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Microextraction of Organophosphorous Pesticides from Environmental Water and Analysis by Gas Chromatography

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Organophosphorous pesticides (OPs) are still commonly used worldwide. They may be analyzed by a variety of methods although the most common involve either gas chromatography (Blaha and Jackson, 1985; Sasaki et al., 1987) or liquid chromatography (Mallet et al., 1990). The use of capillary columns (Kjolholt, 1985) has greatly improved resolution such that many OPs may be analyzed simultaneously with very good limits of detection (order of 0.01 μg) using conventional detectors (NPD, FPD). Also the ion-trap detector (ITD) and the mass selective detector (MSD) offer improved limits of detection and confirmation is possible via mass spectra. Mallet and Mallet (1989a, 1989b) were successful recently in the analysis of OPs in environmental water with a conventional gas chromatograph converted to use megabore columns.

By the traditional method of liquid-liquid extraction, 300 mL and more of organic solvent may be used to process one litre of water (Mallet et al., 1978). Another approach is by solid phase extraction (Brooks et al., 1990) using a proper resin or adsorbent. The introduction of smaller tightly packed columns or disks has reduced the need for large quantities of eluting solvents although conventional procedures may require more than 100 mL of solvent (Mallet et al., 1978).

The use of large quantities of organic solvent for sample extraction constitutes a major drawback in today's world which is more environmentally sensitive. It means more solvent to discard. In addition to the fact that excessive use of solvents is costly, the time involved is considerable. Any potential improvement in these parameters is worth the effort.

In this study it was intended to evaluate the microextraction approach, that is, the use of a small amount of solvent to extract OPs from water. Hopefully, microextraction would offer savings in terms of amount of solvent used and the time required for extraction and thus contribute to reduce the impact on resources and on the environment in general.

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METHODS AND MATERIALS

Organophosphorous pesticides were obtained from Agriculture Canada (Ottawa, Canada). Stock solutions of 1 mg/mL were prepared in ethyl acetate (Anachemia). A complete list of all the pesticides studied is given in Table I.

Table 1. Recovery of Organophosphorous Pesticides (10μg/L) from Water using n-hexane

Common name (trade name)	Chemical name	Recovery (%) (n =2)
Acephate (Orthene)	O,S-Dimethyl acetyl-phosphoroamidothioate	0
Dimethoate (Cygon)	O,O-Dimethyl S-(methyl-carbamoylmethyl)	0
Disulfoton (Disyston)	O,O-Diethyl S-2-(ethylthio)-ethyl phosphorodithioate	53
Ethyl-parathion (Thiophos)	O,O-Diethyl O-p-nitrophenyl phosphorothioate	55
Fenitrothion (Folithion)	O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate	40
Fensulfothion (Dasanit)	O,O-Diethyl O-[4-(methyl-sulphinyl) phenyl]phosphorothioate	0
Fonofos (Dyfonate)	O-Ethyl S-phenyl ethyl-phosphonodithioate	45
Malathion (Mercaptothion)	O,O-Dimethyl S-1,2 di-(ethoxycarbonyl) ethyl phosphorodithioate	24
Methamidophos (Monitor)	O,S-Dimethyl phosphoramidate	0
Phorate (Thimet)	O,O-Diethyl S-(ethylthiomethyl) phosphorodithioate	24
Phosmet (Imidan)	O,O-Dimethyl-S-phthalimidomethyl phosphorodithioate	0
Tetrachlorvinphos (Gardonna)	2-Chloro-1-(2,4,5-trichlorophenyl)vinyl	22
Trichlorfon (Dipterex)	Dimethyl-(2,2,2-trichloro-1-hydroxyethyl phosphonate	0

Conditions: 1 litre of water; 1 mL of n-hexane, direct injection of extract.

A Perkin-Elmer 3920 gas chromatograph with a flame phototometric detector (FPD) was used for some experiments with individual pesticides. The chromatograph was fitted with a glass column (6 mm I.D. x 1.8 m) containing

3.6% OV-101 and 5% OV-210 on CHROMOSORB W, DMCS 80-100 mesh. The chromatograph was set as follows: injection port 230° C; column oven at 220° C, except for disulfoton, 210° C, fonofos, 200° C and phorate 190° C; detector 250° C.

A Tracor 560 gas chromatograph with a Tracor 702 nitrogen-phosphorus detector was used for multiresidue analyses. The chromatograph was equipped with a borosilicate magabore column (Supelco), 30 m x 0.75 mm I.D. containing 1.0-μm SPB-5 (Mallet and Mallet, 1989a).

Into 500 mL of water in a 500-mL erlenmeyer, an appropriate amount of organophosphorous pesticide was added while stirring. A 10-g portion of sodium chloride (commercial) was also added. The contents were transferred into a 500-mL volumetric flask. One mL of organic solvent (e.g., xylene) was added and the solution stirred for 5 minutes. The phases were allowed to separate and a portion of organic extract was removed and placed in a small vial. One μL was injected into gas chromatograph. All extractions were carried out in duplicate and each extract was injected twice. Unless otherwise indicated, the results for said duplicate were averaged but when in doubt a third analysis was performed. For reproducibility studies, the number of replicates was six (for data in Tables 2 and 3). The data in Table 4 were obtained by doing two successive extractions using 2 x 1 mL of solvent, stirring 10 minutes each time and adding a few drops of acetone to break emulsions. The extract was recovered with a Pasteur pipette and the volume adjusted to 2 mL.

RESULTS AND DISCUSSION

In order to develop a microextraction technique for water using a small volume (1 mL) of solvent, the latter has to be lighter than and very insoluble in water. In this case, it may be presumed that the concentration of solute in the solvent which resurfaces is the same as in the solvent which remains dispersed. Thus, direct injection of an aliquot of the extract is acceptable for quantitative purposes. However, when the solvent is slightly soluble in water or when the solubility of the solvent is increased by additives or through the presence of natural dispersing agents, the relation is no longer valid and the extract has to be recovered and diluted or concentrated to a known volume. Both approaches were evaluated in this project and appropriate conclusions drawn.

In preliminary experiments (10 μg/L), it was established that several solvents met the criterion of low solubility. Those retained were n-hexane, isooctane, cyclohexane, xylene, toluene and petroleum ether. However, petroleum ether was left out of the present study because it was too volatile. Also it was established early that a number of OPs namely acephate, dimethoate, fensulfothion, methamidophos, phosmet and trichlorfon could not be extracted using microextraction with n-hexane and were left out (see Table 1). Tetrachlorvinphos was left out due to lack of volatility for gas chromatography. Diazinon was also left out because its retention time interfered with that of disulfoton under isocratic conditions. However further studies have shown that it may be recovered as well as disulfoton under the same conditions and separation is possible using megabore columns (Mallet and Mallet, 1989b).

Nonetheless the recoveries with 1 mL of n-hexane of the remaining OPs from one-litre water samples were not impressive (see Table 1). Therefore, an attempt was made to increase the recoveries by decreasing the volume of the sample. A study of the effect of volume of water on the recovery of fenitrothion showed that the smaller the volume of sample, the better the recovery. With only 250 mL of water, there is almost a twofold increase in recovery. However, this translates into a fourfold drop in limit of quantitation. Therefore at this point we settled for a 500-mL sample for further studies such that the limit of quantitation would only be reduced by half.

The addition of 10 - 20 g sodium chloride also improves recovery of fenitrothion from 57% to 73%. We settled for 10 g (20%) to minimize cost. It was also shown that temperature changes (7° C - 45° C) have little or no effect on the % recovery. Therefore under these standard conditions (1 mL of n-hexane, 500 mL of water and 10 g of salt), the recovery of fenitrothion from water at 10 μg/L was determined to be 73% with a coefficient of variation (n=6) of 3.9% (Table 2) which is quite acceptable for residue analysis.

Table 2. Recoveries (%) of Fenitrothion from Water (10 μg/L) with various solvents

Solvent	% Recovery (n = 6)
hexane	73 ± 3.9
cyclohexane	85 ± 3.2
toluene	129 ± 11
xylene	93 ± 8.1
isooctane	68 ± 5.2

Conditions: 1 mL of solvent, 500-mL sample
10 g NaCl; direct injection of extract.

The results for the other solvents are also presented in Table 2. The best solvent in terms of recovery appears to be xylene but the reproducibility is less because there is a serious emulsion problem which makes it difficult to quantitate properly. However, there is an obvious problem with toluene with recoveries well over 100%. The fact is that toluene is partially soluble in water and that part does not participate in the extraction. As a result the solute is retained by a smaller volume of solvent. This introduces an error in the quantitation step unless the final extract is adjusted to a known volume. In this case an aliquot of the extract was injected directly. With solvents such as n-hexane or cyclohexane which are almost insoluble in water, the concentration of pesticide in the separated layer is expected to be the same as that in the dispersed (or absorbed) state and direct quantitation of the extract provides reliable data. Also reproducibility is better with the alcanes than with either toluene or xylene.

The recoveries of various OPs from water were studied under the same standard conditions, that is, 500 mL of water, 10 g of salt and 1-mL of solvent and no adjustment of the final volume of extract. The results in Table 3 show that the best overall recoveries were obtained with xylene. However,as is the case with toluene, this solvent is slightly soluble in water and quantitation by direct injection is not entirely reliable. There are also some difficulties caused by emulsions, especially with environmental water and centrifugation is sometimes necessary. Hexane and cyclohexane gave good recoveries except for malathion. Isooctane seems to be the least effective. Overall the alcanes resurface very well and are easy to manipulate. Toluene behaved very abnormally as observed earlier.

Table 3. Microextraction of Organophosphorous Pesticides (10 μg/L) from Water

	Percentage Recovery (n = 2)				
Pesticide	Hexane	Cyclohexane	Toluene	Xylene	Isooctane
disulfoton	97	93	136	102	89
ethyl-para.	82	88	132	99	87
fenitrothion	73	85	129	93	68
fonofos	97	90	124	94	85
malathion	37	53	121	99	34
phorate	89	95	126	97	85

Conditions: 500 mL of water; 10 g of salt; direct injection of extract

Problems with direct quantitation of the extract became apparent even with n-hexane, when environmental water (chlorinated water from surface lake used as water supply for the city of Moncton) was used. The volume of solvent coming up to the surface dropped from 87% to 60% presumably because of increased solubility and/or dispersion whilst the percentage recoveries increased as is the case with toluene. It was then decided to recover the extract and dilute to 1 mL but even that was difficult because of emulsions. Finally it was found that better overall recoveries and reproducibilities could be obtained by doing two successive extractions with 1 mL of solvent and diluting the final extract to 2 mL. It was also found that the additon of 2 - 3 drops of acetone to the extract during separation of the phases reduces emulsions considerably.

This new procedure was then used to study the simultaneous recovery of four OPs from water using n-hexane. The results are presented in Table 4. These show excellent recoveries at 10 ppb and 0.1 ppb. with no significant difference in terms of reproducibility between environmental water and distilled water. Thus, it is recommended that this procedure (2x1 mL, 2x10 min) be adopted rather than the one with the direct injection of an aliquot of the extract. Additional experiments have shown that this procedure is also applicable to 1-L water samples although a 15% drop in recovery may be expected.

Table 4. Simultaneous Microextraction of Organophosphorus Pesticides from Water

(n = 6)
% Recovery and Coefficient of Variation

<u>Pesticide</u>	Distilled Water (10 μg/L)	Environ. Water (10 μg/L)	Distilled Water (0.1 μg/L)	Environ. Water (0.1 μg/L)
Phorate	91.6 ± 8.4	94.0 ± 4.7	88.3 ± 3.1	87.8 ± 6.0
fonofos	94.0 ± 6.8	94.0 ± 3.9	96.7 ± 1.1	94.7 ± 3.6
fenitrothion	85.3 ± 7.9	82.1 ± 4.4	93.3 ± 2.8	80.7 ± 8.1
ethyl parathion	95.2 ± 6.4	94.5 ± 4.6	93.8 ± 2.6	95.9 ± 6.8
Average	91.5 ± 7.4	91.2 ± 4.4	93.0 ± 2.4	89.8 ± 6.1

Conditions:500 mL water; 2x1 mL n-hexane,2x10 min.; 10 g of salt; dilution of extract to 2-mL; add. of a few drops of acetone to extract.

Compared to the current procedures in many laboratories where large amounts of solvent (say up to 300 mL) are used for the extraction of OPs in water samples, this procedure provides an alternative for some OPs. The use of only 1 - 2 mL of solvent where applicable is a net improvement in terms of cost and time. In terms of limit of quantitation 0.1 ppb was analyzed with this particular GC-FPD system. However, in principle there is no reason why more than four pesticides could not be analyzed simultaneously as has been demonstrated before with a GC-NPD system using a capillary (or megabore) column at a limit of quantitation better or equal to 0.01 ppb. (Mallet and Mallet, 1989b). The only limitation with OPs is that they be extractable by microextraction.

The method is environmentally friendly and it offers a tremendous potential for laboratories to save in terms of quantity of solvents used and time. It should be readily adaptable to automation. We are presently investigating the extraction of polyaromatic hydrocarbons, polychlorinated biphenyls and organochlorines pesticides using this technique.

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