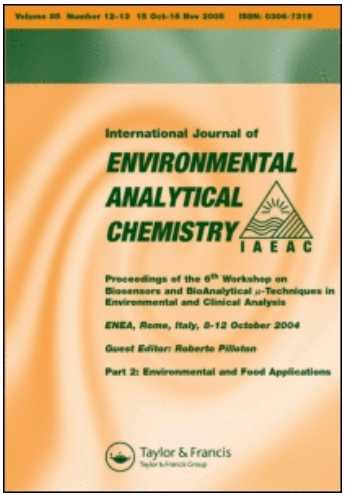

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Developments in the Determination of Organophosphorus Pesticides

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To cite this Article Greve, P. A. and Goewie, C. E.(1985) 'Developments in the Determination of Organophosphorus Pesticides', International Journal of Environmental Analytical Chemistry, 20: 1, 29 — 39
To link to this Article: DOI: 10.1080/03067318508077044
URL: http://dx.doi.org/10.1080/03067318508077044

Intern. J. Environ. Anal. Chem., 1985, Vol. 20, pp. 29–39
0306-7319/85/2002-0029 $18.50/0

Printed in Great Britain

Developments in the Determination of Organophosphorus Pesticides†

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(*Received November 23, 1984*)

In this paper, a review is given of some recent developments in organophosphorus pesticides residue methodology. Enrichment on XAD-resins or C_{18} bonded phases, clean-up by gel permeation and determination by high-performance liquid chromatography (HPLC) with specific detectors can be considered as the major fields of development in the past years.

Despite of the progress made, there is still a further need for procedures which reduce handling time, e.g. by automation, and/or increase specificity of the HPLC determination in matrices of practical interest, such as food, animal and human tissues and environmental samples.

KEY WORDS: Organophosphorus pesticides, residue methodology, HPLC, trace enrichment, XAD-resins, clean-up.

1. INTRODUCTION

Organophosphorus pesticides (OPs) form a large group of compounds with widely different structures and biological activities. The majority of the OPs are esters with insecticidal, acaricidal and/or nematicidal activity, generally based on the principle of

†Presented at the 14th Annual Symposium on the Analytical Chemistry of Pollutants, Barcelona, November 21–23, 1984.

cholinesterase-inhibition. Chemically these esters can be described as phosphates, phosphorothioates, phosphorodithioates, phosphoramidates, phosphonates, pyrophosphates etc. In Table I, some basic structures are given, together with the number of corresponding pesticides, mentioned in the 1983 edition of the Agrochemical Handbook.[1] Table I also gives the number of other OPs (fungicides, herbicides etc.) mentioned in the same publication.

In Table II, examples for each group of compounds are given as an illustration of the large diversity of chemical structures covered by the name organophosphorus pesticides. To the analyst, this diversity poses problems when trying to develop methods which cover as many OPs as possible ("multiresidue methods"). In this paper, main emphasis will be on multiresidue methods, but special (dedicated) methods will be discussed as well. For each aspect of the analysis, viz. enrichment, clean-up and determination, the main developments in the recent years will be illustrated by selected examples.

The paper will deal only with aspects of the analysis at *residue*-level, i.e. at concentrations in the ppm–ppb (mg–μg/kg) range.

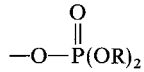
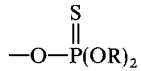
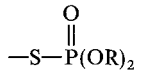
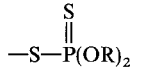
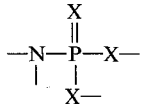
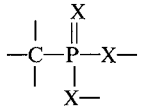
2. ENRICHMENT

2.1. Water samples

Water samples are still widely processed by classical liquid-liquid extraction. Dichloromethane is especially useful to this end, as it is sufficiently polar to extract efficiently most OPs, and its high specific weight is convenient for repeated extractions in a separating funnel. Moreover, its low boiling point makes concentration of the extract possible under mild conditions. Less polar solvents, like hexane,[2] give quantitative extraction for a limited number of OPs only, but when interest is restricted to those compounds, the cleaner extracts obtained in this way can very well be preferred.

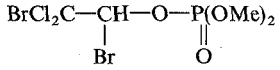
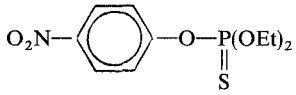
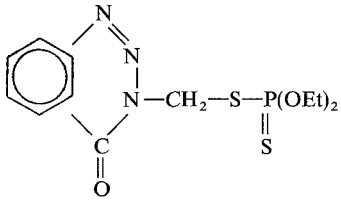
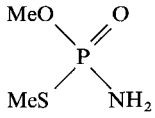
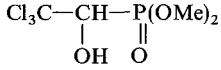
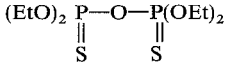
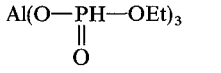
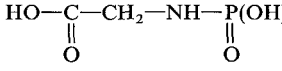
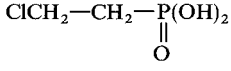
A systematic approach to the extraction of water samples is given by Suffet *et al.*[3] Ionic strength and pH of the aqueous phase have to be standardised carefully in order to achieve reproducible recoveries. A new version of the continuous liquid-liquid extractor has been presented by Wu and Suffet;[4] it must be remembered, however, that in general real water samples (e.g. surface water samples or industrial

TABLE I
Organophosphorus pesticides mentioned in the 1983 edition of The Agrochemical Handbook[1]

INSECTICIDES/ACARICIDES/NEMATICIDES[a]		number of pesticides mentioned
phosphates		10
phosphorothioates		30
	or 	
phosphorodithioates		19
phosphoramidates		7
phosphonates		3
others		5
FUNGICIDES		2
HERBICIDES		4
GROWTH REGULATORS		1
	TOTAL:	81

[a]R = methyl or ethyl. X = O or S.

TABLE II
Examples of organophosphorus pesticides

INSECTICIDES/ACARICIDES/NEMATICIDES		
phosphates		naled
phosphorothioates		parathion
phosphorodithioates		azinphos-ethyl
phosphoramidates		methamidophos
phosphonates		trichlorfon
pyrophosphates		sulfotep
FUNGICIDES		aluminium-fosethyl
HERBICIDES		glyphosate
GROWTH REGULATORS		ethephon

effluents) give problems in such systems due to difficult phase separations caused by silt, detergents etc.

More recently, enrichment by adsorption techniques have come into use also for OPs. The advantage of these techniques is the fact that large volumes of water can be processed (if desired, automatically at the sampling site), thus simplifying transport, improving representativity of the sampling and lowering detection limits. A judicious choice of adsorbents is necessary: XAD-resins[5,6] and C_{18} bonded phases[7] have been used successfully. An excellent review article on solid phase extraction of organic compounds from water samples using porous organic polymers, has been published by Dressler.[8] Enrichment and determination on the same column has been applied by Goretti *et al.*[9] and by Otsuki and Takaku.[10] Although this procedure seems attractive at first sight, it must not be forgotten that the column, when operated with surface water samples or industrial effluents, will readily become fouled and therefore less suited for the determination. Moreover, the most favourable dimensions for an enrichment column are not identical with those for an analytical column and vice versa.[11]

A special problem in the analysis of surface water samples or industrial effluents is the particulate matter often present in such samples. Since many OPs are readily adsorbed by the organic and inorganic components in silt, simple extraction with one solvent of low polarity will not take the more strongly bound residues into account. Theoretically, the best solution is to separate the suspended matter from the aqueous phase and to extract the two phases separately; the solid phase can then be extracted with a more polar, water-miscible solvent, which is in general to be preferred (cf. 2.2). Filtration or centrifugation prior to enrichment over an adsorption column can be necessary if the sample contains so much solids that the column becomes clogged.

Concentration by dry-freezing or freezing-out has been used occasionally,[12] but this technique has not found wide application.

2.2. Solid samples

For the determination of OPs in solid samples, extraction with a liquid is the most practical way of isolating the pesticides. As many OPs are systemic, the solvent must be able to penetrate in the cells,

which means that the solvent must be at least partially miscible with water.

In many modern multi-residue methods, acetone is used for the extraction.[13,14,15] Acetonitrile is also a universal solvent for OPs,[13,15] but its (compared to acetone) higher toxicity and price are practical drawbacks. Methanol is also, but less frequently, used; it is especially useful for the extraction of OPs of high polarity.

Systematic studies on exhaustive extraction of OPs are reported in the Pesticide Analytical Manual.[13] Soxhlet extraction with a 1:1 mixture of chloroform and methanol was found to give the best recoveries of (radio-labelled) "incurred" residues, but this procedure is time-consuming. Other extraction procedures are compared with the chloroform/methanol procedure for appraisal; in this way, blending with acetonitrile was found to give practically the same results as Soxhlet extraction with chloroform/methanol.

Naturally, acetone and other water-miscible solvents extract, apart from the pesticides, many water-soluble compounds together with the water contained in the matrix. These co-extractives (carbohydrates, proteins etc.) must be removed before determination of the pesticides can be carried out. Partitioning, after dilution with water, with e.g. dichloromethane[13,14,15] effectively separates the OPs from the water-soluble co-extractives, but this step can be time-consuming due to difficult phase separations.

Extraction with an only partially water-miscible solvent in the presence of a water-binding salt, e.g. anhydrous sodium sulphate, circumvents the necessity of partitioning. Ethyl acetate, already well studied in connection with clean-up by Sweep-Co distillation (cf. 3), serves this purpose. The sodium sulphate not only effectively binds the water, but also improves the phase separation. Clogging of filters, often occurring with an acetone macerate, is rare with an ethyl acetate/sodium sulphate macerate and, as the volume of ethyl acetate added is known, any suitable aliquot of the filtrate can be used for the analysis. For acetone extracts, either the whole extract must be processed, or corrections for the (estimated) water content of the sample must be applied. In practice, blending with ethyl acetate allows therefore quick processing of a large number of samples.

3. CLEAN-UP

Whenever possible, the extracts obtained by any of the procedures

described above are used for the determination without clean-up, but in still many cases clean-up is necessary. Partitioning between acetonitrile and hexane is effective for the removal of fatty interferences from the extract.[16,17] Without the removal of the fats, the gas chromatographic determination of the OPs is adversely affected due to fouling of the stationary phase.

An important improvement of the clean-up step which has found its way to many pesticide residue laboratories during the last years is gel permeation. Systematic investigations, mainly carried out in the Federal Republic of Germany,[15] have proved the usefulness of this technique, not only for the determination of OPs, but also for many other pesticides. A very important feature of the gel permeation clean-up is the possibility it offers for automation. Apparatus is nowadays commercially available, but assembling from HPLC components is also possible.

Most gel permeation procedures described so far in literature start from an acetone extract, which is subsequently partitioned with dichloromethane (cf. 2.2). At present, a working group in the Netherlands is investigating the applicability of ethyl acetate extraction prior to gel permeation clean-up using ethyl acetate/cyclohexane as mobile phase. It can be expected that gel permeation will in many cases replace the classical column chromatography used so far for clean-up. Scaling-down, in order to minimise solvent consumption, is still desirable however. A possible way to attain this goal would be the one-line interfacing of gel permeation with HPLC, as was recently described by Shepherd.[18]

Sweep-Co distillation[13,15,19] can be automated to a large extent as well; the technique has, maybe due to its specialised nature, not found wide application however.

Normal-phase HPLC on silica has been used by Luchtefeld[20] for the clean-up of food extracts prior to analysis by gas chromatography/mass spectrometry.

4. DETERMINATION

4.1. Gas chromatography

Many OPs are very well amenable to gas chromatography with phosphorus-specific detection (flame-photometric or alkali-flame ionisation). The presence of other atoms, such as N, S or halogen,

gives extra possibilities for detection and/or confirmation, which is, in view of the large number of OPs in use throughout the world, often a delicate task when samples of unknown treatment history are under investigation. It is therefore not surprising that gas chromatography is still widely used for the determination of the OPs. Special techniques are necessary for the more polar compounds, such as the P=O analogues (metabolites) of the P=S compounds: one often used technique is injection of a blank (pesticide-free) extract in order to deactivate the column. Short columns (less than 1 m) are necessary for components of low volatility (e.g. azinphos-ethyl, cf. Table II). Derivatisation can be necessary for very polar compounds, such as ethephon[21] (cf. Table II), where methylation with diazomethane is used. Derivatisation, i.e. oxidation with potassium permanganate to the corresponding sulphones, is also recommendable for OPs which contain a thio-ether group.[22,23] Capillary gas chromatography has been proved useful for confirmation purposes.[24,25,26]

4.2. High-performance liquid chromatography (HPLC)

Developments in HPLC techniques have rapidly taken place in the last years and this has also had its impact on the analysis of OPs. Obviously, primary interest is directed to OPs with low volatility and high UV-absorption, but many other OPs can nowadays be determined at residue-level as well, so that confirmation, after initial preliminary identification by gas chromatography, can be obtained. Fluorescence labelling can be necessary when UV-absorption is too low, e.g. in the case of glyphosate[27,28] (cf. Table II). The former publication mentions pre-column derivatisation; the latter one post-column derivatisation.

Separation of, among others, four OPs on a 3 μm HPLC column has been described by Rice.[29] In this way, the number of analyses could be increased by 300% as compared to conventional analysis on a column with 5 μm particles.

Although measurement by UV at one wavelength is still the most common way of detection, more specific methods for the analysis of OPs are becoming available. Detection based on flame-photometry or alkali-flame ionisation, in analogy to the highly successful detectors in use in gas chromatographic analyses, have been developed

for especially capillary HPLC.[30] Sensitivity was best for the alkali-flame ionisation detector, viz. 2 ng of phosphorus. Home-made capillary columns packed with C_8 and C_{18} bonded phases and organic-aqueous eluents with a flow rate of up to 20 μl/min were used. It must however be stressed that micro-capillary liquid chromatography is still in an experimental stage.

Another device, based on flame-photometry, has been developed by Cope and Townshend.[31] The effluent of the HPLC column falls in this device in cavities drilled in the circumference of a duralumin disk which, by turning, brings the evaporated extracts into a hydrogen/nitrogen/air flame. Sensitivity was here 5 ng of phosphorus.

Also mass spectromentry has been used for the detection of OPs in HPLC effluents.[32] In this publication, negative chemical ionisation mass spectrometry is described; from 1–2 ng of pesticides entering the source, the major fragment ions could be detected in the reconstructed ion chromatograms.

Simultaneous detection at several wavelengths is possible with the recently introduced photodiode array UV-detector, so that a suitable wavelength, free from interferences, can be found. An example of this technique from own experiments is given in Figure 1, where chromatograms obtained at five different wavelengths are given for a mixture of 11 OPs.

5. CONCLUSIONS

In recent years, remarkable progress has been made in a number of aspects of organophosphorus pesticide residue analysis: in the enrichment step, timesaving techniques have been developed for processing water samples, viz. enrichment on XAD-resins or C_{18} bonded phases; in the clean-up step, automation has been introduced in the gel permeation procedure; in the determination step, dramatic developments have taken place in HPLC, with respect to sensitivity as well as specificity. It can be anticipated that all these techniques will be developed further in the years to come; much work remains to be done however as regards applications to food and environmental samples occurring in daily practice.

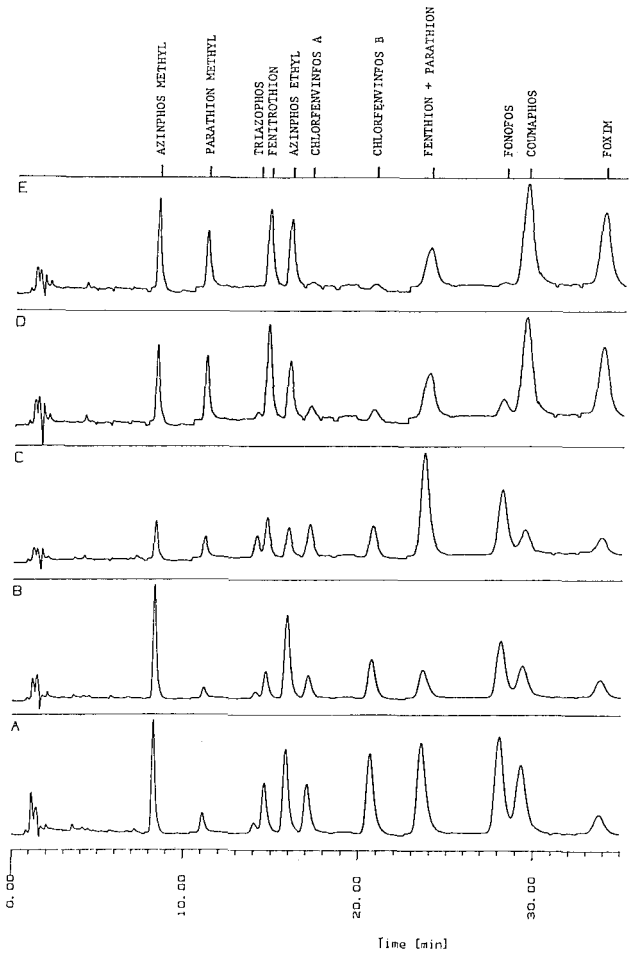


FIGURE 1 Chromatograms obtained at different wavelengths for a mixture of 11 organophosphorus pesticides.

Experimental conditions:

HPLC apparatus: Perkin Elmer Series 4 pump, Rheodyne 7125 injector with 6 one-loop, Hewlet Packard 1040A photodiode array detector
Column: 250 × 2 mm I.D. Ultrasphere ODS (Beckman), 5 m
Eluent: acetonitrile/water, 1:1 (v/v)
Flow rate: 0.2 ml/min
Amounts injected: azinphos-ethyl: 718 ng; azinphos-methyl: 626 ng; chlorfenvinfos A: 524 ng; chlorfenvinfos B: 542 ng; coumaphos; 521 ng; fonofos: 794 ng; foxim: 478 ng; fenitrothion: 817 ng; fenthion: 546 ng; triazophos: 986 ng; parathion: 578 ng; parathion-methyl: 636 ng.
Wavelengths: A: 210 nm; B: 220 nm; C: 250 nm; D: 270 nm; E: 280 nm.

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