

## A Total Phosphorus Technique for Determining Organophosphorus Pesticide Residues Using Schöniger Flask Combustion

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A procedure is described for the determination of organophosphorus pesticide residues based on Schöniger flask combustion and colorimetric measurement of total phosphorus in the combustion products. Efficient conversion during combustion to products responding to the chromogenic reactions is achieved by pretreatment of the paper sample holder with sodium hydroxide in a water-alcohol solution. Phosphorus is determined by a molybdenum blue colorimetric procedure in which the absorbance of the colored product is measured at 820  $m\mu$ . Simple procedures are described for the removal of interfering plant extractives. Sensitivity of 0.1 p.p.m. is attained with recoveries from several crops averaging 90%.

IN THE ANALYSIS of treated crops for pesticide residues, it is sometimes advantageous for the determination to respond to all of the pesticides of a particular type, such as those containing chlorine, sulfur, or phosphorus. One of the more convenient approaches to this problem employs combustion of the sample (combustion tube furnace, Parr bomb, Schöniger flask), followed by determination of the combustion product. Cleanup requirements are often less demanding since most interfering extractives do not survive the combustion step. There are numerous literature citations for residue methods using various combustion techniques for organochlorine and organosulfur pesticides, a few of which are listed here (1, 7-9, 13, 14, 19, 20).

Although references to the determination of phosphorus in various organic materials by Schöniger flask combustion have appeared in the literature (2, 6, 11, 12, 15, 16, 18, 21, 22, 25), there has been only one specialized reference to the use of this technique in determining organophosphorus pesticide residues—that of Cygon residues in hemlock (24). Several excellent total-phosphorus pesticide residue methods have been devised using wet combustion techniques (4, 10, 17, 23), but these methods are time-consuming and require extensive cleanup. It was therefore desirable to develop a general residue procedure utilizing Schöniger flask combustion for organophosphorus pesticides on treated crops.

### Method

**Apparatus.** All glassware and equipment which come in contact with the sample must be rinsed in distilled water and acetone before use. Forceps should be used to handle all small items of apparatus which come in contact with the sample.

Filtering funnels with coarse-porosity, fritted-glass disk, 60-mm. and 20-mm. diameter.

Hypodermic syringes, 10 ml. and 2 ml., each fitted with 6-inch, 20-gauge needle.

Combustion flask, 1000-ml., Thomas-Ogg-type with detachable sample carrier and  $\$$  35/25 ball-joint stopper.

Igniter, safety, Thomas-Ogg-type, utilizing infrared beam igniter.

Kuderna-Danish evaporative concentrator (Kontes Glass Co., Vineland, N. J.).

Colorimeter or spectrophotometer responding at 820  $m\mu$ .

**Reagents.** All reagents are reagent grade unless specified otherwise.

Chloroform. Use any grade which does not contribute to the reagent blank when 200 ml. is evaporated to dryness.

Hyflo Super Cel diatomaceous earth (available from Johns-Manville).

Nuchar C-190-N activated vegetable carbon, unwashed (Industrial Chemical Sales, Division West Virginia Pulp and Paper Co., Covington, Va.). If purification to eliminate phosphorus is required, use procedure of Bertheux (3).

Attaclay attapulugus clay (Minerals and Chemicals Philipp Corp., Menlo Park, N. J.).

Adsorbant mixture. Thoroughly mix two parts Attaclay attapulugus clay with one part Hyflo Super Cel diatomaceous earth.

Acetonitrile, diluted with 10% water. Acidify with a drop of glacial acetic acid and saturate with hexane.

Combustion paper. Schöniger sample holders, A. H. Thomas No. 6471-F.

Sodium hydroxide solution. Dissolve 2.5 grams of sodium hydroxide in 10 ml. of water, add 1 ml. of propylene glycol, and dilute to 100 ml. with methanol.

Ammonium molybdate solution. Dissolve 4 grams of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  in water and dilute to 100 ml.

ANSA reagent. Add 0.25 gram of 1-amino-2-naphthol-4-sulfonic acid to 100 ml. of freshly prepared 15% sodium

bisulfite solution with mechanical stirring. After 15 minutes of stirring, add 0.5 gram of anhydrous sodium sulfite; then after 5 minutes of additional stirring, filter and store at room temperature in a brown bottle. Prepare fresh weekly.

**Procedure.** CLEANUP. Dry 200 ml. of a chloroform solution containing the extractives from 100 grams of a substrate by shaking with 5 to 10 grams of anhydrous sodium sulfate, then add 6 grams of activated carbon and 8 grams of adsorbant mixture, and mix thoroughly. Filter the chloroform solution through a 5-mm. pad of diatomaceous earth in a 60-mm., fritted-glass funnel, and wash with 100 ml. of fresh chloroform. Evaporate the clear solution to dryness in a Kuderna-Danish evaporative concentrator on a steam bath.

With many substrates, the residue is ready for combustion at this point; however, if the residue contains a considerable quantity of waxes or oils, a partition cleanup is required. This procedure consists of heating the residue in the Kuderna-Danish concentrator tube with 4 ml. of aqueous acetonitrile until either complete solution is achieved or a finely divided suspension results. If necessary, filter the cooled suspension through a 5-mm. pad of diatomaceous earth in a 20-mm., fritted-glass funnel; wash with a 4-ml., and then with a 2-ml. portion of fresh acetonitrile. Combine the acetonitrile solutions and wash with 4 ml. of hexane; then transfer the lower acetonitrile phase to a clean tube by means of a hypodermic syringe. Extract the hexane solution with two 1-ml. portions of fresh acetonitrile, combine the acetonitrile solutions, and evaporate to dryness. Dissolve the residue in 0.2 ml. of chloroform.

COMBUSTION. Dip a combustion paper

**Table I. Effect of Various Treatments of the Combustion Paper on the Efficiency of Combustion Conversion of Cygon to Phosphorus Pentoxide**

Treatment	Phosphorus, $\mu\text{g.}$		Recovery, %
	Found	Theoretical	
None	2.50, 2.24, 2.30	4.34	58, 52, 53
NaOH in methanol	3.95, 3.55, 3.42, 3.42	4.34	91, 82, 79, 79
NaOH in methanol, exposed to $\text{CO}_2$ atmosphere while moist	2.37, 2.50	4.34	55, 56
NaOH in 10% aqueous methanol	4.21, 4.34	4.34	97, 100

sample holder into the alcoholic sodium hydroxide solution; then suspend it in a current of heated air. Immediately transfer the chloroform solution of the residue to the combustion paper by means of a micropipet, using two 0.2-ml. portions of fresh chloroform to aid the transfer. Place the folded combustion paper in a platinum cup and suspend in a Schöniger flask containing 10 ml. of 0.75*N* sulfuric acid solution. Displace the air in the flask with oxygen and ignite the paper by means of the infrared igniter. After 1 minute, shake the flask so that the acid solution thoroughly wets all of the inner surfaces of the flask and the platinum basket to absorb the phosphorus combustion products, and transfer 7 ml. of the acid solution to a 10-ml., calibrated test tube.

**COLORIMETRY (23).** Add 1 ml. of 6*N* sulfuric acid solution to the phosphate solution and mix well. Then add, in order with mixing, 0.4 ml. each of ammonium molybdate solution and ANSA reagent. Dilute the solution to 10 ml. with water and heat at 100° C. for 12  $\pm$  2 minutes. Cool, and determine absorbance at 820  $m\mu$  in a colorimeter or spectrophotometer, using water to set the instrument.

### Discussion

Gedansky *et al.* (12) clearly demonstrated the necessity for an oxidizing and fusing medium in the combustion zone to achieve high recoveries of phosphorus by the combustion technique. The use of anhydrous sodium carbonate as recommended by these workers proved inconvenient for the techniques used in this study; therefore, an alternative medium was developed in which the combustion paper was dipped into an aqueous alcoholic sodium hydroxide solution prior to application of the sam-

**Table II. Background Values and Recoveries of Several Organophosphorus Pesticides from Various Substrates**

Organo-phosphorus Pesticide	Found, <sup>a</sup> P.P.M.		Added, P.P.M.		Recovery, <sup>a</sup> %
ALFALFA					
Cygon	0.06 $\pm$ 0.02 <sup>b</sup>	0.00	0.00	0.00	...
	0.09, 0.10	0.10	0.10	0.10	90, 100
	0.17, 0.20	0.19	0.19	0.19	90, 105
	0.48, 0.49	0.48	0.48	0.48	100, 102
	0.71	0.71	0.71	100	
APPLE					
Cygon	0.08 $\pm$ 0.02 <sup>c</sup>	0.00	0.00	0.00	...
	0.17 $\pm$ 0.004 <sup>b</sup>	0.20	0.20	0.20	85 $\pm$ 2
Cygon oxygen analog	0.07, 0.08	0.00	0.00	0.00	...
	0.30, 0.29	0.39	0.39	0.39	77, 74
Parathion	0.12, 0.14	0.00	0.00	0.00	...
	0.49, 0.49	0.54	0.54	0.54	91, 91
ORANGE PEEL					
Cygon	0.10, 0.10	0.00	0.00	0.00	...
	0.15, 0.17	0.20	0.20	0.20	75, 85
SUGAR BEET <sup>d</sup>					
Cygon	0.06, 0.07	0.00	0.00	0.00	...
	0.20, 0.20	0.20	0.20	0.20	100, 100

<sup>a</sup> Corrected for background of untreated control samples.

<sup>b</sup> Based on four samples.

<sup>c</sup> Based on eight samples.

<sup>d</sup> No partition cleanup was required.

ple. The effectiveness of this alkaline treatment and the need for the water in the alcoholic solution are illustrated by the data in Table I.

The colorimetric technique utilized for this procedure is essentially that described by Steller and Curry (23). The sensitivity achieved by combining this colorimetric method with the combustion technique was 1.32  $\mu\text{g.}$  of phosphorus per 0.1 absorbance unit, with constant reagent backgrounds of about 0.3  $\mu\text{g.}$  of phosphorus.

Application of the combustion-colorimetric procedure to the determination in apples of several organophosphorus pesticides of widely differing polarity and of Cygon (trademark, American Cyanamid Co.) insecticide in a variety of crops yielded the data presented in Table II. Efficiency of extraction of the organophosphorus pesticides from these crops was not evaluated. Background values for untreated crops were 0.1 p.p.m. or less in all cases, allowing a practical sensitivity of approximately 0.1 p.p.m., with an over-all average of 90  $\pm$  7%, exclusive of extraction.

As a general method for the determination of organophosphorus insecticides in plant tissues, this procedure has advantages over older procedures of simplicity and economy of operator time, consuming about 3 hours for four samples. Further, it should be readily adaptable for use with paper (5) or thin layer

chromatography, thereby introducing a considerable degree of specificity while retaining its general applicability for a variety of pesticides and crops.

### Literature Cited

- Agazzi, E. J., Peters, E. D., Brooks, F. R., *Anal. Chem.* **25**, 237 (1953).
- Barney, J. E., II, Bergmann, J. G., Tuskan, W. G., *Ibid.*, **31**, 1394 (1959).
- Bertheux, M. H., *Chem. Ind. London* **1957**, p. 1183.
- Caverly, D., Hall, P. S., *Analyst* **86**, 478 (1961).
- Coffin, D. E., "The Metabolism and Persistence of Systox, Diazinon, and Phosdrin on Field-Sprayed Lettuce," presented at the 77th Meeting, Assoc. Offic. Agr. Chemists, Washington, D. C., Oct. 16, 1963.
- Corner, M., *Analyst* **84**, 41 (1959).
- Coulson, D. M., *Adv. Pest. Control Res.* **V**, 153 (1962).
- Coulson, D. M., Cavanagh, L. A., De Vries, J. E., Walther, B., *J. Agr. Food Chem.* **8**, 399 (1960).
- Dunn, C. L., Lisk, D. J., in "Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives," G. Zweig, Ed., Vol. **I**, pp. 253-61, Academic Press, New York-London, 1963.
- Fahey, J. E., Rusk, H. W., "Determination of Organic Phosphorus Residues," U. S. Department of Agriculture, Vincennes, Ind., 1963.
- Fleischer, K. D., Southworth, B. C., Hodecker, J. H., Tuckerman, M. M., *Anal. Chem.* **30**, 152 (1958).
- Gedansky, S. J., Bowen, J. E., Milner, O. I., *Ibid.*, **32**, 1447 (1960).
- Gunther, F. A., Blinn, R. C., "Analysis of Insecticides and Acaricides," pp. 357-78, Interscience, New York-London, 1955.
- Gutenmann, W. H., Lisk, D. J., *J. Agr. Food Chem.* **8**, 306 (1960).
- Haslam, J., Hamilton, J. B., Squirrel, D. C. M., *Analyst* **85**, 556 (1960).
- Kirsten, W. J., Carlsson, M. E., *Microchem. J.* **4**, 3 (1960).
- Laws, E. Q., Webley, D. J., *Analyst* **86**, 249 (1961).
- Liddell, C., *J. Inst. Petrol.* **48**, 221 (1962).
- Lisk, D. J., *J. Agr. Food Chem.* **8**, 119 (1960).
- Lisk, D. J., *Residue Reviews* **1**, 152 (1962).
- Nara, A., Urushibata, Y., Ooe, N., *Bunseki Kagaku* **12**, 294 (1963); *Chem. Abstr.* **59**, 5770h (1963).
- Schöniger, W., "The Oxygen Flask Method," *Facts and Methods for Scientific Research* **1**, No. 2, 1-5, F & M Scientific Corp., New Castle, Delaware, 1960.
- Steller, W. A., Curry, A. N., *J. Assoc. Offic. Agr. Chemists*, in press.
- St. John, L. E., Jr., Wallner, W. E., Weidhaas, J. A., Jr., Lisk, D. J., *J. Econ. Entomol.* **57**, 103 (1964).
- Weaver, E. A., *Analyst* **88**, 736 (1963).

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