and DuTer produced a small but insignificant increase in linoleic acid and a decrease in stearic acid when applied to the Argentine variety in 1970. Treatment effects of Benlate and Bravo on the Florunner variety were similar to those observed with the Argentine variety but were smaller in magnitude. Significant effects (decrease) were observed for palmitic (16:0) and stearic acid. Kocide produced a small but significant decrease (16:0) when applied to the Florunner variety. In the Florigiant variety (Table III), Benlate and Bravo decreased levels of palmitic and linoleic acid and increased levels of stearic and oleic (18:1) acid over controls.

Effect of time of harvest was most pronounced in the Florigiant and Florunner varieties (Tables III and IV). With the Argentine variety, the effect of delayed harvest was occasionally significant for some fatty acids. This effect was small, however, and apparently random in that observed differences did not follow a consistent pattern with respect to time of harvest. Treatment × time-of-harvest interaction was significant only in the Florigiant variety (Table III).

When data from the treatments shown in Tables I and II were combined for both years, an analysis of variance showed highly significant year effects for all fatty acids, significant treatment effects for all fatty acids except arachidic (20:0), and significant year × treatment interaction for all fatty acids except arachidic and eicosenoic (20:1). Date of harvest and other interaction terms were nonsignificant for the combined data.

The differences in fatty acid composition due to treatment observed in this study were no larger than usual year to year differences observed in this and previous studies (Worthington and Hammons, 1971; Worthington *et al.*, 1972) and are probably of little practical importance. The source of these differences is unknown but may be associated with an extended period of plant vigor and a change in the proportions of mature and immature seeds in samples from treated plots as compared with samples from untreated controls.

ACKNOWLEDGMENT

We thank C. T. Young for assistance and advice in preparation and selection of samples, Susan Nolan for technical assistance in gas–liquid chromatography, and H. A. Peacock and J. C. Elrod for assistance in statistical analysis of data.

LITERATURE CITED

Harrison, A. L., *Phytopathology* **59,** 114 (1969).
Jensen, R. E., Boyle, L. W., *Plant Dis. Rep.* **50,** 810 (1966).
Pang, L.-S., "The Influence of Maturity and Time of Harvesting Spanish Peanuts on Peanut Butter Quality," M. S. Thesis, Oklahoma State University, Stillwater, Okla., 1967.
Porter, D. M., *Plant Dis. Rep.* **54,** 955 (1970).
Worthington, R. E., Proceeding of the Fifth National Peanut Research Conference, Research Division, Virginia Polytechnic Institute, Blacksburg, Va., 1969, pp 87–98.
Worthington, R. E., Hammons, R. O., *Oleagineux* **26,** 695 (1971).
Worthington, R. E., Hammons, R. O., Allison, J. R., *J. Agr. Food Chem.* **20,** 727 (1972).
Young, C. T., Mason, M. E., Matlock, R. S., Waller, G. R., *J. Amer. Oil Chem. Soc.* **49,** 314 (1972).

Received for review December 4, 1972. Accepted April 2, 1973. Tables giving other data on treatment effects similar to those given in the tables in this article will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-73-619.

# Reaction Gas Chromatographic Analysis of Pesticides. II. On-Column Transesterification of Organophosphates by Methanol

H. A. Moye

A method is described for the on-column transesterification of four classes of organophosphate pesticides to the corresponding methyl esters, chromatography on Porapak P or Q, and detection by $Rb_2SO_4$ pellet alkali flame ionization detector. Reproducible conversions were obtained for transesterification with methanol. Distinguishing gas chromatographic peaks were also obtained when the organophosphates were injected in ethanol, 1-propanol, and 1-butanol. Chromatographic and detector conditions are given for the simultaneous analysis of a carbamate (Mobam) with the four classes of organophosphate pesticides.

Organophosphate pesticides have been analyzed using gas chromatographic techniques that would be applicable for screening purposes utilizing either the phosphorus-sensitive flame photometric detector or the alkali flame ionization detector (Beroza and Bowman, 1968; Bowman and Beroza, 1970; Watts and Storherr, 1969). Thin-layer techniques have also been employed using both one-dimensional and two-dimensional development (Gardner, 1971). With the approximately 60 organophosphate pesticides that are now marketed, it has become increasingly difficult to verify the identity of a particular gas chromatographic peak or thin-layer spot. Usually multiple columns must be employed in gas chromatography or multiple solvent systems and visualization reagents in thin-layer chromatography. A gas chromatographic method which would give responses characteristic of the various classes of organophosphates would seem to be of value.

The term reaction gas chromatography was coined in 1960 (Drawert *et al.*, 1960) and has evolved to include any structural change of a compound occurring within the gas chromatograph. High temperature pyrolysis of a compound to obtain a fingerprint is the most common technique and is used frequently in the petroleum industry.

Esposito and Swann (1969) formed trimethylsilyl derivatives of some polyols by an on-column reaction. Almost concurrently, Jaglan and coworkers (1969) esterified the dealkyl metabolites of methyl parathion and methyl paraoxon on a gas chromatographic column. Spengler and

Food Science Department, University of Florida, Gainesville, Florida 32601.

**Table I. Representative Organophosphate Pesticides**

| Pesticide | Transesterification product |
|---|---|
| Azodrin [dimethyl *cis*-1-methyl-2-(methylcarbamoyl)-vinyl phosphate] | Trimethyl phosphate |
| Ronnel [O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate] | Trimethyl phosphorothionate |
| Diazinon [O,O-diethyl O-(2-isopropyl-4-methylpyrimidyl) phosphorothioate] | Diethyl monomethyl phosphorothionate |
| Compound 4072 [2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate] | Diethyl monomethyl phosphate |
| Parathion [O,O-diethyl O-*p*-nitrophenyl) phosphorothioate] | Diethyl monomethyl phosphorothionate |

Hamroll (1970) pyrolyzed carbamates and substituted ureas to isocyanates and amines within the injection port of the gas chromatograph.

It has more recently been shown by Moye (1971) that the *N*-methylcarbamate pesticides can be made to undergo a transesterification reaction with methanol in the injection port of the gas chromatograph. This was shown to be an instantaneous reaction which can also occur in the presence of other short-chain alcohols.

The lower molecular weight alkyl esters of phosphoric acid—*i.e.*, *O,O,O*-trimethyl phosphate (TMP); *O,O,O*-trimethyl phosphorothionate (TMTP): *O,O*-diethyl *O*-methyl phosphate (DEMMP); *O,O*-diethyl *O*-methyl phosphorothionate (DEMMTP)—as well as the corresponding thiolates were analyzed by Shafik and coworkers (Shafik and Enos, 1969; Shafik *et al.*, 1970) after the corresponding demethyl compounds were extracted from rat and human urine and methylated with diazomethane using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as a precursor. They used a 12 ft × ¼ in. o.d. aluminum column packed with 20% Versamid 900 on 60/80 Gas Chrom Q. The order of elution they obtained was TMTP, TMP, DEMMTP, and DEMMP.

The work described here investigates the possibility of applying the previously described transesterification approach to the organophosphate pesticides, using as nearly as possible the same experimental conditions. The type reaction to be expected would be

$$ROP(OC_2H_5)_2 + CH_3OH \longrightarrow ROH + CH_3OP(OC_2H_5)_2$$

(O(S) = O or S)

This, if successful, would then give the pesticide chemist a quick screening procedure for total *N*-methylcarbamates and total organophosphates by class.

## EXPERIMENTAL SECTION

A Varian Model 1200 gas chromatograph was used with the rubidium sulfate alkali flame ionization detector, which is specific for organic nitrogen or phosphorus. The column was 6 ft × ¼ in. × 2 mm coiled glass packed with either 80/100 Porapak P or Q, obtained from Waters Associates, Framingham, Mass. The first 6 in. of the column was packed with 80/120 mesh regular glass beads treated by dipping them into an aqueous solution of 2 *N* NaOH and then air drying. In the injection port packing study, other materials were used.

The organophosphate pesticides were obtained from their respective manufacturers in 99%+ pure form. Trimethyl phosphate was obtained in 99%+ pure form from the Ethyl Corporation. Trimethyl thiophosphate was obtained in 99%+ pure form from Mobil Oil Company, as was the potassium diethyl thiophosphate from American Cyanamid Corporation. Diethyl acid phosphate was supplied by Mohammed Shafik, Primate Research Laboratory, Perrine, Fla.

The methyl esters that were not already available were prepared either from the free acid or the potassium salt by the method of Shafik and Enos (1969) using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as an *in situ* precursor for diazomethane.

Table I shows those pesticides that were chosen for study with the predicted transesterification product. Both parathion and diazinon were used as representatives of DEMMTP. The four classes of compounds listed here represent 85% of all commercial and experimental organophosphate pesticides.

## RESULTS

Unless otherwise specified, all measurements were made on the Porapak P column. Only on those occasions where a complete separation of the four transesterification products was desired was the Porapak Q column used. The Porapak P column was never able to separate completely DEMMP from DEMMTP, however the significantly shorter retention times with this column facilitated the various optimization studies that will subsequently be described.

As with the carbamates, four representative organophosphate pesticides—Azodrin, Ronnel, Diazinon, and GC 4072—were injected in MeOH onto the Porapak P column with the injection port containing the 6 in. of NaOH-treated glass microbeads. As expected, peaks of the corresponding methyl esters appeared with the appropriate retention times.

The various parameters which might be involved in the gas phase reaction of organophosphate pesticide with methanol were studied. These were: injection port packing material; injection port temperature; sodium hydroxide concentration of the methanol solutions; presence of other solvents in the methanol solution; and presence of crop coextractives.

The gas chromatographic responses of each pesticide were studied as a function of pesticide concentration. They were also studied for Ronnel and Azodrin in alcoholic solutions other than methanol.

**Injection Port Packing.** Several injection port packing materials were tested: untreated glass microbeads; 2 *N* sodium hydroxide-treated glass microbeads; untreated glass wool; silanized glass wool; Fluoropak; and 0.2 *N* phosphoric acid-treated glass microbeads. The packings were tested with both Ronnel and Azodrin at the 10-ng level. As long as sodium hydroxide was injected along with the methanol they all worked equally well on clean standards. However, when crop extracts were injected, only the sodium hydroxide-coated glass microbeads continued to give high conversion after a large number (50–100) of samples were injected.

**Sodium Hydroxide Concentration.** Figure 1 shows the effect of NaOH concentration in methanol–water standards of the four pesticides at 10 ng/μl. The water was held constant at 1% by volume. Ronnel and Azodrin both approached 95% conversion, whereas parathion and GC

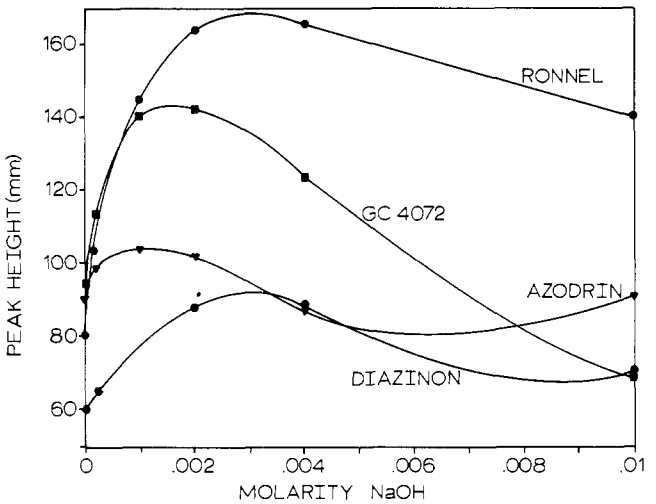


**Figure 1.** Effect of NaOH concentration in 99% methanol on response of 10 ng of each pesticide.

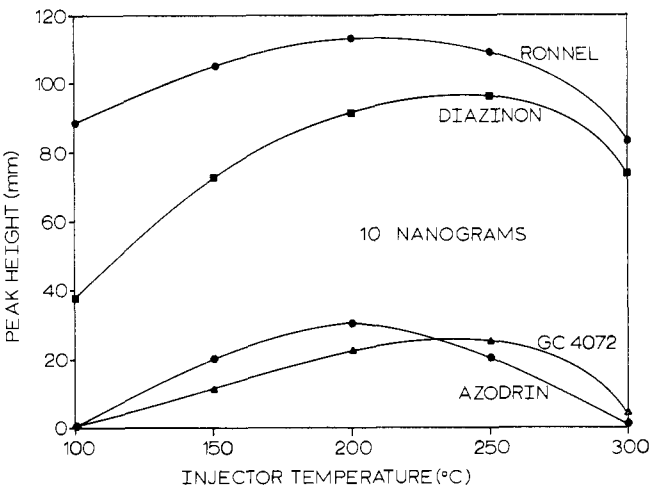


**Figure 2.** Effect of injection port temperature on the conversion efficiency of 10 ng of each pesticide.

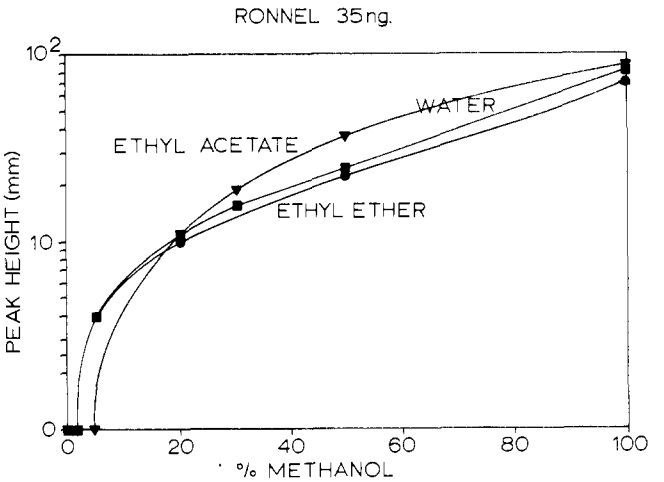


**Figure 3.** Effect of other solvents on Ronnel response in MeOH–NaOH.

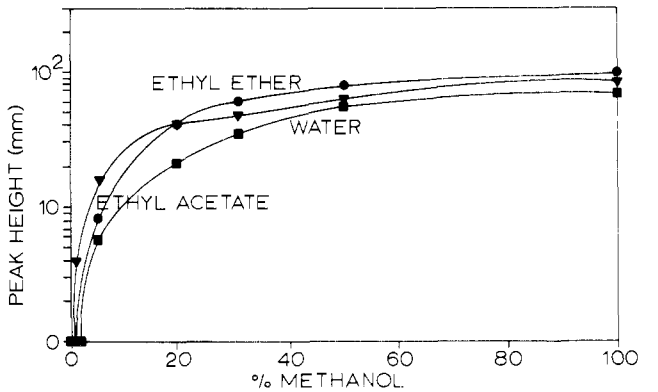


**Figure 4.** Effect of other solvents on Azodrin response in MeOH–NaOH.

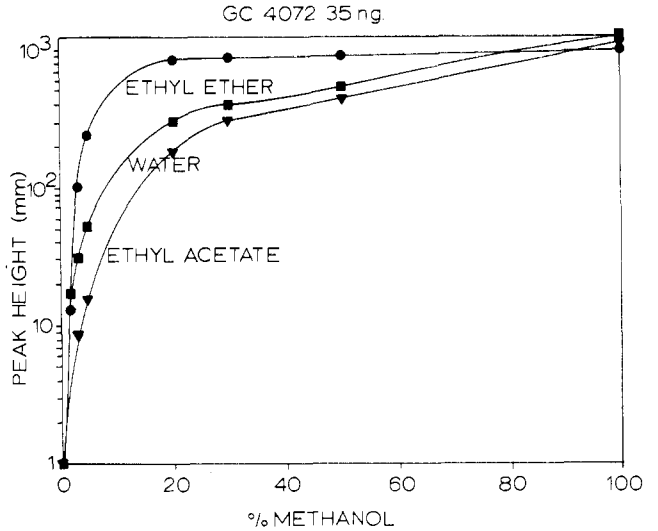


**Figure 5.** Effect of other solvents on compound 4072 response in MeOH–NaOH.

4072 were slightly less but somewhat indeterminant, due to the fact that the procedure used for the preparation of their esters gives only a 15% yield of thionate, which varied randomly (Shafik *et al.*, 1970). Trimethyl phosphate and trimethyl thiophosphate analytical standards were available in pure form and gave identical gas chromatographic retention times under all conditions as the corresponding pesticide injected in methanol. Percent conversions for Azodrin and Ronnel were calculated by comparisons to these standards.

**Injection Port Temperature.** Figure 2 shows the effect of injection port temperature on the responses of the four representative pesticides. Good conversion occurs between 200 and 250° for all four. However, as has been reported before for the carbamates (Moye, 1971), it was sometimes possible to reduce or eliminate crop interferences by operating the injection port at a temperature of 150°. For most analyses, a temperature of 225° was chosen to provide near maximum conversion.

**Solvent Effect.** In the analysis of organophosphate pesticides, solvents other than methanol are generally recommended for extraction. Since methanol is required during the transesterification reaction to give the methyl esters, a study was done to see what effect various extraction solvents would have when mixed with methanol–NaOH containing 35 ng of each of the four representative pesticides. The results are shown in Figures 3–6. They show that dilutions of the methanol with another solvent may be made to the 60% methanol level for the four pesticides studied, while still retaining 80% of the response. For all four pesticides the response was least when dilutions were made with either ethyl ether or ethyl acetate. Water was generally not as good.

Alcohols other than methanol will react with the organophosphate pesticides in the injection port of the chromatograph. Figures 7 and 8 show peaks obtained when

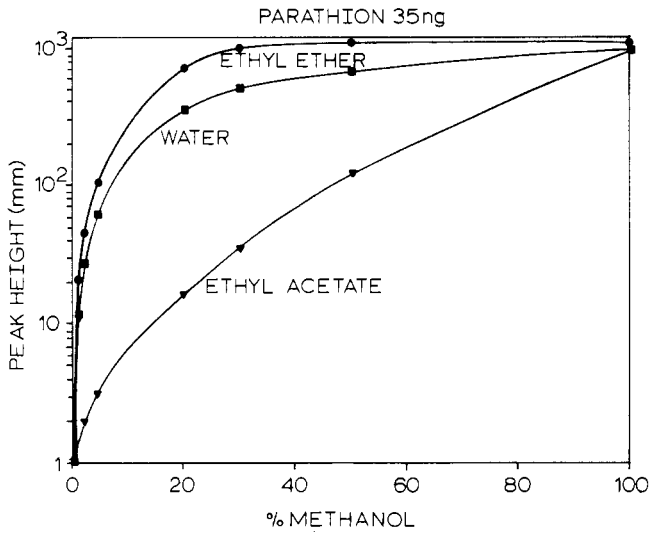


**Figure 6.** Effect of other solvents on parathion response in MeOH–NaOH.

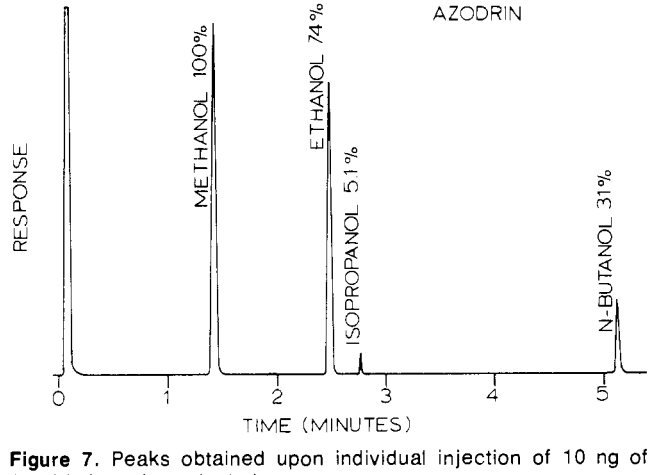


**Figure 7.** Peaks obtained upon individual injection of 10 ng of Azodrin in various alcohols.

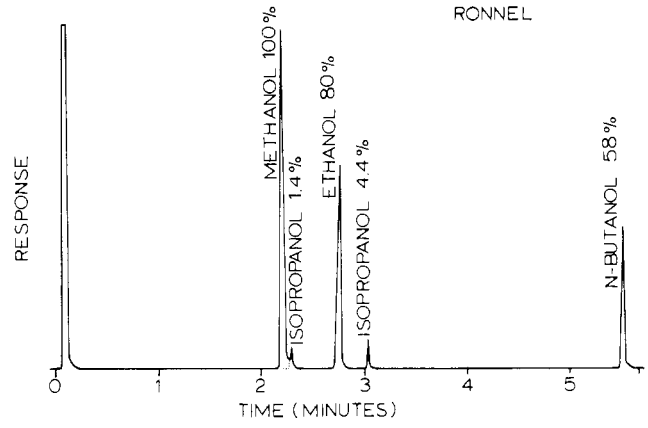


**Figure 8.** Peaks obtained of individual injection of 10 ng of Ronnel in various alcohols.

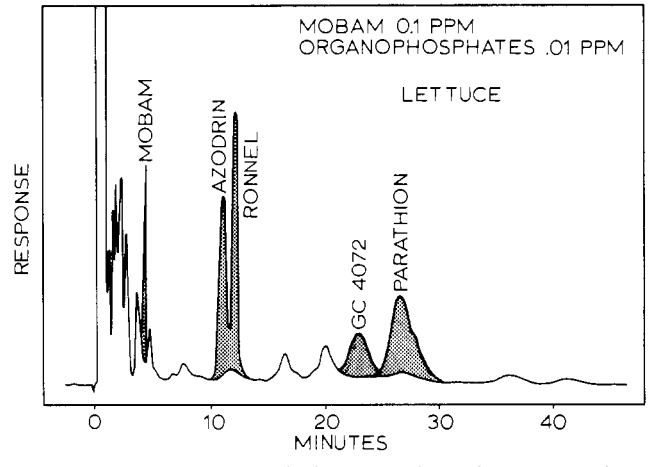


**Figure 9.** Chromatogram of Mobam and the four organophosphates extracted from lettuce.

Azodrin and Ronnel were made up in methanol, ethanol, isopropyl alcohol, and 1-butanol. *tert*-Butanol produced no peaks. For Azodrin these peaks would presumably be trimethyl phosphate, dimethyl monoethyl phosphate, dimethyl monoisopropyl phosphate, and dimethyl mono-*n*-butyl phosphate. For Ronnel, the corresponding thiophosphates would be obtained. It is interesting to note that contrary to the situation for the methylation of a free thioacid (Shafik *et al.*, 1970), there is no apparent production of the thiolate, only the thionate. The thiolate produced after methylation of a free thio acid is easily seen under all gas chromatographic conditions previously described. This peak was never observed upon injection of a thiophosphate pesticide in the transesterification procedure.

**Linearity of Response.** The responses for the four pesticides using the previously optimized conditions were linear over a range of 0.1 to 100 ng.

A 1.0-ng standard of Ronnel was injected six times with a relative standard deviation in peak heights of 2.9%.

**Crop Extract.** Figure 9 shows the chromatogram obtained when lettuce was extracted after spiking with Mobam (a carbamate) at 0.1 ppm and the four organophosphates at 0.02 ppm. Twenty-five grams of untreated lettuce was spiked with 0.1 ppm of Mobam and 0.02 ppm of the four organophosphates and blended with 125 ml of methanol for 5 min. The extract was filtered through fast filter paper and dried with 20 g of $Na_2SO_4$. It was evaporated to 5 ml in a Kuderna–Danish over steam and subsequently evaporated to 0.5 ml with dry nitrogen at room temperature. One drop of 2 *N* NaOH was added before injection on the gas chromatograph. 6 ft × ¼ in. × 2 mm i.d. glass column of 80/100 Porapak Q had the first 6 in. packed with NaOH-treated glass beads. The instrument temperatures were: injection, 225°; detector, 240°; and column, 215°. The gas flow rates were: He (carrier), 20 ml/min; $H_2$, 25 ml/min; and air, 188 ml/min. The electrometer was operated at $4 \times 10^{-12}$ AFS.

## DISCUSSION

To verify that the transesterification was occurring in the injection port and not at room temperature in the MeOH–NaOH solution, the following experiment was performed. After making a MeOH–NaOH standard of an organophosphate pesticide, the standard was allowed to sit at room temperature for 30 min. Large amounts of water were added and the MeOH–$H_2O$ solution was partitioned with ethyl ether. The ethyl ether layer was dried with $Na_2SO_4$ and analyzed on a 5% SE30 column for the unreacted pesticide. In all cases, nearly 100% of the pesticide was recovered.

A great deal of time and effort was spent in attempts to trap or collect the pesticide reaction products at the end of the gas chromatographic column and characterize them by mass spectrometry, nmr, ir, and thin-layer chromatography. Also, an attempt was made to couple the gas chromatograph to a Beta VII Plasma Chromatograph (PC), Franklin G.N.O. Corporation, West Palm Beach, Fla.,

and compare positive and negative ion spectra with those of the methyl esters.

Essentially no success resulted from attempts to trap and characterize either the methyl esters or the methanol-pesticide reaction products. It was first throught that this difficulty was due only to the high volatility of the methyl esters. However, when the Plasma Chromatograph was coupled to the gas chromatograph through a 10-in. long piece of heated $\frac{1}{16}$-in. stainless steel tubing, it was obvious that essentially no material was getting through to the PC other than solvent, even when 100-ng amounts of methyl ester standard were injected into the gas chromatograph. The PC was easily able to detect 1 ng of pesticide or methyl ester standard using the direct probe approach. Either the organophosphate pesticides and methyl esters were irreversibly absorbed onto the metal surfaces or were decomposed to products that the PC could not detect.

Thin-layer chromatography of both the pesticides and methyl esters after trapping from the gas chromatographic column showed a large number of spots containing phosphorus. This occurred even when the transfer line running from the end of the column to the liquid $N_2$ trap was an 8 in. × $\frac{1}{8}$ in. piece of heated glass tubing. However, the Porapak columns themselves appeared to produce no detectable breakdown when the detector was in place, other than a diminution of peak heights when the column temperature ranged over 230°.

The somewhat unexpected high precision with which retention times of both the ester standards and products from pesticide injections could be measured enhanced the credibility of structure assignments. Retention times were timed with a stopwatch from point of injection. Peaks with retention times as long as 8.5 min could be reproduced upon five serial injections within 2 sec. Retention times on the order of 5 min or less could be reproduced within 1 sec. The retention times for Azodrin, Ronnel, and compound 4072 and their corresponding esters were all coincident within ±1 sec of variation. Parathion and diazinon, however, consistently gave retention times 2 sec later than diethyl monomethyl thiophosphate when the Porapak Q column was operated at 60 ml/min and 250°. By operating the column at 20 ml/min and 260°, the DEMMTP peak split into two components, the second coinciding exactly with the 8-min 24-sec peak of parathion and diazinon. The first peak was probably a decomposition product of the thiolate, since it increased in size with increasing column temperature while the thiolate peak diminished.

The coupling of the rubidium sulfate AFID, which is highly sensitive to organic nitrogen and phosphorus, to an on-column transesterification step and subsequent separation of the products would seem to be of value as a one-step screening technique for the presence of both organophosphate and carbamate pesticides.

LITERATURE CITED

Beroza, M., Bowman, M. C., *Environ. Sci. Technol.* **2**, 450 (1968).
Bowman, M. C., Beroza, M., *J. Ass. Offic. Anal. Chem.* **53**, 499 (1970).
Drawert, F., Felgenhauer, R., Huffer, G., *Angew. Chem.* **72**, 555 (1960).
Esposito, G. G., Swann, M. H., *Anal. Chem.* **41**, 1118 (1969).
Gardner, A. M., *J. Ass. Offic. Anal. Chem.* **54**, 517 (1971).
Jaglan, P. S., Gunther, F. A., March, R. B., *Anal. Chem.* **41**, 1671 (1969).
Moye, H. A., *J. Agr. Food Chem.* **19**, 452 (1971).
Shafik, M. T., Enos, H. F., *J. Agr. Food Chem.* **17**, 1186 (1969).
Shafik, M. T., Bradway, D., Biros, F. J., Enos, H. F., *J. Agr. Food Chem.* **18**, 1174 (1970).
Spengler, D., Hamroll, B., *J. Chromatogr.* **49**, 205 (1970).
Watts, R. R., Storherr, R. W., *J. Ass. Offic. Anal. Chem.* **52**, 513 (1969).

Received for review November 16, 1972. Accepted March 19, 1973.

# Human Exposure to Organophosphorus Pesticides. A Modified Procedure for the Gas–Liquid Chromatographic Analysis of Alkyl Phosphate Metabolites in Urine

Talaat Shafik,* Diane E. Bradway, Henry F. Enos, and Anne R. Yobs[1]

A previously described method for analysis of alkyl phosphates (Shafik and Enos, 1969) has undergone a major modification. The new method is more suitable for use in a monitoring program designed to determine human exposure to organophosphate pesticides. Improvements in the methodology include preparation of a less volatile ester derivative of the alkyl phosphate and selection of gas chromatographic columns and conditions which are more compatible with systems used in a routine chlorinated hydrocarbon pesticide residue program. The average recovery of the six dialkyl phosphates from human urine fortified at the 0.1-ppm level was 98.3%. The amyl derivatives of the dialkyl phosphates showed a remarkable increase in sensitivity compared to the methyl and ethyl derivatives on two gas chromatographic columns normally used in pesticide residue analysis. Data illustrating the application of the new method to a human monitoring program are presented.

The gas chromatographic determination of various dialkyl phosphates has been based on preparation of their respective methyl and ethyl ester derivatives and analysis by gc employing a phosphorus sensitive detector (Askew *et al.*, 1960; Shafik *et al.*, 1971; St. John and Lisk, 1968). The available methods are lengthy, especially the gas chromatographic step, the stability of the derivatives is questionable, and urinary inorganic phosphate interferes with the quantitation of low levels of the derivatized dialkyl phosphates. The application of these methods to large numbers of urine samples indicated the urgent need for certain modifications in order that the method could be used in a monitoring program.

The purposes of this investigation were to speed up gas chromatographic analysis, find a more suitable column packing for the separation of dialkyl phosphate derivatives, prepare more stable derivatives, increase the num-

Perrine Primate Laboratory, Environmental Protection Agency, Perrine, Florida 33157.
[1] Present address: Environmental Protection Agency, Chamblee, Georgia 30341.