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ORGANOPHOSPHORUS PESTICIDES

A GAS CHROMATOGRAPHIC SCREENING TECHNIQUE BASED ON THE DETECTION OF METHYLATED HYDROLYSIS PRODUCTS*

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SUMMARY

A screening method applicable to the detection of many organophosphorus pesticides and their metabolites in foodstuffs is described. The procedure involves the hydrolysis of the pesticides under alkaline conditions and gas chromatographic determination of the methylated hydrolysis products. Most pesticides hydrolyzing to O,O-dimethyl phosphorothionate, O,O-diethyl phosphate and O,O-diethyl phosphorothionate can be determined at residue levels.

INTRODUCTION

The need for rapid screening methods capable of detecting residues of organophosphorus pesticides in foodstuffs has attracted the attention of many analysts[1]. Methods described in the literature involve a total phosphorus determination following clean-up to remove naturally occurring phosphorus compounds[2,3], cholinesterase inhibition techniques[4], or gas chromatography using detectors showing phosphorus selectivity[5,6]. The latter technique is currently attracting much attention as it is rapid, and selective detectors such as the thermionic and flame photometric detectors enable analyses to be made with minimal clean-up.

In our experience the gas chromatographic technique has limitations for screening purposes. For example, some parent pesticides and many of their naturally produced oxidation products are difficult to detect gas chromatographically[7] and may remain undetected in a sample. In addition, those pesticides which can be chromatographed vary so widely in their properties that temperature programming or isothermal runs at two or more temperatures is necessary if a complete scan of retention times is to be achieved. Using thermionic detectors high levels of coextractives not containing phosphorus can also produce chromatographic peaks, and in such cases

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recourse to two stage detectors is necessary if a correct interpretation of the chromatogram is to be made[8].

Most of the organophosphorus pesticides now in use, together with many of their oxidation products, can be hydrolyzed to produce dialkyl phosphates or O,O-dialkyl phosphorothionates, which on methylation yield products that can be separated and detected by gas chromatography. Such compounds are unlikely to arise in nature and the method provides the basis for a pesticide screening technique overcoming some of the weaknesses of a direct chromatographic method. ST. JOHN AND LISK[9] have utilised this approach to detect some five pesticides but did not produce a method capable of general application. This paper describes a method applicable to the screening of foodstuffs for residues of many organophosphorus pesticides and their metabolites.

EXPERIMENTAL

Sample extraction

Numerous procedures have appeared in the literature for the extraction of organophosphorus pesticides from foodstuffs[1]. Any of these procedures can be used but the extract so obtained will contain a mixture of biologically active pesticides and oxidation products together with their naturally produced, but non-toxic, hydrolysis products. A partition between water and a solvent such as chloroform will generally separate the non-active and active materials prior to characterisation of the latter by the described method.

Hydrolysis conditions

By following the alkaline hydrolysis of carbophenothion, shown in a previous study to hydrolyze at a slower rate than most other pesticides, conditions applicable to general screening were found. Methanol, being miscible with 5 *N* sodium hydroxide, was selected as the solvent and a hydrolysis time of 15 min at 70° was found adequate for hydrolysis of all the pesticides examined with the possible exception of schradan.

Under the conditions used the products formed indicate that the major site of cleavage for most pesticides occurs at the bond linking the phosphorus atom with the oxygen or sulphur atom adjoining the non-alkyl side chain. Thus two products, a dialkyl phosphate or O,O-dialkyl phosphorothionate are formed from the four types of organophosphorus pesticide commonly encountered.

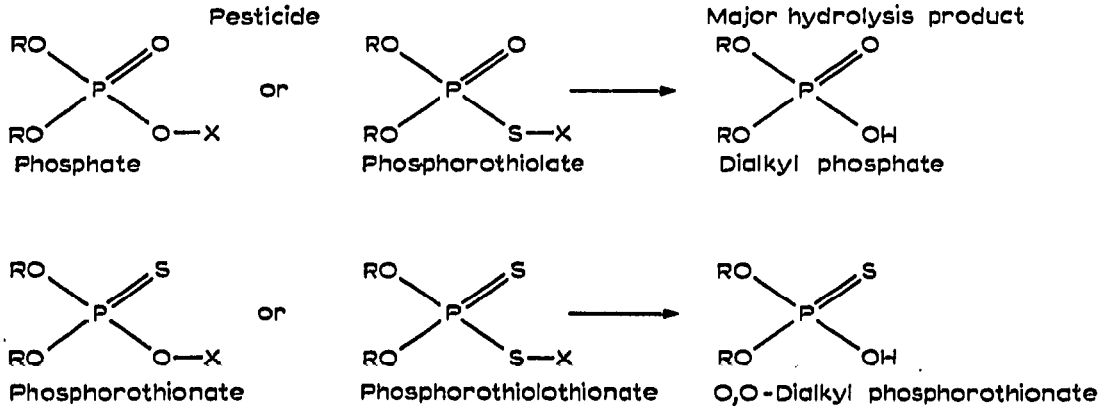

TABLE I

DERIVATIVE FORMATION AS A FUNCTION OF HYDROLYSIS TIME

Hydrolysis time at 70° (min)	*Weight of derivative (in μg) produced by hydrolysis of 1 μg of pesticide*[a]			
	Phorate	*Carbophenothion*	*Menazon*	*Chlorfenvinphos*
15	0.22	0.19	0.28	0.24
30	0.19	0.20	0.27	0.24
45	0.22	0.21	0.30	0.19
60	0.19	0.19	0.30	0.23

[a] Hydrolysis and extraction conditions were as described in the section METHOD.

Although each pesticide hydrolyzes at a different rate the reaction effectively halts at the dialkyl phosphate (or thionophosphate) stage as subsequent hydrolysis proceeds at a much slower rate. This is illustrated in Table I which shows that the yield of methylated derivative, and hence yields of dialkyl phosphate or O,O-dialkyl phosphorothionate, remain unchanged over a long period.

The products normally found in practice are dimethyl and diethyl phosphates and the corresponding O,O-dialkyl phosphorothionates. In the course of our work, however, it becomes apparent that certain naturally occurring phosphorus compounds were also hydrolyzing to phosphoric acid under these conditions and that on subsequent methylation, trimethyl phosphate was formed. This phenomenon precludes detection of those pesticides which hydrolyze to dimethyl phosphate as this in turn methylates to give trimethyl phosphate.

Extraction of hydrolysis products

The alkyl phosphates and phosphorothionates are extracted into chloroform

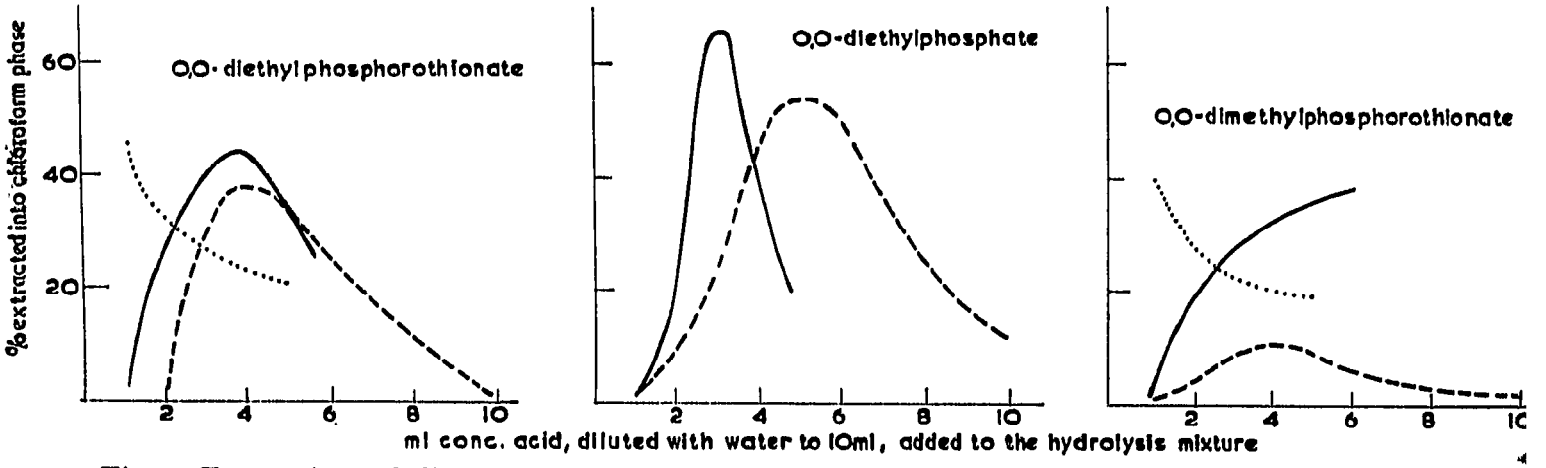

Fig. 1. Extraction of dialkyl phosphates and phosphorothionates from aqueous solution with chloroform as a function of acid type, concentration and salt saturation. (———) concentrated sulphuric acid; (— — — —) concentrated hydrochloric acid; (· · · · ·) concentrated sulphuric acid and solution saturated with sodium sulphate. Hydrolysis mixture: 2 ml 5 *N* NaOH + 2 ml ethanol.

after acidification of the aqueous hydrolysis mixture. Fig. 1 shows that the quantity extracted is dependent upon the acid used, its concentration and the presence of saturating salts. Acidification with 40% sulphuric acid was chosen as offering an optimum condition suitable for screening.

Many coextractives can be effectively separated from the hydrolysis products by partition into hexane following acidification, the hydrolysis products being retained in the aqueous phase and subsequently removed with chloroform.

Methylation and gas chromatography

Methylation of the hydrolysis products was carried out with diazomethane using the procedure of SCHLENK AND GELLERMAN[10]. With pesticide blanks methylation is complete in 2–3 min but where co-extractives obscured the yellow colour indicating excess diazomethane, a time of 10 min was adopted. Formic acid was used to remove excess diazomethane prior to gas chromatography, otherwise the diazomethane gives a large peak at a retention time close to that of trimethyl phosphate.

A Versamid 900 column was selected to separate the methylated derivatives, this giving better separation than the Ucon Polar[9] or Carbowax 20 M[11] stationary phase used by other workers. Table II shows the relative retention times of derivatives

TABLE II

RELATIVE RETENTION TIME OF THE METHYLATED HYDROLYSIS PRODUCTS DERIVED FROM ORGANOPHOSPHORUS PESTICIDES GAS CHROMATOGRAPHED ON A VERSAMID 900 COLUMN

Chromatographic conditions: column, 150 cm glass, 3 mm O.D.; packing, 4% Versamid 900 on Chromosorb G, acid washed, dimethylchlorosilane treated 80–100 mesh; temperature, oven 120°, injector 160°, detector 180°; instrument, Varian Aerograph 205-B with phosphorus detector; gas flow rates, nitrogen 25 ml/min, hydrogen 22 ml/min, air 200 ml/min.

Pesticide derivative	*Relative retention time*
Trimethyl phosphate (TMP)	41
Diethyl methyl phosphate (DEMP)	75
Trimethyl phosphorothionate (TMPT)	100 (2.30 min)
Diethyl methyl phosphorothionate (DEMPT)	175
Di-2-chloroethyl methyl phosphate	200
Di-dimethylamino methyl phosphate	240

encountered in our screening work. Using a thermionic detector (Aerograph phosphorus detector) the derivatives could be detected at a level down to 0.1 ng. Under the chromatographic conditions described only one pesticide gave a chromatographic peak in the range shown by the derivatives, this being dimefox (relative retention time 81). All other pesticides emerged much later and in general remained undetected if at low level.

Derivative standards

Pure samples of the derivatives encountered in this work are not readily available but were prepared in the course of the work by hydrolysis of the pesticides chlorfenvinphos, parathion, and parathion-methyl, giving on subsequent methylation diethyl methyl phosphate (DEMP), O,O-diethyl-O-methyl phosphorothionate (DEMPT) and O,O,O-trimethyl phosphorothionate (TMPT) respectively. Quantities

of 10 mg of these pesticides were treated according to the method described later. After methylation the solutions were streaked on silica gel thin layer plates (250 μ thick) and developed in diethyl ether until the solvent front had travelled 10 cm. The relevant portions of the plate (R_F values: trimethyl phosphate (TMP) 0.15, DEMP 0.25, TMPT 0.50, DEMPT 0.70) were removed and the derivative eluted from the silica gel with chloroform. The derivatives were examined by GLC to show that only one component was present, the phosphorus content was determined colorimetrically[3] and an examination was made by I.R. spectroscopy to confirm the structure of the derivatives. Suitable sub-standards for GLC were prepared by dilution in acetone after removal of chloroform.

METHOD

Macerate 100 g of vegetable, meat or fish with 200 ml of acetone and filter the suspension through a cotton wool plug using vacuum if necessary. Re-extract the solid material with a further 200 ml of acetone. Add the combined acetone extracts to 600 ml of water containing 20 g of anhydrous sodium sulphate and extract with two 100 ml portions of chloroform. Dry the combined chloroform extract by passage down a column containing granular anhydrous sodium sulphate and evaporate to low volume using a Kuderna–Danish evaporator. Remove final traces of chloroform by gently blowing and redissolve the residue in methanol. Adjust the volume of the solution with more methanol so that each 2 ml of solution is equivalent to 10 g of sample.

Pipette a 2 ml aliquot of the solution into a 10 ml pear-shaped flask, add 2 ml of 5 *N* sodium hydroxide, insert a reflux condensor and heat in a water bath at 70° for 15 min. Transfer the hydrolyzed extract to a 100 ml separator, wash the flask with 10 ml of 40% sulphuric acid and add the washings to the separator. Where coextractives are present add 25 ml of hexane to the solution, shake vigorously for 30 sec and discard the hexane layer. Extract the aqueous phase with two 25 ml portions of chloroform, shaking for 30 sec. Dry the chloroform extracts by passage through a column containing 20 g of granular anhydrous sodium sulphate and wash the column with a further 25 ml of solvent. Evaporate the combined eluates to low volume using a Kuderna–Danish evaporator fitted with a 10 ml graduated test tube. Insert a boiling chip into the tube and fit a micro-Snyder column[12]. Evaporate the remaining liquid to about 1 ml and remove the final traces of chloroform with a gentle stream of air. Redissolve the residue in 2 ml of diethyl ether containing 10% methanol.

Methylate the sample with diazomethane using the procedure of SCHLENK AND GELLERMAN[10], adequate ventilation and the normal safety precautions associated with the use of this reagent should be employed. (A charge of about 50 mg of the diazomethane intermediate N-methyl-N-nitrosotoluene-*p*-sulphonamide was generally used for each sample.) Allow the stream of nitrogen containing the diazomethane to bubble through the solution until a yellow colour persists in the sample (with highly coloured samples a ten minute period was routinely used). Add 10 μl of formic acid to the sample using a microlitre syringe and after adjusting the solution to a known volume inject 5 μl on to the chromatographic column (see Table II). Determine the content of derivative by comparison with a series of standards.

TABLE III

HYDROLYSIS OF PESTICIDES FOR 15 min AT 70°—YIELDS OF METHYLATED PRODUCTS DERIVED FROM 1 μg QUANTITIES OF PESTICIDE

Pesticide	*1*	*2*	*3*[a]	*Pesticide*	*1*	*2*	*3*[a]
Pesticides yielding O,O,O-trimethyl phosphorothionate (TMPT)							
Azinphos-methyl	0.55	0.12	22	Fenitrothion	0.56	0.38	68
Bromophos	0.43	0.21	49	Formothion	0.61	0.24	40
Dimethoate	0.68	0.32	47	Malathion	0.47	0.04	8
Ethoate-methyl	0.64	0.36	56	Menazon	0.55	0.30	55
Fenchlorphos	0.49	0.32	65	Morphothion	0.55	0.40	73
Pesticides yielding O,O-diethyl-O-methyl phosphorothionate (DEMPT)							
Azinphos-ethyl	0.53	0.04	8	Parathion	0.63	0.16	25
Carbophenothion	0.54	0.20	37	Phenkapton	0.49	0.18	37
Coumaphos	0.51	0.06	12	Phorate	0.71	0.20	28
Diazinon	0.60	0.28	47	Phosalone	0.50	0.12	24
Dichlofenthion	0.63	0.26	42	Pyrimithate	0.55	0.14	25
Disulfoton	0.67	0.36	54	Sulphotep	1.14	0.64	56
Ethion	0.96	0.16	17	Thionazin	0.74	0.23	32
Mecarbam	0.56	0.18	32				
Pesticides yielding diethyl methyl phosphate (DEMPT)							
Chlorfenvinphos	0.47	0.22	47	Phorate o.a. sulphone	0.61	0.36	59
Demeton S	0.66	0.30	45	TEPP	1.16	0.68	52
Paraoxon	0.61	0.26	43	Thionazin-O-analogue	0.68	0.36	53
Phorate-O-analogue	0.69	0.35	51				

[a] 1 = Theoretical weight of derivative (in μg) if hydrolysis and methylation proceed to yield a single product; 2 = weight of derivative found after hydrolysis, extraction and methylation; 3 = % of theory recovered.

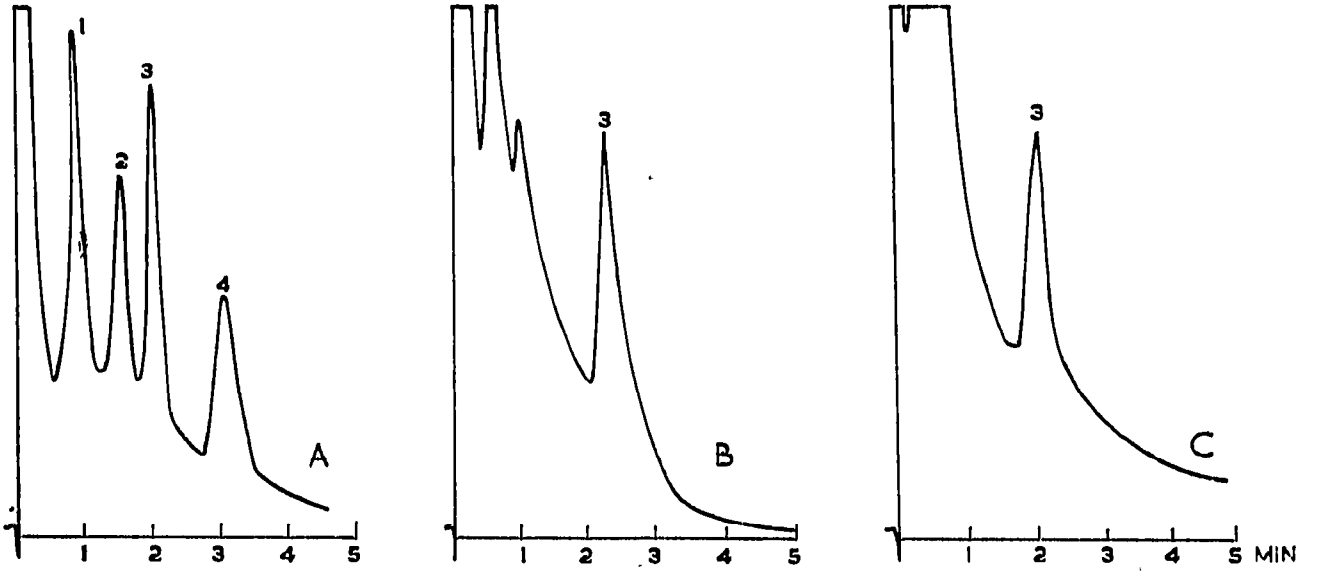

Fig. 2. Chromatograms of trialkyl phosphates and thiophosphates. (1) = TMP, (2) = DEMP, (3) = TMPT, (4) = DEMPT. (A) Mixture of derivatives. Each peak = 0.25 ng material. (B) Fish extract (= 10 g) spiked with 1 μg Menazon, hydrolyzed, extracted and methylated. (C) Runner bean extract (= 10 g) spiked with 1 μg Menazon, hydrolyzed, extracted and methylated.

RESULTS

Table III shows the yields of derivative produced when the described method was applied to 1 μg quantities of pesticides. The pesticides haloxon and dimefox gave derivative peaks of relative retention time 200 and 240; these derivatives were believed to be di-(2-chloroethyl)methyl phosphate and di-(dimethylamino)methyl phosphate respectively. The pesticides schradan and crufomate failed to give detectable derivatives.

The derivatives gave linear standard curves over the range studied (0–1 ng) and the weight of derivative produced by the method was directly proportional to the weight of pesticide hydrolyzed. Extracts from vegetable, fish and meat samples before hydrolysis gave no chromatographic peaks over the range of interest with the exception of cabbage extracts in which a compound was detected with relative retention time 52. After hydrolysis and methylation all these samples gave a single large peak corresponding in retention time to TMP. Samples spiked with pesticides gave the anticipated derivatives in yields similar to that obtained from the corresponding pesticide controls (values of 80–100% of the blank valve were encountered in all the samples). The analysis time for an individual sample was about 45 min. Fig. 2 shows typical chromatograms encountered with spiked samples.

DISCUSSION

The results in Table III indicate that the method is widely applicable. However, the yield of derivative from each pesticide when expressed as a percentage of that theoretically predicted vary somewhat widely—TMPT 40–70%, DEMPT 20–50%, DEMP 40–60%—but this spread is at least partly explicable by the variations in the purity of the pesticide standards used. Where yields well below the ranges indicated were encountered, samples were examined under gas chromatographic conditions capable of detecting the parent compounds and their absence was taken as confirmation that hydrolysis had occurred. It seems probable that in these cases the hydrolysis paths are differing from that normally encountered because of the nature of the side chains. Thus malathion is probably cleaving on the ester grouping of the side chain and in the case of azinphos-methyl where two derivative peaks were encountered (relative retention time 100 ≡ TMPT, 80 ≡ O,O,S-trimethyl phosphorodithionate) it is apparent that the side chain is influencing the point of hydrolytic attack, leading to low yields of the expected derivative.

With few exceptions therefore the method appears to be applicable to most pesticides and oxidation products which yield TMPT, DEMPT or DEMP including a number of compounds which we have not as yet been able to detect by gas chromatography (*e.g.* menazon and phorate-O-analogue sulphone). The detection limit using extractives from 10 g of sample falls in the range of 0.01 to 0.1 p.p.m.

The method does not provide a complete screening test however, as the important group of pesticides and metabolites which break down to form TMP cannot be differentiated from the derivatives produced from naturally occurring phosphorus compounds. Pesticides which give TMP on hydrolysis include demeton-S-methyl, dichlorvos, mevinphos, oxydemeton-methyl, phosphamidon, trichlorphon and vamidothion. It is possible that this group of pesticides could be detected by ethylation

following hydrolysis (ethanol as solvent could replace the methanol used in the current procedure) to give ethyl dimethyl phosphate, whereas the natural material would be expected to yield triethyl phosphate.

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