A Method for Confirmation of Organophosphorus Compounds at the Residue Level

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It is generally recognized that the analysis of pesticides in environmental samples should not be based solely on the results obtained by using a single column-detector system in a gas chromatograph (1,2). When at least two columns of different polarities are used in the determination, the results are more reliable and, in most cases, give more information about the possible identity of the compound in question. Moreover, the use of two different types of detectors can provide even more convincing evidence. The dual flame photometric detector, which monitors both sulfur and phosphorus simultaneously, is quite useful for this purpose (3).

Another tool for the confirmation of pesticide residues in environmental samples is chemical derivatization. A rapid method for the analysis of organophosphorus insecticides has been described (4) which is based on their hydrolysis to the corresponding dialkyl phosphate, methylation, and subsequent quantitation of the trialkyl derivative by gas chromatography. This method has been applied to Abate®, Dursban®, Perfar®, dasanit, and Guthion®. However, there are certain limitations to the method which do not permit its applicability to a broad spectrum of organophosphorus compounds. The conditions specified for hydrolysis and extraction are not optimum for all compounds of interest and the gas chromatographic column used does not resolve all of the components of a mixture of trialkyl phosphate derivatives.

It has been reported recently that methylated products resulting from the alkaline hydrolysis of organophosphorus compounds can be detected by a gas chromatographic screening technique (5). The same conditions of hydrolysis were applied to all of the compounds studied. Most pesticides which hydrolyze to form dimethyl phosphorothionate, diethyl phosphate, and diethyl phosphorothionate could be determined at submicrogram levels. Those hydrolyzing to dimethyl phosphate could not be analyzed by this procedure because of interference from inorganic phosphate which yields trimethyl phosphate under the conditions of analysis.

Many of these limitations have been resolved in a recent publication on the determination of alkyl phosphates and thiophosphates in human urine (6). The gas chromatographic conditions and derivatization procedures reported there were selected to obtain the complete resolution of all the dialkyl phosphates that might result from the metabolism or hydrolysis of the common organophosphorus insecticides.

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The purpose of this investigation was to determine the optimum conditions necessary to obtain maximum hydrolysis of a wide variety of organophosphorus insecticides to their corresponding dialkyl phosphates or thiophosphates. The gas chromatographic procedure described above (6) coupled with the appropriate hydrolysis conditions should result in a very useful confirmatory procedure.

Experimental

Glassware, apparatus, reagents, and standard solutions were the same as those described by Shafik and Enos (6) except that a 12' glass column was used instead of the aluminum column.

Methods

Three methods for the hydrolysis and alkylation of insecticides containing alkyl phosphate groups are presented below. Since the conditions for analysis vary among the different groups of organophosphate insecticides, general procedures are given. Specific details concerning the normality of base needed, and the time and temperature of hydrolysis are given in Tables 1 and 2.

The products resulting from each of the hydrolysis procedures are identified and quantitated by the gas chromatographic method previously described (6).

Method 1

(a) Pipet an aliquot of the organophosphorus insecticide in hexane (10-20 μg) into a 13-ml glass-stoppered centrifuge tube and evaporate the hexane with a gentle stream of filtered nitrogen. Add 0.1 ml of aqueous NaOH, mix, and let stand for the designated time. If the sample is to be hydrolyzed at 50°C, attach a reflux condenser with circulating ice-cold water.

After the hydrolysis is completed, acidify the solution with enough HCl for an excess equivalent to about 0.04 ml of 5 N HCl in the final solution. (If 2 N or 5 N NaOH is used in the hydrolysis, then 10 N HCl is used for acidification. If the normality of the base is less than 1, acidify with 5 N HCl.) Add 0.4 ml of alcohol to the solution, using methanol if the solution is to be methylated or ethanol if the solution is to be ethylated, and enough diazoalkane to give a persistent yellow color. Allow the mixture to stand for 20 minutes, remove the excess diazoalkane by bubbling filtered nitrogen through the solution, and adjust the volume with the alcohol used in alkylation so that the final concentration is about 1 μg/ml.

(b) The reaction is carried out in a small tube prepared by cutting the narrow end of a disposable Pasteur pipet to a length of 1/2 inch and sealing. Pipet an aliquot of the insecticide in hexane (100 ng) into the tube and evaporate the hexane with a stream of nitrogen. Add 50 μl of aqueous NaOH, mix, and let stand. After the hydrolysis is completed, acidify the solution with 15 μl of 5 N HCl and add, dropwise with vigorous mixing, enough diazoalkane to give a persistent yellow color to the ether layer. Allow to stand for 20 minutes with occasional mixing. Remove excess diazoalkane by bubbling nitrogen through the solution. Evaporate any remaining ether, and add 35 μl of the appropriate alcohol so that the final volume is about 100 μl.

Method 2

(a) Pipet an aliquot of the pesticide in hexane (10-20 μg) into a 25-ml glass-stoppered concentrator tube (Kontes No. 570050) and evaporate the solvent with a stream of filtered nitrogen. Add 1 ml of aqueous NaOH, mix, and allow to stand. If the sample is to be hydrolyzed at 50°C, attach a reflux condenser with circulating ice-cold water. After the hydrolysis is completed, add 4 ml of distilled water, 5 g of NaCl, 10 ml of the extracting solvent (1:1 ethyl ether-acetonitrile), and enough HCl so that the final solution contains about 1 ml of 5 N HCl in excess of that needed to neutralize the base. Mix for 1 minute on a Vortex mixer, and allow the layers to separate. Pipet an aliquot of the organic extract into a centrifuge tube and add enough diazoalkane to give a persistent yellow color to the solution. After 20 minutes, remove the excess diazoalkane by bubbling nitrogen through the solution. Adjust the volume with the appropriate alcohol so that the final concentration is about 1 μg/ml.

(b) Pipet an aliquot of the solution containing the pesticide (100 ng) into a 13-ml glass-stoppered centrifuge tube. Evaporate the solvent, add 50 μl of aqueous NaOH, mix, and allow to stand. If the sample is to be hydrolyzed at 50°C, attach a reflux condenser with circulating ice-cold water. After the hydrolysis is completed, add 0.5 ml of distilled water, 0.5 g of NaCl, 0.5 ml of extracting solvent (1:1 ethyl ether-acetonitrile), and 0.5 ml of 5 N HCl. Mix for 4 minutes on a Vortex mixer, allow the layers to separate, and pipet an aliquot into another 13-ml centrifuge tube. Alkylate as previously described, adjusting the final volume to 0.1 ml.

Method 3

This method was adapted from the WHO procedure for the analysis of malathion (7). Pipet an aliquot of the pesticide (10-20 μg per 0.25 ml in absolute methanol or ethanol) into a 13-ml glass-stoppered centrifuge tube. Add 0.02 ml of aqueous 0.5 N NaOH. Swirl gently for 5 to 10 seconds. (DO NOT SHAKE!) Allow to stand for 120 ±10

TABLE 1

Alkaline hydrolysis of organophosphorus insecticides containing DMP, DMTP, DMPTh, and DMDTP moieties[a]

Compound	Method[b]	Hydrolysis Conditions Normality of NaOH	Time, min	Temp., °C[c]	Product	% of Theoretical Yield
Containing DMP Moiety						
Bidrin®	1(a)	2	60	RT	DMP	47
Dichlorovos	1(a)	2	60	RT	DMP	88
					MMP	6
Dichlorovos	1(b)	1	30	RT	DMP	89
Phosdrin®	1(a)	2	120	RT	DMP	51
Trichlorfon	1(a)	2	60	RT	DMP	62
					MMP	3
Trichlorfon	1(b)	1	30	RT	DMP	92
CIBA-9491 oxon	1(a)	2	120	RT	DMP	79
Fenthion oxon	1(a)	2	120	RT	DMP	93
Fenthion oxon	1(b)	1	15	RT	DMP	22
Ronnel oxon	1(a)	2	60	50	DMP	70
Ronnel oxon	1(b)	1	60	50	DMP	55

TABLE 1 (Continued)

Compound	Method[b]	Hydrolysis Conditions Normality of NaOH	Time, min	Temp., °C[c]	Product	% of Theoretical Yield
<u>Containing</u> <u>DMTP</u> <u>Moiety</u>						
Abate®	1(a)	5	120	50	DMTP	50
Abate®	1(b)	2	120	50	DMTP	57
CIBA-9491	1(a)	1	120	RT	DMTP	68
Fenthion	1(a)	5	60	50	DMTP	51
					DMP	56
Fenthion	1(b)	2	60	50	DMTP	57
					DMP	<5
Ronnel	1(a)	5	60	RT	DMTP	83
Ronnel	1(b)	1	15	50	DMTP	47
Methyl parathion	1(a)	5	60	RT	DMTP	87
Methyl parathion	1(b)	1	60	RT	DMTP	88
Sumithion®	1(a)	5	60	RT	DMTP	82
Sumithion®	1(b)	1	60	RT	DMTP	64

TABLE 1 (Continued)

Compound	Method	Hydrolysis Conditions			Product	% of Theoretical Yield
		Normality of NaOH	Time, min	Temp.,[c] °C		
Containing DMPTh Moiety						
Guthion® oxon	1(a)	1	60	RT	DMP	47
Malaoxon	1(a)	1	60	RT	DMP	54
					DMTP	18
Meta-Systox-R®	1(a)	1	60	RT	DMP	18
Containing DMDTP Moiety						
Dimethoate	1(a)	0.1	30	50	DMTP	44
Guthion®	3	0.5	2.5	RT	DMDTP	63
Malathion	3	0.5	2.5	RT	DMDTP	88

[a] Abbreviations: DMP, dimethyl phosphate; DMTP, dimethyl thiophosphate; DMPTh, dimethyl phosphorothiolate; DMDTP, dimethyl dithiophosphate; MMP, monomethyl phosphate.

[b] Method (a) for 10-20 µg; Method (b) for 100 ng.

[c] RT is room temperature.

TABLE 2

Alkaline hydrolysis of organophosphorus insecticides containing DEP, DETP, DEPTh, and DEDTP moieties[a]

Compound	Method[b]	Hydrolysis Conditions Normality of NaOH	Time, min	Temp., °C[c]	Product	% of Theoretical Yield
<u>Containing DEP Moiety</u>						
Coumaphosoxon	2(a)	5	60	RT	DEP	68
Coumaphosoxon	2(b)	1	60	50	DEP	62
Diazoxon	2(a)	5	60	RT	DEP	63
Diazoxon	2(b)	1	60	RT	DEP	99
Paraoxon	2(a)	5	60	RT	DEP	73
Paraoxon	2(b)	1	60	RT	DEP	96
TEPP	2(a)	5	60	RT	DEP	84
<u>Containing DETP Moiety</u>						
Diazinon	1(b)	1	30	50	DETP	62
Dursban®	2(a)	1	60	50	DETP	49
Parathion	1(b)	1	30	50	DETP	85
Sulfotepp	1(b)	2	120	50	DETP	130[d]

TABLE 2 (Continued)

		Hydrolysis Conditions				
Compound	Method[b]	Normality of NaOH	Time, min	Temp., °C[c]	Product	% of Theoretical Yield
<u>Containing DEPTh Moiety</u>						
Systox® (84% Thiol)	2(a)	1	60	RT	DEP	78
					DETP	23
Systox® (84% Thiol)	2(b)	1	60	RT	DEP	80
Trioxon	2(a)	1	60	RT	DEP	68
Trioxon	2(b)	1	60	RT	DEP	100
<u>Containing DEDTP Moiety</u>						
Di-Syston®	1(a)	5	60	RT	DETP	23
Ethion	1(a)	1	60	50	DETP	19
					DEDTP	46
Ethion	1(b)	1	60	RT	DETP	62
Phorate	1(a)	1	60	RT	DETP	26
Trithion®	1(a)	1	60	RT	DETP	25

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[a] Abbreviations: DEP, diethyl phosphate; DETP, diethyl thiophosphate; DEPTh, diethyl phosphorothiolate; DEDTP, diethyl dithiophosphate.

[b] Method (a) for 10-20 μg; Method (b) for 100 ng.

[c] RT is room temperature.

[d] NMR spectrometry of this compound indicated the presence of more than one compound.

seconds. Add 0.75 ml of HCl and mix well by swirling. Transfer a portion (not larger than 0.2 ml) into a 13-ml centrifuge tube. Add 1 ml of ethanol or methanol and about 4 ml of the diazoalkane. Proceed with the determination.

Results and Discussion

A major limitation to the use of alkaline hydrolysis as a route to confirmation of the identity of organophosphorus pesticides has been the inability to find a GLC column which satisfactorily resolves all of the trialkyl phosphates of interest. A solution to this problem was recently reported by Shafik and Enos (6); the chromatograms in Figure 1 were obtained by using the column and conditions described in that paper. Baseline separation is achieved for both methylated and ethylated mixtures of the six dialkyl phosphates. Since DETP isomerizes to the thiolate upon alkylation (8), DEPTh appears in the standard mixtures in place of DETP. All calculations involving this compound are made from DEPTh.

By systematically varying the conditions of hydrolysis, conditions can be selected which produce either the most complete hydrolysis or the most characteristic products. By using a 20% Versamid column for separating mixtures of alkyl phosphates, a more complete picture of the hydrolysis products of organophosphorus pesticides can be obtained.

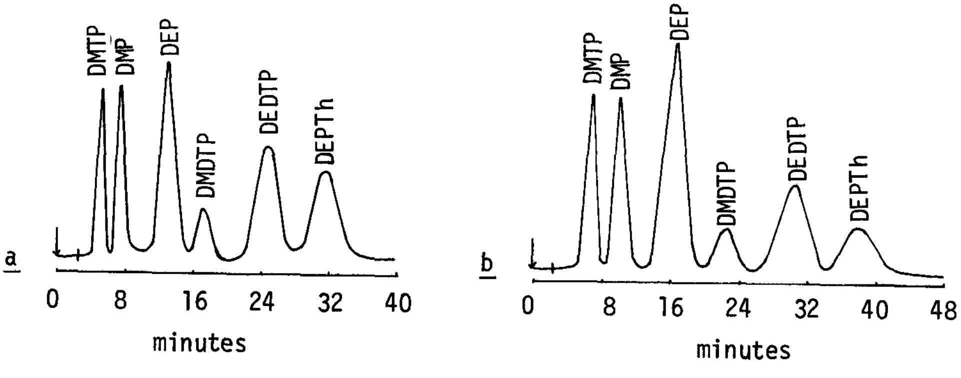

Figure 1. Chromatograms of mixed standards of trialkyl phosphates using a phosphorus filter, 526 mμ. a - Methylated: DMTP 3.3 ng; DMP 10 ng; DEP 6 ng; DMDTP 25 ng; DEDTP 6 ng; DEPTh 8.5 ng. b - Ethylated: DMTP 10 ng; DMP 10 ng; DEP 6 ng; DMDTP 25 ng; DEDTP 5 ng; DEPTh 5 ng.

Tables 1 and 2 show the hydrolysis conditions which produced the most satisfactory results for each insecticide. The compounds are listed in groups according to the dialkyl phosphate moiety. Within each group, one or two sets of conditions are reported which seem to give the most generally satisfactory results. Although no one method or set of conditions may be applied to all organophosphorus insecticides, consistency was noted within each group.

Three basic procedures were found which could be applied to nearly all insecticides studied. Method 1 is the most generally applicable. It involves hydrolysis of the organophosphate under various conditions and alkylation of the entire hydrolysate and can be applied to both microgram and nanogram quantities of most dimethyl-substituted organophosphorus insecticides, as well as many of the diethyl-substituted compounds. The only compounds which did not give satisfactory hydrolysis by this procedure were in those groups containing DEP and DEPTh moieties and the three compounds Dursban, malathion and Guthion.

Although most compounds were hydrolyzed by both Method 1(a) and 1(b), three insecticides in the group containing the DETP moiety (diazinon, parathion and sulfotepp) hydrolyzed only at the nanogram level. All attempts to hydrolyze microgram quantities of these compounds, under a wide variety of conditions, produced only insignificant amounts of the alkyl phosphate.

A few compounds were hydrolyzed by Method 1 to give results which were characteristic of the individual insecticide. Most notably, ethion hydrolyzed to a mixture of DETP and DEDTP by Method 1(a) and was the only compound examined which produced DEDTP. Fenthion yielded an equimolar mixture of DMTP and DMP by Method 1(a), but Method 1(b) gave predominantly DMTP at 50% of the theoretical amount; this behavior was unique to fenthion. With Abate, as the conditions of hydrolysis were made more stringent, the yield of DETP passed through a maximum at 50% of theoretical and then declined. The conditions listed in Table 2 were selected to give the maximum yield of DETP.

Method 2 was developed to treat those compounds which could not be satisfactorily hydrolyzed by Method 1. In Method 2, a larger volume is used for hydrolysis and the alkyl phosphate products are separated from the aqueous hydrolysate by extraction. Many compounds which were hydrolyzed only slightly or not at all by Method 1 could be hydrolyzed successfully by Method 2. Those compounds in the groups containing DEP and DEPTh moieties gave satisfactory results at both nanogram and microgram levels.

Method 3, an adaptation of the WHO procedure for the analysis of malathion (7), was modified to handle microgram quantities, but its applicability was limited. Although this method was tried with every insecticide containing a thiolate sulfur, it gave satisfactory hydrolysis only with malathion and Guthion.

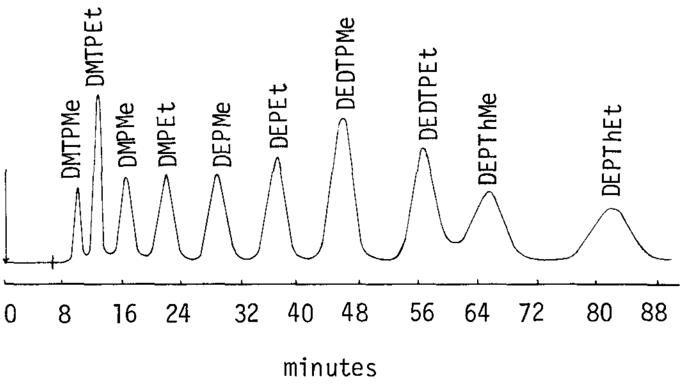

Figure 2. Chromatogram of trialkyl phosphate esters using the phosphorus filter, 526 mμ, and the 16' Versamid column.

A special attempt was made, using all three methods, to obtain the alkyl phosphorothiolate through hydrolysis of the S-R bond. Those compounds containing dimethyl or diethyl phosphorothiolate moieties hydrolyzed without exception at the P-S bond, yielding only DEP or DMP, respectively. In a few cases involving dialkyl phosphorodithioate compounds, some hydrolysis occurred at the S-R bond to produce the corresponding dithioate. In these compounds at least, the S-R bond appears to be somewhat labile.

During these investigations, a further study was made of the gas chromatographic system. It was found that a mixture of all 12 alkyl phosphates could be resolved on a 16' x 1/4 ", 20% Versamid column at 160°, as shown in Fig. 2. For this reason, the 16' column has found some application in the analysis of mixtures complicated by the presence of desalkyl phosphates formed either by metabolism or during hydrolysis. However, because of the increased time required for analysis it is not used routinely.

Conclusions

Additional information concerning the identity of organophosphorus insecticides may be obtained using the hydrolysis procedures described in this paper. A variety of conditions were used to produce the most favorable hydrolysis. Limitations encountered in other methods have been largely eliminated through improved conditions of hydrolysis and the use of a more efficient gas chromatographic system for resolving trialkyl phosphates.

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