THE GAS CHROMATOGRAPHIC DETERMINATION OF ORGANO-PHOSPHORUS PESTICIDES

PART II. A COMPARATIVE STUDY OF HYDROLYSIS RATES*,**

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INTRODUCTION

The use of pesticides in agricultural practice is now firmly established and the quantities used are increasing. Despite the undoubted advantages accruing from the use of these materials, concern has been expressed at the potentially harmful effects of using pesticides which are extremely stable and can accumulate in man and his environment[1]. Of the materials, in current use the organophosphorus pesticides show the most promise where biological activity and relatively low persistance are concerned. Most of the organophosphorus pesticides are esters of phosphoric or phosphorothioic acids which, unlike their organochlorine counterparts, are hydrolyzed under natural conditions with the ultimate formation of non-toxic residues.

The number of organophosphorus pesticides in use is quite large—in this country, for example, 24 pesticides are approved for agricultural application[2]. Many of these compounds can react to give toxic metabolites by oxidation and isomerization before hydrolysis and detoxification occurs[3], and the analyst preparing to determine residues in crops or polluted water is confronted with a problem of great complexity[4]. A knowledge of the hydrolysis rates of pesticides and their metabolites is of great interest to the residue analyst as it is ultimately hydrolysis which determines whether toxic residues will be present in a sample or not.

In this laboratory we have been concerned with building up background data on the chemistry of organophosphorus pesticides of interest particularly to the analyst. We have noticed that very few comparative hydrolysis studies of commercially important phosphorus esters have been made, although hydrolysis studies on individual pesticides are not uncommon[5,6]. O'BRIEN[3] has reviewed much of the literature up to 1959, and FAUST AND SUFFET[7] have recently reviewed this aspect of organophosphorus chemistry. The only comparative study in any depth was that made by MÜHLMANN AND SCHRADER[8] in 1957.

The techniques that have been used to follow hydrolysis include radioactive labelling[9], titrimetry[10], colorimetry[11] and conductimetry[12]. With the recent devel-

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opment of sensitive phosphorus-specific detectors for gas chromatography[13,14] a further analytical tool has become available. Most of the compounds now used as pesticides can be determined by gas chromatography, and in a previous publication the gas chromatographic conditions used to detect nanogram quantities of many organophosphorus compounds and some of their oxidation products were described[15].

In the present study gas chromatography using a "specific" phosphorus detector has been used to study the hydrolysis of pesticides.

EXPERIMENTAL

Choice of hydrolysis conditions

The hydrolysis studies were carried out in a mixture of ethanol–pH 6.0 buffer solution (20:80) at 70°, with the pesticides initially present at a concentration of 6 µg/ml. Choice of these conditions depended on the following considerations.

A reaction temperature of 70° was selected as this enabled measurable hydrolysis of most pesticides to occur during a four-day period. The rate of hydrolysis is strongly temperature dependent and Table I compares the rates for three pesticides at two temperatures. Table II shows the effect of ethanol on the rate of hydrolysis.

The hydrolysis rates of organophosphorus pesticides are strongly pH dependent; MÜHLMANN AND SCHRADER[8] have reported large changes in the range pH 3.0–9.0.

TABLE I

COMPARISON OF THE HYDROLYSIS RATES AT 20° AND 70° IN ETHANOL–pH 6.0 BUFFER SOLUTION (20:80)

Pesticide	*Half life (h)*	
	20°	*70°*
Phorate-O-analogue	192	0.50
Dichlorvos	260	1.35
Phorate	450	1.75

TABLE II

INFLUENCE OF ETHANOL ON THE HYDROLYSIS RATES AT 70°

Pesticide	*Half life (h)*		
	in de-ionized water	*in ethanol–water (20:80)*	*in ethanol–pH 6.0 buffer (20:80)*
Phorate-O-analogue	0.11	0.63	0.50
Phorate	0.31	1.90	1.75

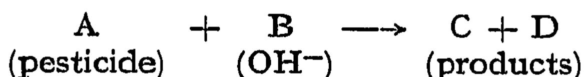
It is essential for comparative studies to be made under identical pH conditions. Choice of a pH 6.0 buffered solution was made after consideration of the conditions likely to be encountered in the field. Within plants, for example, hydrolysis is likely to occur under essentially neutral conditions, whereas on plant surfaces and in river waters more acid conditions could operate. River waters generally range from pH 5.5 to 8.5. A pH of 6.0 was felt to offer a suitable compromise with rates determined at this acidity being the slowest likely to be encountered (hydrolysis rates increasing with decreasing acidity).

Choice of extracting solvent

The extraction of unhydrolysed pesticide from the reaction mixture was carried out with chloroform in these studies. This solvent had favourable extraction characteristics for all the pesticides examined, including the "watersoluble"[16] pesticides such as dimefox and dimethoate. Unfortunately chloroform extinguishes the flame in the "phosphorus" detector and replacement of this solvent with an inflammable solvent was essential before gas chromatographic analysis. No satisfactory inflammable extracting solvents could be found; hexane, capable of extracting many pesticides, being unsuitable for the "water-soluble" pesticides.

Presentation of data

Nucleophilic substitutions such as the hydrolysis of phosphate esters can be expected to show second order kinetics. Representing the reaction as:



Then the rate equation can be represented as: $dx/dt = k_2 (a - x) (b - x)$
where: a and b are the initial concentration of reactants A and B; x is the decrease in concentration after time t.

In conditions where one reactant is in large excess or where its concentration is held constant, as is the case in our study where the hydroxyl ion concentration is constant, then the rate equation reduces to: $dx/dt = k_2 (a - x) (b)$.

This is the rate equation for a first order reaction. Under these conditions the rate can be represented by the half-life of the ester, *i.e.* the time in which half the ester originally present has hydrolyzed. Half-lives are used in this paper to indicate the experimentally determined rates.

METHOD

Reagents

Pesticides: These were obtained from manufacturers and were dissolved in acetone to give stock solutions containing 1000 μg/ml. Purity was claimed to be 95 % or better for most of these materials.

pH 6.0 buffer: Dissolve 17.5 g of anhydrous disodium hydrogen orthophosphate and 8.0 g citric acid monohydrate in water and dilute to 1 l.

Acetone chloroform and ethanol: analytically pure grades were used where possible.

Sodium sulphate (anhydrous).

Apparatus

Varian Aerograph Gas Chromatograph model No. 205-B, fitted with the Aerograph phosphorus detector.

Water bath with thermostatic control.

Volumetric flask, preferably with plastic stoppers.

Separating funnels.

Graduated test tubes.

Procedure

Hydrolysis and extraction. Pipette an aliquot containing about 600 μg of pesticide from the stock solution into a 100 ml volumetric flask. Reduce the solution to dryness with a gentle stream of air, add 20 ml of ethanol and sufficient pH 6.0 buffer to bring the volume of the solution up to 100 ml. Insert a plastic stopper in the flask, shake well and place in a water bath maintained at 70°. Remove aliquots at suitable time intervals and treat as follows.

Remove 10 ml of the solution and add to 25 ml of water in a 250 ml separator. Add 25 ml of chloroform and shake for 1 min. Remove the lower chloroform layer after 5 min swirling to bring down droplets still on the water surface, and pass down a column containing 10 g of granular anhydrous sodium sulphate. Collect in a Danish-Kuderna flask fitted with a 10 ml pear-shaped flask. Wash the column with a further 2 × 25 ml of chloroform: collect the washings and add to eluate. Evaporate the solution to low volume on a steam bath. Transfer to a 10 ml graduated test tube and blow the solution to dryness. Add acetone to the sample for subsequent gas chromatographic analysis (samples for colorimetric analysis can be left dry). Store the tube in a refrigerator until all the samples obtained during the course of the experiment are available.

Gas chromatographic determination

A Varian Aerograph model 205-B gas chromatograph fitted with two 150 cm coiled glass columns of 5 mm outer diameter was used in this study. Only one of the two columns present was used, this being packed with an Apiezon L, Epikote 1001 coated Chromosorb G. The column preparation and chromatographic operating conditions have been previously described[15]. The detector used was a Varian Aerograph Phosphorus detector. The procedure for pesticide determination was as follows.

Adjust the oven temperature of the gas chromatograph for the pesticide being determined, Table III shows the optimum column temperature and retention time relative to parathion for the pesticides studied. British Standard names of pesticides are used where applicable[17].

Add acetone to the samples in the tubes to give a suitable concentration (1–6 μg/ml) of the pesticide. Select a detector signal amplification which gives the largest on-scale response when a 5 μl aliquot from the first and most concentrated of the pesticide extracts is injected on the chromatographic column. Make a 5 μl injection of each of the samples. Measure the height of the chromatographic peak corresponding to the pesticide. Inject 5 μl aliquots from a standard series of the pesticide covering the range encountered experimentally. Plot a standard curve of peak height *versus* pesticide concentration and calculate the pesticide content of each sample in the hydrolysis series. Plot graphically log μg of pesticide against time.

TABLE III

GAS CHROMATOGRAPHIC DETERMINATION OF PESTICIDES ON AN APIEZON, EPIKOTE 1001 COATED CHROMOSORB G

Pesticide	*Optimum column temp.(°C)*	*Relative retention time*	*Pesticide*	*Optimum column temp.(°C)*	*Relative retention time*
Azinphos ethyl	190	1375	Mecarbam	190	122
Azinphos methyl	190	1160	Mevinphos	165	8.5, (11.5)
Carbophenothion	190	450			
Chlorfenvinphos	190	140	Morphothion	190	285
Demeton-S-methyl	165	21	Parathion	165	100
Diazinon	165	32	Parathion-O-analogue	165	89
Dichlorvos	125	2.6	Parathion methyl	165	67
Dimefox	125	1.6	Phenkapton	190	825
Dimethoate	165	50	Phorate	165	25
Disulfoton	165	42	Phorate-O-analogue	165	19
Ethion	190	265	Phosphamidon	165	46, (66)
Fenchlorphos	165	81	Schradan	165	106
Fenitrothion	165	82	Thiometon	165	28
Fenthion	165	103	Thionazin	165	16
Formothion	165	67	Thionazin-O-analogue	165	18
Malathion	165	67	Vamidothion	190	318
Malathion-O-analogue	165	62			

Draw the line which best fits the experimental data. Subtract 0.30 from the value of log μg at time zero, the time at which this concentration was reached corresponds to the half life.

Colorimetric determination

Some pesticides will not give chromatographic peaks under the conditions described. Data on the hydrolysis rates of these compounds were determined by carrying out a conventional colorimetric phosphorus determination after oxidation of the extracted pesticide. The technique used was that described by ABBOTT *et al.*[18].

RESULTS

All the pesticides examined decomposed by a pseudo first order reaction. The results are shown in Table IV. Formothion was found to undergo reactions with ethanol prior to hydrolysis and its hydrolysis rate could not be determined. Under natural conditions this compound breaks down to dimethoate, this was also the case when refluxed with water.

Azinphos ethyl decomposed to a compound having a shorter retention time peak (relative retention time 1180) during the first 24 h of hydrolysis.

All the other compounds remained unchanged during the course of the hydrolysis and no new chloroform extractable–GLC detectable compounds were formed.

Table V shows the hydrolysis rates of four pesticides in two river waters and an aqueous extract of brussel sprouts prepared by macerating 100 g of sprouts with 100 ml of water and filtering: 20 % ethanol was used to improve pesticide solubility.

TABLE IV

HYDROLYSIS RATES OF PESTICIDES AT 70° IN ETHANOL–pH 6.0 BUFFER SOLUTION (20:80)

Pesticide	*Half life (h)*	*Pesticide*	*Half life (h)*
Phorate-O-analogue	0.5	Phosphamidon peak II	14.0
Dichlorvos	1.35	Thiometon	17.0
Phorate	1.75	Oxy-demeton methyl*	17.1
Trichlorphon*	3.2	Demeton-S	18.0
Mevinphos peak I	3.7	Morphothion	18.4
Mevinphos peak II	4.5	Fenthion	22.4
Demeton-S-methyl sulphone*	5.1	Vamidothion	25.4
Mecarbam	5.9	Menazon*	27.6
Malathion-O-analogue	7.0	Parathion-O-analogue	28.0
Demeton-S-methyl	7.6	Thionazin	29.2
Malathion	7.8	Disulfoton	32.0
Thionazin-O-analogue	8.2	Diazinon	37.0
Parathion methyl	8.4	Ethion	37.5
Fenchlorphos	10.4	Parathion	43.0
Azinphos methyl	10.4	Phenkapton	92.0
Phosphamidon peak I	10.5	Chlorfenvinphos	93.0
Fenitrothion	11.2	Carbophenothion	110.0
Dimethoate	12.0	Dimefox	212
		Schradan	Neglible hydrolysis in 96 h

* These compounds were studied using a colorimetric determination.

TABLE V

HYDROLYSIS RATES AT 70° IN RIVER WATERS AND AN AQUEOUS BRUSSEL SPROUT EXTRACT; 20% ETHANOL PRESENT

Pesticide	*Half-life (h)*		
	*in River Thames water**	*in River Irthing water***	*in Brussel sprouts extract*
Dimethoate	22	18	18
Thionazin	50	54	56
Parathion	65	68	60
Chlorfenvinphos	104	116	70

* Sampled at Windsor, pH 8.0, ammoniacal nitrogen 0.35 mg/l, total hardness as $CaCo_3$ 314 mg.

** Sampled at Gilsland, pH 7.5, ammoniacal nitrogen 0.047 mg/l, total hardness as $CaCO_3$ 42 mg.

DISCUSSION

The results shown in Table IV indicate that the pesticides currently in use have widely different hydrolysis rates. The two phosphoramidates studied, schradan and dimefox, were extremely resistant to hydrolysis. O'BRIEN[3] has attributed this to

Schradan — $[(CH_3)_2N]_2P(=O)\text{–}O\text{–}P(=O)[N(CH_3)_2]_2$

Dimefox — $[(CH_3)_2N]_2P(=O)F$

the very weak electrophilic effect of the —$N(CH_3)_2$ group; as a result the central phosphorus atom is much less electropositive than is the case with the more typical phosphorus esters and the resistance to attack by the nucleophilic —OH group is correspondingly increased.

The above two compounds are atypical, however, as most of the pesticides have the general structure:

$$(RO)_2P(=O(S))\text{–}O(S)\text{–}X$$

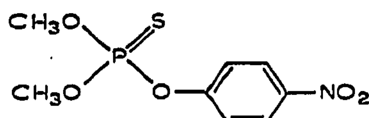
where R = an alkyl group; X = organic radical.

Hydrolysis, involving a nucleophilic substitution generally proceeds with the splitting off of the OX or (SX) radical.

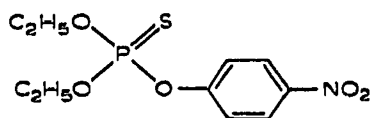
$$(RO)_2P(=O(S))\text{–}O(S)\text{–}X + OH^- \rightarrow (RO)_2P(=O(S))\text{–}O\text{–}H + XO^-_{(S)}$$

Under comparable conditions the rate is dependent on variations in the substituents of the phosphorus compound with substituents rendering the phosphorus atom more electropositive increasing the hydrolysis rate unless steric effects interfere.

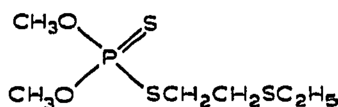
The effect of varying R is well illustrated in the results; those pesticides where R is a methyl group hydrolyzing more rapidly than their ethyl analogues. This being due to the greater electrophilic character of the methyl group.



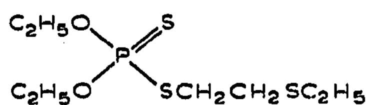
Parathion methyl
8.4



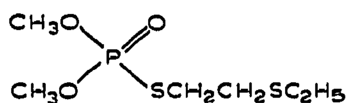
Parathion
43.0



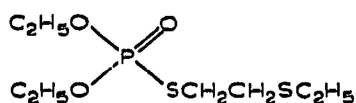
Thiometon
17.0



Disulfoton
32.0

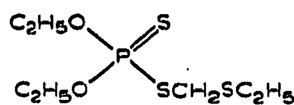


Demeton-S-methyl
7.6

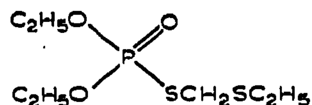


Demeton-S
18.0

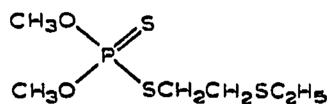
In an analogous manner the substitution of P = O for P = S should result in a molecule of greater hydrolyzability. The results support this in most instances.



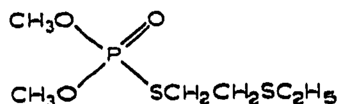
Phorate
1.75



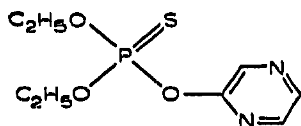
Phorate-O-analogue
0.5



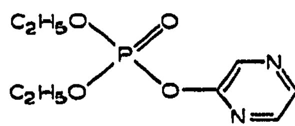
Thiometon
17.0



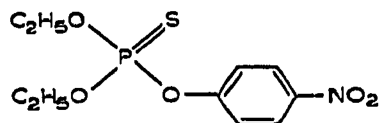
Demeton-S-methyl
7.6



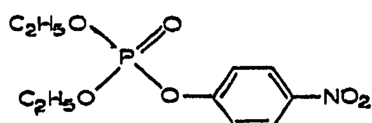
Thionazin
29.2



Thionazin-O-analogue
8.2

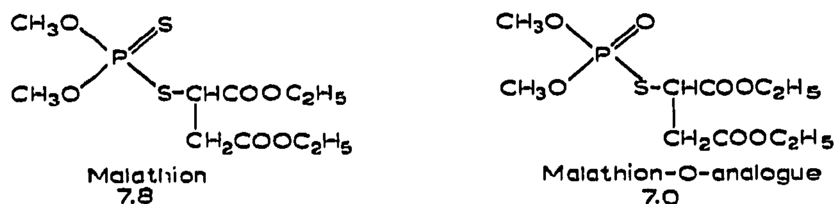


Parathion
43.0

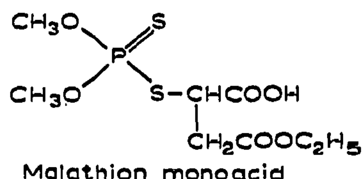


Parathion-O-analogue
28.0

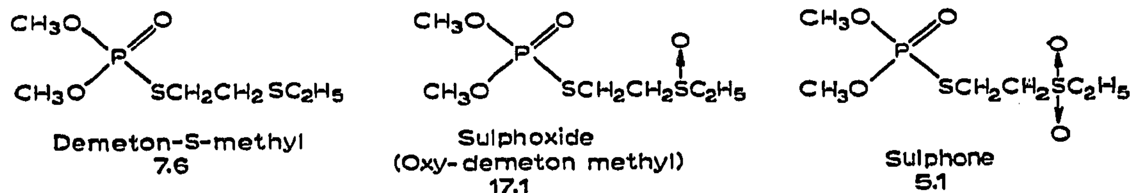
In the case of malathion, however, the oxidation made very little difference to the hydrolysis rate.



This is explicable if the predominant hydrolysis was occurring in the side chain, where the inductive effect of the —P=O bond would be expected to only marginally influence the hydrolysis rate. Malathion monoacid has been characterised as a naturally occurring hydrolysis product[19].

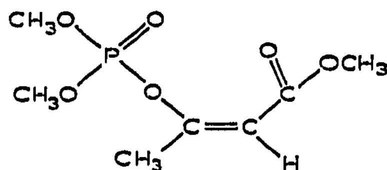


Where the pesticide contains a side chain with a thioether group present as is the case with demeton S, demeton-S-methyl, disulfoton, fenthion, phenkapton, phorate, thiometon and trithion there exists the possibility of oxidation to the sulphoxide and sulphone. Such oxidation has been observed to occur naturally[20,21], and would be expected to increase the hydrolysability of the molecule by increasing the electrophilic character of the side chain. The only series of samples we had available were the oxidation products of demeton-S-methyl. The results obtained were as shown; a similar pattern was reported by MÜHLMANN AND SCHRADER[8].

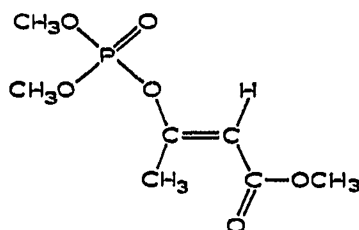


Only the parent compound could be determined by gas chromatography. The possibility that chloroform extractable impurities were present in the sulphoxide and sulphone samples cannot be ruled out. Such impurities would contribute to the total phosphorus found and could lead to erroneous results. This does, however, illustrate the advantage of the gas chromatographic method whereby a distinct characterisation of the chloroform extractables can be made.

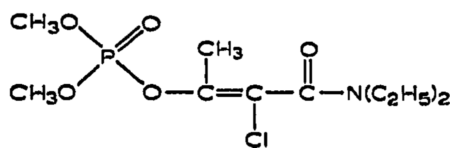
Another interesting feature brought out in the results is the way that *cis-trans* isomerisation can influence the hydrolysis rate of a pesticide. The compounds mevinphos and phosphamidon both show this type of isomerisation[22].



Mevinphos *trans*-isomer



Mevinphos *cis* isomer



Phosphamidon

Both compounds give two peaks on gas chromatography. In the case of mevinphos there is sufficient information available to deduce that peaks I and II in Table IV correspond to the *cis* and *trans* forms respectively. With phosphamidon, however, we have been unable to characterize each peak although peak II may be the major component of the mixture.

The results of hydrolysis of four pesticides in river waters and a plant extract (Table V) are interesting in that their rates are, with one exception, all slower than in the buffered systems. A similar effect was found with de-ionised water (see Table **II**). This change in rate is either due to the ionic strength being greater in the buffer solution or a catalytic effect due to one of the component ions of the buffer. The increase in rate is probably due to the latter as a rate increase due to an increase in the ionic strength of a solution, *i.e.* the so-called primary salt effect, generally only occurs in reactions between two ions. One would perhaps expect the hydrolysis rates in the plant extract to be accelerated by enzymatic action but only in the case of chlorfenvinphos was an increased rate noted. The high temperature and the presence of ethanol could, however, result in deactification of the enzymes present. It is hoped to investigate this aspect of pesticide hydrolysis further, under more normal temperature conditions.

SUMMARY

The hydrolysis rates of organophosphorus pesticides are dependent upon their chemical structure and in pH 6.0 buffered solutions at 70° range from half lives of 0.5 h to 4.0 days and longer still for phosphoramidates. At 20° the rates are several hundred times slower. Oxidation of pesticides, where possible, usually results in decreased stability. Compared to the highly stable organochlorine pesticides the toxic phosphorus esters are undoubtedly less persistant, however, the rates of decompo-

sition under uncatalyzed conditions are still relatively slow. Contamination of surface waters by accident or misuse could result in long-lived and potentially hazardous residues.

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