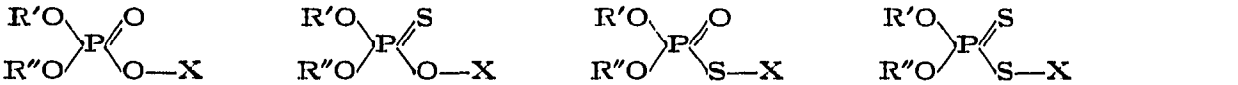
THE GAS-CHROMATOGRAPHIC EXAMINATION OF ORGANOPHOSPHORUS PESTICIDES AND THEIR OXIDATION PRODUCTS*

J. RUZICKA, J. THOMSON AND B. B. WHEALS
Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E. 1 (Great Britain)
(Received March 1st, 1967)

As organophosphorus pesticides become more widely used in agriculture, increased attention is being paid to the examination of foodstuffs and the environment for residues of these often highly toxic materials[1,2]. In 1966, for example, 23 organophosphorus pesticides were approved for agricultural use in this country by the Ministry of Agriculture, Fisheries and Food[3]. Paper, thin-layer and gas chromatography have all been applied to the problem of organophosphorus pesticide residue analysis and the developments have been reviewed by ABBOTT AND THOMSON[4] up to the end of 1965. During the last two years the development and improvement of selective phosphorus detectors for gas chromatography[5-7] has considerably stimulated interest in this technique of residue analysis.

The sodium thermionic detector developed by GUIFFRIDA[5] has high specificity and sensitivity to phosphorus-containing compounds but the experience of this laboratory has been that it is somewhat unreliable in operation and not ideally suited to routine residue analysis. Recently, however, a thermionic detector has been developed by HARTMANN[8] which largely overcomes the drawbacks previously associated with this type of detector. The detector described by HARTMANN consists of a conventional flame ionisation detector in which the flame burns on a tip containing caesium bromide and an inert filler moulded under high pressure. A detector of this type has been in use in this Laboratory for several months and has proved to be most satisfactory.

When analysing foodstuffs for organophosphorus pesticides the presence of toxic metabolites in addition to the parent compounds must be considered[9]. The majority of organophosphorus pesticides are esters of phosphoric, phosphorothionic, phosphorothiolic and phosphorothiolothionic acids:



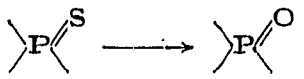
Phosphates — Phosphorothionates — Phosphorothiolates — Phosphorothiolothionates

where R', R" are alkyl groups,
X is a side chain.

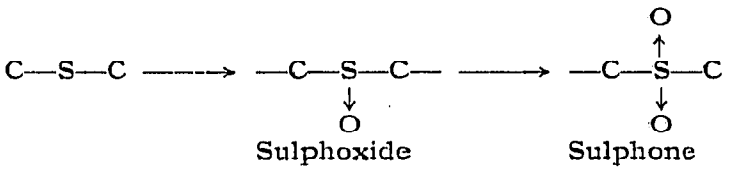
Hydrolysis, occurring fairly rapidly under natural conditions, results in ionic water-soluble derivatives which are generally considered to be non-toxic. Oxidation,

* Permission to publish this work has been given by the Government Chemist.

however, produces toxic metabolites which are extractable from aqueous solution by solvents such as hexane, chloroform and benzene. Oxidation of the organophosphorus esters usually involves sulphur atoms present in the molecule. Thus the thiono–sulphur atom may oxidize as follows



and where a thio-ether link is present in the side chain the sulphoxide and sulphone may occur as additional oxidative metabolites



In this study the gas chromatographic behaviour of the approved organophosphorus pesticides and a limited number of their oxidation products has been investigated using the thermionic detector. In addition simple chemical oxidations of the pesticides have been carried out and gas chromatography used to separate and where possible detect oxidation products in the reaction mixtures.

EXPERIMENTAL

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. Glass columns are preferable to metal columns for the gas chromatography of organophosphorus compounds as they minimize on-column breakdown. One column was packed with acid washed, dimethyldichlorosilane treated Chromosorb W (80 to 100 mesh) coated with a stationary phase of 10% w/w SE 30 and 1% Epikote 1001 by a conventional evaporation technique. The other column was packed with acid washed dimethyldichlorosilane treated Chromosorb G (70 to 80 mesh) coated with Apiezon L and Epikote 1001 using the following filtration technique: 10 g of the support was dried by heating at 120° for 30 min. The cooled support was added to 50 ml benzene containing 1.50 g Apiezon L and 0.10 g Epikote 1001. The slurry was swirled gently for 5 min and filtered rapidly by suction through a sintered glass filter funnel. After drawing air through the packing for about 10 min it was transferred to the oven at 120° for final drying. Although Chromosorb G has the desirable characteristic of being much less friable than Chromosorb W it was not found possible to prepare a satisfactory SE 30 column using the former support. The filtration technique used above is a good way of preparing low-loaded packings, but cannot be used where a coating as high as 10% is required.

Two Varian Aerograph phosphorus detectors, as described by HARTMANN[8], were used in these experiments, the amplified signals being fed simultaneously into a Westronics dual-channel recorder. Brass collars were constructed which fitted over the detector-base raising the detector housing so that a gap of 4 mm between the bottom of the ignitor coil and top of the caesium bromide tip was obtained. The

TABLE I

RELATIVE RETENTION TIMES AND SENSITIVITIES OF ORGANOPHOSPHORUS PESTICIDES

Pesticide	*Column temperature (°C)*	*Retention time relative to parathion at the temperature shown**		*Sensitivity*** *($g \times 10^{-9}$)*	
		SE 30 column	*Apiezon column*	*SE 30 column*	*Apiezon column*
Azinphos methyl	190	1190	1160	65	55
Demeton-O-methyl		No sample available			
Demeton-S-methyl	165	33	21	1.0	0.5
Diazinon	165	38	32	0.5	0.5
Dichlorvos	125	4.5	2.6	1.0	0.5
Dimefox	125	2.9	1.6	1.0	0.5
Dimethoate	165	78	50	1.5	1.0
Disulfoton	165	41	42	0.5	0.5
Ethion	190	244	265	1.0	1.0
Formothion	165	91	67	1.5	1.5
Malathion	165	91	67	1.5	1.0
Mecarbam	165	142	122	2.5	2.0
Menazon	190	Not detected	Not detected	—	—
Mevinphos	165	14	8.5	1.0	0.5
Morphothion	190	325	285	6.0	3.5
Oxydemeton-methyl	190	Not detected	Not detected	—	—
Parathion	165	100	100	1.0	1.0
Phenkapton	190	510	825	4.0	4.0
Phorate	165	25 (One peak)	25 (Two peaks)	0.5	0.5
Phosphamidon	165	121	46,66	—	
Schradan	165	Not detected	106	—	5.0
Trichlorphon	125	Not detected	Not detected	—	—
Vamidothion	190	Not detected	318	—	65

* The retention times for parathion at the various temperatures were as follows:
SE 30 column: 190°—3.25 min; 165°—8.80 min; 125°—43 min.
Apiezon column: 190°—2.25 min; 165°—6.30 min; 125°—30 min.

** Weight of pesticide producing a peak with a height equivalent to 10 % of full scale deflection. The noise level at the amplification used corresponding to approximately 5 % of full scale deflection.

hydrogen and air supplies to the detectors were from cylinder sources. The hydrogen stream was split and the flow to each flame controlled by means of a flow restrictor. Flow control was achieved by adjusting the cylinder regulator—a flow rate of about 20 ml/min to each detector being obtained with a regulator-pressure of 14 lb./sq.in. The air flow rate to each detector was adjusted by a needle valve and measured with a floating-ball flowmeter. The gas flow rates to the two detectors were maintained at the following constant levels:

Detector 1 (SE 30 column): H_2 20 ml/min; N_2 (carrier-gas) 25 ml/min; air 200 ml/min.

Detector 2 (Apiezon column): H_2 20 ml/min; N_2 (carrier-gas) 22 ml/min; air 300 ml/min.

The air flow rate was arrived at by selecting the minimum flow rate at which the flame remained alight after injection of a 5 μl sample of hexane. Unless adequate air is supplied the flame blows out on injection.

GAS CHROMATOGRAPHY OF THE APPROVED PESTICIDES

Pesticide solutions were prepared in hexane or a mixture of hexane and acetone, 5 μl injections were made into the glass liner of the independently heated injection block, which was maintained at a temperature of 165°. The column temperature was varied to suit the particular compound injected. The detector, also heated independently of the column, was maintained at a temperature of 200°.

RESULTS

Table I shows the retention times relative to parathion on the two columns, the column temperature used and the sensitivity for most of the pesticides on the approved list. No sample of demeton-O-methyl was available and a commercial sample of demeton methyl believed to contain a mixture of the O- and S-isomers gave only a single peak corresponding to demeton-S-methyl. The sensitivities attained are not as good as those reported by HARTMANN[8], but flame noise, which in part determines the detection limit, requires high quality flow control to be reduced to a minimum and the control used in this study was not of comparable quality to that used by the previous author. The sensitivity reached for most of the pesticides, however, is quite adequate for residue work. The sensitivity to azinphos methyl could probably be increased by using a shorter column but menazon, oxy demeton-methyl and trichlorphon did not give any detectable chromatographic peaks.

Linearity and specificity were found to be much as previously reported[8].

PREPARATION AND GAS CHROMATOGRAPHY OF OXIDATION PRODUCTS

Pesticides containing an oxidizable thiono-sulphur atom and those containing an oxidizable side chain are listed in Table II.

In order to supplement the limited number of oxidation products obtainable from pesticide manufacturers, recourse was made to carrying out chemical oxidations on the pure pesticides listed below. Three oxidizing solutions were used:

I. Peracetic acid, prepared by adding 1 ml of 100 volume hydrogen peroxide to 5 ml of glacial acetic acid immediately before use.

II. A saturated aqueous solution of bromine.

III. A saturated solution of potassium permanganate in acetone, prepared daily.

Approximately 100 μg of pesticide was deposited in a 10 ml test tube by

TABLE II

Pesticides containing a thiono-sulphur atom	*Pesticides with an oxidizable side-chain*
Azinphos methyl, Demeton-O-methyl	Demeton-O-methyl
Diazinon, Dimethoate	Demeton-S-methyl
Disulfoton, Ethion	Disulfoton
Formothion, Malathion	Phenkapton
Mecarbam, Menazon	Phorate
Morphothion, Parathion	Vamidothion
Phenkapton, Phorate	

evaporating a solution of the pesticide to dryness. To the tube was added 6 ml of oxidant I or 5 ml of either oxidant II or III. Oxidation was allowed to proceed for up to 15 min at room temperature or 50°. After the requisite time, the contents of the tube were poured into a 250 ml separator containing 25 ml of a saturated solution of sodium sulphite. For oxidations carried out with oxidants II and III, 5 ml glacial acetic acid was also added to the separator. After mixing, excess acid was neutralised

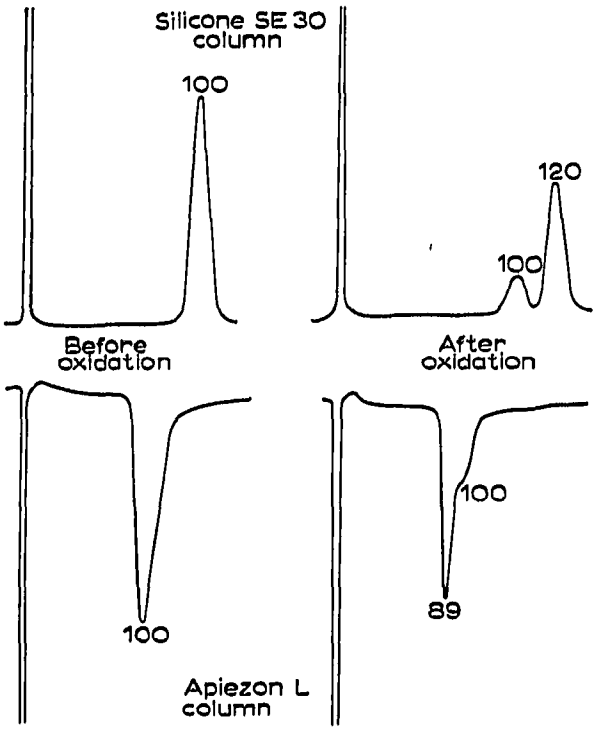


Fig. 1.

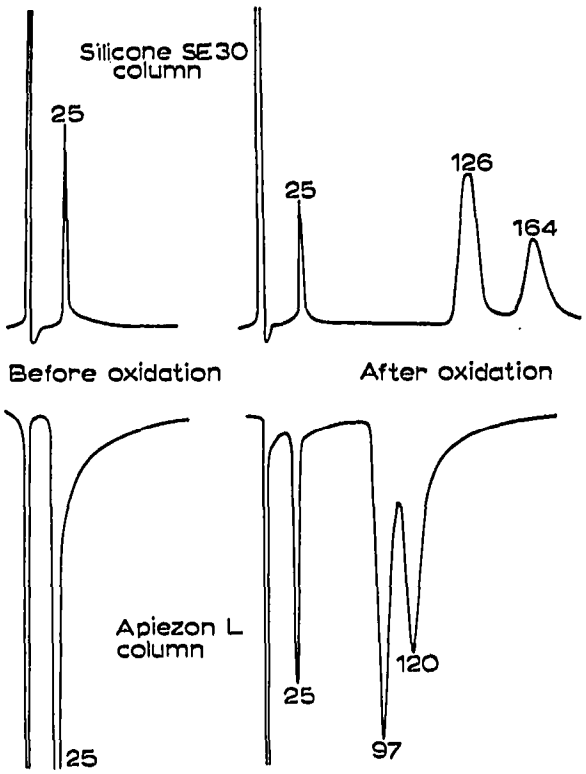


Fig. 2.

Fig. 1. Typical chromatograms of the oxidation of parathion with bromine water for 15 min at 50°. Numbers refer to retention times relative to parathion (= 100).

Fig. 2. Typical chromatograms of the oxidation of phorate with potassium permanganate in acetone for 5 min at 20°. Numbers refer to retention times relative to parathion (= 100).

by addition of 25 ml of a saturated solution of sodium bicarbonate. The solution was extracted with 25 ml hexane and the hexane extract dried by passage through a column containing 10 g anhydrous sodium sulphate. The solution was evaporated to low volume in a Danish Kuderna flask, transferred to a graduated test tube and the volume adjusted to 1 ml. 5 μl aliquots of the solution were injected on the gas chromatographic columns previously described. The chromatograms obtained were used to deduce information about the oxidation products. Figs. 1 and 2 shows typical chromatograms obtained for parathion and phorate using bromine water and potassium permanganate as their respective oxidizing solutions.

RESULTS OF OXIDATION

Listed in Table III are the relative retention times and sensitivities of standard samples of pesticide oxidation products. In Table IV the relative retention times of

TABLE III

RELATIVE RETENTION TIMES OF STANDARD OXIDATION PRODUCTS

Oxidation product	*Column temperature (°C)*	*Retention time relative to parathion at temperature shown*		*Sensitivity ($g \times 10^{-9}$)*	
		SE 30 column	*Apiezon column*	*SE 30 column*	*Apiezon column*
Demeton-S-methyl sulphone	165	500 ng Not detected	500 ng Not detected	—	—
Dimethoate-O-analogue	165	200 ng Not detected	48	—	20
Malathion-O-analogue (Malaoxon)	165	102	62	24	3.5
Parathion-O-analogue (Paraoxon)	165	120	89	11	3.5
Phorate-O-analogue	165	24	19	0.5	0.5
Phorate-O-analogue sulphone	165	200 ng Not detected	93	—	24

oxidation products produced by the above oxidation procedure are recorded. The most suitable oxidant and oxidizing conditions are also listed.

Oxidation of azinphos methyl, demeton-S-methyl, dimethoate, formothion, morphothion and vamidothion, although judged to be occurring by disappearance of the chromatographic peak corresponding to the parent compound, did not give rise to any detectable oxidation products.

A comparison of Tables III and IV indicates that the oxidation products produced from malathion and parathion are the expected oxygen analogues. By

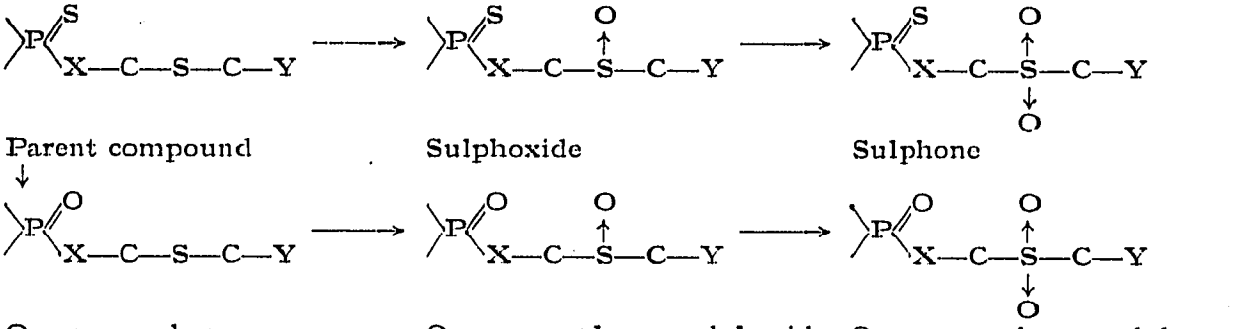
TABLE IV

RELATIVE RETENTION TIMES OF PREPARED OXIDATION PRODUCTS OF ORGANOPHOSPHORUS PESTICIDES

Parent compound	*Column temperature (°C)*	*Retention time of oxidation products relative to parathion*		*Oxidant**	*Oxidizing conditions (reaction time and temperature)*
		SE 30 column	*Apiezon column*		
Diazinon	165	46	33	II	15 min
Disulfoton	165	25	19	III	5 min
		280	207	III	5 min
		Not detected	290	III	5 min
Ethion	190	Not detected	200	I and II	15 min 50°
Malathion	165	Not detected	62	II	5 min
Mecarbam	165	174	108	I	15 min 50°
Parathion	165	120	89	I and II	15 min 50°
Phenkapton	190	Not detected	600	I	5 min 50°
		1160	1500	I	15 min 50°
Phorate	165	126	97	III	5 min
		164	120	III	5 min

* Oxidant: I = Peracetic acid; II = aqueous bromine; III = potassium permanganate in acetone.

analogy the oxidation products of diazinon and mecarbam are probably also the oxygen analogues. Where side chain oxidation can occur in addition to thionosulphur oxidation there are five possible oxidation products, and characterisation of a peak



corresponding to a particular oxidation product is difficult. This is the case with the oxidation products of disulfoton, phenkapton and phorate. However, in the case of phorate pure samples of the oxygen analogue and oxygen analogue sulphone were available and the two oxidation products listed in Table IV corresponded to neither of these compounds. Oxidation of the phorate oxygen analogue failed to produce detectable oxidation products and it can be assumed that the two compounds producing chromatographic peaks were the sulphoxide and sulphone of the parent compound. BACHE AND LISK[10] reported that they could not find a column suitable for the gas chromatography of phorate-oxygen analogue sulphoxide and sulphone although they were able to separate and detect the other oxidation products. The similarity between the molecular structure of phorate and disulfoton renders it likely that the two long retention time peaks produced by oxidation of the latter compound are also the sulphoxide and sulphone. In the case of phenkapton and ethion it is not possible to deduce by analogy the structures of those oxidation products giving peaks.

It is apparent from the results of this study that greater sensitivity for organophosphorus pesticides is obtained with the Apiezon column in most cases. However, an Apiezon column does not adequately resolve oxygen analogues from their parent compounds, although the former compounds generally have a slightly shorter retention time, probably by virtue of their lower molecular weight. The silicone column usually provides a good separation of oxygen analogue and parent compound, presumably with the substitution of the P=O for the P=S inducing a greater polarity in the molecule.

The study of the organophosphorus pesticides and their oxidation products is the first part of a programme covering hydrolysis products, effect of cooking, irradiation effects and thermal decomposition of this very interesting and useful group of pesticides.

SUMMARY

A considerable number of organophosphorus pesticides have been approved for use in agriculture and increasing attention is being paid to the examination of foodstuffs and the environment for residues of those often highly toxic materials. This

paper deals with the gas–liquid chromatographic separation of some organophosphorus pesticides and their metabolites using a thermionic detection system. Many of the oxidation products were prepared and optimum conditions for oxidation of the pesticides are reported.

REFERENCES

1 D. C. ABBOTT, N. T. CROSBY AND J. THOMSON, *Proc. S.A.C. Conf.*, 1965, p. 121.
2 J. A. R. BATES, *Analyst*, 90 (1965) 453.
3 Ministry of Agriculture, Fisheries & Food, *Agricultural Chemicals Approval Scheme, List of Approved Products, 1965, for Farmers and Growers.*
4 D. C. ABBOTT AND J. THOMSON, *Rept. Progr. Appl. Chem.*, 50 (1965) 216.
5 L. GUIFFRIDA, *J. Assoc. Offic. Agr. Chemists*, 47 (1964) 293.
6 U. P. BURCHFIELD AND R. J. WHEELER, *J. Assoc. Offic. Agr. Chemists*, 49 (1966) 651.
7 A. J. MCCORMACK, S. S. C. TONG AND W. D. COOKE, *Anal. Chem.*, 37 (1965) 1470.
8 H. C. HARTMANN, *Bull. Environ. Contamination Toxicol.*, 1 (1966) 159.
9 R. D. O'BRIEN, *Toxic Phosphorus Esters: Chemistry, Metabolism, and Biological Effects*, Academic Press, New York, London, 1960.
10 C. A. BACHE AND D. J. LISK, *J. Assoc. Offic. Agr. Chemists*, 49 (1966) 647.