Organo-Phosphorus Pesticides in Sewage Sludge; Organo-Phosphorus Pesticides in River and Drinking Water, an addition, 1985

Methods for the Examination of Waters and Associated Materials

London Her Majesty's Stationery Office

Organophosphorus Pesticides in Sewage Sludge 1985 Organophosphorus Pesticides in River and Drinking Water, an addition, 1985.

Methods for the Examination of Waters and Associated Materials

This booklet contains a primary Extraction Gas Chromatographic procedure for the analysis of Sewage Sludge and a confirmatory Thin Layer Chromatography procedure. It also contains a short note to be read in conjunction with the 1980 booklet for Waters on the determination of traces of pesticides such as Carbophenothion.

Contents

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About this Series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, short reviews of the more important analytical techniques of interest to the water and sewage industries.

In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as series of booklets on single or related topics; thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods and notes being issued when necessary.

The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users — the senior technical staff to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is a committee of the Department of the Environment set up in 1972. Currently it has seven Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General non-metallic substances
6.0 Organic impurities
7.0 Biological methods
9.0 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee.

The names of those associated with this method are listed inside the back cover. Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in that booklet.

L R PITTWELL
Secretary

1 July 1986

Warning to Users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary.

Local Safety Regulations must be observed.

Laboratory procedures should be carried out only in properly equipped laboratories.

Field Operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried.

Care should be taken against creating hazards for one's self, one's colleagues, those outside the laboratory or work place, or subsequently for maintenance or waste disposal workers. Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

Lone working, whether in the laboratory or field, should be discouraged.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Guide to Safe Practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No. 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection', Public Health Laboratory Service Monograph 6, HMSO, London.

It cannot be too strongly emphasized that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radio-chemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected. If an ambulance is called or a hospital notified of an incoming patient give information on the type of injury, especially if poisoning is suspected, as the patient may be taken directly to a specialized hospital.

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Organophosphorus Pesticides in Sewage Sludges

A. Primary Gas Chromatographic Procedure

A1 Performance Characteristics of the Method

A1.1	Substances determined	Organophosphorus pesticides and some organophosphorus pesticide degradation products.
A1.2	Types of sample	Sewage sludges
A1.3	Basis of method	Sequential extraction into hexane and dichloromethane, followed by gas–chromatographic separation and detection by a flame photometric or flame thermionic detector.
A1.4	Range of application	Typically up to 100 $\mu g kg^{-1}$ wet weight.
A1.5	Calibration curve	Range of linearity depends on the detector in use. For all the pesticides listed in Table 1 the instrument used gave a linear response over the range 0–2.5μg injected.
A1.6	Standard deviation	See Table 1.
A1.7	Limit of detection	See Table 2. All the pesticides tested gave a limit of detection of < 1 $\mu g kg^{-1}$ wet weight.
A1.8	Sensitivity	Dependent on the determinand and the instrument in use.
A1.9	Bias	Extraction efficiencies are normally less than 100%. Bias will vary with the extraction efficiency of any particular determinand. See Table 1.
A1.10	Interferences	Any co-extracted material which has a similar GC retention time to any organophosphorus pesticide and which gives a detector response will interfere.
A1.11	Time required for analysis	4 hours per sample

A2 Principle

The organophosphorus (O-P) pesticides are extracted sequentially into hexane and dichloromethane (DCM). This helps to substantiate the identification of some O-P pesticides. The separate extracts are dried and evaporated to a suitable volume. The hexane extract is cleaned up with an alumina column before injection into a gas chromatograph fitted with either a flame photometric or flame thermionic detector.

A3 Interferences

The detectors are designed to be selective for phosphorus compounds although thermionic detectors may also respond to compounds containing sulphur or nitrogen. A variety of organophosphorus compounds are used in industry in flame retardant formulations; these have been found to interfere with the gas chromatographic analysis of organophosphorus pesticides. Gas chromatography using two or more columns may assist in

differentiating O-P pesticides from interfering peaks on the chromatogram. Table 3 gives a list of columns which have been used for this analysis with the relative retention times of most O-P pesticides currently in use.

A4 Hazards

Hexane and acetone are flammable and DCM is narcotic. Organophosphorus pesticides in the undiluted state are *very* toxic. Extreme caution must be exercised when preparing the stock solutions. The balance should be placed in a fume cupboard, and the operator should be protected by an overall, plastic gloves and face shield. Skin contact, ingestion and inhalation must be avoided. Symptoms of poisoning may include excessive sweating, headache, faintness and giddiness, nausea, stomach pains, vomiting, small pupils, blurred vision and muscle twitching. If any of these symptoms occur, the operator should stop work, remove contaminated clothing and summon medical assistance. See also 'Warning to Users' at the front of this booklet. Sewage sludge may contain pathogenic bacteria. Chromic cleaner is extremely corrosive and protective clothing and a face shield must be worn.

A5 Reagents

All reagents must be of sufficient purity that they do not give rise to significant interfering peaks in the gas chromatographic analysis of the extracts. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Reagents should be stored in all-glass containers; the use of plastic can result in contamination.

A5.1 Hexane—fraction from petroleum. Boiling range not less than 95% between 67°C and 70°C.

A5.2 Dichloromethane—DCM AR grade.

A5.3 Sodium sulphate—anhydrous, granular, neutral. Some batches of sodium sulphate have been found to be alkaline; in these circumstances wash with methanol containing 0.5 ml of concentrated HCl per litre and dry on a steam bath before roasting in a muffle furnace at 500 ± 20°C for 4 h ± 30 min.

A5.4 Acetone—redistilled or MFC grade.

A5.5 Alumina (aluminium oxide)—Woelm W200 neutral or an equivalent of the same mesh size. Heat at 500°C ± 20°C for 4 hours ± 30 min in a muffle furnace. Allow to cool to approximately 200°C in the furnace and then transfer to a desiccator containing a suitable desiccant (magnesium perchlorate or equivalent alternative). After cooling to ambient temperature transfer to a sealable all-glass container and add 7 ± 0.2% w/w distilled water. Seal and agitate for at least 30 min to ensure uniformity. Store in a sealed all-glass container. Storage time is normally about one week. Batches may be reprocessed as above after the maximum storage time.

A5.6 Defatted Cotton Wool—washed with hexane/acetone (1:1 mixture) and stored in a glass container.

A5.7 Liquid Paraffin—BP grade.

A5.7.1 Keeper solution in DCM—1% w/v liquid paraffin in DCM.

A5.8 Standard solutions of organophosphorus pesticides.

WARNING: ORGANOPHOSPHORUS PESTICIDES IN THE UNDILUTED STATE ARE VERY TOXIC. EXTREME CAUTION MUST BE EXERCISED WHEN PREPARING THE STOCK SOLUTIONS. SKIN CONTACT, INGESTION AND INHALATION MUST BE AVOIDED.

A5.8.1 Stock Solutions—these may be prepared by dissolving pure or certified materials in acetone. A suitable concentration is 50 mg/100 ml.

A5.8.2 Working Standards—these may be prepared from stock solutions using microlitre syringes which are reserved solely for this purpose. Some useful O-P working standards are given below:

Dichlorvos	0.5 μg/ml
Chlorpyrifos	0.5 μg/ml

Dimethoate	0.5 μg/ml
Pyrimiphos-methyl	0.5 μg/ml
Malathion	0.5 μg/ml
Parathion	0.5 μg/ml
Chlorfenvinphos	0.5 μg/ml
Carbophenothion	1.0 μg/ml
Fenitrothion	0.5 μg/ml
Azinphos-methyl	5.0 μg/ml

A5.8.3 Disposal of unwanted standards and stock solutions.

Acetone solutions of organophosphorus pesticides and small quantities of the pure compounds may be rendered innocuous by hydrolysis. The compounds or its solution should be added to a large excess of an aqueous 1 molar potassium hydroxide solution and left for at least 24 h. The solution may then be washed down the sink with liberal quantities of water.

A6 Apparatus

All apparatus and glassware should be shown to be free from contamination. Glassware can be cleaned by soaking overnight in chromic acid. Alternatively, a suitable detergent may be used followed by an acid rinse (0.1 M HCl). After soaking and rinsing, glassware should be rinsed with distilled water and dried in an oven at 60°C. (Volumetric glassware should not be dried at a temperature exceeding 60°C). Immediately before use, rinse with acetone. This assists in freeing glassware from possible contamination.

A6.1 Sample Bottles—All glass (wide neck) capable of holding 500 ml. Each bottle should be checked for contamination by rinsing with a small volume of hexane and examining the rinsings by gas-liquid chromatography.

A6.2 Mixer/homogenizer—Ultra-Turrax T45/N or similar.

A6.3 Drying Tubes—Glass tubes approximately 130 mm long by 5–6 mm internal diameter fitted with a reservoir at the top and a jet at the bottom. The clean-up column (A6.11) may be used for drying. The jet should be loosely plugged with cotton wool (A5.6) and the tube half filled with sodium sulphate.

A6.4 Centrifuge Tubes—Glass, 50 ml capacity with a screw cap.

A6.5 Centrifuge—Capable of 3000 rev.min^{-1}.

A6.6 High-speed Mixer—X10/20 laboratory disperser or similar (sparkproof).

A6.7 Pipettes—20 ml, 2.0 ml and 1.0 ml capacity.

A6.8 Round-bottomed Flasks—Glass—Quickfit, glass stoppered, 25 ml capacity.

A6.9 Kuderna-Danish evaporator (Figure 1).

A6.10 Micro-Snyder column (Figure 2).

A6.11 Clean-up columns—Glass, 130 mm long by 5–6 mm internal diameter with reservoir and PTFE stopcock.

A6.12 Pasteur or Teat Pipettes—Glass, 5 ml capacity.

A6.13 Graduated Centrifuge Tubes—Glass 10 ml, 0.1 ml graduations tapered, glass stoppered.

A6.14 Microlitre-syringes—10 μl, 5 μl and 1 μl capacity.

A6.15 Alumina clean-up column—Plug a glass column (A6.11 above) with cotton wool and fill the column with hexane. Add 2.0 g ± 0.1 g deactivated alumina (A5.5 above) and allow to settle. Cap with 0.5 cm of sodium sulphate (A5.3). The column should be prepared immediately before use. Standard solutions of the determinands of interest to the analyst should be carried through the clean-up procedure in order to estimate recovery from the alumina column, and also to determine the exact volumes of solvent required to elute the compounds of interest. This procedure should be carried out for each new batch of partially deactivated alumina.

A6.16 Anti-bumping granules—Washed with acetone.

A6.17 Modified Werner-Schmidt apparatus (Figure 3)

A6.18 Gas Liquid Chromatograph (GC)

A gas chromatograph with either a flame photometric or flame thermionic detector and temperature programming facility is required, to be operated in accordance with the manufacturer's instructions. On-column or glass-lined injection systems should be used. Many different columns have been used for pesticide analysis; some suitable and versatile columns are listed in Table 3. Capillary columns are also suitable, e.g. 25–50 m OV1 or SE54 glass or fused silica, wall-coated open tubular.

A7 Sample Storage

O-P pesticides can degrade rapidly in an aqueous environment, and the sample should be extracted as soon as possible after sampling. (See Note A8.3.1.d.).

A8 Analytical Procedure

Step	Procedure	Notes
A8.1	**Homogenization of sludge sample**	
A8.1.1	Insert the probe of the homogenizer (6.2) into approximately 250 ml of sample contained in the sample bottle and homogenize for 15 min ± 5 min. (See Note (a)).	(a) The probe must be cleaned thoroughly between samples to avoid cross-contamination.
A8.2	**Extraction of OP insecticides**	
A8.2.1.	Weigh a subsample of homogenized sludge (5.0 ± 0.1 g) in a glass centrifuge tube. Add 20 ml ± 0.2 ml hexane and mix for 5 min ± 0.5 min using the high-speed blender (6.6).	
A8.2.2.	Centrifuge the tube and contents at 3000 rev/min for 3 min ± 0.5 min to separate the phases. Transfer the hexane layer to the drying column using the Werner-Schmidt apparatus and collect the eluate in a Kuderna-Danish evaporator fitted with a graduated centrifuge tube. Add a further 10 ± 1 ml hexane to the tube containing the homogenized sludge sub-sample and transfer this solvent to the same drying column collecting the eluate in the same Kuderna-Danish evaporator and graduated centrifuge tube (note (b)).	(b) Care must be taken not to disturb the solids layer. Hydrolysis of O-Ps may occur if the sodium sulphate is alkaline. This should be checked (see A5.3).
A8.2.3.	Re-extract the same sludge sub-sample as in A8.2.1 and A8.2.2 using DCM in place of hexane as the solvent and transfer the DCM layer to a second drying column. Collect the eluate in a second set of Kuderna-Danish evaporator and graduated centrifuge tube. Add a further 10 ± 1 ml DCM to the tube containing the homogenized sludge sub-sample and transfer this solvent to the same drying tube used for the initial DCM extract collecting the eluate in the same evaporator and tube used for the initial DCM eluate. (See notes (c) and (d)).	(c) Separate sequential extractions into hexane and DCM helps to substantiate the identification. Some O-P pesticides which are insoluble in hexane are: Trichlorphon, Phosphamidon, Demeton-S-Methyl, Merinphos, Dimethoate, Vamidothion

Step	Procedure	Notes
		(d) The DCM layer may be transferred using a Pasteur pipette.
A8.3	**Concentration**	
A8.3.1.	Concentrate each extract to approximately 5 ml on a steam bath. (Note (e)).	(e) Sample extracts may be stored in a spark-proof refrigerator at this stage if necessary.
A8.3.2.	Further concentrate the extracts to 1 ml ± 0.05 ml using a micro-Snyder column or a gentle stream of dry nitrogen in a fume cupboard.	
A8.4	**Clean-up Procedure**	
A8.4.1.	Prepare an alumina column as described in Section A6.15. Run off the surplus solvent until meniscus reaches the top of the sodium sulphate and then add the concentrated hexane extract, using a Pasteur or treat pipette. Wash the sample vessel with 1 ml ± 0.1 ml hexane and add the washings to the column. Elute the column with 15 ml ± 1 ml hexane and collect the eluate in a Kuderna-Danish evaporator fitted with a 10 ml graduated centrifuge tube containing a boiling chip. (Notes (f), (g) and (h)).	(f) During the additions to the column do not allow the meniscus of the solvent to fall below the surface of the alumina. (g) The volume of solvent required should be determined (See section A6.15). (h) Clean up of the DCM extract is not required.
A8.4.2.	Concentrate the extracts as in A8.3.1. and A8.3.2.	
A8.4.3.	To the DCM extract add 1 ml ± 0.1 ml liquid paraffin keeper solution (A5.7.1) and evaporate off the solvent with a gentle stream of dry nitrogen. (See Note (i)). Dissolve the residue in 1 ml ± 0.05 ml acetone.	(i) The presence of 1 ml of the keeper solution reduces the loss of certain compounds e.g. fenitrothion and parathion but has been found to affect retention times on capillary columns.
A8.5	**Gas Chromatography**	
A8.5.1.	Inject 1 µl of the mixed working standard solution (A5.8.2) into the gas chromatograph programmed from 140°C to 260°C at 4°C per minute (Notes (j) and (k)).	(j) These standards act as markers and serve to give guidance on sensitivity adjustments.
A8.5.2.	Inject 1 µl (Note (l)) of each extract into the chromatograph using the same programme parameters and compare the chromatograms obtained with that of the standard. The elution order of O-P compounds given in Table 4 may assist in tentatively identifying any peaks found.	(k) Some workers have used heating rates up to 20°C/min. (l) Larger injection volumes may be used.
A8.5.3.	If the retention time data indicate an O-P pesticide may possibly be present, an isothermal GC run should be performed to check the exact retention times of the standard compound and the suspected peak (Note (m)). Further evidence of the identity of the suspected compound may be obtained by repeating the chromatographic examination using columns of different polarities. If the standard and unknown peaks give identical retention times on at least two columns (see Table 3) calibration standards should be prepared and the pesticide quantified. Generally when the pesticide concentration in the sample exceeds 1 mg per litre confirmation	(m) Evidence suggesting the presence of O-P pesticides may also be obtained from electron-capture chromatograms if these are available. Electron capture detectors respond to many O-P pesticides.

Step	Procedure	Notes

of its identity may be obtained from thin layer chromatography (See Section B).

A8.6 Calculation of Concentration

A8.6.1. Using the same gas chromatographic conditions as for the sample extracts inject 1 μl aliquots of the appropriate standard such that the concentration of the compound in the sample extract falls within the range of injected standards. Prepare a calibration graph of peak height or area plotted against the weight of material injected (ng). At least four points should be plotted.

A8.6.2. Measure the peak height or area of the sample extract peaks obtained. Read off the weight present in the injected volume from the calibration chart. Calculate the concentration of O-P pesticide in the extracts from:

$$E = \frac{V.A.}{I} \ \mu g/ml$$

Where E = concentration in extracts (Hexane or DCM) μg/ml
V = volume of final extract (ml) (Hexane or DCM)
A = weight of substance in the injected volumes extracts. (ng)
I = volume injected (μl)

A8.6.3. Calculate the concentration of each pesticide in the original sample form:

$$C = \frac{(E_{Hexane} + E_{DCM})}{W} \times 1000 \ \mu g/kg \text{ wet wt.}$$

Where C = concentration of pesticide in sample (μg/kg wet wt.)
E_{Hexane} = concentration of pesticide in Hexane extract (μg/ml)
E_{DCM} = concentration of pesticide in DCM extract (μg/ml)
W = weight of original sample taken (g).

A8.6.4 If it is desired to express the concentration as μg of pesticide per g of sludge dry solids then a total solids determination should be carried out on the sewage sludge and the following formula used:

$$D = C \times \frac{100}{DSM} \ \mu g/kg$$

Where DSM = % dry solid matter in original sample.

D = concentration of pesticide in μg/kg dry solids.

Confirmatory Procedure for Organophosphorus Pesticides by Thin Layer Chromatography (TLC)

B1 Performance Characteristics

B1.1	Substances determined	Organophosphorus pesticides and some organophosphorus pesticide degradation products.
B1.2	Types of sample	Sewage sludge.
B1.3	Basis of method	The extracts from the gas-chromatographic (GC) procedure are examined by TLC.
B1.4	Limit of detection	0.5–10 μg depending on the compound present.
B1.5	Interference	Any co-extracted compounds which are visualized by the reagent and having similar relative retention (Rf) values as the compounds of interest.
B1.6	Time required for analysis	Six sample extracts can be examined in 2 hours.

B2 Principle

The extracts from the gas-chromatographic procedure are applied to the TLC plate and chromatographed using a mixed solvent system. The compounds are located using 2,6-dibromo-p-benzoquinone-4-chlorimine (2,6-D-Q) as a chromogenic spray. The Rf values are compared with those of standard materials.

B3 Interferences

Substances usually present in sewage sludge do not interfere at their normal concentrations.

B4 Hazards

Hexane, acetone and ethyl acetate are flammable. 2,6-D-Q is potentially explosive and should be stored in a sparkproof refrigerator. Plates should be sprayed in a well ventilated fume cupboard to avoid inhalation hazards. Sample extracts from the GLC examination must be stored in a sparkproof refrigerator.

WARNING: ORGANOPHOSPHORUS PESTICIDES IN THE UNDILUTED STATE ARE VERY TOXIC. EXTREME CAUTION MUST BE EXERCISED WHEN PREPARING SOLUTIONS. SKIN CONTACT, INGESTION AND INHALATION MUST BE AVOIDED.

B5 Sample Preservation

Samples must be extracted in accordance with the procedure for GC examination. The extract must be stored in a sparkproof refrigerator.

B6 Reagents

B6.1 Hexane—fraction from petroleum. Boiling range not less than 95% between 67°C and 70°C.

B6.2 Acetone—(GPR).

B6.3 Ethyl acetate—analytical grade.

B6.4 Solvent System i): Hexane—acetone 4:1 v/v.

B6.5 Solvent System ii): Hexane—ethyl acetate 3:1 v/v

B6.6 Solvent System iii): Hexane—acetone 3:1 v/v

B6.7 Spray reagent—dissolve 1 g ± 0.1 g of 2.6 dibromo-p-benzoquinone chlorimine (GPR) in hexane and dilute to 100 ml. This reagent should be freshly prepared before use.

B6.8 Hydrochloric acid—concentrated—(AR)

B6.9 Standards—the stock solutions of the GLC method may be used, reagent A5.8.1.

B7 Apparatus

B7.1 TLC Plates—200 mm × 200 mm glass plates coated with silica gel to a thickness of 0.25 mm. Prepared plates (Merck 60F. 254) have been found to be suitable.

B7.2 Pipettes or syringes—to deliver up to 10 µl of liquid onto the TLC plate.

B7.3 TLC developing tank—glass with lid.

B7.4 Tank lining paper—Whatman No. 1 or equivalent.

B7.5 TLC spray system—glass.

B7.6 Laboratory oven.

B8 Analytical Procedure

Step	Procedure	Notes
D8.1	**Preparation of Tank**	
B8.1.1	Line the walls of the tank with the lining paper (B7.4).	
B8.1.2	Pour sufficient solvent (see Table 1) into the tank to give a 15 mm depth of solvent (see Note (a)).	(a) Three solvent systems are used: i) Hexane: Acetone 4:1 v/v ii) Hexane: Ethyl Acetate 3:1 iii) Hexane: Acetone 3:1 Any one or all three systems with fresh plates in each case, can be tried (Table 4).
B8.1.3	Replace the tank lid and rock gently to wet the walls and lining paper. Allow the system to equilibrate for at least 30 minutes.	
B8.2	**Spotting of Plates**	
	10 µl of the appropriate standard solutions and sample extracts (see Note (b)), are spotted onto the TLC plate in a straight line 20 mm from the bottom of the plate and 25 mm apart. The line of spots should be about 20 mm from each edge of the plate.	(b) Further concentration of the sample extract may be needed if the original sample apparently contained less than 0.05–1 mg/l.
B8.3	**Development of Plate**	
B8.3.1	Carefully lower the plate into the tank, replace the lid and allow the solvent front to develop to a height of 150 mm ± 10 mm from the origin of the spots. (See Notes (c) and (d)).	(c) It is advantageous to make a small mark on the edges of the plates 150 mm from the line of spots. (d) A plate holder may be used for convenience.
B8.3.2	Remove the plate from the tank and allow it to air dry in a fume cupboard (see Note (e)).	(e) All naked lights and sources of ignition must be absent.

Step	Procedure	Notes
B8.4	**Visualization**	
B8.4.1	Spray the plate with the spray reagent (B6.7) in a well ventilated fume cupboard (see Note (e)).	
B8.4.2	Expose the plate to hydrochloric acid vapour for 20 ± 5 s (see Note (f)).	(f) Avoid inhalation of fumes.
B8.4.3	Place the plate in an oven at $90°C \pm 5°C$ for $5 \text{ min} \pm 1 \text{ min}$ (see Note (g)).	(g) Heating improves the reliability and repeatability of spot development.
B8.4.4	Compare the Rf values of the sample extract spots with those of the standards (see Note (h)).	(h) The use of more than one system improves the confirmation.

Table 1 Percentage recovery of three representative organophosphorus pesticides from sewage sludge and standard deviation

Compound	Spike Concentration µg/kg	Concentration (Blank) µg/kg	Mean* Concentration Recovered µg/kg	Recovery %	S_t** µg/kg	Range*** µg/kg
Diazinon	100	ND	71.7	71.7	5.8	64.6–80.0
	10	ND	7.43	74.3	1.27	6.18–9.47
Malathion	100	ND	80.8	80.8	8.0	70.3–89.2
	10	ND	6.33	63.3	0.35	5.83–6.66
Ethyl parathion	100	ND	80.7	80.7	10.1	68.8–95.8
	10	ND	5.49	54.9	0.4	5.0–6.05

* of four replicates
** S_t is total standard deviation with three degrees of freedom
*** Range of four individual results in four separate batches
ND = Not detected

Table 2 Limits of detection for organophosphorus pesticides

Compound	Absolute limit of detection[1] ng	Analytical limit of detection[2] µg/kg
Diazinon	0.2	4.0
Malathion	0.3	6.0
Ethyl parathion	0.4	8.0

[1] Assuming a signal to noise ration of 2.5.:1 for the flame photometric detector output.
[2] Assuming that 5 ml sewage sludge is extracted, final extract volume is 0.5 ml, injection volume 5 µl. These detection limits have not been statistically derived. They are based upon the minimum amount discernible (2.5 × baseline noise) in a spiked sludge sample.

Table 3 Retention times (relative to malathion = 100) of some organophosphorus pesticide

Pesticide	4% SE30 (1.5 m)	2% Apiezon L (1.5 m)	1.3% Apiezon L (1.0 m)	10% DC200 (1.0 m)	1.3% Butandiol succinate (1.0 m)	3% OV210 (1.5 m)
Azinphos-ethyl	1141*	1508*	—	—	—	—
Azinphos-methyl	1023*	1273*	956*	956*	—	648
Bromophos	120	204*	212	129	100	—
Carbophenothion	318*	583	—	—	—	—
Chlorfenvinphos	165	195	—	—	—	130
Cruformate	193	—	—	—	—	—
Demeton S	25	29	—	—	—	—
Demeton-S-methyl	39	33	41	35	—	41
Demeton-S-methyl sulphone	—	—	—	92	—	—
Diazinon	48	58	65	54	21	30
Dibrom	7	39	—	—	—	—
Dichlorofenthion	68	100	—	—	—	—
Dichlorvos	5†	5†	6†	3†	—	14
Dimefox	2†	2†	—	—	—	—
Dimethoate	75	65	62	50	293	78
Disulfoton	53	71	80	65	36	39
Ethion	259*	339*	381*	257*	214*	—
Ethoate-methyl	80	71	—	—	—	—
Fenchlorphos	82	130	140	92	64	—
Fenitrothion	103	123	129	100	150	117
Formothion	96	94	—	—	—	—
Iodofenphos	—	—	362	192	178	—
Malathion	100	100	100	100	100	100
Mecarbam	149	177	—	—	—	—
Mevinphos	15†	12†	—	—	—	—
Morphothion	335*	321*	—	—	—	—
Omethoate	—	—	—	37	—	—
Parathion	118	152	150	112	150	136
Parathion-methyl	100	100	106	83	157	—
Parathion-O-analogue	—	132	—	—	—	—
Phenkapton	494*	970*	—	—	—	—
Phorate	34	47	55	43	21	28
Phorate-O-analogue	—	28	—	—	—	—
Phosalone	706*	1106*	—	—	—	—
Phosphamidon	79 and 107	62 and 83	75	—	—	—
Pyrimithate	93	118	—	—	—	—
Pyrimiphos-methyl	—	100	112	100	50	52
Schradan	153	118	—	—	—	—
Sulfotep	31	29	—	—	—	—
Thionazin	29	32	—	—	—	—
Thionazin-O-analogue	—	27	—	—	—	—

Column 1 = SE30, 4%, 1.5 m) Retention times determined at 195°C except where
Column 2 = Apiezon L 2%, 1.5 m) marked * = 220°C and † = 150°C
Column 3 = Apiezon L 1.3%, 1.0 m) Retention times determined at 220°C
Column 4 = DC200 10%, 1.0 m) except where marked * = 260°C and † = 180°C
Column 5 = Butan-diol succinate 1.0 m)
Column 6 = OV210 3%, 1.5 m Retention time determined at 195°C
Additional data on columns and retention times are given in Ref. 2.

Table 4 Rf values of some organophosphorus pesticides

Compound	System i	System ii	System iii
Chlorpyrifos	153 (0.46)	75 (0.58)	157 (0.52
Chlorfenvinphos	37 (0.11)	66 (0.30)	—
Diazinon	123 (0.37)	87 (0.39)	—
Dimefox	20 (0.06)	—	18 (0.06)
Dimethoate	10 (0.03)	4 (0.02)	9 (0.03)
Ethion	127 (0.38)	118 (0.53)	—
Fenitrothion	73 (0.22)	80 (0.36)	76 (0.25)
Malathion	70 (0.21)	69 (0.31)	69 (0.23)
Mecarbam	80 (0.24)	73 (0.33)	82 (0.27)
Parathion	100 (0.30)	100 (0.45)	100 (0.33)
Carbophenothion	136 (0.41)	124 (0.56)	—

All values are relative to parathion; parathion = 100
Rf values given in parentheses.
Details of the three solvent systems are given in B.6.4–B.6.6

Figure 1 Kuderna-Danish evaporator system for concentration of pesticide solutions

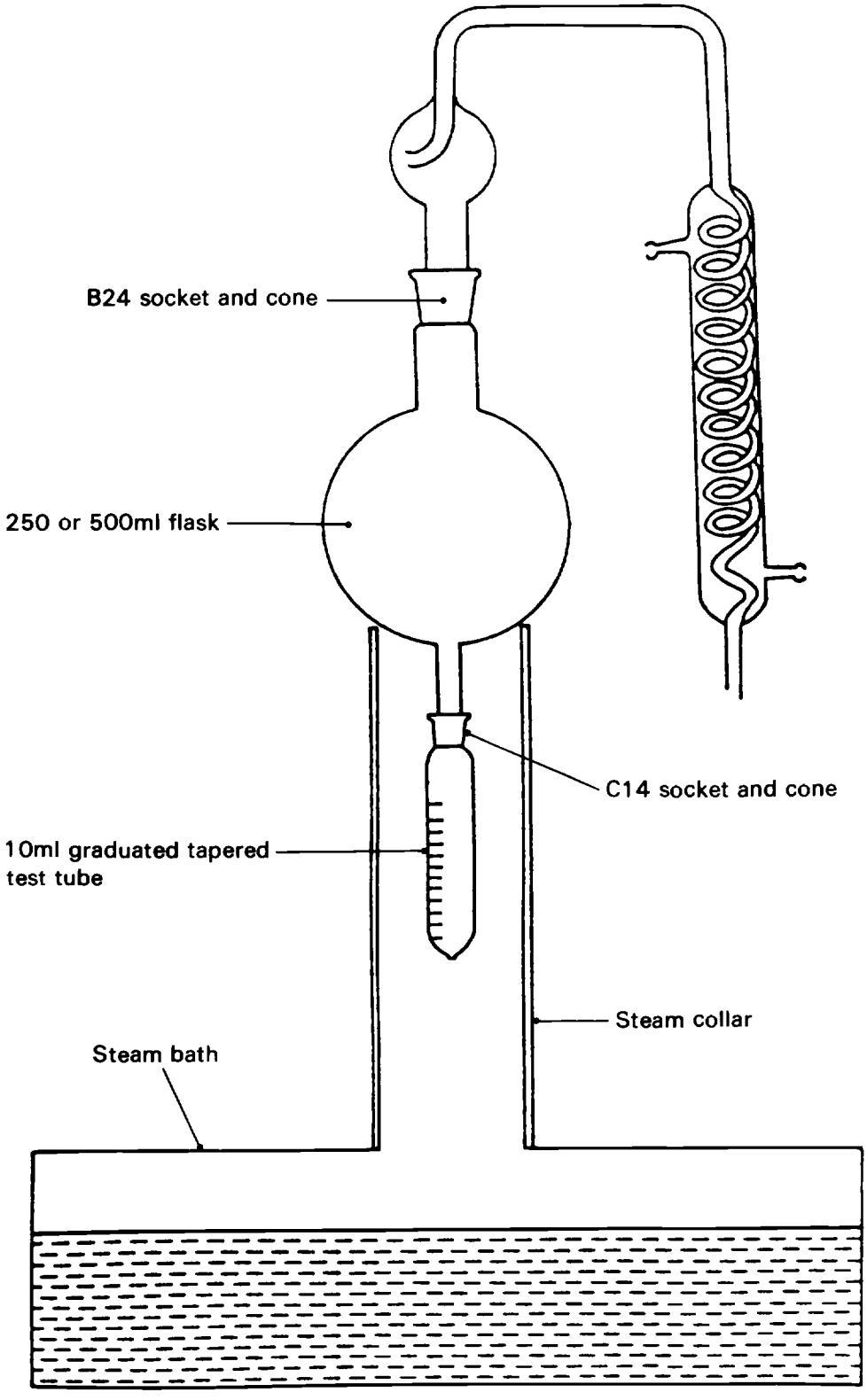
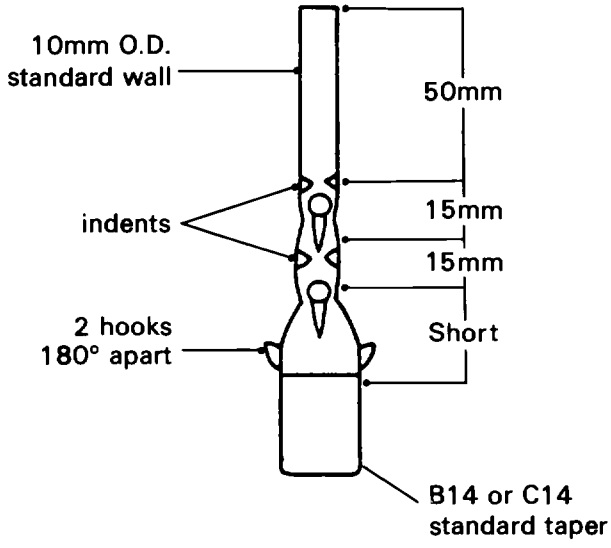
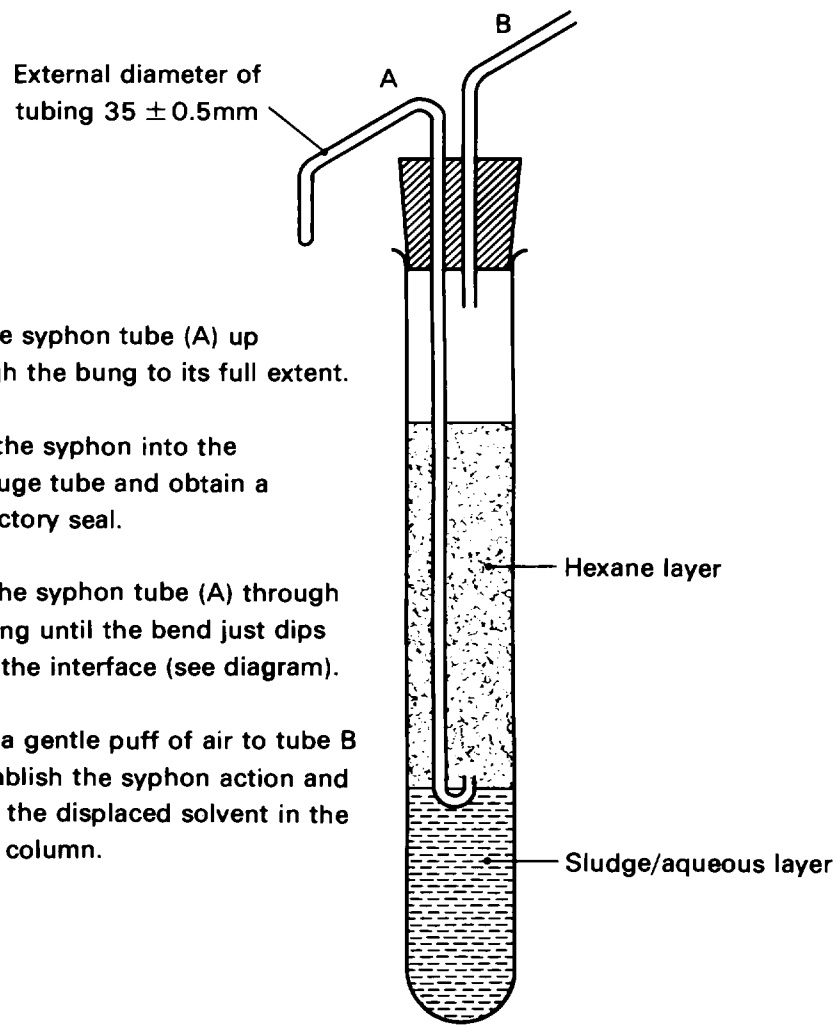


Figure 2 Micro Snyder column



(J.A. Burke, P.A. Mills & D.C.Bostwick
J.Assoc. Offic. Anal. Chem., 1966, 49, 999)

Figure 3 Werner Schmidt syphon tube



1. Pull the syphon tube (A) up through the bung to its full extent.

2. Insert the syphon into the centrifuge tube and obtain a satisfactory seal.

3. Push the syphon tube (A) through the bung until the bend just dips below the interface (see diagram).

4. Apply a gentle puff of air to tube B to establish the syphon action and collect the displaced solvent in the drying column.

Organophosphorus Pesticides in River and Drinking Water, an addition, 1985

Interference effects which lower the sensitivity of the Gas Chromatographic Procedure—Method A in the 1980 booklet (3) may often be reduced or eliminated, especially for Carbophenothion:

(a) by use of an electron capture detector (step A8.33 note (h)) in that method. Other substances with similar retention times tend not to be detected by this detector;

(b) by use of the capillary columns as mentioned in Section A6.18 of this booklet.

Using both modifications together gives a typical measurable limit of detection for Carbophenothion of 0.05 μg/l. (data from SAC Scientific).

Analytical Quality Control

Once the methods have been put into routine operation, many factors may subsequently adversely affect the accuracy of the analytical results. It is recommended that experimental tests to check sources of inaccuracy should be made regularly. Many tests are possible and they should be used as appropriate. As a minimum, it is suggested that at least one sample of suitable concentration in each batch of samples be analysed at least in duplicate. Inclusion of a quality control standard of concentration unknown to the actual operator is also useful. Plots of the deviation between multiplicate samples, or of the control standard result, will facilitate detection of inadequate precision and allow the standard deviation of routine analytical results to be estimated. For further information see Refs. 1 and 2.

References

(1) British Standards 5700 to 3703 inclusive

(2) Davey, D. J. and Hunt, D. T. E. The use of Cumulative Sum Charts in Analytical Quality Control WRC. Technical Report TR174. Water Research Centre, Medmenham, 1982.

(3) Organo-Phosphorus Pesticides in River and Drinking Water 1980. HMSO, London, in this series.

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However thoroughly a method may be tested, there is always the possibility of a user discovering a hitherto unknown problem. Users with information on this booklet are requested to write to:

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The Standing Committee of Analysts
The Department of the Environment
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