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Organo-Phosphorus Pesticides in River and Drinking Water 1980 Tentative Method

Methods for the Examination of Waters and Associated Materials

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About this method

This method is divided into two parts. The first part is concerned with the separation and measurement of the amount of the various substances present. The second part, gives a method for verifying that the various peaks obtained by the procedure given in the first part have been correctly identified. This is followed by information on ways of estimating the accuracy of analyses made by this method, and the usual end papers.

Chromatographic methods are very sensitive to minor physical and chemical variations in the quality of the materials and apparatus used. Hence this method mentions the actual materials used for the evaluation tests. This in no way endorses these materials as superior to other similar materials. Equivalent materials are acceptable, though it must be understood that the performance characteristics may be different, and can vary with batch. It is left to the senior supervising analyst to evaluate and choose from the appropriate brands available.

Only the common names for pesticides have been used throughout this method. For full information on the chemical names and formulae, see *The Pesticide Manual*, Martin H, and Worthington C R. British Crop Protection Council, Droitwich, Worcester, England.

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

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for 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.

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. It cannot be too strongly emphasised that prompt first aid, decon-

tamination, 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.

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.

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. 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.

About this series

This booklet is part of a series intended to provide recommended methods for the determination of water quality. In addition, the series contains 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 individual methods, 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 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 analytical chemist, biologist, bacteriologist etc, 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 one of the joint technical committees of the Department of the Environment and the National

Water Council. It has nine 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
- 2.0 Instrumentation and on-line analysis*
- 3.0 Empirical and physical methods
- 4.0 Metals and metalloids
- 5.0 General nonmetallic substances
- 6.0 Organic impurities
- 7.0 Biological methods
- 8.0 Sludge and other solids analysis*
- 9.0 Radiochemical methods.

The actual methods etc 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, and the current status of publication and revision will be given in the biennial reports of the Standing Committee of Analysts.

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 for booklets in this series are given in the Reports of The Standing Committee of Analysts, published by the Department of the Environment but sold by the National Water Council, 1 Queen Anne's Gate, London SW1H 9BT. 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 the booklet.

* These two working groups are in process of being wound up. Their tasks are being redistributed among the other Working Groups.

T A DICK
Chairman

L R PITTWELL
Secretary

25 September 1981

Organo-Phosphorus Pesticides In River and Drinking Water

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	River and drinking water.
A1.3	Basis of method	Extraction separately into hexane and dichloromethane, followed by gas-chromatographic separation and detection by a flame thermionic or flame photometric detector.
A1.4	Range of application	Typically up to 1 mg/l.
A1.5	Calibration curve	Range of linearity depends on the detector in use. For all the pesticides listed in Table 2 the instrument used gave a linear response over the range 0–1 µg injected.
A1.6	Standard deviation	See Table 2.
A1.7	Limit of detection.	See Table 3. All the pesticides tested gave a limit of detection of <1 µg/l.
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 2.
A1.10.	Interferences	Any co-extracted material which has a similar GC retention time to any organophosphorous pesticide and which gives a detector response will interfere.
A1.11.	Time required for analysis	4 hours per sample.

A2 Principle

The organophosphorus pesticides are extracted in sample bottles separately into hexane and dichloromethane (DCM). The separate extracts are dried and evaporated to a suitable volume before injection into a gas chromatograph fitted with either a flame thermionic or flame photometric detector. No 'clean-up' is employed.

A3 Interferences

The detectors are designed to be selective for phosphorus compounds although compounds containing nitrogen, or sulphur may also respond. Gas chromatography using two or more columns may assist in differentiating O-P pesticides from interfering peaks on the chromatogram. Table 1 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. Organo-phosphorus 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 overall, plastic gloves and a 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.

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.

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

A5.2. **Dichloromethane** – 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 $500 \pm 20^\circ\text{C}$ for $4 \text{ h} \pm 30 \text{ min}$.

A 5.4. **Acetone** – redistilled or MFC grade.

A 5.5. **Liquid Paraffin** – BP grade.

A 5.5.1. *Keeper solution in DCM* – 1% w/v liquid paraffin in dichloromethane.

A 5.6. **Standard Solutions of Organo-phosphorus 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.

A 5.6.1. *Stock Solutions* – these may be prepared by dissolving pure or certified materials in acetone. A suitable concentration is 50 mg/100 ml.

A5.6.2 *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

A 5.6.3. *Disposal of unwanted standards and stock solutions.*

Acetone solutions of organo-phosphorous pesticides and small quantities of the pure compounds may be rendered innocuous by hydrolysis. The compound 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

Glassware should be clean and dry. Rinsing with acetone just before use assists in freeing glassware from possible contaminants.

A6.1 Sample Bottles – these should be of all glass construction capable of holding 1.2 litres and calibrated at 1 litre. Each bottle should be checked for contamination by rinsing with a small volume of hexane and examining the rinsings by gas chromatography. Bottles showing evidence of contamination should be rejected.

A6.2 Drying columns – 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 jet should be loosely plugged with acetone-washed cotton wool and the tube half filled with sodium sulphate.

A6.3 Separating funnels – glass 1 litre capacity with ungreased glass or PTFE taps.

A6.4 Bottle shaking machine

A6.5 Kuderna-Danish evaporator (Figure 1).

A6.6 Micro-Snyder column (Figure 2).

A6.7 Graduated centrifuge tubes – glass 10 ml 0.1 ml graduations, tapered, glass stoppered.

A6.8 Gas-Liquid Chromatograph (GC)

A gas chromatograph with either a flame thermionic or flame photometric 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:

- i Glass column 1.5 m x 3 mm ID packed with 80–100 mesh AW-DMCS Chromosorb W supporting 4% by weight of OV1 or SE30 stationary phase.
- ii An identical column as in (i) but supporting 5% by weight of OV210 (QF1).

Details of some suitable columns are given in Table 1. Glass or fused silica capillary columns may also be used.

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. If it is impractical to analyse the samples immediately, the appropriate solvent should be added to the sample bottles immediately, followed by shaking and subsequent storage in a spark-proof refrigerator. The sample bottles should be protected from contamination by covering the top and shoulders with polythene sheeting. Alternatively the whole bottle may be sealed in a polythene bag. Samples should not be placed in close proximity to concentrated solutions of O-P pesticides.

FIG 1 KUDERNA-DANISH EVAPORATOR SYSTEM FOR CONCENTRATION OF PESTICIDE SOLUTIONS

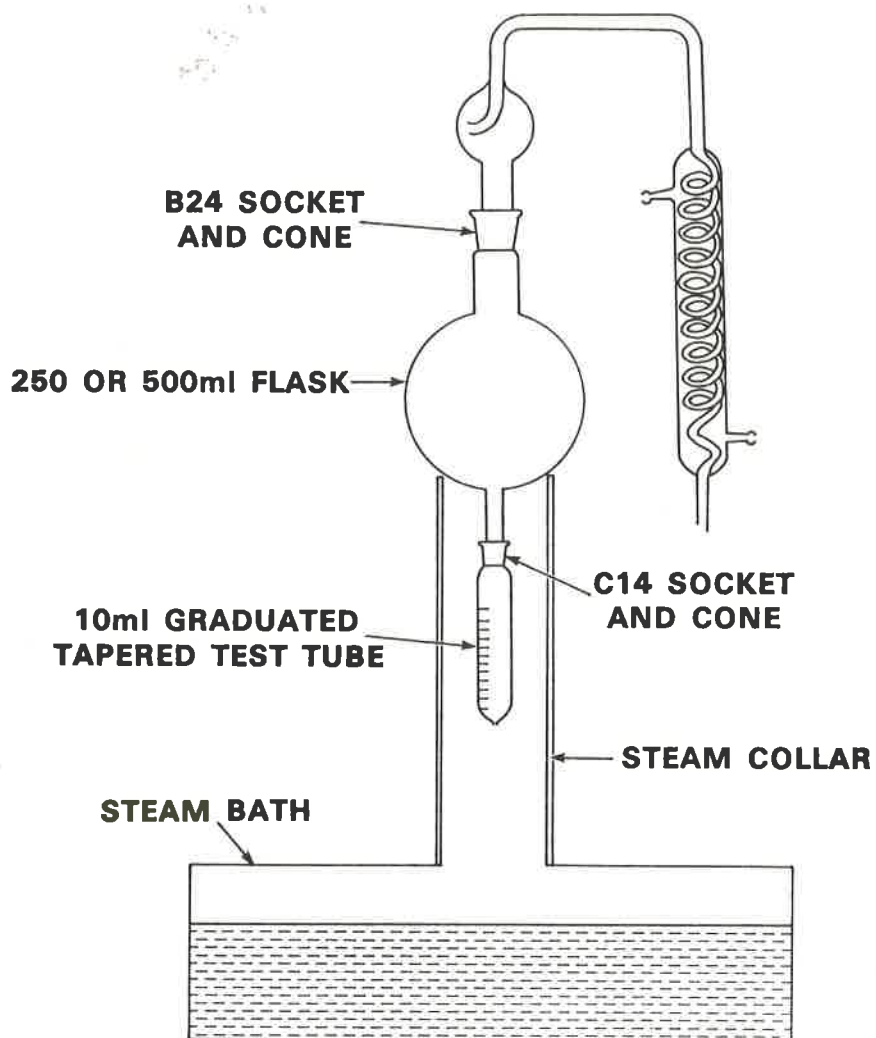
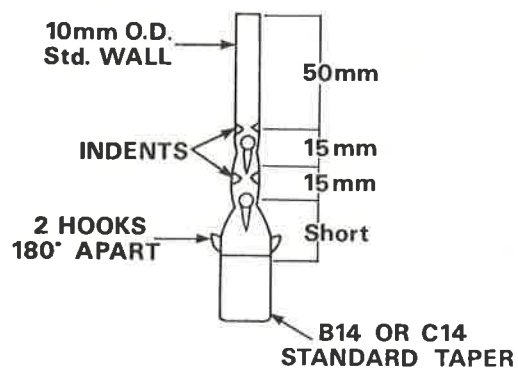


FIG. 2 MICRO SNYDER COLUMN



A8 Analytical Procedure

Step	Procedure	Notes
A8.1	Extraction of Organo-phosphorus pesticides	
A8.1.1	Add 25 ml \pm 5 ml hexane to approximately 1 l of sample, re-stopper and shake vigorously for 2 minutes (note a). Transfer the sample and hexane extract to a 1 l separating funnel and allow the phases to separate.	(a) A shaking machine may be used but the shaking period must be extended to at least ten minutes.
A8.1.2	Run off the aqueous layer into a measuring cylinder and record the volume (V) of sample and discard.	
A8.1.3	Pass the hexane layer through a drying column (6.2) (note b) and collect the eluate in a Kuderna-Danish evaporator fitted with a graduated centrifuge tube. Wash the column with 10 ml \pm 1 ml hexane and collect the washings in the same evaporator.	(b) Hydrolysis of O-Ps may occur if the sodium sulphate is alkaline. This should be checked see A3
A8.1.4	Add 50 ml \pm 5 ml DCM to a separate sample of approximately 1 l volume and shake vigorously for two minutes (see note a). Transfer the sample and DCM extract to a second 1 l separating funnel and allow the phases to separate. (note c)	(c) The use of the two solvents allows differentiation to be made between some pesticides ¹ .
A8.1.5	Run off the DCM extract into a second drying tube (note b) and collect the eluate in a second Kuderna-Danish evaporator. Wash this column with 10 ml \pm 1 ml DCM and collect the washings in the same evaporator.	
A8.1.6	Run the aqueous layer from the 1 l separating funnel of 8.1.4 into a measuring cylinder. Record the volume of sample and discard.	
A8.2	Concentration	
A8.2.1	Concentrate each extract to 5 ml \pm 1 ml on a steam bath.	
A8.2.2	Further concentrate the extracts to 1 ml \pm 0.05 ml using a micro-Snyder column or a gentle stream of dry nitrogen in a fume cupboard.	
A8.2.3	To the DCM extract add 1 ml \pm 0.1 ml liquid paraffin keeper solution (5.5.1) and evaporate off the solvent (note d). Dissolve the residue in 1 ml \pm 0.05 ml acetone (note e).	(d) The presence of 1 ml of the keeper solution reduces the loss of certain compounds e.g. fenitrothion and parathion. (e) Step 8.2.3 may be omitted if a flame photometric detector is to be used.
A8.3	Gas Chromatography	
A8.3.1	Inject 1 μ l of the mixed working standard solution (5.6.2) into the gas chromatograph programmed from 140°C to 260°C (note f) at 4°C per minute.	(f) These standards act as markers and serve to give guidance on sensitivity adjustments.

Step	Procedure	Notes
A8.3.2	Inject 1 µl (note g) 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 1 may assist in tentatively identifying any peaks found.	(g) Larger injection volumes may be used.
A8.3.3.	If the retention time data indicate that 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 h). 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 1) calibration standards should be prepared and the pesticide quantified. Generally when the pesticide concentration in the sample exceeds 1 mg per litre confirmation of its identity may be obtained from thin layer chromatography (note i).	(h) Evidence suggesting the possible 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. (i) For some O-P pesticides the TLC procedure may be applicable when the concentration exceeds 50 µg per litre. (See Confirmatory Procedure)
A8.4.	Calculation of Concentration	
A8.4.1	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 the injected standards. Prepare a calibration graph of peak height or area plotted against the weight of material injected (ng).	
A8.4.2	Measure the peak height or area of the sample extract peaks obtained under the same GC conditions as the standards. Read off the weight present in the injected volume from the calibration chart. Calculate the concentration of O-P pesticide in the original sample from:-	

$$C = \frac{1000 \cdot E \cdot A}{I \cdot V} \mu\text{g/l}$$

Where C = concentration in sample (µg/l)
E = volume of final extract (ml)
A = weight of substance in the injected volume of extract (ng)
I = volume injected (µl)
V = volume of original sample (ml)

If exactly 1 litre of sample is used and the volumes stated in the method adhered to, the calculation is simplified to:-

$$C = A \mu\text{g/l}$$

Table 1 Retention times (relative to malathion = 100) of some organo-phosphorus pesticides

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 % Butan- diol succinate (1.0 m)	5% OV210 (1.5 m)
Azinphos-ethyl	1141*	1508*	—	—	—	—
Azinphos-methyl	1023	1273*	956*	956*	—	648
Bromophos	120	204*	212	129	100	—
Carbophenothion	313*	533	—	—	—	—
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	—	—	—	—
Dichlofention	68	100	—	—	—	—
Dichlorvos	5 ⁺	5 ⁺	6 ⁺	3 ⁺	—	14
Dimefox	2 ⁺	2 ⁺	—	—	—	—
Dimethoate	75	65	62	50	293	73
Disulfoton	53	71	30	65	36	39
Ethion	259*	339*	231*	257*	214*	—
Ethoate-methyl	80	71	—	—	—	—
Fenchlorphos	32	130	140	92	64	—
Fenitrothion	103	123	129	100	130	117
Formothion	96	94	—	—	—	—
Iodofenphos	—	—	362	192	173	—
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	62	75	—	—	—
	and	and				
	107	83				
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%, 1.5m

Column 2 = Apiezon L 2%, 1.5 m

Retention times determined at 195°C except where marked* = 220°C and + = 150°C

Column 3 = Apiezon L 1.3%, 1.0 m

Column 4 = DC200 10%, 1.0 m

Column 5 = Butan-diol succinate 1.0 m

Retention times determined at 220°C except where marked * = 260°C and + = 180°C

Column 6 = OV210 5%, 1.5 m Retention time determined at 195°C

Additional data on columns and retention times are given in Ref. 2.

Table 2 Recoveries and estimates of total standard deviation (S_t)

Pesticide	Solvent	Mean recovery % (with 95% confidence limits)	Spike* ($\mu\text{g}/\text{l}$)	S_t ($\mu\text{g}/\text{l}$)
Dichlorvos	DCM	71 \pm 10	4.5	0.60
Dichlorvos	DCM	70 \pm 21	0.5	0.14
Dimethoate	DCM	63 \pm 5	4.5	0.48
Dimethoate	DCM	73 \pm 13	0.5	0.09
Malathion	Hexane	71 \pm 5	4.5	0.30
Malathion	DCM	79 \pm 13	4.5	0.81
Malathion	Hexane	66 \pm 9	0.5	0.06
Malathion	DCM	72 \pm 12	0.5	0.09
Parathion	Hexane	92 \pm 7	4.5	0.43
Parathion	DCM	87 \pm 12	4.5	0.74
Parathion	Hexane	85 \pm 10	0.5	0.07
Parathion	DCM	74 \pm 12	0.5	0.08
Pirimiphos-methyl	Hexane	92 \pm 11	4.5	0.71
Pirimiphos-methyl	DCM	59 \pm 9	4.5	0.57
Pirimiphos-methyl	Hexane	59 \pm 13	0.5	0.09
Pirimiphos-methyl	DCM	60 \pm 11	0.5	0.08
Chlorpyrifos	Hexane	80 \pm 11	4.5	0.71
Chlorpyrifos	DCM	68 \pm 9	4.5	0.57
Chlorpyrifos	Hexane	43 \pm 10	0.5	0.07
Chlorpyrifos	DCM	38 \pm 8	0.5	0.05
Fenitrothion	Hexane	89 \pm 10	4.5	0.64
Fenitrothion	DCM	71 \pm 14	4.5	0.85
Fenitrothion	Hexane	100 \pm 17	0.5	0.12
Fenitrothion	DCM	82 \pm 13	0.5	0.09
Chlorfenvinphos	Hexane	80 \pm 10	4.5	0.61
Chlorfenvinphos	DCM	75 \pm 23	4.5	1.4
Chlorfenvinphos	Hexane	93 \pm 17	0.5	0.12
Chlorfenvinphos	DCM	85 \pm 21	0.5	0.15
Carbophenothion	Hexane	57 \pm 7	4.5	0.44
Carbophenothion	DCM	43 \pm 18	4.5	1.2
Carbophenothion	Hexane	80 \pm 24	0.5	0.17
Carbophenothion	DCM	53 \pm 9	0.5	0.06

These data were obtained at The Polytechnic of North London, Holloway, London N7 8DB. A flame photometric GC detector was used with nitrogen as the carrier gas. Ten replicate analyses were made for each estimate of the total standard deviation. Further tests were performed by Severn-Trent Water Authority on 10 replicate samples of river water containing up to 100 mg/l of suspended solids spiked at a level of 50 $\mu\text{g}/\text{l}$ with seven pesticides. These indicated that there was no significant difference in recovery between water with high suspended solids and water with low suspended solids.

* The pesticides were added to river water in a minimal volume of acetone solution.

Table 3 Limits of detection

Pesticide	Limit of detection			
	Determined by spiking*		Determined by baseline fluctuation**	
	ED µg/l	Degrees of freedom	LD µg/l	Degrees of freedom
Dichlorvos	NT	–	0.04	7
Dimethoate	NT	–	0.40	14
Malathion	NT	–	0.13	14
Parathion	NT	–	0.10	14
Fenitrothion	0.37	18	0.03	30
Chlorfenvinphos	0.54	16	0.24	30
Carbophenothion	0.78	18	0.15	30
Pirimiphos-methyl	0.16	19	NT	–
Chlorpyrifos	0.17	19	NT	–

NT = Not tested

* These tests were carried out using pairs of river water samples spiked at low levels with the pesticides and the within batch of standard deviation assumed to be the same as that for a true blank. The limit of detection was calculated from:-

$$LD = 2\sqrt{2} tS_w$$

Where t = one sided value of t at 95% confidence for ∞ degrees of freedom.

S_w = estimate of the within batch standard deviation.

This method of calculation will tend to over-estimate the true limit of detection.

** These tests were carried out by decreasing the attenuation of the GC amplifier until the baseline noise became measurable. The estimate of the standard deviation for blanks was calculated by converting this baseline fluctuation to an equivalent concentration of pesticide at points with the appropriate retention time for each pesticide for each pair of tests and calculating the variation.

NB Under normal working conditions the response of the flame photometric detector used in these tests was zero when no O-P compounds were present. This resulted normally in a flat baseline, when the true detection limit was the minimum discernible variation in this baseline. The above table represents efforts to quantify the variation and to obtain an estimate of the limit of detection. In these tests, because of the flat baseline, no blank correction was applied. In this situation the limit of detection is given by $LD = 2 tS_w$. In other circumstances however a blank correction may be necessary, when the use of $LD = 2\sqrt{2}tS_w$ is likely to give a more realistic estimate of the limit of detection.

B

Confirmatory Procedure for Organophosphorus Pesticides by Thin-Layer Chromatography

B1 Performance Characteristics

B1.1	Substances determined	Organophosphorus pesticides and some organophosphorus pesticide degradation products.
B1.2	Type of sample	River water and drinking water.
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. This represents the minimum amount which will give a discernible spot on the TLC plate.
B1.5	Interferences	Any co-extracted compounds which are visualised by the reagent and having similar relative retention (R _f) 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-dibromoquinone-4-chlorimide (2,6-DQ) as a chromogenic spray. The R_f values are compared with those of standard materials.

B3 Interferences

Substances usually present in river water and drinking water do not normally interfere.

B4 Hazards

HEXANE, ACETONE AND ETHYL ACETATE ARE FLAMMABLE. 2,6-DQ IS POTENTIALLY EXPLOSIVE AND SHOULD BE STORED IN A SPARK-PROOF REFRIGERATOR. PLATES SHOULD BE SPRAYED IN A WELL-VENTILATED FUME CUPBOARD TO AVOID INHALATION HAZARDS. SAMPLE EXTRACTS FROM THE GC EXAMINATION MUST BE STORED IN A SPARK-PROOF REFRIGERATOR.

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

See also Warning to Users at the front of this booklet.

B5 Sample Preservation

Samples must be extracted in accordance with the procedure for GC examination. The extracts must be stored in a spark-proof 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** – AR

B6.4 **Hexane: acetone 4:1 v/v**

B6.5 **Hexane: ethyl acetate 3:1 v/v.**

B6.6 **Spray reagent** – dissolve 1 ± 0.1 g of 2,6-DQ (GPR) in hexane and dilute to 100 ml. This reagent should be freshly prepared before use.

B6.7 **Hydrochloric acid** – concentrated – (AR)

B6.8 **Standards** – the stock solutions of the GC method may be used, reagent A5 6.1

B7 Apparatus

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

B7.2 **Pipettes or syringes** – to deliver up to 10 μ l of liquid on to 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.

B8 Analytical Procedure

Step	Procedure	Notes
B8.1	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 4) into the tank to give a 15 mm depth of solvent (note a).	(a) Two solvent systems are suggested:- (i) Hexane: Acetone 4:1 v/v (ii) Hexane: Ethyl Acetate 3:1 v/v Either system can be tried. Use fresh plates in each case.
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 min.	

Step	Procedure	Notes
B8.2	<p>Spotting of Plates</p> <p>10 μl of the appropriate standard solutions and sample extracts (note b) are spotted onto the TLC plate in a straight line 20 mm from the bottom of the plate and 25 mm apart.</p> <p>The line of spots should not be closer than 20 mm from each edge of the plate.</p>	(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 \pm 10 mm from the origin of the spots (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 (note e).	(e) All naked lights and sources of ignition must be absent.
B8.4	Visualisation	
B8.4.1	Spray the plate with the spray reagent (B6.6) in a well ventilated fume cupboard (note e).	
B8.4.2	Expose the plate to hydrochloric acid vapour for 20 s \pm 5 s (note f).	(f) Avoid inhalation of fumes.
B8.4.3	Place the plate in an oven at 90°C \pm 5°C for 5 min \pm 1 min (note g).	(g) Heating improves the reliability and repeatability of spot development.
B8.4.4	Compare the R _f values of the sample extract spots with those of the standards (note h).	(h) The use of more than one system improves the reliability of the confirmation.

References

1. Askew, J., Ruzicka, J. H. and Wheals, B.B., *Analyst* **94**, 275–283 (1969). A General Method for the Determination of Organophosphorus Pesticide Residues in River Waters and Effluents by Gas, Thin-Layer and Gel Chromatography.
2. *Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples*. Ed J. F. Thomson. US Environmental Protection Agency, 1977.

Table IV R_f values of some organo-phosphorus pesticides

Compound	System i	System ii
Chlorpyrifos	0.46	0.58
Chlorfenvinphos	0.11	0.30
Diazinon	0.37	0.39
Dimefox	0.06	—
Dimethoate	0.03	0.02
Ethion	0.38	0.53
Fenitrothion	0.22	0.36
Malathion	0.21	0.31
Mecarbam	0.24	0.33
Parathion	0.30	0.45
Carbophenothion	0.41	0.56

Checking the Accuracy of Analytical Results

Once the methods have been put into normal routine operation many factors may subsequently adversely affect the accuracy of the analytical results. It is recommended that experimental tests to check certain sources of inaccuracy should be made regularly. Many types of tests are possible and they should be used as appropriate. As a minimum, however, it is suggested that at least one sample, of suitable concentration, in each batch of analyses be analysed in duplicate. The results obtained should then be plotted on a quality control chart which will facilitate detection of inadequate precision and allow the standard deviation of routine analytical results to be estimated.

The routine checking of the recovery of the procedure is also desirable but time consuming.

Addresses for Correspondence

1 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 method are requested to write to:

The Secretary
The DOE/NWC Standing Committee of Analysts
The Department of the Environment
Romney House
43 Marsham Street
LONDON, SW1P 3PY
England

2 At the present time, though based on work in several laboratories, thorough test data is only available from a few laboratories. Additional test data would be welcomed. Results should be sent to:

The Secretary
Working Group 6
The DOE NWC Standing Committee of Analysts
The Department of the Environment
Romney House
43 Marsham Street
LONDON, SW1 3PY
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- 1 Member of Main Committee (from May 1973 unless stated).
- 2 Member of Working Group 6 (from January 1976).
- 3 Member of the Pesticides Panel.

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