Determination of Hydrolytic Metabolites of Organophosphorus Insecticides in Cow Urine Using an Improved Thermionic Detector

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A gas chromatographic method is described for determination of dimethyl or diethyl phosphate, thiophosphate, and dithiophosphate as hydrolysis products of organophosphorus insecticides in cow urine. The method involves ether extraction of acidified urine followed by methylation of the extracted acids. The methyl esters are chromatographed using the alkali-thermionic detector. The thermionic detector was modified to improve its stability during extended use. The method is sensitive to about 0.2 p.p.m. of the metabolites in urine.

A common metabolic reaction of organophosphorus insecticides in mammals involves hydrolysis and urinary excretion of salts of dimethyl or diethyl phosphate, thiophosphate, or dithiophosphate. Previous procedures for analysis of these products have included characterization by paper chromatography (Chamberlain, 1964a, 1964b) or ion exchange chromatography (Plapp and Casida, 1958; Dauterman *et al.*, 1959). Detection has been performed by counting isotopically labeled compounds or by spectrophotometric methods. Methyl, ethyl, and butyl phosphates have been prepared as pure standards and analyzed by gas chromatography (Stanley, 1966). In the work reported, dimethyl and diethyl phosphates, thiophosphates, and dithiophosphates are extracted from cow urine into ether following acidification and salt saturation of the urine sample. After methylation in ether, the compounds are characterized and determined by gas chromatography using an improved thermionic detector.

EXPERIMENTAL PROCEDURE

A recovery curve was developed for dimethyl phosphate, dimethyl thiophosphate, dimethyl dithiophosphate, diethyl phosphate, diethyl thiophosphate, and diethyl dithiophosphate by the following procedure.

A volume of 0.5 ml. of the compound [as a standard solution of the salt in water or the acid in methanol (Table I)] was added to each of a series of 25-ml. volumetric flasks containing 4 ml. of cow urine. The concentrations of the respective standard solutions were prepared to cover the ranges shown for the recovery curve of each compound in Figure 1. One milliliter of 5*N* hydrochloric acid (1 ml. of 5*N* sulfuric acid was used for methyl phosphate) and 5 ml. of diethyl ether were added, the contents were made to volume with saturated sodium chloride solution, and each flask was shaken vigorously for 1 minute. A 2-ml. aliquot of the ether layer was transferred to a 10-ml. volumetric flask, 0.22 ml. of methanol was added, and the solution was methylated with diazomethane following the procedure of Schlenk and Gellerman (1960). After removal of excess diazomethane with air as described, the flasks were made to volume with diethyl ether and mixed. Up to 5 μl. of the ether solution were analyzed by gas chromatography.

A Barber-Colman Model 10 gas chromatograph equipped with a Model 5121 hydrogen flame ionization detector modified as a thermionic detector was used. This detector, as described previously (Giuffrida *et al.*, 1966), was modified. A coil made of 20-gage Nichrome wire was used in place of platinum. A small plug of steel wool was inserted in the upper helices of the wire spiral, and this

Table I. Retention Times and Limits of Detection of the Compounds Studied

Compound Methylated	Retention Time, Min.	Sensitivity, Ng.
Dimethyl phosphate[a]	1.6	0.05
Dimethyl thiophosphate[b]	3.7	0.20
Dimethyl dithiophosphate[c]	3.1	0.30
Diethyl phosphate[c]	1.7	0.05
Diethyl thiophosphate[b]	6.0	0.15
Diethyl dithiophosphate[c]	4.2	0.30

[a] As barium salt.
[b] As monopotassium salt.
[c] As monoacid.

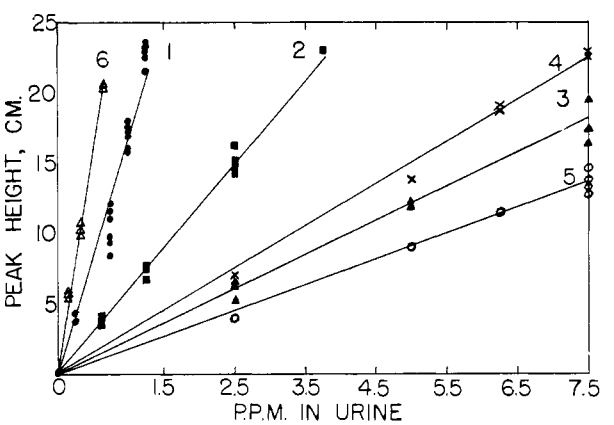

Figure 1. Recovery curves of compounds added to urine in increasing concentrations

△ Dimethyl phosphate (6)
○ Dimethyl thiophosphate (5)
× Dimethyl dithiophosphate (4)
● Diethyl phosphate (1)
■ Diethyl thiophosphate (2)
▲ Diethyl dithiophosphate (3)

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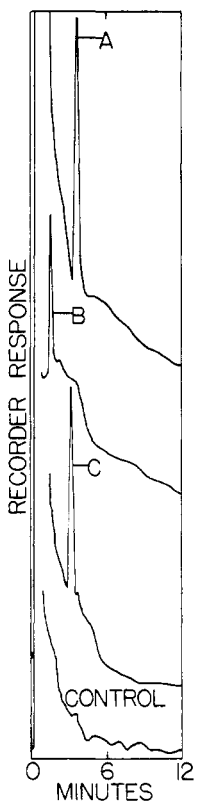

Figure 2. Chromatograms of methyl esters added to urine and control urine

A. 5 p.p.m. of dimethyl thiophosphate (0.8 ng.)
B. 0.16 p.p.m. of dimethyl phosphate (0.05 ng.)
C. 2.5 p.p.m. of dimethyl dithiophosphate (0.8 ng.)

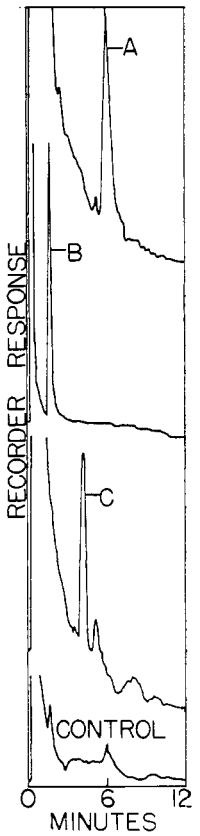

Figure 3. Chromatograms of methyl esters added to urine and control urine

A. 1.25 p.p.m. of diethyl thiophosphate (0.4 ng.)
B. 0.75 p.p.m. of diethyl phosphate (0.24 ng.)
C. 2.5 p.p.m. of diethyl dithiophosphate (0.8 ng.)

upper portion containing the wool was dipped in saturated potassium sulfate solution. After burning off the excess salt, the wire was affixed on the hydrogen flame jet. After igniting the jet, the detector was allowed to attain thermal equilibrium for 24 hours before use. The detector was sensitive and stable and retained its sensitivity and stability for 2 months of continuous use without further attention.

A borosilicate glass column, 6 feet long, containing 2% Ucon Polar on 80- to 100-mesh Gas Chrom Q, was used. The operating temperatures of the column, flash heater, and detector were 105°, 140°, and 280° C., respectively. Prior to analysis, the column was conditioned at 180° C. for 12 hours and allowed to equilibrate at 105° C. for 24 hours. The flow rates used for nitrogen (carrier gas), hydrogen, and air were 60, 33, and 350 cc. per minute, respectively.

RESULTS AND DISCUSSION

The recovery curves for the methyl esters of dimethyl or diethyl phosphate, thiophosphate, or dithiophosphate were developed (Figure 1). In several instances, a standard of given concentration was analyzed in replicate on the same day or on different days as a measure of reproducibility of the procedure. All of these points are included in Figure 1. Figures 2 and 3 show chromatograms for the various methylated phosphorus compounds in urine.

Table I lists the compounds studied, their retention times, and limits of detection. The sensitivity of the method to each is estimated as the amount in nanograms injected to produce at least a 10% full-scale pen deflection (or a peak height of about 2.5 cm.). The sensitivities are sufficient to permit determination of these hydrolytic products at the feeding levels employed in most metabolic studies. Thus, in a study of the fate of Dursban [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] insecticide fed to a cow at a very low feeding level (5 p.p.m. in the feed), the method was successfully used to determine the presumed hydrolysis products of Dursban (diethyl phosphate and diethyl thiophosphate) (Gutenmann *et al.*, 1968).

The method is rapid with a minimum of preliminary isolation necessary. This is due largely to the selectivity and sensitivity of the detector. The method described would also permit esterification and detection of desmethylated or desethylated hydrolytic phosphorus-containing metabolites. Thus, standard solutions of monomethyl phosphate were methylated and chromatographed, and a linear standard curve resulted. To characterize these (desmethyl or desethyl) metabolites by the method, one could conceivably devise a scheme whereby desethylated compounds could be methylated and desmethylated could be ethylated. A longer column possibly could be used then to separate these products adequately from the corresponding (and very similar) diester metabolites which had not undergone dealkylation.

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LITERATURE CITED

Chamberlain, W. F., *J. Econ. Entomol.* **57,** 119 (1964a).
Chamberlain, W. F., *J. Econ. Entomol.* **57,** 329 (1964b).
Dauterman, W. C., Casida, J. E., Knaak, J. B., Kowalczyk, T., J. AGR. FOOD CHEM. **7,** 188 (1959).
Giuffrida, L., Ives, N. F., Bostwick, D. C., *J. Assoc. Offic. Agr. Chemists* **49,** 8 (1966).
Gutenmann, W. H., St. John, L. E., Jr., Lisk, D. J., J. AGR. FOOD CHEM. **16,** 45 (1968).
Plapp, F. W., Casida, J. E., *Anal. Chem.* **30,** 1622 (1958).
Schlenk, H., Gellerman, J. L., *Anal. Chem.* **32,** 1412 (1960).
Stanley, C. W., J. AGR. FOOD CHEM. **14,** 321 (1966).

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