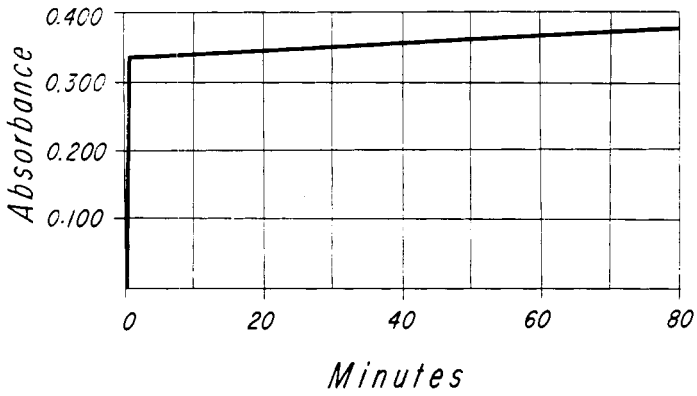

Figure 1. Rate of increase of Phygon-dimethylamine color *vs.* time

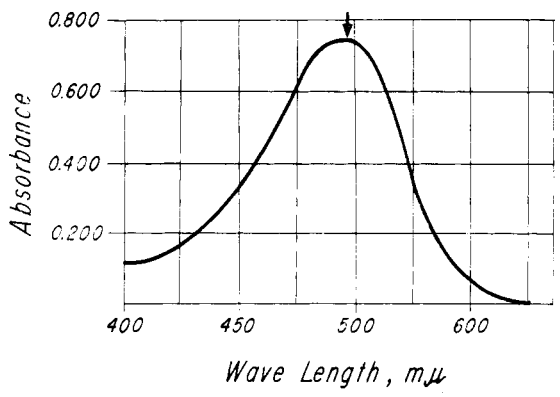

Figure 2. Absorption spectrum of orange color produced by reaction of Phygon with dimethylamine

Table I. Recovery of Knowns

	P.P.M. Added	P.P.M. Recovered[a]	% Recovery
Peaches	0.46	0.39	85
	0.46	0.44	91
	0.46	0.46	100
	0.43	0.41	94
	0.43	0.42	98
	0.43	0.40	93
Apples	0.46	0.47	101
	0.46	0.45	97
	0.46	0.46	99
	0.46	0.47	101
	0.43	0.41	95
	0.43	0.44	101
Strawberries	0.43	0.39	92
String beans	0.50	0.45	89
	1.0	0.88	88
	1.0	0.84	83
Tomatoes	0.50	0.48	97
	0.50	0.45	91
		Average	91

[a] Corrected for interference due to untreated sample.

lute to 40 ml. with anhydrous dimethylamine and mix thoroughly. At the same time, dilute a second 38 ml. of the filtered solvent wash to 40 ml. with benzene (technical grade) and mix thoroughly. Against benzene as the reference, measure the absorbance of both the prepared color solutions at 495 mμ on a Beckman Model DU spectrophotometer in 10-cm. Corex cells. The color solutions should be measured 5 to 20 minutes after the addition of dimethylamine. The absorbance difference between the portion diluted with dimethylamine and the portion diluted with benzene is that at 495 mμ used in Equation 1.

Calculations.

$$\text{P.p.m.} = 2.91 \times (\text{absorbance difference at 495 m}\mu) \quad (1)$$

P.p.m. (due to dimethylamine coloration of interferences present) = p.p.m. check (with dimethylamine) − p.p.m. check (no dimethylamine) (2)

P.p.m. treated sample (corrected)

$$\frac{(A - B - C) \times 100}{\%\text{ recovery of known}} \quad (3)$$

where A = p.p.m. of sample treated with dimethylamine, B = p.p.m. of sample treated without dimethylamine, C = p.p.m. due to crop interference, from Equation 2.

Equation 1 has worked well in this laboratory, but it is advisable to run standard curves for approximately 2 weeks prior to instituting analysis. This will standardize the method to each laboratory's equipment, before formulation of an equation similar to Equation 1.

Recovery Experiments

Two means of adding known amounts of Phygon to untreated samples were used. Initially, the untreated sample and benzene were placed in a gallon jar, the known amount of Phygon was added, and the sample was tumbled on an automatic rolling device. Later, to simplify analysis, the untreated sample was washed first with benzene alone. To an aliquot of the recovered solvent, the known amount of Phygon was added, and analysis was made. Equally good recoveries were obtained from each addition and no distinction is made in Table I.

Acknowledgment

The author is indebted to the Agricultural Chemical Group of Naugatuck Chemical for their assistance and cooperation in making this work possible and to Dorothy R. Hill for much of the experimental data in this paper.

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FUNGICIDE RESIDUES

Spectrophotometric Determination of Pentachloronitrobenzene on Food and Forage Crops

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A SPECTROPHOTOMETRIC METHOD developed to detect residues of pentachloronitrobenzene (Terraclor, Olin Mathieson Chemical Corp.) has yielded good recoveries with extracts containing 10 to 50 γ, or as little as 0.02 p.p.m. Terraclor, also field tested as PCNB and Compound 275, is an effective soil fungicide for control of many root-

A spectrophotometric method for organic nitro compounds was modified to detect residues of pentachloronitrobenzene in treated vegetables, food products, and forage crops for registration of this soil fungicide with federal and state agencies. Quantities in the 10- to 50-γ range, as low as 0.02 p.p.m., can be detected. Analyses of samples from numerous test plots, treated at the recommended levels, show no significant residues at harvest time.

rotting and damping-off diseases, and has been registered for use on many plants through evidence accumulated by this method.

Initially, the crops were ground with a suitable solvent. The extracts were processed to remove pigments, waxes, and oils according to the procedures described for the various crops. The photometric analysis of 2,3,5,6-tetrachloronitrobenzene by Canback and Zajaczkowska (*1*) was modified to determine trace quantities of Terraclor in the extracts. This consists in alkaline hydrolysis to potassium nitrite, which in turn is used to diazotize procaine hydrochloride. The diazotized product is coupled with 1-naphthylamine to give a magenta colored solution, the maximum absorption occurring at 525 mμ.

Reagents

General. Petroleum ether, Merck's reagent petroleum benzin, 30°–60° C. Prewash with 1 to 1 hydrochloric acid, then with distilled water until neutral; dry with anhydrous sodium sulfate; filter and store in glass bottles.

Ethanolic potassium hydroxide, 0.5*N*. Allow to stand overnight; filter and store in dark. Discard when yellow.

Color reagent. Dissolve 0.350 gram 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 distilled water until neutral. Dry at 100° C.

Reference standard, pentachloronitrobenzene. Recrystallize from 95% ethyl alcohol. Filter while hot, cool the filtrate, and recover crystals by filtration. Dry under a heat lamp. The pure product melting point is 143.6° C.

For Procedure A. Adsorbant Mix 1. Heat magnesium oxide (Westvaco No. 2641) at 200° C. for 1 hour. Cool in a sealed jar. Transfer 100 grams of this material to a mortar, add 6 ml. of distilled water, grind, transfer to a jar, seal, and mix for 30 minutes. Add an equal volume of Johns-Manville Hyflo-Super Cel and mix for 30 minutes. Age for 24 hours. Discard after 4 days.

For Procedure B. Adsorbant Mix 2. Mix equal volumes of Celite 545 with Attaclay (Attapulgus Division, Minerals and Chemicals Corp., Philadelphia, Pa.). Store in a sealed jar.

Adsorbant Mix 3. Prepare the same as adsorbant mix 1, adding Celite 545 in place of Hyflo-Super Cel.

For Procedure C. Redistilled petroleum ether. Reflux reagent petroleum benzin (30° to 60° C.) with stirring, over concentrated sulfuric acid for 5 minutes, then distill. Store in glass bottles.

Apparatus

General. Deionizing column. Use either a commercial kit or a mixture of regenerated ion exchange resins (H^+ and OH^-) in a column, throuhg which distilled water is passed from a constant level reservoir.

Mechanical shaker, wrist action, Burrell Corp., 2223 Fifth Ave., Pittsburgh 19, Pa.

Clarification equipment. Straight sealing tube with coarse fritted disk (Corning Glass No. 39570) fitted to a rubber stopper in a 1-liter suction flask.

Spectrophotometer, Beckman Models DU or B, with 5-cm. matched Corex cells.

For Procedure A. Blendor, Waring or Osterizer (replace rubber gaskets with Teflon gaskets).

Acetylization flasks, borosilicate glass, $\overline{\S}\,^{24}/_{40}$, 150 ml., pear-shaped.

Reflux apparatus, West-type, water-cooled condenser, no-drip tip.

Chromatographic column, 30 × 280 mm. medium porosity fritted glass disk with vacuum sidearm below disk. $\overline{\S}\,^{24}/_{40}$ joint at base.

For Procedure B. Jars, 80-ounce, 65-mm. mouth, screw cap with removable pure tin disk liners.

Mill, Wiley or Ball and Jewell, $^3/_{16}$-inch screen.

Blender, Patterson-Kelley, Twin-Shell, DD series.

Chromatographic column, refrigerated, similar to column described above with circulating jacket, 38 × 220 mm., surrounding it from a point 20 mm. above the fritted glass disk. Methanol, used as a coolant, is circulated by a pump from the coil in a methylene dichloride–dry ice bath.

For Procedure C. Mill, Mikro-Sampl-Mill, $^1/_{16}$-inch screen.

Preparation of Standard Calibration Curve

Prepare a standard test solution containing 10 γ of Terraclor per 1.00 ml. from the reference standard pentachloronitrobenzene in petroleum ether. Store at 20° C. or lower.

Transfer 1, 2, 3, 4, and 5 ml. of the test solution to 150-ml. acetylization flasks. Adjust the volume of each to 5 ml. Add 2.0 ml. of 0.5*N* ethanolic potassium hydroxide and 1.0 ml. of ACS grade acetone to each test solution and to a petroleum ether blank. Heat exactly 7 minutes at 80° C. in a water bath, with the bottoms of flasks immersed only $^1/_4$ to $^1/_2$ inch in the water.

Cool in a cold water bath. Add 20.0 ml. of the color reagent. Transfer the solutions to 100-ml. beakers. Adjust to pH 2.0 to 2.5 with concentrated hydrochloric acid. Return to the same flasks and add 25 ml. of petroleum ether. Stopper and shake mechanically for 15 minutes. Then transfer to 60-ml. separatory funnels. Pass each aqueous layer, portionwise, through 1 × 1 cm. treated Celite in a medium porosity filter stick, collecting filtrates in test tubes.

Measure absorbance, *A*, of the solutions through 5-cm. cells in a suitable spectrophotometer at 525 mμ. The color produced conforms to Beer's law within the range of 10 to 50 γ when plotted as a straight-line calibration curve.

Determine the concentration of apparent pentachloronitrobenzene from such a curve or by the following formula:

$$\text{Apparent } \gamma \text{ of PCNB} = \frac{A \text{ of crop sample} - A \text{ of crop blank}}{\dfrac{A \text{ of PCNB std.} - A \text{ of reagent blank}}{\gamma \text{ of PCNB in std.}}} \qquad (1)$$

Procedures

Procedure A. Extract lettuce, green beans, peppers, potatoes, celery, and tomatoes by the following method. (Slight modifications are necessary due to physical characteristics of the crops.)

Clean the representative subsamples by accepted domestic or commercial procedures. Use only those portions of the crops normally consumed by humans or animals which furnish food products to man. Cut samples into small pieces.

Blend 200 grams of the crop subsample with 250 to 300 ml. of methylene dichloride. Control blending speed by means of a transformer to minimize emulsions. Separate the extracts from the pulp in a basket centrifuge lined with No. 2 Whatman filter paper. Transfer any aqueous material remaining in the basket to a separatory funnel. Re-extract the pulp and paper matrix, and recover the extracts as above.

Combine the aqueous layers and wash with methylene dichloride. Pool these washes with the extracts. Dry the extracts by shaking in a separatory funnel with sodium sulfate. Pass the extracts carefully through the sulfate layer, portionwise, into a 150-ml. acetylization flask as the solvent is evaporated on a hot water bath. Remove the last traces of

solvent by means of a gentle stream of clean, dry air.

Add 25 ml. of petroleum ether to the residue. Attach the flask to a condenser and reflux for 15 minutes. Maintain an even flow of condensate down the side walls of the flask to wash continuously any residue adhering to the side walls.

Prepare a chromatographic column with 2 cm. of anhydrous, granular sodium sulfate on the fritted glass disk, 6 cm. of adsorbant mix 1, and top with 2 cm. of sodium sulfate. Pack, with slight suction, by tamping with a blunt rod.

Prewash with 100 ml. of petroleum ether and discard this filtrate. Deliver the extract to the column and collect the filtrate in a 150-ml. acetylization flask. Add 25-ml. petroleum ether washes four times to the column as each preceding batch passes into the upper sulfate layer. Do not permit the column to be drawn dry.

Concentrate the filtrate to 5 ml., and analyze it as above. The average recoveries of Terraclor, from in vitro standards, at various concentration levels, are presented in Table I.

Procedure B. The forage crops (alfalfa, clover, timothy, and mixtures of these) contain an abundance of plant wax and fats which interfere with the spectrophotometric method to such an extent that the method of extraction and clean-up was modified.

Air dry forage crops in the field or in a protected, uncontaminated area. Mill representative samples and blend before taking 300-gram subsamples. Weigh the subsample into an 80-ounce jar, and add 1800 ml. of *n*-hexane. Secure the tin-lined screw cap. Extract by mechanical tumbling for 45 minutes. Remove the jar from the tumbler, and allow solids to settle. Decant the supernatant liquid through a double thickness of cotton gauze lining on a large glass funnel. Invert the jar over the funnel. Collect the filtrate in a 2-liter separatory funnel.

Dry the extracts with sodium sulfate, add 10 grams of adsorbant mix 2, and swirl. Filter the extracts through this mixture and collect a 900-ml. aliquot.

Evaporate the *n*-hexane from the extracts in a 150-ml. acetylization flask, as described in procedure A. Add 25 ml. of petroleum ether to the residue and reflux for 15 minutes.

Prepare a refrigerated chromatographic column with 2 cm. of sodium sulfate, 3 cm. of adsorbant mix 2, 3 cm. of adsorbant mix 3, and 2 cm. of sodium sulfate. Level and pack each layer with a heavy blunt rod. Chill the methanol to be circulated through the jacket to −10° C. in a methylene dichloride–dry ice bath. Chill the column and prewash it with 100 ml. of petroleum ether. Allow the ether to cool before drawing it off with a slight suction. After discarding the filtrate, add about 2 grams of Celite 545 to the extract, swirl, and deliver to the column. Allow to cool several minutes, thus permitting plant waxes and fats to solidify on the suspended Celite. Add four 25-ml. petroleum ether washes after each preceding volume is drawn into the upper sulfate layer. Remove the source of suction after each volume is drawn into the column and allow the next volume to chill before drawing it into the packing.

Concentrate the filtrate to 5 ml. for analysis as described above. After hydrolysis some waxes solidify, and, after adding the color reagent, it is necessary to warm the flask for about 3 minutes in the 80° C. bath to free all the nitrite from the waxes.

Average recoveries of Terraclor from forage crops are presented in Table I.

Procedure C. The high oil content of the extracted cottonseeds required development of another clean-up procedure for selective recovery of Terraclor as well as a slight modification of the analytical procedure.

Grind delinted cottonseed in a Mikro-Sampl-Mill through a $^1/_{16}$-inch screen, blend, and obtain 100-gram subsamples. Blend the milled cottonseed for 5 minutes with 200 ml. of petroleum ether, specially redistilled from sulfuric acid. Filter the extracts from the slurry through an asbestos mat on a Büchner funnel. Rinse quantitatively.

Transfer the filtrate to a 1-liter separatory funnel and add 50 ml. of concentrated sulfuric acid. Rotate to obtain maximum contact of extracts with acid. After a 15-minute separation period, drop the acid-sludge through two 100-ml. lots of petroleum ether. Allow another 15-minute separation. Then decant petroleum ether washes and extracts into a clean funnel. Wash successively with 50-ml. lots of deionized water, 2% aqueous sodium bicarbonate, and deionized water, until the water wash is neutral. Dry the extracts with sodium sulfate.

Concentrate the extracts to 5 ml. for analysis as described above. Check the pH of the mixture just before hydrolysis; some entrained acid in the extract may necessitate using more than the recommended 2 ml. of ethanolic potassium hydroxide. If the magenta dye appears at the interface after shaking, add 1 ml. of butyl alcohol to partition the dye into the aqueous layer.

Average recoveries of Terraclor from cottonseed are presented in Table I.

Table I. Recovery of Terraclor from Food and Forage Crops

Crop	Extraction Procedure	Sample Wt., Grams	Added, P.P.M.	Av. Blank, Apparent P.P.M.	Av. Recovery, %
Forage crops	B	300	0.07	0.07	80
			0.13	0.09	82
			0.17	0.10	79
			0.20	0.05	84
			0.25	0.08	95
			0.27	0.07	80
			0.33	0.07	89
Green beans	A	400	0.05	0.02	84
		450	0.11	0.03	78
		400	0.13	0.02	96
		450	0.22	0.02	79
		450	0.44	0.03	80
		225	0.88	0.04	80
Cottonseed	C	100	0.50	0.04	49
			0.40	0.04	70
			0.30	0.00	80
			0.20	0.01	62
			0.10	0.07	71
Lettuce	A	400	0.05	0.04	86
		540	0.05	0.02	90
		540	0.09	0.05	71
		200	0.15	0.02	90
		540	0.18	0.01	84
		200	0.25	0.02	89
Peppers	A	400	0.05	0.01	85
			0.08	0.01	82
			0.10	0.02	89
			0.13	0.01	81
Potatoes	A	200	0.05	0.03	64
			0.10	0.02	86
			0.15	0.02	71
			0.20	0.02	81
			0.25	0.01	74
Tomatoes	A	400	0.08	0.06	56
			0.10	0.03	80
			0.13	0.05	50
			0.15	0.01	86
			0.20	0.02	85
			0.25	0.04	71

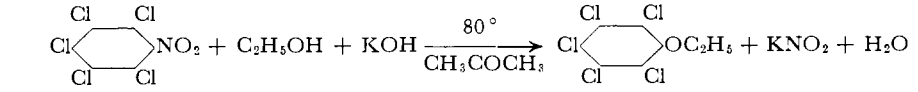
Discussion

Analysis Reactions. The spectrophotometric determination of Terraclor is based upon the following reactions:

Table II. Typical Terraclor Residues in Crops Receiving Treatment

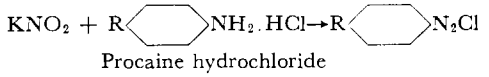
Crop	Source of Sample	Methods of Application	Dosage Rate, Lb. Active/ Acre	Av. Residue, Net P.P.M.
Forage	N. J.	Foliar spray	6.0	0.05
	Ohio and N. J.		12.0	0.08
	N. J.		24.0	0.05
Green beans	Fla. and S. C.	Mixed with soil by cultivation	3.5	0.02
	S. C.		14.0	0.03
	Calif.		25.0	0.00
	Calif.		31.5	0.03
	Calif.		50.0	0.00
Cottonseed	La.	Spraying seed in open furrow	5.9	0.00
	Calif.		6.7	0.02
	Calif.		6.7	0.00
	Mo.		6.7	0.01
Lettuce	Fla.	Mixed with soil by cultivation	4.5	0.00
	Calif.		15.0	0.02
Peppers	Md.	In transplant water	47.0	0.00
	Md.		94.0	0.00
Potatoes	Mich.	Dusted on soil, then cultivated	45.0	0.07
	Me.		50.0	0.05
	Wash.		60.0	0.07
Tomatoes	Del.	Foliar spray	3.0	0.02
	Tenn.	Drench or dust soil	10.0	0.01
	Tex.	Soil spray	50.0	0.01

HYDROLYSIS



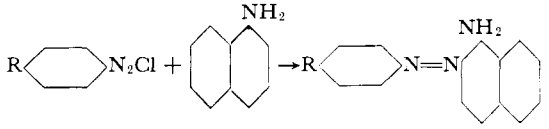
Pentachloronitrobenzene — Pentachlorophenetole

DIAZOTIZATION



Procaine hydrochloride

COUPLING



1-Naphthylamine

It has been demonstrated that the hydrolysis of pentachloronitrobenzene, under the conditions described, does not proceed to completion. The production of potassium nitrite from this compound has consistently been shown to be 50% of the theoretical yield. The optimum temperature for hydrolysis is 80° ± 0.1° C.; the optimum time is 7 minutes.

The diazotization of nitrite is conventionally performed as a separate reaction, under controlled conditions, and then followed by coupling, also under controlled conditions. The procedure described in this paper for successive diazotization and coupling in the same step was developed with the cooperation of the Food Research Laboratories, Inc., Long Island City, N. Y. (*2*).

Maximum color development occurs within a pH range of 2.0 to 2.5, with an appreciable decrease in development of color above pH 3.0. The spectral absorbance curve (Figure 1) shows that maximum absorption occurs at a wave length of 525 mμ under the conditions specified.

The filtration process, described for removal of turbidity or fine suspensions from the azo dye solution before observing absorbances, must be performed through an inert, neutral material.

Crop blanks and in vitro recovery standards are analyzed with each set of treated samples. Such in vitro standards are prepared by addition of known quantities of purified pentachloronitrobenzene, in the appropriate solvent, to untreated samples just before initial extraction.

Calculation of Terraclor in Crops. The absorbance obtained for untreated samples is subtracted from the absorbance obtained for the in vitro standards and the treated samples. The concentration of apparent micrograms of Terraclor may then be determined from the standard calibration curve or by calculation according to Formula 1. This concentration is corrected by the per cent recovery to obtain the net amount of Terraclor residue.

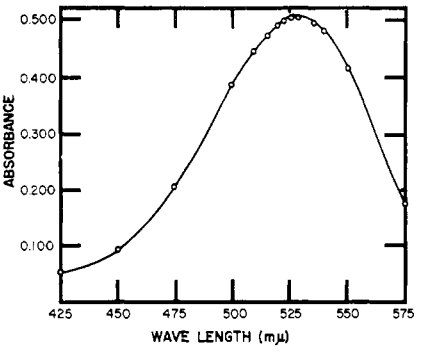


Figure 1. Spectral absorbance curve for azo dye from analyses of 50 γ of pentachloronitrobenzene

Typical Terraclor Residues. In Table II, typical Terraclor residues in treated crops are presented. The different methods of application and dosage rates show little effect on quantities of Terraclor detected. In all tests where the fungicide was applied as directed, residues in the food and forage crops were of very low concentrations.

Conclusions

It was shown that Terraclor does not translocate from the soil to any of the crops whose edible portions grow above the ground. The data also indicate that Terraclor is not translocated, or left as a residue, on potatoes grown in soil treated at the recommended application level and by the recommended method of application.

Acknowledgment

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