Rapid, Sensitive Residue Determination of Organophosphorus Insecticides by Alkali Thermionic Gas Chromatography of Their Methylated Alkyl Phosphate Hydrolytic Products

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A rapid method is described for residue analysis of organophosphorus insecticides. Following alkaline hydrolysis, the cleaved alkyl phosphates are partitioned into ether from an acidified solution. The derivative is methylated and determined by alkali thermionic gas chromatography. No further isolation is required. The method has been applied to the analysis of Guthion in grapes, Abate in soil, and other insecticides. As little as 0.1 p.p.m. of an organophosphorus insecticide residue may be detected.

The analysis of residues of organophosphorus insecticides has been aided greatly by the development of gas chromatography with selective detectors. The alkali thermionic detector (Giuffrida, 1964; Karmen, 1964) is sensitive, selective, and the simplest to construct. It has been used for analysis of the majority of these compounds commonly used in agriculture.

For successful application of any detector the compound must be able to be chromatographed and ideally should elute in a discrete symmetrical peak. Certain organophosphorus compounds (Guthion and others) cannot be chromatographed, or only with difficulty after much column conditioning owing to their polarity, nonvolatility, or instability. Polar oxygen analogs or sulfoxides and sulfones of organophosphorus insecticides are also examples.

The alkyl phosphate hydrolytic products of virtually all commonly used organophosphorus insecticides or their oxidized metabolites (dimethyl or diethyl phosphate, thiophosphate or dithiophosphate) have been methylated and successfully chromatographed and determined as possible metabolites in cow urine using alkali thermionic detection (St. John and Lisk, 1968). In the work reported, organophosphorus insecticides are hydrolyzed in dilute alkali and the resulting alkyl phosphates are extracted into ether from an acidified solution. The derivative is methylated and chromatographed using alkali thermionic detection.

EXPERIMENTAL PROCEDURE

Standard curves were developed for Abate (*O,O,O',O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate), Dasanit [*O,O*-diethyl *O*-*p*-(methylsulfinyl)phenyl phosphorothioate], Dursban (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate), Prefar [*N*-beta-*O,O*-diisopropyldithiophosphorylethyl)-benzene sulfonamide], and Guthion [*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate] as follows: zero, 0.2, 0.4, 0.6, and 0.8 ml. of standard solutions of the insecticides (50 μg. per ml. in acetone) were transferred to a series of 10-ml. volumetric flasks. For Abate, the concentration of the standard solution was 25 μg. per ml. One milliliter of *N* sodium hydroxide (for Guthion, 10*N* alkali was used) was added to each. A 10/30 standard taper male ground joint (with the full 14.5-cm. length of glass tubing attached) was placed in the top (as an air condenser). The resulting aqueous-acetone solution was gently refluxed in a hot water bath for 15 minutes (for Dursban, the solution was instead allowed to stand for 30 minutes at room temperature) and the acetone was then evaporated with a gentle air stream. The condenser was rinsed down with 4 ml. of *N* hydrochloric acid and an additional 4 drops of concentrated acid were added with mixing. (For Guthion, 3 ml. of *N* acid and 1 ml. of concentrated acid were used.) Five milliliters of diethyl ether was added to the flask and the contents were shaken for 1 minute. A 2-ml. aliquot of the ether layer was transferred to a 10-ml. volumetric flask containing 0.2 ml. of absolute methanol 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 flask was made to volume with ether and mixed. Up to 5 μl. of the ether solution was analyzed by gas chromatography. The gas chromatographic column, modified alkali thermionic detector, and operating parameters were identical to those reported previously (St. John and Lisk, 1968).

The procedures for the recovery of Abate in soil (Bath gravelly silt loam) and Guthion in Concord grapes were as follows:

Fifty-gram portions of dry, well mixed soil were shaken mechanically for 30 minutes with 100 ml. of acetone in a 250-ml. Erlenmeyer flask. Fifty-gram portions of grapes were blended with 100 ml. of acetone for 2 minutes. Progressive amounts of Abate and Guthion were added in acetone to represent zero to 0.8 p.p.m. of these insecticides, respectively, in soil and grapes. The soil extract or grape puree was filtered and rinsed with acetone until a total volume of 100 ml. of filtrate was obtained. Ten grams of anhydrous sodium sulfate were added to the filtrate and mixed. After settling, 10 ml. of the filtrate were transferred

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to a 10-ml. volumetric flask and were concentrated with rinsing to approximately 2 ml. The remainder of the procedure was conducted as described for development of standard curves beginning with addition of alkali and hydrolysis.

RESULTS AND DISCUSSION

Figure 1 illustrates the standard curves obtained for the insecticides. Peak height is plotted against equivalent nanograms of the parent insecticide injected. Table I lists the respective presumed hydrolysis products of the insecticides and the retention times of their methyl esters. With the exception of the hydrolysis product of Prefar, these products were characterized by their retention times being identical to those of methylated pure standards. The methylated hydrolysis product of Prefar had a different retention time than that of methylated diisopropyl dithiophosphate, and therefore the product may be diisopropyl thiophosphate. About 0.5 nanogram of the parent insecticides is detectable.

Figures 2 and 3 show, respectively, the recovery curves and typical chromatograms from which they were obtained. The chromatograms appeared remarkably free of other extraneous peaks considering the modest degree of prelimi-

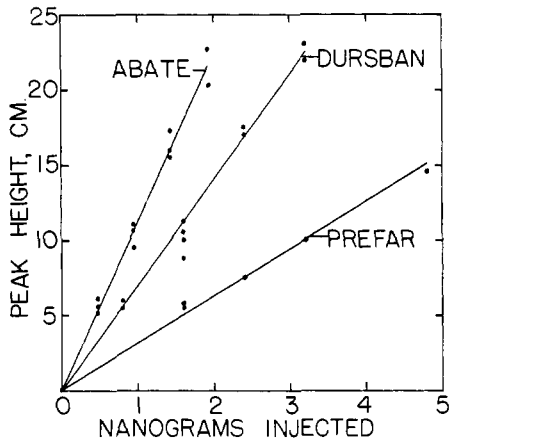

Figure 1. Insecticide standard curves

Top. Abate, Dursban, and Prefar
Bottom. Dasanit and Guthion

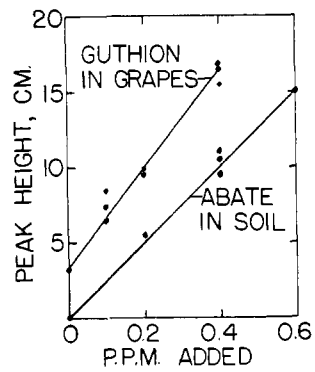

Figure 2. Insecticide recovery curves

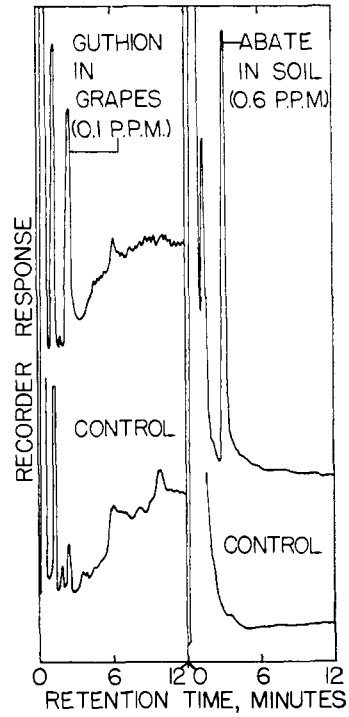

Figure 3. Chromatograms of insecticide recoveries

Table I. Retention Times of Presumed Insecticide Hydrolytic Products

Insecticide	Presumed Hydrolysis Product	Retention Time of Methylated Product, Minutes
Abate	Dimethyl thiophosphate	3.3
Dasanit	Diethyl thiophosphate	4.6
Dursban	Diethyl thiophosphate	4.6
Prefar	Diisopropyl thiophosphate	4.2
Guthion	Dimethyl dithiophosphate	2.7

nary chemical isolation applied to the samples. Clean chromatograms were also obtained when a similar isolation (acidification, salt saturation, ether extraction, and methylation) procedure was used for the analysis of diethyl phosphate and diethyl thiophosphate as the metabolic cleavage products of Dursban in cow urine (Gutenmann *et al.*, 1968).

The advantages of the method are obvious. Since the methylated alkyl phosphate hydrolytic products of organophosphorus insecticides (or oxidized metabolites) can be chromatographed (St. John and Lisk, 1968), a gas chromatographic method is therefore available for analysis of the parent compound even though it per se cannot be chromatographed. If the insecticide can be chromatographed, its identity can be based on its retention time as well as that of its hydrolyzed, methylated derivative.

Obviously the method will not distinguish between two or more organophosphorus insecticides which may be present in the same sample and which all yield the same hydrolysis product. Nor will it distinguish between an insecticide and its hydrolysis product if the latter is already present in the sample prior to extraction.

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