Organochlorine Insecticides and Polychlorinated Biphenyls in Waters 1978

Methods for the Examination of Waters and Associated Materials

London Her Majesty's Stationery Office £1.25 net

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Tentative Method

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Contents

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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 a properly equipped laboratory. 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 others. 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 specification. 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. One such publication is 'Code of Practice for Chemical Laboratories' issued by the Royal Institute of Chemistry, London. Where the Committee has 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, 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 radiochemical 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.

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 check-list, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of the correct protective clothing or goggles, removal of toxic fumes and wastes, containment in the event of breakages, 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 a hazard may exist and take reasonable precautions than to assume that no hazard exists until proved otherwise.

First published 1979

ISBN 0 11 751373 3

About this series

This booklet is one of a series intended to provide recommended methods for the determination of water quality. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, has 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 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 non-metallic 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.

TA DICK
Chairman

LR PITTWELL
Secretary

20 *July* 1977

About this method

0.1 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, the Appendix, gives methods 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.

0.2 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.

0.3 Only the common names for organochlorine 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. Two hundred and nine chlorinated biphenyls are theoretically possible; the commercial material usually consists of a mixture of tri- to heptachlorobiphenyls; over twenty compounds usually being present in significant amounts with more in trace quantities ranging from the mono- to decachloro-compounds. Not all isomers have the same thermodynamic probability of occurrence. Pure compounds are not readily available.

Organochlorine Insecticides and Polychlorinated Biphenyls in Waters Tentative Method (1978 version)

1 Performance Characteristics of the Method

1.1	Substances determined	Organochlorine insecticides and polychlorinated biphenyls (PCB).
1.2	Type of sample	Natural waters, drinking water and sewage effluents.
1.3	Basis of method	Extraction into hexane and removal of extraneous materials using a column of alumina – silver nitrate. Separation of most chlorinated insecticides from PCB by column chromatography on silica gel, followed by gas-liquid chromatography using an electron capture detector.
1.4	Range of application	Typically up to 250 ng/l.
1.5	Calibration Curve	Range of linearity depends on the detector in use. The instrument used in the performance tests gave a linear response over the following ranges: γ-HCH 0–250 pg Aldrin 0–250 pg Dieldrin 0–375 pg pp′ DDE 0–375 pg pp′ TDE 0–750 pg pp′ DDT 0–1500 pg Aroclor 1260* 0–10 ng
1.6	Standard Deviation	See Tables II and III.
1.7	Limit of Detection	See Table II.
1.8	Sensitivity	Dependent on determinand and instrument in use.

*Polychlorinated biphenyls are sold under a variety of trade names, of which Aroclor mentioned here is only one. The following is a list of the principal trade names used for PCB-based dielectric fluids which are usually classified as Askarels: Aroclor (UK, USA), Pyroclor (UK); Inerteen (USA); Pyranol (France); Clophen (Germany); Apirolio (Italy); Kaneclor (Japan); Solvol (USSR). Other names were used for PCB products intended for different applications no longer in current use; these include: Santothern FR (UK, prior to 1972 for heat transfer); Therminol FR (USA, prior to 1972 for heat transfer); Pydraul (USA, prior to 1972 for hydraulic applications); Phenoclor (France) and Fenclor (Italy). The trade names Santothern, Therminol and Pydraul are still in use but they now refer to non-chlorinated products.

1.9	Bias	The recoveries of insecticides are variable and seldom quantitative, typically about 50%, depending upon extraction efficiency which may vary with sample and determinand (see Table III). Poor extraction and use of standards made up in the extraction solvent cause negative bias. For practical reasons, the convention is to report the biased results thus obtained without correction for recovery.
1.10	Interference	Any electron-capturing material which passes through the procedure and has similar gas chromatographic characteristics to the determinand.
1.11	Time required for analysis	Assuming all reagents prepared and the instrument already calibrated, extraction and clean-up 2 hrs, gas chromatography up to a further 2 hrs depending on determinand and instrument in use. Total time for analysis including preparation of reagents, apparatus etc and confirmation of identity of determinands: approximately 6 samples per man-week.

2 Principle

The insecticides and PCB are extracted into an organic solvent. The extract is cleaned up on an alumina-silver nitrate column (1). If PCB isomers are present in a sample they will give peaks which appear at the same retention times as some of the insecticides; this can lead to gross misinterpretation of the chromatographic results. A separation of PCB isomers from the majority of the insecticides can be achieved by column chromatography on silica gel prior to gas-liquid chromatography.

3 Interferences

In principle, any substance capable of producing a response on the electron capture detector at a retention time indistinguishable from a chlorinated insecticide will interfere. In practice many potentially interfering substances will be removed during the clean-up and extraction procedures. The use of the two gas chromatographic columns recommended will be suitable for the organochlorine insecticides used in the standards; however, it must be accepted that in the absence of positive identification by other means the concentrations determined in the final extract must be considered as maxima. It is possible that there will be cases where a more definite identification is required or where the presence of a less common organochlorine insecticide is suspected. The information given in Table I and the Appendix will provide the analyst with a further choice of column and identification techniques. The information given in references 2, 3, and 5 will also be of value.

4 Hazards

Organochlorine Insecticides can be toxic, some more than others. Avoid ingestion. Care must be taken when handling solids and concentrated solutions. Wear gloves, do not re-use gloves, clean up all spillages as soon as possible.

The solvents used are flammable and may be narcotic. The use of naked flames must be prohibited. When it is necessary to store standard solutions and sample extracts a spark proof refrigerator must be used. Ventilation must be adequate to remove all fumes. Mouth pipettes must not be used, avoid inhalation of solvent vapours and insecticide dusts.

5 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 processed extract. This must be checked for each batch of material by running procedural blanks with each batch of samples analysed.

Reagents may become contaminated by contact with air and with materials, particularly plastics. Therefore storage should be in tightly sealed all-glass containers or other vessels found to be suitable.

5.1 **Acetone** – redistilled using a 300 mm Dufton spiral fractionation column or equivalent. Some batches of solvent may be acceptable without redistillation.

5.2 **Diethyl ether** – redistilled from potassium hydroxide using the apparatus described in 5.1.

5.3 **Hexane – fraction from petroleum.** Boiling range not less than 95% between 67°C and 70°C. Redistilled as in 5.2 (hereafter called hexane).

5.4 **Water** – distilled in an all-glass apparatus and stored under hexane in an all-glass aspirator.

5.5 **Cotton wool** – wash with hexane and ether before use.

5.6 **Sodium sulphate** – analytical reagent grade, granular, anhydrous. Heat to 500°C ± 20°C for 4 hours ± 30 minutes, then cool to about 200°C in the muffle furnace. Cool to ambient temperature in a desiccator containing magnesium perchlorate. Store in the desiccator.

5.7 **Silver nitrate** – analytical reagent grade.

5.8 **Alumina** – Woelm W200 neutral or an equivalent of the same mesh size. Heat at 500°C ± 20°C for 4 hours ± 30 minutes in a silica dish in a muffle furnace. Cool to about 200°C in the furnace and then to ambient temperature in a desiccator (as in 5.6). Weigh a portion into a sealable all-glass container and add 7% ± 0.2% w/w water. Seal and agitate for at least 2 hours to ensure uniformity. Store in a sealed glass container. Once the seal has been broken storage time is normally about one week. After the maximum storage time batches may be reprocessed as above.

5.9 **Alumina-silver nitrate** – Dissolve 0.75g ± 0.01g silver nitrate in 0.75 ml ± 0.01 ml water (use a microburette). Add 4.0 ml ± 0.2 ml acetone followed by 10 g ± 0.2 g dry alumina. Mix thoroughly by shaking in an open-topped conical flask, protected from light. Allow the acetone to evaporate at room temperature, warming with the hand to prevent condensation. Store in the dark and use within four hours of preparation.

5.10 **Anti-bumping granules** – wash with acetone before use.

5.11 **Silica gel** – Merck 7754 (available from BDH Chemicals Ltd) or an equivalent of the same mesh size. Heat to 500°C ± 20°C in a silica dish in a muffle furnace for at least 2 hours. Cool to 200°C in the furnace and then to ambient temperature in a desiccator containing magnesium perchlorate or equivalent alternative where it may be stored for up to a fortnight.

5.12 **Prepared silica gel** – To a suitable weight of dry silica gel (section 5.11) in an all-glass container, add 3.5% ± 0.1% w/w water and stopper tightly. Agitate for at least 2 hours to ensure uniformity and store in the stoppered glass container. This silica gel, partially hydrated in the manner prescribed, should be prepared daily.

5.13 **Standard solutions**

HAZARD – see Section 4.

Individual standards may be made up in hexane and diluted to give peaks within the linear range of the detector. It is more convenient to use a mixed insecticide standard for routine work. The mixed standard should be made up such that the individual peaks are of approximately equal heights. The concentrations needed to achieve this will vary with each instrument but the following procedure has been found to give satisfactory results:

5.13.1 *Primary stock solutions*

Dissolve the following quantities (x mg) of each material in acetone in separate glass-stoppered 100 ml volumetric flasks to give primary stock solutions:

Standard material	Weight (x mg) dissolved in 100 ml acetone
γ-HCH	40
Aldrin	60
Dieldrin	100
pp' DDE	100
pp' TDE	200
pp' DDT	400
Aroclor 1260	2000

5.13.2 *Individual standards*

Take 100μl of a primary stock solution (5.13.1) in a 100 μl or 250 μl syringe and dilute to 100 ml in a calibrated flask with hexane. Take a 100 μl aliquot of the diluted solution and make up to 10 ml with hexane. A 5 μl aliquot of the final diluted solution contains $\frac{x}{2}$ pg of the material.

Separate syringes should be used for the successive dilutions at different concentration levels.

5.13.3 *Composite standards of insecticides* (must not contain PCB)

Composite standards of insecticides which must not contain PCB may be made by placing 100 μl of each of the primary insecticide stock solutions in a 100 ml calibrated flask and diluted with hexane to the mark. 100 μl of this solution is then further diluted to 10 ml with hexane to give a composite standard solution containing $\frac{x}{2}$ pg of each insecticide per 5 μl.

Further calibration standards may be diluted from the above solutions using either syringes, pipettes or burettes. (Note: Pipettes may give significant drainage errors). Standards should be diluted to cover the linear range of the detector in use, in steps of 1, 2, 5 and 10 pg per 5 μl γ – HCH or corresponding quantities of other insecticides.

5.13.4 *Silica gel test solutions*

5.13.4.1 Prepare a solution in hexane such that 1 ml contains dieldrin 0.05 μg, pp' DDE 0.05 μg, and pp' DDT 0.2 μg.

5.13.4.2 Prepare a solution in hexane such that 1 ml contains 1 μg Aroclor 1260.

5.14 **Storage**

Most organochlorine insecticides are sufficiently stable to be stored in organic solvent solution for a number of years. The following procedures have been found to be satisfactory:

5.14.1 Primary stock solutions should be stored in the dark in a spark-proof refrigerator.

5.14.2 Hexane and Acetone are very volatile. Significant loss may occur on prolonged storage due to diffusion at ground glass joints. This may be minimised by storage under refrigeration. To compensate for such losses, always record the gross weight of the container plus solution prior to storage. Before subsequent use, allow the material to warm up to room temperature, reweigh the container plus solution, replace any evaporation loss by fresh solvent, stopper and mix thoroughly.

Insecticides and particularly PCB may concentrate around the sides and neck of the bottle due to co-distillation. This may be reduced by wrapping the top of the bottle in foil to reduce air circulation. Mix well before use.

5.14.3 The working standards must be stored in a spark-proof refrigerator and should be discarded when their volumes are depleted by 5%, unless used at once. To avoid hazardous waste do not prepare excessive amounts of these solutions.

6 Apparatus

All apparatus should be checked for contamination. A suitable cleaning procedure for glassware is as follows:

Wash thoroughly to remove all organic matter, rinse with insecticide – free water and air dry at 60–120°C and cool. A proprietary detergent may be used, provided that the analyst is satisfied that it does not cause interference with the determinands at the levels which are of interest. Immediately before use, rinse with acetone and hexane and allow to drain.

Apparatus which has been in contact with high concentrations of insecticide or PCB should be rejected unless shown to be contamination free after cleaning.

6.1 General apparatus

6.1.1 *Glass Columns* – 130 mm long by 5–6 mm internal diameter (see fig 1).

6.1.2 *Kuderna-Danish evaporator* (fig 2).

6.1.3 *Micro-Snyder column* (fig 3). See Reference 9.

6.1.4 *Graduated centrifuge tubes* (10 ml, 0.1 ml graduations) tapered, glass stoppered.

6.1.5 *Air or nitrogen line* – the supply of gas must be cleaned by passage through a column of $\frac{1}{16}$ in pellets of type 13X molecular sieve and 15–40 mesh silica gel ending in a fine jet of glass or metal controlled such that the gas jet just indents the meniscus of the solvent being evaporated (typically 200 ml/min from a jet of internal diameter 0.5 mm at a distance of 20 mm from the liquid).

6.1.6 *Separating funnel* 3l capacity with a glass tap (without grease) or a PTFE* tap.

6.1.7 *Bottle rolling machine* suitable for rolling bottles at 60 rpm.

6.2 Special apparatus

6.2.1 *Alumina – alumina/silver nitrate column*

Plug a glass column (6.1.1) with cotton wool. Put in 15 ± 1 ml hexane then add 1.0 g ± 0.2 g alumina/silver nitrate (see Section 5.9) and allow to settle. Then add 2.0 g ± 0.2 g alumina (7% moisture) (see Section 5.8) and again allow to settle. Add sufficient sodium sulphate to give a layer 5 mm deep on top of the column. The whole column should be prepared immediately before use.

6.2.2 *Silica Gel column*

6.2.2.1 Preparation

Plug a glass column (6.1.1) with cotton wool. Add 2.00 g ± 0.01 g prepared silica gel (see Section 5.12). Weigh using a rapid weighing top pan balance in order to avoid changes in the activity of the gel during contact with the atmosphere. Cap with a layer of sodium sulphate. A fresh column must be prepared before each determination.

6.2.2.2 Checking of activity

The activity of each batch of silica gel must be checked as follows:

Prepare a silica gel column as in Section 6.2.2.1. Add 1.0 ml ± 0.1 ml of insecticide solution (see Section 5.13.4.1), when the column will be partially wetted. Wash the insecticide solution into the column using 1 ml ± 0.1 ml hexane, allowing the liquid to penetrate the column but retaining the meniscus just above the column packing. Elute with 10 ml ± 0.1 ml hexane, separately collecting 1.0 ml ± 0.1 ml fractions in marked conical centrifuge tubes until nine fractions have been collected. Change the receiver and allow the rest of the hexane to flow through the column until the meniscus just reaches the top of the silica gel. Elute the column with 12 ml ± 1.0 ml of 10% v/v diethyl ether in hexane into the same receiver. Concentrate the final eluate to 1.0 ml ± 0.2 ml using a Kuderna-Danish evaporator and finally by blowing with a gentle stream of dry air or nitrogen (see Section 6.1.5).

Examine each of the solutions by gas chromatography. All the dieldrin should be in the ether-hexane solution. Ideally the pp'DDT should not have been eluted before the seventh hexane fraction and all the pp'DDE should have emerged in the first six

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* PTFE: Polytetrafluoroethylene.

hexane fractions. This may not always be the case, and where deviation is experienced the volume of hexane needed to effect separation of pp'DDE from pp'DDT should be noted and this volume used in the analytical procedure (see Section 8 Step 4) instead of the 7 ml suggested. Most PCB isomers will appear in the first 3 to 4 ml of hexane; this can be checked by repeating the procedure using the Aroclor solution (see Section 5.13.4.2).

6.3 Gas Chromatography

A gas chromatograph with electron capture detector is required. This should be operated in accordance with manufacturer's instructions. On-Column or glass lined injection systems should be used. Many different columns have been used for pesticide analysis. Two suitable and versatile columns are

(i) Glass column, 1.5 m long by 3 mm internal diameter, packed with 80–100 mesh AW-DMCS Chromosorb W supporting 2% OV-1 plus 3% QF-1 by weight. Operated at 190°C and 50 ml/min N_2.

(ii) Glass column, 1.2 m long by 3 mm internal diameter, packed with 80–100 mesh AW-DMCS Chromosorb G supporting 1.3% by weight Apiezon L. Operated at 190°C and 50 ml/min N_2.

A further selection of suitable columns is given in Table 1. Columns should have an efficiency of better than 1,000 theoretical plates and give mimimal pp'DDT breakdown. Extraneous peaks produced by injection of pure pp'DDT standards should not be greater than 10% of the pp'DDT peak. Some PCB isomers may take up to 2 hrs to emerge from some columns. Electron capture detectors give a linear response over a limited range. This range must be determined for each detector and set of conditions. Contamination of the detector will cause a loss of linearity and change in calibration. These effects vary with the instrument and analysis being performed, and in unfavourable cases will necessitate daily full calibration with check standards being run between every two or three sample injections. The weights of insecticides in the standards and test solutions injected must be chosen or adjusted such that the detector is operating in its linear range. This is best accomplished by maintaining a fixed injection volume (5 μl) and adjusting the strength of the standard solution and the volume to which the extract is finally made up.

7 Sample Storage

Sample bottles should be all-glass with glass stoppers, cleaned in the manner described for glass apparatus (see Section 6). Each bottle should be checked by rinsing with a small volume of hexane and examining the rinsings by electron capture gas chromatography. Bottles showing contamination should be rejected. At the levels of concentration likely to be encountered it is essential that the sample be protected from contamination. The stopper and shoulder of the bottle should be protected by covering with a polythene sheet tied in place (other plastics may give interference problems); alternatively the whole sample bottle may be sealed in a polythene bag. Samples should not be placed in close proximity to concentrated insecticide or PCB solutions or solids. Water samples may be stored in a refrigerator at 4°C but it is recommended that all samples be extracted as soon as possible to prevent decomposition of the insecticides after sampling. The solvent extracts may be stored in a refrigerator at 4°C and at this temperature are stable for months or even years.

Evaporation of the solvent may still occur even under refrigeration. Extracts must not be allowed to dry out and the volume of solvent must be restored to the original volume before proceeding with the analysis.

8 Analytical Procedure

READ SECTION 4 ON HAZARDS BEFORE STARTING THIS PROCEDURE

Step	Experimental Procedure	Notes
8.1	Extraction of Insecticides and PCB	
8.1.1	To approximately 2 l of sample in a 2.5 l bottle, add 50 ml ± 2 ml hexane. Stopper and shake vigorously for at least 5 mins. Transfer sample and extract to a 3 l separating funnel. Allow the phases to separate for at least 5 mins. When good separation has been achieved run off the lower aqueous phase and measure its volume (V ml) to ± 20 ml. Run the hexane layer into a suitable small glass stoppered vessel (notes a, b, c, and f).	(a) Most pesticides will be associated with the solid material. If appreciable amounts of settleable solids are present separate extractions of solid and liquid should be made. (b) If a smaller volume of sample is taken reduce the volume of solvent proportionally. (c) Vigorous shaking produces emulsions with some samples. These may be broken by the addition of acids or salts, or by centrifugation. Care is needed to avoid contamination. If a solvent water interfacial cuff forms this may either be included and water removed at the drying step, or discarded and the volume of solvent recovered noted and a suitable adjustment made in the final calculations. The separating funnel may be rinsed with a further 5 ml of hexane and the rinsings added to the main extract. A second extraction may be applicable if a large emulsion layer is formed.
8.1.2	Alternative procedures for waters not containing sediment: To 2 l of sample contained in a 2.5 l bottle, add 50 ml ± 2 ml hexane. Stopper and roll at 60 ± 5 rpm for at least 2 hrs. Transfer the sample and extract to a 3 l separating funnel avoiding mixing of the phases. Allow the phases to separate for at least 2 mins. Run off the lower aqueous phase and measure its volume V to ± 20 ml. Run the hexane layer into a small glass stoppered vessel (notes d, e, and f).	(d) Step 8.1.2 avoids the formation of emulsions. It has been used successfully with turbid river waters, however it is not suitable where heavy sediment in the sample lies on the bottom of the bottle and is not stirred to contact the solvent during extraction. (e) Bottle stoppers may be sealed using PTFE sleeves or 2 turns of PTFE jointing tape. In step 8.1.2 stoppers must be held into the bottles in some suitable manner (eg ref 3). (f) Alternative methods of separating the phases have been found suitable which avoid transference to a second vessel (ref 3 and 4).
8.2	Concentration	
	Dry the hexane extract by passing it through a column containing 5 g ± 1 g anhydrous sodium sulphate supported by a small plug of cotton wool. Collect the dried extract in a Kuderna Danish type evaporator. Wash the column with 10 ml ± 1 ml hexane and collect in the evaporator. Add two anti-bumping granules and evaporate to 5 ml ± 1 ml on a steam bath. Further concentrate the extract to 1 ml ± 0.1 ml using a micro Snyder column or a gentle stream of dry air or nitrogen with a tube placed in a warm bath (not exceeding 40°C) (notes g and h).	(g) Larger quantities of drying agent may be needed if water from emulsions or cuffs is included in the extract. (h) Various methods of solvent evaporation have been used. All need care to avoid loss of the more volatile insecticides and contamination during the procedure.

Step	Experimental Procedure	Notes
8.3	Clean-up Procedure	
	Prepare an alumina-alumina/silver nitrate column as in Section 6.2.1. Run off the surplus hexane. When the hexane level reaches the top of the column add the concentrated sample extract. Wash the sample vessel with 2 ml ± 0.5 ml hexane and add the washings to the column. Elute the column with 30 ml ± 1 ml hexane and collect the eluate in a Kuderna-Danish evaporator. Concentrate the extract to 1 ml ± 0.1 ml as in step 2 (notes i, j, and k).	(i) During the additions to the column do not allow the meniscus of the hexane to fall below the surface of the alumina. (j) If the alumina/silver nitrate column blackens along its entire length prepare a fresh column and repeat step 8.3. If total blackening is a common occurrence, larger columns may be used but additional hexane will be required to elute dieldrin (see Appendix: Table 1). (k) The examination of a 5 μl aliquot of the concentrated cleaned-up extract by gas chromatography at this stage will give a qualitative guide to the compounds present.
8.4	Separation of Insecticides and PCB	
	Prepare a silica gel column as in section 6.2.2.1. Add the concentrated sample extract (1.0 ml ± 0.1 ml) to the column (note 1). Wash the container with 1.0 ml ± 0.1 ml hexane, add this to the column retaining the meniscus just below the top of the silica gel (note 1). Add 10 ml ± 0.2 ml hexane and collect the first 7 ml eluate (fraction 1) (note m). This eluate contains PCB (note n). Change the receiver and collect the remaining hexane eluate. Add 12 ml ± 1.0 ml 10% v/v diethyl ether in hexane to the column and collect all the eluate into the same receiver (fraction 2) (note n). Concentrate each fraction to 1.0 ± 0.1 ml as in step 8.2.	(l) The liquid will penetrate the column, retain the meniscus just below the silica gel surface. Subsequently do not let the meniscus drop below the surface of the silica gel. (m) 7 ml or a volume as determined in section 6.2.2.2. (n) Fraction 1 should contain if present PCB, pp′ DDE, Heptachlor, Aldrin and part of any op′ DDT present. Fraction 2 should contain the other organochlorine insecticides and their metabolites.
8.5	Gas chromatography	
8.5.1	Calibration of gas chromatograph. Inject 5 μl aliquots of each working standard into the chromatograph at amplifier attenuations such that the peak heights of all but the lowest standards are between one quarter and three quarters full scale deflection. Construction calibration graphs of peak height or peak area against the relevant quantity of material (note o).	(o) Initially it will be necessary to inject each standard solution separately to obtain the relative retention times (Rt). Subsequently composite standards may be used.
8.5.2	*Examination of extracts* Inject 5 μl of the extract onto each column and identify the components by comparison of their retention times with those of calibration standards (notes p and q).	(p) It is essential that the identity of peaks be confirmed by the use of at least two GC columns of different polarities. Particular circumstances may indicate the need for further confirmation of identity (ref 1). (q) A complete reagent blank should be carried out.

Step	Experimental Procedure

8.6 Calculation of Concentration

8.6.1 Insecticides

Measure peak areas or heights and read off the quantity of each insecticide (A pg) from the calibration graph. Calculate the concentration (C ng/l) of each insecticide as follows:

$$C = \frac{200\,vA}{V}\ \text{ng/l}$$

where v = final volume of extract in ml
V = volume of sample in ml
A = weight of substance (pg) in the 5 μl portion of extract chromatographed.

8.6.2 PCB

Aroclor 1260 is used as the standard material in the method but other PCB formulations may be more appropriate dependent on the sample. All peaks prior to DDE should normally be discounted. If the material is degraded several peaks can be measured and compared with those of a standard material most resembling the sample or the peak areas can be calculated as DDE. The most definitive method involves perchlorination (see Appendix, Section A5.7).

9 Abbreviated Technique

Experience may dictate that with some samples the procedure may be shortened.

CAUTION: If the clean-up procedure is not employed and substantial amounts of interfering materials or high levels of insecticides are present, it is very probable that the detector will become contaminated. The time to return to the base line will be very long and the detector may be permanently damaged. Subsequent recalibration will be very time consuming and it may be considered prudent always to employ the clean-up procedure until sufficient background knowledge of the samples has been gained.

10 References

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Table I Retention Lines of Organochlorine Insecticides relative to Dieldrin, on some useful G.L.C. columns (†)

Insecticide	Column packing								
	1% Apiezon M or L	2.5% methyl silicone (eg OV-1)	2.5% phenyl methyl silicone (eg OV-17)	2.5% cyano silicone gum rubber XE-60	5% trifluoropropyl silicone oil QF-1 (FS-1265)	1% neopentylglycol succinate (NPGS)	1% FFAP	1.5% QF-1 + 1% OV-1	2.0% OV-1 3.0% QF-1
α-HCH	0.20	0.19	0.17	0.23	0.17	0.23	0.20	0.19	0.22
γ-HCH (Lindane)	0.26	0.23	0.22	0.35	0.22	0.37	0.32	0.24	0.26
β-HCH	0.31	0.21	0.22	1.11	0.28	1.63	0.79	0.25	0.28
δ-HCH	0.35	0.26	0.43	1.09	0.31	1.34	0.27	0.29	0.31
Chlordane	—	—	—	—	—	—	—	—	0.34*
Heptachlor	0.36	0.41	0.36	0.23	0.23	0.22	0.22	0.35	0.36
Aldrin	0.49	0.53	0.46	0.26	0.28	0.22	0.25	0.43	0.44
Heptachlor Epoxide	0.60	0.66	0.63	0.64	0.60	0.64	0.60	0.64	0.65
Endosulfan A	0.88	0.85	0.80	0.69	0.79	0.73	0.67	0.83	—
Endosulfan B	1.45	1.16	1.35	2.33	1.66	2.69	2.39	1.32	—
Dieldrin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
pp'DDE	1.25	1.06	1.01	0.83	0.65	1.01	1.09	0.88	0.85
Endrin	1.49	1.28	1.55	0.71	1.22	1.12	1.07	1.26	1.15
op'TDE	1.23	1.04	1.07	1.37	0.87	1.81	1.72	0.96	0.95
pp'TDE	1.83	1.30	1.40	2.44	1.21	3.39	3.03	1.30	1.24
op'DDT	1.62	1.38	1.42	1.12	0.94	1.40	1.69	1.17	1.12
pp'DDT	2.40	1.72	1.85	2.07	1.32	2.84	2.99	1.60	1.51

* Chlordane is a multipeak compound with other smaller peaks at RRT 0.37, 0.69, 0.76 and 1.23.
Glass Columns 1 m by 3 mm Internal Diameter 60 to 80 mesh acid-washed DMCS-treated Chromosorb W support at 180°C and 20 to 40 ml N_2/Min.
(Based on TP.90 Water Research Association)

†See page 17

Table II Blanks and Limits of Detection (†)

Insecticide	Mean ng/l	S_w	S_b	S_t	Limit of Detection** (ng/l)	Criterion of Detection* (ng/l)
γ-HCH	0.21	0.320 (7)	NS (6)	0.320 (13)	12	0.9
Aldrin	0.04	0.032 (7)	0.060 (6)	0.063 (6)	3	0.1
Dieldrin	0.28	0.205 (7)	0.430 (6)	0.480 (7)	14	0.6
pp'TDE	0.42	0.570 (7)	NS (6)	0.70 (11)	8	1.5
pp'DDT	0.03	0.125 (7)	NS (6)	0.125 (13)	15	0.3
PCB	2.8	1.8 (7)	3.2 (6)	3.7 (8)	106	4.8

These results were obtained by examining pairs of blanks on seven separate occasions.

(n) figures in brackets indicate number of degrees of freedom.

S_w = Within-batch standard deviation
S_b = Betweeen-batch standard deviation
S_t = Total standard deviation

* Criterion of Detection: that concentration which is unlikely to be exceeded unless the sample contains the determinand (95% confidence level).

**Limit of Detection: that concentration for which there is a desirably small probability that the result will be less than the criterion of detection (see ref. 8).

† See page 17

11. Waste Disposal

All these compounds are notifiable wastes. Solvents are rarely recovered, but could be cleaned by an absorption procedure similar to the method itself.
Disposal of solids and other than the most dilute solutions should be done through a qualified contractor, either by burning at a high temperature in a licensed plant, or sent to a licensed toxic waste disposal site.
Material containing more than 0.1% of PCB must be burnt as above. For further information write to:

Toxic Wastes Section
Directorate of Wastes
Department of the Environment
London SW1P 3EB.

Table III Estimates of Total Standard Deviation (S_t) (Results expressed in ng/l) (†)

	γ-HCH			Aldrin			Dieldrin			pp'TDE			pp'DDT			PCB		
	Spike	Mean	S_t	Spike	Mean	S_t	Spike	Mean	S_t	Spike	Mean	S_t	Spike	Mean	S_t	Spike	Mean	S_t
Distilled water plus 10% spike	0.50	0.50	0.29 (4)	0.50	0.50	0.20 (4)	0.75	0.40	0.33 (4)	1.5	1.85	0.5 (4)	3.0	2.20	1.47 (4)	40	42	22 (5)
Distilled water plus 90% spike	4.5	2.70	0.75 (9)	4.5	2.60	0.85 (9)	6.75	3.80	2.05 (9)	13.5	11.6	2.25 (9)	27	15.2	4.3 (9)	360	228	62 (5)
River water	—	—	—	—	0.05	0.07 (7)	—	1.30	1.13 (7)	—	0.35	0.40 (7)	—	0.15	0.20 (7)	—	6.0	7.0 (8)
River water plus 10% spike	0.5	4.70	2.84 (7)	0.5	0.85	0.7 (7)	0.75	1.15	1.05 (7)	1.5	3.00	2.20 (7)	3.0	2.75	3.4 (7)	40	25	21 (5)
River water plus 75% spike	3.75	6.10	4.19 (7)	3.75	1.80	0.74 (7)	5.625	2.30	2.30 (7)	11.25	7.5	1.38 (7)	22.5	10.4	5.25 (7)	300	133	49 (5)

S_t = estimate of total standard deviation. Figures in brackets indicate numbers of degrees of freedom.

(†) These data were obtained at Southern Water Authority, Sussex Area Resource Planning Laboratory.

The spike levels are based on the ranges normally encountered in River Waters and cover the lower, upper and centre of this range.

FIG.1 **ADSORPTION COLUMN**

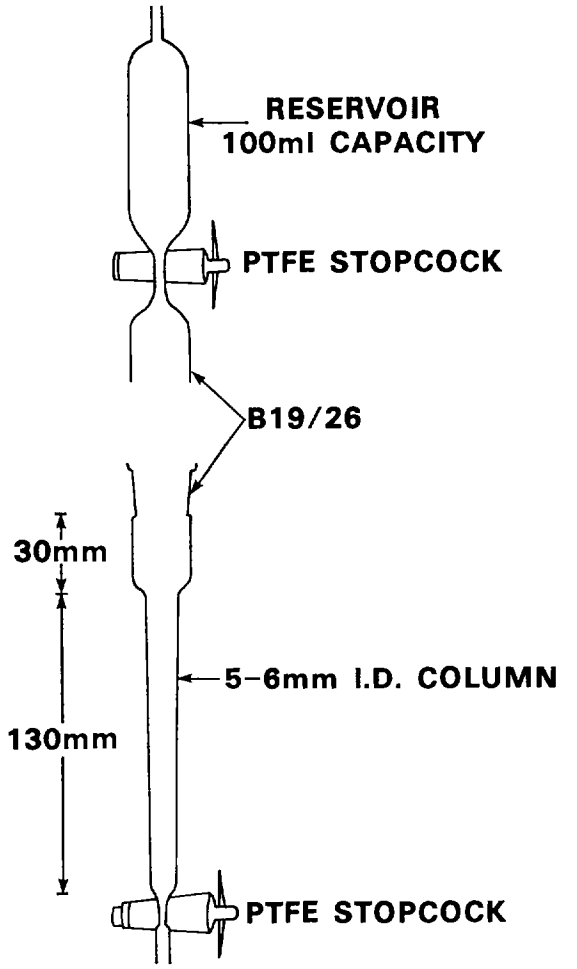


FIG.2 **KUDERNA-DANISH EVAPORATOR SYSTEM FOR CONCENTRATION OF PESTICIDE SOLUTIONS**

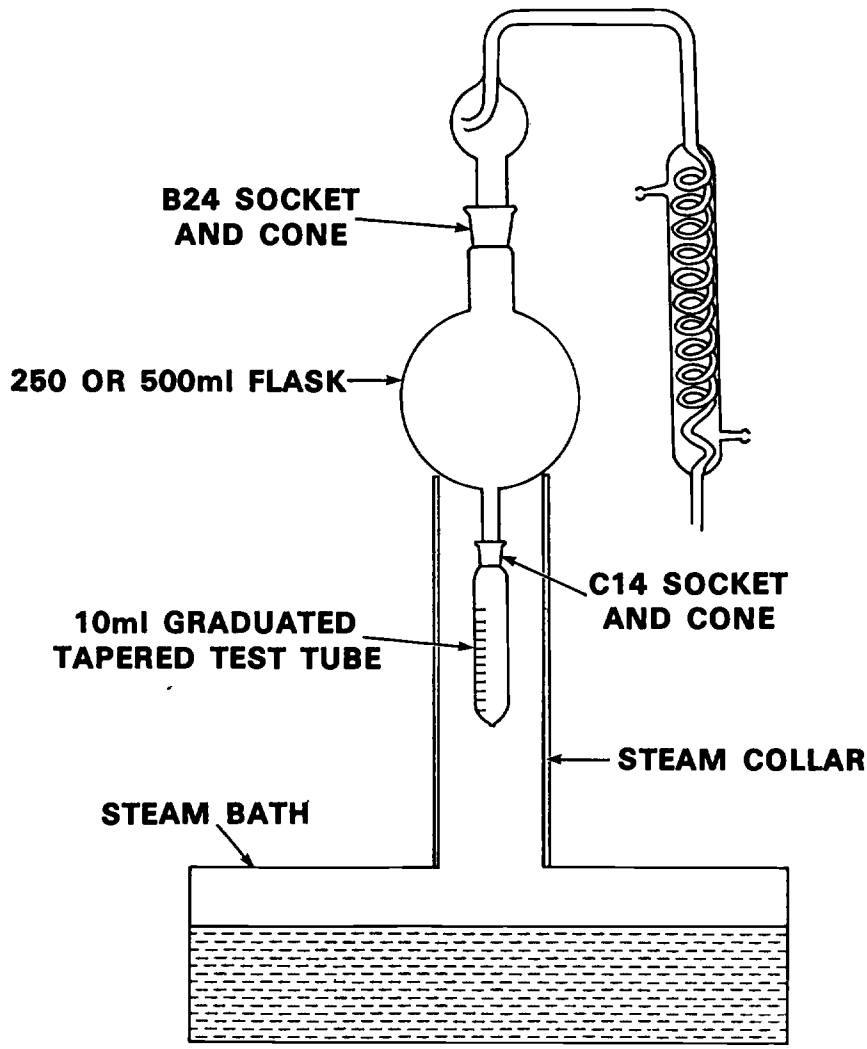
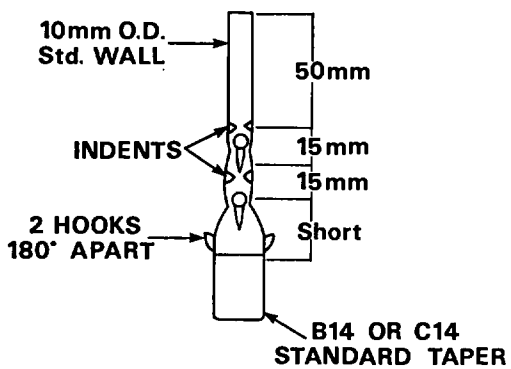


FIG.3 **MICRO SNYDER COLUMN**



Appendix

The Confirmation of the Identity of Chlorinated Insecticide Residues and Polychlorinated Biphenyls

A1 Introduction

As normally employed, gas-liquid chromatography does not give an absolute identification of residues found in a sample extract. Additional peaks may appear in the chromatograms due to other organic compounds which have the same retention times as the sought organochlorine compounds.

Confirmation of identity may be obtained at times by the use of other techniques such as infra-red or mass and nuclear magnetic resonance spectrometry; but unfortunately the sensitivities of these techniques are often insufficient to deal with the very small amounts of residues which occur in many samples. It is however possible to corroborate the identity of residues at nanogram and picogram levels by preparing suitable derivatives from the residues and repeating the gas chromatogram. Proof of identity is then based on the disappearance of the Peaks(s) for the parent compound from the chromatogram plus the appearance of the new peak(s) due to the derivative at the correct retention time(s).

A2 Isolation of the Residues

Ideally each residue should be separated from all the others present in the sample extract before a derivative is prepared, but in many cases this is impracticable and even unnecessary, for example where the compound gives a derivative which is well resolved from the other residues or where the concentration of the compound is so much greater than that of the other residues that its reaction product will not be masked by their presence. Where separation is required, thin-layer chromatography or alumina column fractionation (see Table A1) may be tried, but the combination of residues in samples is so variable that a standard procedure for their separation cannot be given.

A3 Additional hazards

(ADDITIONAL TO THOSE IN SECTION 4)

Antimony pentachloride must be used carefully. It gives off harmful vapours and is very poisonous. The acetic, hydrobromic and hydrochloric acids used are all corrosive fuming liquids. They may cause eye irritation and impair respiration. Sodium hydroxide and alcoholic potassium hydroxide solutions are strong alkalis. When alkalis are neutralised by acids heat is generated, which may cause the reactants to boil. The reaction vessel must be kept cool and facial protection should be worn when carrying out operations with these reagents. Chromium trioxide is a strong oxidising agent which may assist fire and cause skin irritation. Bleaching powder can liberate gaseous chlorine if it comes into contact with acids. The vapours of chloroform and toluene must not be inhaled.

A4 Additional reagents and apparatus

A4.1 **Alcoholic potassium hydroxide solutions**

A4.1.1 *Alcoholic potassium hydroxide, 0.5M*

Dissolve 2.8 g $\pm$ 0.1 g of potassium hydroxide (A.R. grade) in not more than 5 ml distilled water in a glass beaker (CAUTION: heat is evolved). Cool, transfer to a 100 ml calibrated flask, dilute with ethanol (absolute alcohol) almost to the mark, mix and make up to the mark with ethanol.

A4.1.2 *Alcoholic potassium hydroxide, 0.01M*

Dilute 2 ml $\pm$ 0.02 ml of alcoholic potassium hydroxide solution 0.5M (see section A4.1.1) to 100 ml with ethanol (absolute alcohol) in a calibrated flask and mix. This solution should be prepared freshly as required each time the method is used.

A4.2 **Glacial acetic acid** – redistilled in all-glass apparatus.

A4.3 **Sodium hydroxide solution, 5M**

Dissolve 20 g ± 2 g of sodium hydroxide (A.R. grade) in distilled water in a glass beaker (CAUTION: heat is evolved). Cool, transfer to a 100 ml calibrated flask and dilute to the mark with distilled water.

A4.4 **Chromium trioxide** – Analytical Reagent grade

A4.5 **Hydrobromic acid/glacial acetic acid – 1:1 V/V mixture**

A4.6 **Antimony pentachloride** – Reagent Grade

A4.7 **Hydrochloric acid (d_{20} 1.18)** – Analytical Reagent grade

A4.8 **Bleaching powder** – commercial grade

A4.9 **Sodium hypochlorite solution** – containing approximately 10% available chlorine in water.

A4.10 **Chloroform** – redistilled

A4.11 **Sodium sulphate solution** 2% W/V

Dissolve 2 g ± 0.2 g sodium sulphate (see Section 5.6) in 100 ml insecticide-free water.

A4.12 **Toluene** – Analytical Reagent grade

A4.14 **Perchlorination tube** – see Figure A1

A4.15 **Gas Chromatograph** – slight changes may be needed for some of the tests (see A5.7.12).

A4.16 **Solutions of organochlorine compound derivatives.**

These are prepared by the procedures which follow in Section A5, starting with known samples of pesticide, etc. (In a few instances solutions of the commercially available derivative, prepared according to Section 5.13 may be used). (See A5.1.9. A5.2.2, A5.3.9, A5.4.9, A5.5.8, A5.6.5, A5.7.12 and note d to the latter).

A5 Analytical Procedures for Confirmation of Identity

As a general rule, the amount of derivative which is injected on to the gas chromatographic column is of the order of 1 ng, but the range can be from 0.1 ng to 10 ng depending on the operating characteristics of the gas chromatograph and the properties of the derivative.

READ SECTION A.3 ON HAZARDS BEFORE STARTING THE FOLLOWING PROCEDURES

A5.1 pp'-DDT (Ref A6.1)

Step	Experimental Procedure	Notes
A5.1.1	Transfer the solution to be examined to a 10 ml graduated test-tube, add 2 anti-bumping granules and fit a micro-Snyder column to the tube.	
A5.1.2	Carefully reduce the volume nearly to dryness by heating the tube in steam (note a).	(a) Do not boil the solvent too rapidly nor take the solution completely to dryness – loss of residue may occur.
A5.1.3	Remove the micro-Snyder column and introduce 1.0 ml $\pm$ 0.1 ml of freshly prepared 0.5 M alcoholic potassium hydroxide solution (Section A4.1.1). Replace the micro-Snyder column.	
A5.1.4	Heat the tube for 5 mins in boiling water (note b).	(b) pp'-DDT is converted to pp'-DDE.
A5.1.5	Cool to room temperature, add 1.0 ml $\pm$ 0.1 ml of hexane, stopper the tube and shake vigorously for 30 s.	
A5.1.6	Add 10 ml $\pm$ 1 ml of 2% w/v sodium sulphate solution, stopper, shake and allow the two layers to separate.	
A5.1.7	Inject 5 μl of the upper hexane layer on to two different glc columns (note c), and compare the traces so obtained with the original traces and with either a standard amount of pp'-DDT treated in the same manner or a standard amount of pp'-DDE (note d).	(c) Do not take up any of the alkaline layer into the syringe. (d) Concentrate or dilute the sample solution as necessary.
A5.1.8	Carry out a complete 'blank' test on all the reagents at the same time as the sample extract is being examined.	
A5.1.9	Disappearance of the pp'-DDT peak from the chromatograms and the appearance of a pp'-DDE peak confirms the presence of pp'-DDT in the sample extract (note e).	(e) The Retention Time (Rt) of pp'-DDE relative to that of pp'-DDT is about 0.5 on a dimethyl silicone column.

A5.2 pp'-TDE

Step	Experimental Procedure	Notes
A5.2.1	Use the procedure as outlined in A5.1 but extend the heating period in Step A5.1.4 to 15–20 minutes. Substitute a standard solution of pp'-DME for pp'-TDE in the same manner (note a, b).	(a) pp'-DME has poor electron-capturing power and gives small peaks. (b) pp'-DME is sometimes known as pp'-DDMU.
A5.2.2	Disappearance of pp'-TDE from the chromatograms and the appearance of pp'-DME confirms the presence of pp'-TDE in the sample extract (note c).	(c) The Rt of pp'-DME relative to that of pp'-TDE is about 0.5 on a dimethyl silicone column.

A5.3 **pp'-DDE** (Ref A6.2)

Step	Experimental Procedure	Notes
A5.3.1	Transfer the solution to be examined to a 10 ml graduated test-tube, add 2 anti-bumping granules and fit a micro-Snyder column to the tube.	
A5.3.2	Carefully reduce the volume almost to dryness by heating the tube in steam (note a).	(a) Do not allow the solvent to boil too rapidly nor take the solution completely to dryness – loss of residue may occur.
A5.3.3	Remove the micro-Snyder column and introduce 2.0 ml $\pm$ 0.2 ml of glacial acetic acid and 100 mg $\pm$ 10 mg of chromium trioxide.	
A5.3.4	Replace the micro-Snyder column and heat the tube in boiling water for 15 mins (note b).	(b) The reaction mixture should remain brown – if green, add more chromium trioxide and continue heating.
A5.3.5	Cool the tube to room temperature, add 2.0 ml of hexane, stopper the tube and shake vigorously for about 15 s.	
A5.3.6	Carefully, with cooling and agitation, add enough 5M sodium hydroxide solution to the tube to neutralise the acid (note c). Invert the tube several times and then set it aside until two layers separate.	(c) CAUTION: heat will be generated by the addition of the sodium hydroxide. About 7 ml will be required.
A5.3.7	Inject 5μl of the upper hexane layer on to two different glc columns and compare the chromatograms with those obtained by treating a known amount of pp'-DDE in the same manner.	
A5.3.8	Carry out a complete 'blank' test on all the reagents at the same time as the sample extract is being examined.	
A5.3.9	Disappearance of pp'-DDE from the chromatograms and appearance of the reaction product – dichlorobenzophenone (DBP) – confirms the presence of pp'-DDE in the sample extract (note d).	(d) DBP has a poor electron-capturing power and gives small peaks. The Rt of DBP relative to that of pp'-DDE on a dimethyl silicone column is about 0.75.

A5.4 **Dieldrin, Endrin, Heptachlor epoxide** (Ref A6.1)

Step	Experimental Procedure	Notes
A5.4.1	Transfer the solution to be examined to a 10 ml graduated test-tube, add 2 anti-bumping granules and fit a micro-Snyder column to the tube.	
A5.4.2	Carefully reduce the volume nearly to dryness by heating the tube in steam (note a).	(a) Do not allow the solution to boil too rapidly nor take the solution completely to dryness – loss of residue may occur.
A5.4.3	Cool the tube and introduce 0.5 ml of a 1 + 1 solution of concentrated hydrobromic acid and glacial acetic acid (see Section A.4.5).	

Step	Experimental Procedure	Notes
A5.4.4	Replace the micro-Snyder column and heat the mixture in boiling water for 10 mins.	
A5.4.5	Cool to room temperature. Add 1.0 ml ± 0.1 ml hexane, stopper the tube and shake it vigorously for 30 s.	
A5.4.6	Dilute the solution with 10 ml of 2% sodium sulphate solution and set the tube aside until the two layers separate.	
A5.4.7	Inject 5 μl of the upper hexane layer on to 2 different glc columns and compare the traces with those obtained from a known amount of standard insecticide treated in an identical manner (note b).	(b) Concentrate or dilute the final solution to give a suitable peak for comparison.
A5.4.8	Carry out a complete 'blank' determination on all the reagents at the same time as the sample and standard are being examined.	
A5.4.9	The disappearance of the parent peak from the chromatograms and the appearance of two derivate peaks (note c) affords the proof of identity of the compound being examined.	(c) Some columns cannot resolve the heptachlor epoxide derivatives and only a single peak is seen. The approximate retention times of the bromination products relative to dieldrin on a dimethyl silicone column, are: heptachlor epoxide 1.8; dieldrin 3.45, 3.2; endrin 3.10, 1.9.

A5.5 γ-HCH

Step	Experimental Procedure	Notes
A5.5.1	Transfer the solution to be examined to a 10 ml graduated test-tube, add 2 anti-bumping granules and 1 ml ± 0.1 ml of freshly prepared 0.01 M alcoholic potassium hydroxide solution (see Section A4.1.2).	
A5.5.2	Carefully evaporate the hexane layer using a gentle stream of dry air or nitrogen.	
A5.5.3	Fit a micro-Snyder column to the tube and then heat the tube in boiling water for 5 mins.	
A5.5.4	Cool to room temperature, add 1.0 ml ± 0.1 ml of hexane, stopper the tube and shake vigorously for 15 s.	
A5.5.5	Dilute the mixture with about 8 ml of water and set the tube aside until the two layers have separated.	
A5.5.6	Inject 5 μl on to a gas chromatographic column (note a), and compare the trace so obtained with that given by a known amount of γ-HCH treated in a similar manner or a known amount of 1, 2, 4-trichlorobenzene.	(a) The temperature of the column should be 120°C. Flow rate of gas may be adjusted to give a suitable retention time.

Step	Experimental Procedure	Notes
A5.5.7	Carry out a complete 'blank' test on all the reagents at the same time as the sample is being examined.	
A5.5.8	The disappearance of the γ-HCH peak and the appearance of a peak at the retention time of 1, 2, 4-trichlorobenzene confirms the presence of the HCH isomer.	

A5.6 **Aldrin** (Ref A6.1)

Step	Experimental Procedure	Notes
A5.6.1	Suspend 0.25 g of bleaching powder in 10 ml of water in a 50 ml separating funnel. Add 10 ml of chloroform and 0.5 ml of hydrochloric acid. Shake the mixture thoroughly and allow the layers to separate. Run the lower chloroform layer through a small filter containing 1.0 g of anhydrous sodium sulphate into a 10 ml graduated test-tube. Stopper the tube (note a).	(a) Prepare the reagent freshly for each batch of samples. 0.5 ml $\pm$ 0.05 ml of 10% aqueous solution of sodium hypochlorite (Section A4.10) may be used in place of the bleaching powder.
A5.6.2	Evaporate the solution of aldrin carefully to dryness in a 10 ml graduated test-tube, using a micro-Snyder column or a gentle stream of dry air or nitrogen.	
A5.6.3	Dissolve the residue in 0.5 ml of chloroform and add 0.1 ml of the reagent prepared in Step A.5.6.1.	
A5.6.4	Set the mixture aside for 5 mins at room temperature, and, after evaporating the chloroform to dryness, dissolve the residue in 1.0 ml $\pm$ 0.1 ml of hexane.	
A5.6.5	Examine the hexane solution by GC and compare the resulting peak on the chromatogram with that formed from a standard solution of aldrin by the same reaction (note b).	(b) The retention time of the chloroaldrin derivative relative to that of dieldrin on a dimethyl silicone column is about 1.3.
A5.6.6	Carry out a complete 'blank' test on all the reagents at the same time as the sample and standard are being examined.	

A5.7 **Polychlorinated biphenyls (PCB)**

NOTE: The presence of biphenyl in the sample will interfere seriously with this test. Biphenyl has been detected in some sludges. It may be removed by oxidation to benzoic acid using chromium trioxide in glacial acetic acid (see Step A5.3.3); the polychlorinated biphenyls remain unchanged by this procedure.

Step	Experimental Procedure	Notes
A5.7.1	Transfer the solution to be examined to a thick-walled glass tube, which is fitted with a side arm capable of being sealed with a screw-threaded PTFE stopper (perchlorination tube, Fig A1).	

Step	Experimental Procedure	Notes
A5.7.2	Evaporate the solvent very carefully using a stream of dry air or nitrogen (note a).	(a) There must be no moisture in the tube or it will react with the antimony pentachloride.
A5.7.3	Add 1 g ± 0.2 g of antimony pentachloride to the tube and close the PTFE tap (note b).	(b) Carry out these operations in a fume cupboard.
A5.7.4	Place the lower end of the tube in an oilbath, or heating block which has been pre-heated to 200°C and leave it at this temperature for 1 hr.	
A5.7.5	Remove the tube from the bath and allow it to cool. Point the side-arm away from the face and carefully open the tap and remove the stopper.	
A5.7.6	Introduce 2 ml ± 0.2 ml of hydrochloric acid and shake the tube gently to mix the liquids (note c).	(c) The hydrochloric acid forms a complex with the residual antimony trichloride.
A5.7.7	Add 1.0 ml ± 0.1 ml of toluene to the tube, close the tap and shake the mixture vigorously.	
A5.7.8	Allow the layers to separate and pipette off the upper layer into a 50 ml separating funnel. Extract the aqueous layer in the tube with two 2.5 ml portions of hexane, transferring these washings to the funnel (note d).	(d) Do not pipette by mouth.
A5.7.9	Make the volume of the extracts up to 10 ml ± 1 ml by adding more hexane and, after introducing 10 ml of water, shake the mixture for 1 min. Allow to separate and discard the lower layer.	
A5.7.10	Pass the hexane through a column containing about 5 g of anhydrous sodium sulphate and collect the eluate in a Kuderna-Danish evaporator. Wash the separating funnel with 10 ml of hexane and pass this washing through the sodium sulphate column into the evaporator. Finally wash the sodium sulphate column with a further 10 ml of hexane.	
A5.7.11	Concentrate the extract and washings to a suitable volume for gas-liquid chromatography.	
A5.7.12	Compare the resulting chromatogram with that produced by the treatment of a known amount of a standard solution of PCB, for example Aroclor 1260 (note e). Because of the long retention time of the derivative, decachlorobiphenyl, under normal GC conditions, either the column temperature will have to be increased to 230°C – 250°C or a short column, eg 0.3 m, will have to be used at 200°C in order to enhance the sensitivity and shorten the time of analysis.	(e) Some batches of antimony pentachloride contain bromide as an impurity which results in the formation of bromononachlorobiphenyl; this appears is a small secondary peak following that of the decachlorobiphenyl and can be ignored when comparing with a standard solution of PCB treated with the same reagent.

A.6 References

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%RECOVERY OF CHLORINATED PESTICIDE RESIDUES FROM A 10g ALUMINA COLUMN (7% MOISTURE)

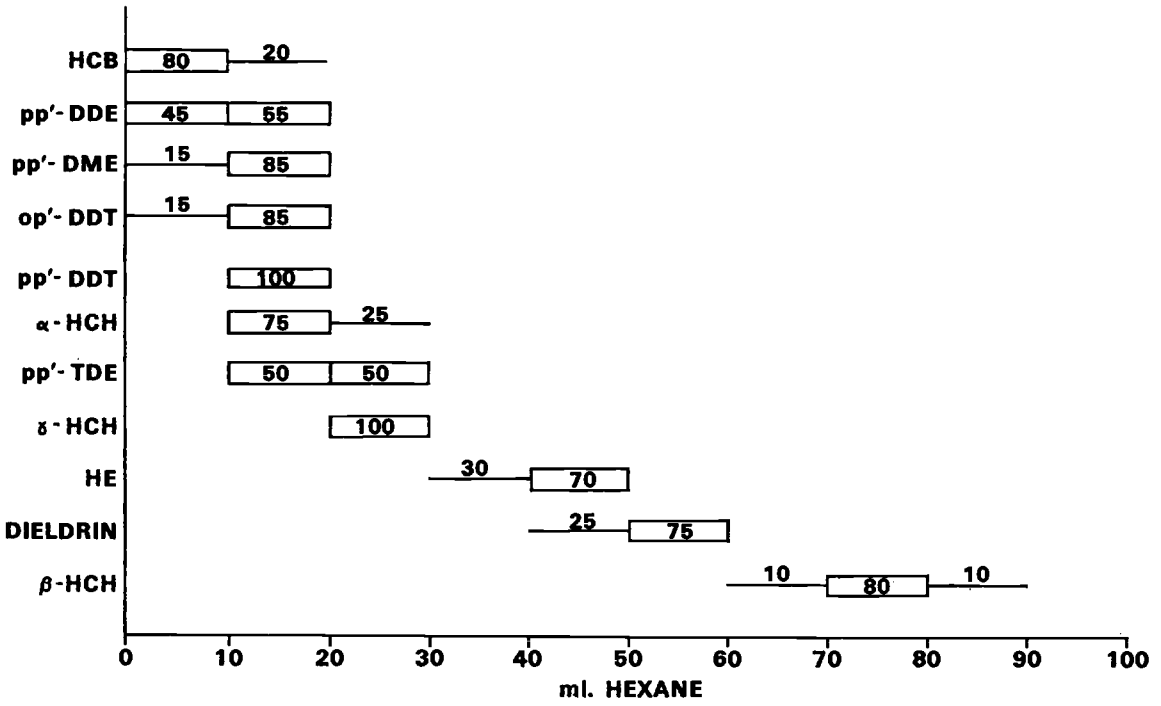
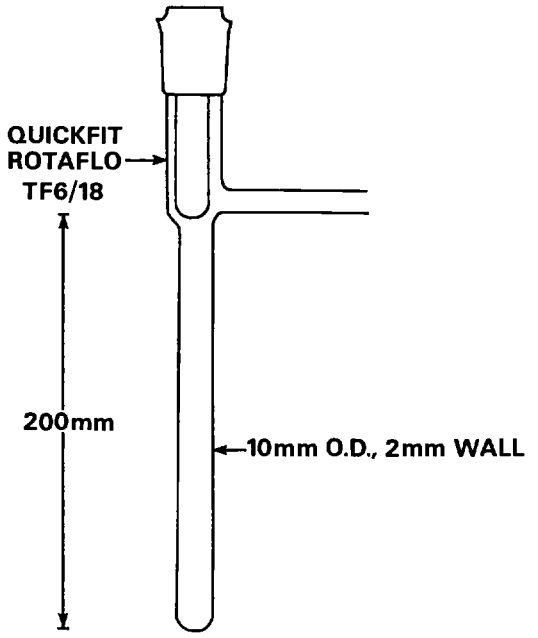


TABLE A1

FIG. A1 **PERCHLORINATION TUBE**



B1 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. This subject will be discussed in a subsequent publication.)

B2 Addresses for Correspondence

B2.1 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
2 Marsham Street
LONDON SW1P 3EB
England

B2.2 At the present time, though based on work in several laboratories, thorough test data is only available from one laboratory, hence the tentative status of the method. 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
2 Marsham Street
LONDON
SW1P 3EB
England

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Dd 597644 K24 4/79

Standing Committee of Analysts

Members of the Committee Responsible for this Method:

Mr BT Ashurst	[3, 4]
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Mr M McEvoy	[4]
Mr D Meek	[2, 3, 4]
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Mr P Morries	[1](after June 1975)
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Mr JF Palframan	[3]
Dr AT Palin	[1](until June 1975)
Dr SJ Patterson	[1]
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Mr LD Purdie	[1](after June 1975)
Mr BD Ravenscroft	[1](after June 1975)
Mrs SM Rawson	[3]
Prof JP Riley	[1](after August 1975)
Dr EA Simpson	[2]
Mr R Sinar	[1]
Mr PAH Sperring	[1](until January 1976)
Dr S Torrance	[3]
Mr BT Whitham	[1](after June 1975), [2]
Mr AL Wilson	[1]
Dr R Wood	[1](June 1975 to March 1978)

[1]Member of Main Committee (from May 1973 unless stated).

[2]Member of the Oil and Pesticides Panel (October 1973–November 1975 unless stated.)

[3]Member of Working Group 6 (from January 1976).

[4]Member of the Chloro-Pesticides and PCBs Panel (from December 1975).

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