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DETERMINATION OF TRACE AMOUNTS OF ORGANOPHOSPHORUS PESTICIDES AND RELATED COMPOUNDS IN SOILS AND SEDIMENTS USING CAPILLARY GAS CHROMATOGRAPHY AND A NITROGEN-PHOSPHORUS DETECTOR

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SUMMARY

A multi-residue method for the determination of a series of organophosphorus compounds in soils and sediments is presented. Homogenized samples are subjected to Soxhlet extraction with acetone–*n*-hexane and the extract is partitioned between methylene chloride and water. The extract of the combined methylene chloride phases is cleaned up by adsorption chromatography and analysed by capillary gas chromatography using a nitrogen–phosphorus detector. Recoveries at the 10 ppb level are between 54.6 and 82.4% with standard deviations between 3.1 and 8.9%. Detection limits are between 95 and 220 ng/kg. The influence of a number of variables on the extraction efficiency and interference problems due to the presence of elemental sulphur are discussed.

INTRODUCTION

The serious ecological consequences of the use of organochlorine pesticides for the protection of crops are well known. Among the alternatives that have taken their place are organophosphates, which have been found useful for a broad range of applications. Further, this group of compounds is believed to be easily degraded in the environment[1-4]. It has been pointed out, however, that persistent residues may occur under unfavourable conditions such as the absence of organic matter or microbial activity[5-8]. It is known that to a great extent parathion pesticides are not actually degraded but are irreversibly bound mainly to the organic fraction of soils[2,7,9,10] and it seems that this process is very rapid. In an experiment using [14C]-labelled parathions, 49% of aminoparathion and 1.6% of parathion itself were adsorbed on a loam soil within only 2 h[9]. Not all organophosphorus compounds are bound at the same rate or equally tightly to soil; in general, the adsorptivity decreases with increasing water solubility of the compounds[11].

As parathions and other organophosphorus pesticides are generally considered to be easily degradable in soils, most published analytical methods have dealt with the determination of residues in fruits and vegetables, usually at the ppm level.

Methods using gel permeation or adsorption chromatography for clean-up prior to analysis by gas chromatography (GC) with nitrogen–phosphorus (NPD) or flame-photometric detection have been reported[12–14].

Little has been published on the determination of trace amounts of organophosphates in soils and sediments, except for a number of investigations on degradation and adsorption of single compounds, mainly parathion[2,6–8,10,15]. Data on the related compounds, methyl and ethyl esters of phosphoric acid and thio- and dithiophosphoric acid, are virtually absent as most studies on degradation pathways have focused on the resulting phenols.

The aim of this paper is to present a sensitive and selective method (ppb–ppt levels*) for the simultaneous determination of a series of organophosphorus pesticides and related compounds in soils and sediments. Some problems and variables that must be considered when performing such analyses are discussed.

EXPERIMENTAL

Materials

All pesticides and related compounds of at least 95% purity were supplied by Cheminova (Harboøre, Denmark). Dichlorvos of 99.4% purity was purchased from Dansk Shell (Copenhagen, Denmark). The standards were used without further purification.

Acetone, *n*-hexane, methylene chloride, cyclohexane and toluene of HPLC grade were purchased from Rathburn Chemicals (Walkerburn, Scotland, U.K.) and ethyl acetate and propylene glycol (analytical-reagent grade) from Merck (Darmstadt, F.R.G.). For the use of the latter a 50% solution in acetone was prepared.

Tetrabutylammonium hydrogen sulphate of at least 99% purity was purchased from Fluka (Buchs, Switzerland) and sodium sulphite (>96% purity) from May & Baker (Dagenham, U.K.). All other chemicals were of analytical-reagent grade and tested in blank procedures.

For the mixture for adsorption chromatography, Nuchar S-N C-77 activated carbon was purchased from Fischer Scientific (Fair Lawn, NJ, U.S.A.), magnesium oxide from BDH Chemicals (Poole, U.K.) and Celite 545 from Bie & Berntsen (Rødovre, Denmark). These ingredients were pre-treated according to the modified Watts *et al.* method[13] and homogenized in the ratio 1:4:8.

All other chemicals were of analytical-reagent grade and tested in blank procedures.

Extraction and clean-up procedure

The sediment was homogenized and an aliquot (*ca.* 20 g dry weight) was transferred into an extraction thimble, acidified with 1 ml of 4 *M* hydrochloric acid and subjected to Soxhlet extraction overnight with 200 ml of acetone–*n*-hexane (4:1). The extract was reduced to *ca.* 25 ml on a rotary evaporator and transferred into a 250-ml separating funnel with 25 ml of methylene chloride. Deionized water (100 ml) was added and the pH was adjusted to 6 with 0.5 *M* sodium hydroxide solution. After shaking for 2 min, the methylene chloride was collected and dried with anhydrous

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* Throughout this article the American billion (10^9) and trillion (10^{12}) are meant.

sodium sulphate. The aqueous phase was partitioned with two further 25-ml volumes of methylene chloride. The combined methylene chloride extracts were evaporated almost to dryness after the addition of 0.5 ml of propylene glycol solution and the residue was dissolved in 5 ml of ethyl acetate.

A 10-g amount of the mixture for adsorption chromatography was transferred into a column (25 × 1.5 cm I.D.) and washed with 50 ml of ethyl acetate–acetone–toluene (1:1:2). The sample extract was then transferred to the column by means of 120 ml of ethyl acetate saturated with water and was eluted with a further 150 ml of ethyl acetate–acetone–toluene (1:1:2) at 5–6 ml/min.

The total eluate was collected and evaporated to dryness, then the residue was dissolved in 1 ml of cyclohexane containing the internal standard (dichlorvos was used at a concentration of 100 ng/ml) prior to analysis by GC–NPD.

Removal of elemental sulphur

A solution of 3.39 g (0.01 mol) of tetrabutylammonium hydrogen sulphate in 100 ml of water was prepared and then saturated with 25 g (0.2 mol) of sodium sulphite. A 10-ml volume of this solution was shaken for 1 min with the concentrated extract from the Soxhlet procedure (25 ml). If the precipitated sodium sulphite disappeared, more was added in 100-mg portions until a solid residue remained after repeated shaking. Both phases were then transferred into the separating funnel and cleaned up as described above.

Gas chromatography

A Packard Model 433 gas chromatograph with a nitrogen–phosphorus detector and a Packard Model 612 PND controller were used.

A 25 m × 0.2 mm I.D. fused-silica capillary column (film thickness 0.33 μm) coated with 5% phenyl methyl silicone (SE-54) (Hewlett-Packard, Böblingen, F.R.G.) was used. The following temperature programme was used: 0.7 min at 75°C, increased at 5°C/min to 135°C and at 8°C/min to 250°C, the last temperature being held for 2 min.

A 1-μl volume of sample was injected in the splitless mode in 0.7 min.

RESULTS AND DISCUSSION

The method described was tested on two soils and four marine sediments from Danish coastal areas. Sediments from such areas are often rich in material of biological origin such as partly degraded plants or aquatic organisms. Freeze-drying of biological material causes destruction of many cell membranes and hence the contact between the matrix and an extraction solvent is generally improved by this procedure. For this reason, the influence of freeze-drying on extraction effiency was studied using a sediment known to be polluted with some of the compounds in question. Freeze-drying did not, however, improve the recovery of the pesticides compared with "wet" extraction (Table I) and, as interference from other substances increased considerably, freeze-drying prior to extraction was discontinued.

These findings indicate that the organophosphorus compounds in the sediment have not entered biological cells, or at most only in small amounts. The heavy impurities at the beginning of the chromatogram did not in themselves interfere with

TABLE I

QUANTIFICATION OF ORGANOPHOSPHORUS COMPOUNDS IN A POLLUTED SEDIMENT
SAMPLE WITH AND WITHOUT FREEZE-DRYING BEFORE EXTRACTION

Compound	Concentration in sample extracted directly (ppb)	Concentration in sample freeze-dried before extraction (ppb)
Triethyl phosphate	1.5	1.5
O,O-Diethyl-S-methyl thiophosphate	2.5	3.2
O,O,S-Triethyl thiophosphate	2.5	2.3
O,O,S-Triethyl dithiophosphate	5.9	4.9
Sulfotep	12.2	12.2
Malathion*	–	–
Parathion	4800	5900

* Malathion was present in the sample but impossible to quantify because of interference from the
large parathion peak.

the compounds to be analysed, but caused an unfavourable and varying displacement
of up to 1 min of the relevant peaks when compared with a standard. In practice this
prevented the identification of peaks in samples of complex composition. For this
reason, further clean-up was crucial and, as illustrated in Fig. 1, the introduction of
an adsorption chromatographic step markedly reduced the impurities.

The influence of pH on extraction efficiency in the partition step was studied
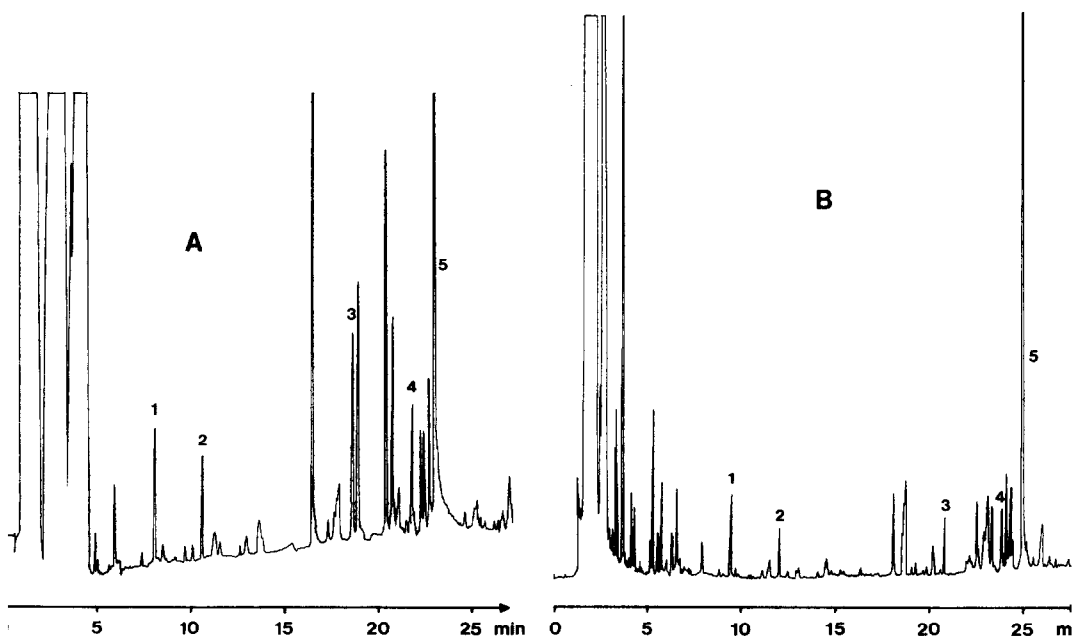

Fig. 1. A, Chromatogram of a 20-g sample of polluted sediment analysed without any adsorption chro-
matographic clean-up. B, Sample of the same sediment cleaned up by adsorption chromatography before
the GC analysis. Injection volume, 1 μl. 1, Triethyl phosphate; 2, dichlorvos (internal standard); 3, sulfotep;
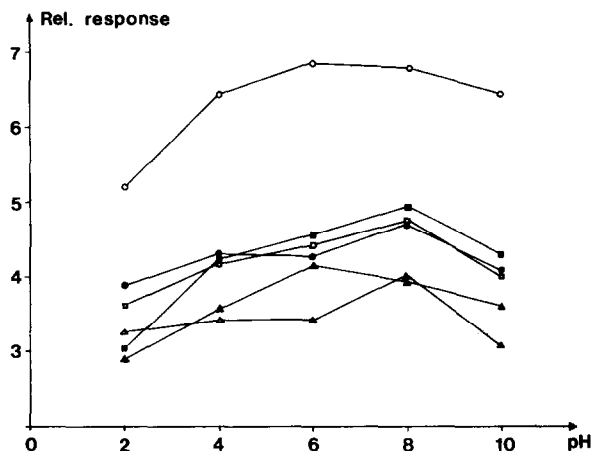4, parathion methyl; 5, parathion.

Fig. 2. Efficiency of extraction by partition between water and methylene chloride as a function of pH. ○, Sulfotep; ●, O,O-diethyl-S-methyl thiophosphate; □, parathion methyl; ■, dimethoate; △, O,O,S-triethyl thiophosphate; ▲, malathion.

(Fig. 2). A pH of 7–8 was found to be optimal, but in the pH range 4–8 the exact value was not critical. If, however, the pH was raised to 10 or lowered to 2 a markedly reduced extraction efficiency was observed. To secure uniform conditions from sample to sample we tried to carry out the partitioning using a phosphate buffer. However, this caused emulsification of the methylene chloride and it was therefore decided to adjust the pH with sodium hydroxide solution. A pH of 6 was finally chosen because of a stronger tendency for emulsification to occur at pH 7–8.

Emulsions may still be formed, especially in samples that are rich in organic matter. If it is not possible to break the emulsion in those instances by adding, *e.g.*, sodium chloride the sample must be centrifuged, after which the methylene chloride phase is collected and the aqueous phase is poured back into the separating funnel.

Recovery after extraction and clean-up was studied by spiking samples of 20

TABLE II

RECOVERIES AT 10 ppb LEVEL AND DETECTION LIMITS FOR ORGANOPHOSPHORUS COMPOUNDS IN SOIL AND SEDIMENT

| Compound	Recovery (%)	Standard deviation (n = 6) (%)	Detection limit* (ng/kg)
Triethyl phosphate	70.0	8.9	115
O,O-Diethyl-S-methyl thiophosphate	72.6	3.4	130
O,O,S-Triethyl thiophosphate	79.6	6.8	205
O,O,S-Triethyl dithiophosphate	66.2	6.4	220
Sulfotep	82.4	3.6	95
Dimethoate	54.6	4.6	130
Parathion methyl	71.3	3.1	170
Malathion	62.1	6.0	205
Parathion	79.3	5.8	185

* Based on experiments at the 1 ppb level (n = 4).
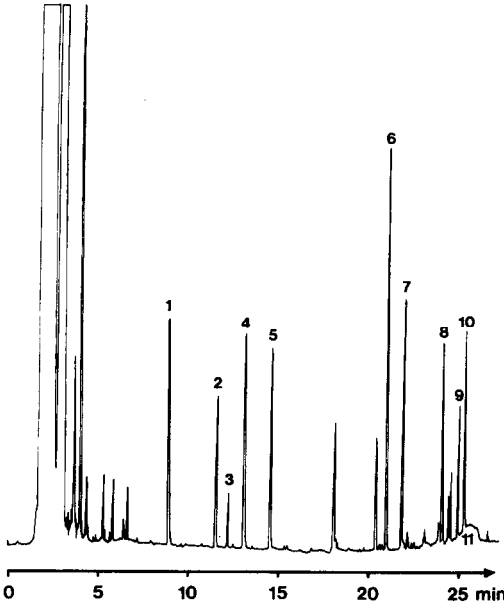
Fig. 3. Chromatogram of a soil sample from a salt marsh spiked with 10 μg/kg of each of the following compounds: 1, triethyl phosphate; 2, O,O-diethyl-S-methyl thiophosphate; 3, dichlorvos (internal standard); 4, O,O,S-triethyl thiophosphate; 5, O,O,S-triethyldithiophosphate; 6, sulfotep; 7, dimethoate; 8, parathion methyl; 9, malathion; 10, parathion; 11, elemental sulphur (present in the sediment). Sample size, 20 g (dry weight); injection volume, 1 μl.

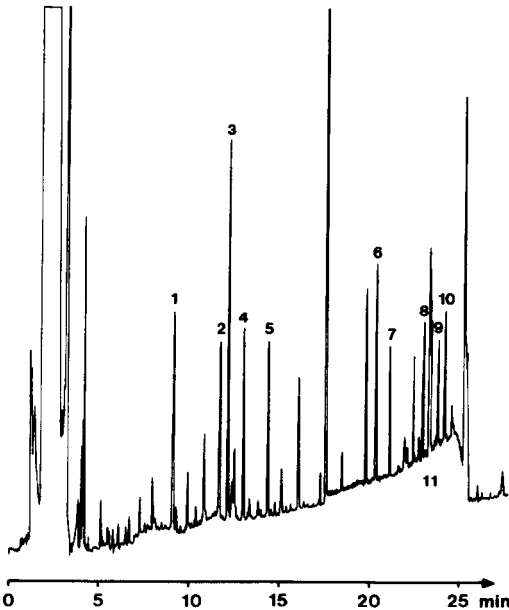Fig. 4. Chromatogram of a sediment sample from Limfjorden, Denmark, spiked with 1 μg/kg of each of the following compounds: 1, triethyl phosphate; 2, O,O-diethyl-S-methyl thiophosphate; 3, dichlorvos (internal standard); 4, O,O,S-triethyl thiophosphate; 5, O,O,S-triethyl dithiophosphate; 6, sulfotep; 7, dimethoate; 8, parathion methyl; 9, malathion; 10, parathion; 11, elemental sulphur (present in the sediment). Sample size, 20 g (dry weight); injection volume, 1 μl.

g dry weight with a mixture of standards at a concentration of 10 ppb (Table II). The samples were left to stand for 2 h before Soxhlet extraction was commenced. The recoveries were satisfactory and, as the standard deviations for all the compounds were low and of the same order, the method permits quantitative work on a series of organophosphorus compounds. Figs. 3 and 4 show chromatograms of a soil and a sediment spiked with 200 and 20 ng, respectively, of each compound. The detection limits for all nine compounds investigated are in the ppt range (ng/kg sediment, dry weight) when analysing samples of 20-g dry weight. The values shown in Table II were calculated by extrapolation of the results from experiments at the 1 ppb level and stated for a signal-to-noise ratio of 3.

A notable problem in the analysis of organic pollutants in sediments is caused by the elemental sulphur that is often present in such materials. This has been reported, e.g., in the analysis of polychlorinated biphenyls in sediment and sewage sludge[16], and also when determining organophosphorus pesticides by GC with thermoionic detection. Elemental sulphur affects the chromatogram by creating a hump over the whole range from sulfotep to parathion although most severely affecting the determination of malathion and the parathions. A number of reagents, such as mercury[17], Raney nickel[18] and tetrabutylammonium sulphite[16], have been proposed for the removal of sulphur from environmental samples. As the results of experiments
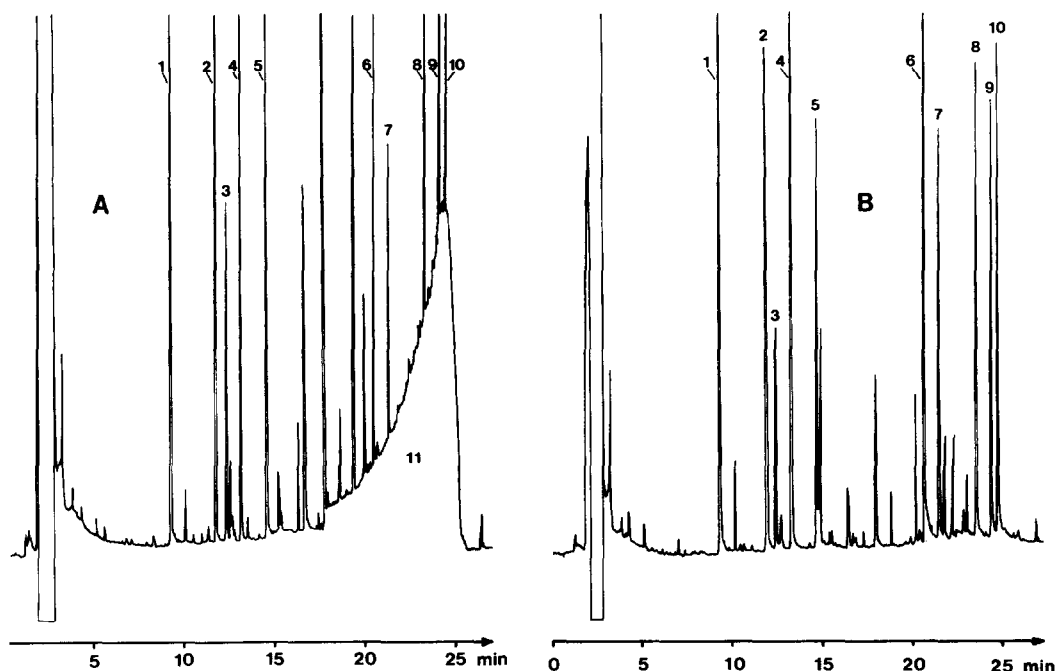

Fig. 5. Chromatograms of a sediment sample from the Baltic Sea spiked with 10 µg/kg of each of the compounds listed below. A was cleaned up according to the ordinary procedure and removal of elemental sulphur was included in the clean-up of B. 1, Triethyl phosphate; 2, O,O-diethyl-S-methyl thiophosphate; 3, dichlorvos (internal standard); 4, O,O,S-triethyl thiophosphate; 5, O,O,S-triethyl dithiophosphate; 6, sulfotep; 7, dimethoate; 8, parathion methyl; 9, malathion; 10, parathion; 11, elemental sulphur (present in the sediment). Sample size, 20 g (dry weight); injection volume, 1 µl.

performed in our laboratory with copper were not completely satisfactory, it was decided to implement the last procedure, in which sulphur is removed according to the reaction

$$(TBA^+)_2 \, SO_3^{2-} \, + \, S(s) \, \underset{(\leftarrow)}{\overrightarrow{}} \, 2 \, TBA^+ \, + \, S_2O_3^{2-}$$
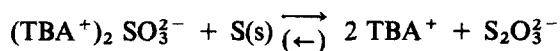
where TBA^+ = tetrabutylammonium ion.

Applying the sulphur-removing step immediately after the Soxhlet extraction and subjecting the whole reaction mixture to the subsequent clean-up procedure efficiently removed sulphur and gave no significant losses of the compounds of interest (Fig. 5). The recoveries of all compounds after extraction and clean-up by the extended procedure ($n = 2$) were within the limits given in Table II for the procedure without the sulphur-removal step. It therefore seems that the sulphur-containing compounds of interest in this study are not affected by the TBA reagent, or at least not to any significant extent.

In conclusion, a sensitive, selective and relatively precise method has been developed for the determination of organophosphorus compounds in matrices with high contents of organic or biological material, and has been tested on soil and sediment samples. With minor adjustments the method is applicable to waste water and foodstuffs, for which the Soxhlet extraction step is not needed. The method is currently being used in our laboratory in a study of the dispersal of pollutants from a point source to a marine inlet.

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