lend much significance to this slight variation.

Table I shows the amount of heptachlor epoxide residue (p.p.m.) in the omental fat of the dairy steers grazing 30 days after each was placed on treated plots. The earlier an animal was placed on a plot after treatment with heptachlor, the higher the level of residue in the omental fat. In general, the results followed the same residue pattern as was obtained in the butterfat of lactating dairy cows grazing the same plots (*6*).

Table II shows the heptachlor epoxide residues in the omental fat of beef animals grazing on aerially treated pasture for various lengths of time. The highest levels of residues were present in the 30-day samples, regardless of the age of the animals. After 125 days of grazing, heptachlor epoxide residues were found in all fat samples.

The levels of heptachlor epoxide residues in the omental fat of the animals grazing on aerially treated pasture for 30 days were much higher than the level found in the dairy steer grazing on surface-treated pasture. Since the beef animals were on pasture during the aerial application of heptachlor, as is usually practiced, apparently some of the insecticide may have been inhaled through the respiratory tract or absorbed through the skin in addition to ingestion of treated forage. Also, the fact that animals were grazing the same day of treatment may account for the higher level in the omental fat of these animals.

Residues in Organs of Beef Animals. Samples of brain, kidney, liver, and meat were obtained from three animals after grazing on treated pasture for 125 days to find out whether heptachlor epoxide might concentrate in these organs.

No residue was found in any of the organs of these animals although their fatty tissues showed approximately 2.5 p.p.m. of heptachlor epoxide. Since the residue concentrates in fatty tissue, any variation in amounts found in organs and tissues reflects a variation in fat content. Less than 1 p.p.m. of residue was present in raw and cooked meat (Table III). The increase in concentration of the heptachlor epoxide residue in cooked meat over raw meat is no doubt due to the dehydration of meat after cooking.

Literature Cited

(1) Entomology Research Division, U. S. Dept. Agr., *J. Econ. Entomol.* **52,** 1206 (1959).
(2) Gannon, N., Decker, G. C., *Ibid.*, **53,** 411 (1960).
(3) Meyer, C. F., Malina, M. A., Polen, P. B., J. AGR. FOOD CHEM. **4,** 694 (1956).
(4) Murphy, R. T., Barthel, W. F., *Ibid.*, **8,** 442 (1960).
(5) Radeleff, R. D., *Vet. Med.* **45,** 125 (1950).
(6) Rusoff, L. L., Waters, W. H., Gholson, J. H., Frye, J. B., Jr., Newsom, L. D., Burns, E. C., Barthel, W. F., Murphy, R. T., J. AGR. FOOD CHEM. **10,** 377 (1962).
(7) *U. S. Dept. Agr., Plant Pest Control Div., Control Manual* **807-25.6100,** (1960).

Received for review May 31, 1962. Accepted September 4, 1962.

INSECTICIDE RESIDUES

Method for Phosphamidon Residue Analysis

RUDOLF ANLIKER
Ciba, Ltd., Basle, Switzerland
R. E. MENZER[1]
Department of Entomology, University of Maryland, College Park, Md.

A paper chromatographic method has been developed for the determination of phosphamidon in plant tissue. After chromatography with a selective solvent system, phosphamidon and its metabolites were detected with a specific blue tetrazolium dye. After elution into test tubes with methanol, a total phosphate analysis was carried out by the phosphomolybdenum blue method. The sensitivity of the method is 0.2 to 0.4 p.p.m. depending on the dilutions and aliquots used.

PHOSPHAMIDON (1-chloro-1-diethylcarbamoyl-1-propen-2-yl dimethyl phosphate) and its metabolites (*2*) were extracted from plant material by maceration in a Waring Blendor with methylene chloride. An aliquot of the extract was evaporated to dryness, taken up in a small measured quantity of methylene chloride, and applied to Whatman No. 1 filter paper. The extracts were chromatographed by a solvent system developed by Bush (*4*). After chromatography, the papers were developed and eluted with methanol into test tubes. A blue solution, the absorbance of which could be read on a spectrophotometer, was developed through the use of the phosphomolybdenum blue method, based on the modifications made by Bartlett (*3*).

[1] Present address: Department of Entomology, University of Wisconsin, Madison 6, Wis.

Reagents

Methylene chloride, technical, redistilled.

Chromatography solvent system, made by mixing by volume 5 parts petroleum ether (b.p. 90°-100° C.), 5 parts toluene, 7 parts methanol, and 3 parts water, and separating the two phases. All solvents were redistilled.

Blue tetrazolium solution [3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-bis(2,5-diphenyl-2H-tetrazolium chloride)], (Matheson, Coleman and Bell No. 7596), 0.1%, stored in a dark bottle.

Hydrogen peroxide, 30%, analytical grade.

Potassium permanganate solution, 0.01*N*, made up fresh weekly.

Sodium oxalate solution, 0.01*N*, made up fresh weekly.

Ammonium molybdate solution, 5%, made up fresh weekly.

1-Amino-2-naphthol-4-sulfonic acid, 0.2%, containing 12% sodium metabisulfite, and 1.2% anhydrous sodium sulfite, made up fresh daily.

Apparatus

Chromatography cabinet, Research Specialities Co., No. B-550, or equivalent. Chromatography jars were found to give unsatisfactory results because the at-

Table I. Recovery of Added Phosphamidon

Plant	Added, P.P.M.	Recovered P.P.M.	Recovered %
Peas	4.4	4.8	109
	4.4	4.4	100
Spinach	5.0	4.9	98
	5.0	4.7	94
Potatoes	4.4	4.4	100
	4.4	4.4	100
Snap beans	20.0	17.2	86
	20.0	18.4	92
Lima beans	11.4	11.2	98
Tomatoes	5.0	4.0	80
	10.0	11.8	118
Alfalfa	5.0	4.0	80
	2.5	2.4	96
Apples	5.0	4.8	96
	2.5	2.4	96
Asparagus	3.3	3.0	91
	6.5	5.2	80
Watercress	5.0	3.2	64
	2.5	1.6	64
Parsley	10.0	4.8	48

mosphere could not be sufficiently stabilized.

Spectrophotometer, Beckman Model B, or equivalent, reading at a wavelength of 790 mμ.

Procedure

Preparation of Standard Curve. A solution of phosphamidon in methylene chloride of 1000 μg. per ml. was prepared, using analytical grade material. Ten, 20, 30, 40, and 50 μl. of this solution were placed in duplicate on lines approximately 4 inches from the top of pieces of Whatman No. 1 filter paper approximately $1^1/_2 \times 22^1/_2$ inches. (It was found necessary to extract the paper in a giant Soxhlet extraction apparatus for 3 days with methanol and 3 days with chloroform to remove impurities.) The solution must be distributed evenly over the $1^1/_2$-inch line. The papers were conditioned by leaving them in the chromatography cabinet overnight. The atmosphere of the chromatography cabinet was saturated by placing trays containing the lower phase of the solvent system and several beakers containing the upper phase in the bottom. The following day the papers were developed by the use of the descending technique with the upper phase of the solvent system. Care was taken that the atmosphere should be disturbed as little as possible when the solvent was added to the troughs. After the papers had been allowed to run about 4 hours, they were removed from the cabinet and dried upside down in a hood. One set of the papers was dipped in a solution of 1 volume of the 0.1% blue tetrazolium solution and 9 volumes of a 2*N* sodium hydroxide solution. A blue smear appeared, indicating the presence of phosphamidon. The R_f value of the phosphamidon varied slightly with the quantity used. The other set of papers was compared with the developed set and cut off just below the R_f value of the phosphamidon and somewhere below the starting line so as to be of a convenient length for elution. Thus, impurities below the R_f value of phosphamidon, including plant pigments, and those at the starting line are eliminated in this step. These cut-off papers were then eluted overnight in a large chromatography jar into 25 × 240 mm. test tubes with redistilled methanol. Approximately 2 ml. of methanol was sufficient for quantitative extraction. The test tubes could be left several days at this point before proceeding with the analysis.

To complete the analysis, the test tubes were removed from the closed jar, 0.5 ml. of concentrated sulfuric acid and a glass bead were added, and the test tubes were placed on a sand bath at 300° C. until the methanol had boiled off. A slight charring resulted from this heating. To decolorize the solution, 2 or 3 drops of hydrogen peroxide were added to each of the test tubes, and they were returned to the sand bath for 5 minutes. It was often necessary to repeat this step several times until the solution was completely colorless. The final heating was for 20 minutes. The tubes were cooled and 5 ml. of distilled water was added down the sides. To ensure complete decomposition of the hydrogen peroxide, potassium permanganate solution was added drop by drop until the pink color persisted; the pink color was removed by adding a drop of sodium oxalate solution. The tubes were then heated for 20 minutes in a boiling water bath. The test tubes were cooled again, and 5 ml. more of distilled water was added down the sides. Then 0.5 ml. of the ammonium molybdate solution and 0.5 ml. of the 1-amino-2-naphthol-4-sulfonic acid solution was added to each of the test tubes, and they were heated again for 20 minutes in a boiling water bath to develop the blue color. After the tubes were removed from the water bath, they were cooled in ice. Exactly 4.0 ml. of *n*-butanol was added to each tube and the contents mixed well by shaking. After the phases were allowed to separate, the top layer was transferred to cuvettes and the absorbance measured at 790 mμ against a standard of pure *n*-butanol. With each set of samples, a reagent blank was run. If the color was too dark to be read on the spectrophotometer, a further dilution of the solution was made with a larger amount of *n*-butanol for the preparation of the standard curve. In this way, several curves were made with different amounts of *n*-butanol. This allowed very dark unknown solutions to be read on the spectrophotometer by a simple dilution rather than repeating the entire analysis of a single sample with a smaller aliquot of extract.

Analysis of Plant Material. A representative sample of the plant material to be analyzed weighing 400 grams was taken for analysis and macerated in a Waring Blendor with only as much water as necessary to achieve efficient maceration. The plant material was transferred to a 1-gallon jar using small quantities of water to achieve efficient transfer. The jars containing the plant material were placed at 0° F. and left overnight. The next morning the jars were removed from the freezer and allowed to thaw. After thawing was complete, 400 ml. of redistilled methylene chloride was added to the jar, and the contents mixed thoroughly by shaking for 1 minute. It was then allowed to stand overnight. The next morning the mixture was tumbled for 5 minutes, and the liquid decanted through several layers of cheesecloth into a 1000-ml. separatory funnel. If at this point an emulsion was formed which would not break immediately, 20 ml. of 1*N* hydrochloric acid was added and the contents shaken for 1 minute, and the phases were allowed to separate. The clear extract was filtered through a plug of glass wool into a storage bottle containing approximately 10 grams of anhydrous sodium sulfate. The plant extracts were stored in a refrigerator at 40° F., if it was not convenient to use them immediately.

An aliquot of the extract of either 50 or 100 ml., depending on the amount of phosphamidon expected to be present, was transferred to a beaker and allowed to evaporate to dryness at room temperature. The residue was taken up in 2.0 ml. of methylene chloride and 50 μl. of this solution was applied by micropipet to papers in the same fashion as in the preparation of the standard curve. Since it was desired to run the samples in duplicate through the elution and total phosphate analysis steps, three applications from the same extract were made. From this point on, the procedure was the same as for the preparation of the standard curve.

Discussion

To determine the accuracy of the method, aliquots of a standard solution of phosphamidon in methylene chloride were added to unsprayed plant material before extraction and the extracts carried through the procedure. Recoveries of added phosphamidon by this procedure were very good, except those for watercress and parsley, and the method is suitable for the analysis of most plant materials (Table I).

According to Anliker *et al.* (*1*, *2*), both phosphamidon and its three identified metabolites can be located on the paper chromatograms with the blue tetrazolium dye. They list the following R_f values: phosphamidon, 0.38; metabolite

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.

Literature Cited

(1) Anliker, R., Geiger, M., unpublished data.
(2) Anliker, R., Beriger, E., Geiger, M., Schmid, K., *Helv. Chim. Acta* **44,** 1622 (1961).
(3) Bartlett, G. R., *J. Biol. Chem.* **234,** 466 (1959).
(4) Bush, I. E., *Biochem. J.* **50,** 370 (1952).

Received for review June 19, 1962. Accepted September 24, 1962. Scientific Article Number A991, Contribution Number 3376 of the Maryland Agricultural Experiment Station, Department of Entomology. Part of a thesis presented to the Graduate School, University of Maryland, by the second author in partial fulfillment of the requirements for the Master of Science Degree.

NEMATOCIDE RESIDUES

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

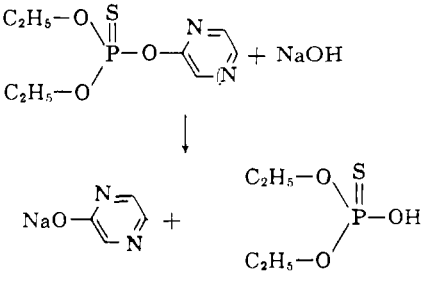
ULO KIIGEMAGI and L. C. TERRIERE

Department of Agricultural Chemistry, Oregon State University, Corvallis, Ore.

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