the polarographic determination of the fungicide pentachloronitrobenzene on forage. The chemical is extracted by tumbling with Skellysolve B. The extract is filtered and dried, and part of the extractives are removed by freezing and absorption on Attaclay. Chromatography of the concentrated extract using Florisil removes the remainder of the interfering substances. After chromatography the solvent is evaporated and the residue dissolved in isopropyl alcohol. Sodium acetate and acetic acid are added as a supporting electrolyte and after the solution has been deoxygenated, the polarogram is recorded from 0.00 to −1.15 volts against a saturated calomel electrode. Comparison data are presented from analysis of samples by this procedure and a spectrophotometric method.

PENTACHLORONITROBENZENE (PCNB) will control fungus diseases such as Sclerotinea crown rot and clubroot in forage. The method of Ackermann *et al.* (*1*) has been used here for the colorimetric determination of residues of the fungicide on red clover–timothy forage. Because of difficulties in the chromatographic cleanup, more than one analysis was required with some samples to obtain reliable results.

Webster and Dawson (*4*) developed a polarographic method for the determination of tetrachloronitrobenzene residues on potatoes. In the work reported, PCNB is determined polarographically by an adaptation of the Webster and Dawson procedure, after interfering substances have been removed by freezing and adsorption on Attaclay and Florisil.

Analysis of Forage

Using the method of Ackermann *et al.* (*1*), extract 150 grams of finely ground forage material with 900 ml. of Skellysolve B by tumbling for 45 minutes. Filter the extract through a double thickness of cheesecloth into a separatory funnel. Dry the extract, using sodium sulfate. Add 5 grams of an equal-volume mixture of Celite 545–Attaclay to the separatory funnel and shake for about 1 minute. Allow the solid material to settle and drain off the filtrate through the sodium sulfate layer. Discard the first 50 ml. Take an aliquot of the extract up to 300 ml. and concentrate it to 20 ml. by distillation through a Snyder column. While warm, transfer the solution quantitatively to a 16 × 50 mm. test tube. Remove plant waxes by immersing the tube in a dry ice–isopropyl alcohol bath (about −50° C.) for 10 minutes according to the method of Ordas, Smith, and Meyer (*3*). The addition of a small amount of Celite will keep the precipitate dispersed and facilitate washing. Filter the cold extract through S. & S. 595 paper and wash the precipitated waxes with three 5-ml. portions of *n*-hexane previously cooled to −50° C. Combine the filtrate and washings, and concentrate the solution to 10 ml. by distillation through a Snyder column.

Pack a 15 × 300 mm. chromatographic column with 15 grams of unactivated Florisil. Wet the column with about 25 ml. of *n*-hexane and add the extract to the column. Elute the PCNB with 100 ml. of *n*-hexane and collect the eluate in a 125-ml. acetylization flask. Distill off the hexane through a Snyder column and remove the last traces with a gentle stream of air. Add 2.5 ml. of 0.04*N* acetic acid, 2.5 ml. of 0.04*N* sodium acetate, and 5 ml. of C.P. isopropyl alcohol. Reflux the mixture for 5 minutes, using a boiling water bath and an Allihn condenser. Remove the water bath and immerse the flask, with condenser attached, in cold water. After the solution has cooled, filter it through S. & S. 595 paper into the polarographic cell.

The polarographic determination was carried out in an H-cell similar to that of Lingane and Laitinen (*2*), except

Table I. Recovery of PCNB from Alfalfa–Clover–Bird's-foot Trefoil Forage

Added, P.P.M.	Recovery, %
0.2	130
0.2	120
0.2	110
0.5	98
1.0	83
2.0	100
2.0	76
2.0	78.5

Check (average of 4 analyses), 0.34 p.p.m.

Table II. Determination of PCNB on Forage[a]

Days after Application	Residue, P.P.M. Spectrophotometric	Polarographic
7	32.0	30.0
	2290.0	1950.0
	725.0	744.0
	416.7	522.0
21	15.6	12.8, 13.2
	62.0	69.6
	3.6	7.7
	31.3	70.0, 73.5
	2.4	4.6, 3.8
	48.8	79.8, 78.0
35	1.8	3.3, 3.3
	35.6	35.2, 38.0
	18.8	17.4, 17.4
	14.3	11.9
	28.8	18.0, 18.6
	49.0	43.5

[a] Dry subsamples of same field sample.

that the outside diameter of the sample portion of the cell was reduced to 18 mm. to accommodate a smaller sample. A stopcock was attached to the base of the sample holder to facilitate cleaning. The entire sample was added to the H-cell and deoxygenated for 10 minutes with nitrogen. The polarogram was determined over the range of 0.00 to −1.15 volts against a saturated calomel electrode. The diffusion current was measured manually with a Fisher Elecdropode, which has a sensitivity range of 0.01 to 0.00001 equivalent per liter. The half-wave potential is −0.47 volt.

Standard Curve. Prepare a standard curve by adding 2-, 3-, 10-, 20-, and 30-ml. portions of a hexane solution of PCNB (5 μg. per ml.) to a series of 125-ml. acetylization flasks. Distill off the hexane through a Snyder column and remove the last traces with a gentle stream of air. Add 2.5 ml. each of the sodium acetate and acetic acid solutions and 5 ml. of isopropyl alcohol. Reflux for 5 minutes, cool, filter, and record the polarogram as in the analysis of forage.

Results and Discussion

The method was used to recover PCNB added to forage before extraction. Table I shows the recoveries obtained.

On May 6, 1957, PCNB was applied to clover-timothy forage plots for control of Sclerotinea crown rot. The rate of application was 75 pounds per acre, which was about 10 times the recommended rate. The PCNB was applied as a 75% wettable powder in 400 gallons of water per acre from a hand sprayer. The object was to study the disappearance of the fungicide with time after a very high rate of application. Samples were taken for residue analysis 7, 21, and 35 days after application. Table II shows PCNB found by the method of Ackermann *et al.* (*1*) and by the polarographic procedure.

Although very good agreement was obtained between the two methods, large differences can be observed among samples from the same field treatment. This variability might be due to errors in calibration of the hand sprayer, lack of agitation of the spray formulation during application, and variations in plant composition (differences in the percentage of weeds present) at harvest. Unfortunately, a satisfactory sample of check material from this experiment could not be obtained.

The half-wave potential shifted to a limit of about −0.53 volt during analysis of forage material. The magnitude of this shift was roughly proportional to the size of the sample. This did not affect interpretation of the polarogram, as no other waves occur in this region.

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Literature Cited

(1) Ackermann, H. J., Baltrush, H. A., Berges, H. H., Brookover, D. O., Brown, B. B., J. AGR. FOOD CHEM. **6,** 747–50 (1958).
(2) Lingane, J. J., Laitinen, H. A., *Ind. Eng. Chem., Anal. Ed.* **11,** 504 (1939).
(3) Ordas, E. P., Smith, V. C., Meyer, C. F., J. AGR. FOOD CHEM. **4,** 444 (1956).
(4) Webster, J. G., Dawson, J. A., *Analyst* **77,** 203–5 (1952).

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FUNGICIDE RESIDUES

Colorimetric Estimation of Dodecylguanidine Acetate Residues

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THE FUNGICIDE *n*-dodecylguanidine acetate, Cyprex 65W [American Cyanamid's 65% dodine (coined name) formulated as a wettable powder], has been proved useful for control of specific plant diseases. It is especially effective against apple scab [*Venturia inaequalis* (Cke.) Wint.], pear scab (*Venturia pirina* Aderh.), and cherry leaf spot (*Coccomyces hiemalis* Hig.) Additional uses are being developed.

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In conjunction with the field research which established its utility, a satisfactory analytical method was required to obtain data on the rate of disappearance of *n*-dodecylguanidine acetate (dodine) residues and to establish that its use would not result in hazardous residues at harvest.

A method was developed for determining dodine residues on apples using a surface extraction procedure to remove the chemical from the fruit. Subsequently, Hamilton and Szkolnik (*7*) reported local penetrant properties for the fungicide into apple leaves. When this property of dodine was first recognized, a macerate extraction procedure for total residues was developed to evaluate the efficacy of the surface extraction technique for determining total dodine residues.

Many high molecular weight nitrogenous bases have been shown to form salt-like addition products with acidic dyes in buffered aqueous solution (*1, 4, 12*). These addition products partition favorably into water-immiscible organic solvents, such as chloroform and benzene.