The Determination of Organochlorine Insecticides and Polychlorinated Biphenyls in Sewages, Sludges, Muds and Fish 1978

Organochlorine Insecticides and Polychlorinated Biphenyls in Water, an addition, 1984

Methods for the Examination of Waters and Associated Materials

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 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 whether for one's self, one's colleagues in the laboratory, outsiders, or subsequently for maintenance workers. 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 manufacturer's 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 emphasized that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radio-chemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected.

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

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About this series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, short reviews of the more important analytical techniques of interest to the water and sewage industries. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as 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 a committee of the Department of the Environment set up in 1972. Currently it has seven Working Groups, each responsible for one section or aspect of water cycle quality analysis.
They are as follows:

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

The actual methods and review are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover. Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5.

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

L R PITTWELL
Secretary

31 October 1983

About these methods

This booklet contains a method for the analysis of organochlorine insecticides and polychlorinated biphenyls in sewages, sludges, muds and fish. It also contains an addition to the already published booklet on the determination of the same substances in water, which should be read in conjunction with that earlier booklet.

The main 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.

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 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 heptachloro-biphenyls; 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.

The Determination of Organochlorine Insecticides and Polychlorinated Biphenyls in Sewages, Sludges, Muds and Fish

1 Performance Characteristics of the Method

1.1	Substances determined	Organochlorine insecticides and polychlorinated biphenyls (PCBs).
1.2	Type of sample	Sewages, sludges, muds and fish
1.3	Basis of method	Extraction into hexane, removal of extraneous materials using a column of alumina /Ag NO_3, separation of most insecticides from PCBs by column chromatography on silica-gel, followed by gas liquid chromatography using electron-capture detection. (Ref. 1).

1.4 Range of application

Insecticide	Primary sewage ng/l	Settled sewage ng/l	Activated sludge g/g dry wt	Mud g/g dry wt	Fish g/g wet wt
Lindane	100	100	360	12	3.2
Aldrin	150	150	530	19	4.8
Dieldrin	250	250	890	30	8.0
pp'-DDE	250	250	890	30	8.0
pp'-TDE	500	500	1,800	60	16.0
pp'-DDT	1,000	1,000	3,600	120	32.0
PCB	400	400	1,800	60	16.0

1.5 Calibration curve

Range of linearity depends upon the detector in use. The instrument used for performance tests on a similar method for water gave a linear response over the following ranges

Lindane	0–200	pg	weights of insecticide injected into the chromatogram
Aldrin	0–250	pg	
Dieldrin	0–500	pg	
pp'-DDE	0–500	pg	
pp'-TDE	0–1000	pg	
pp'-DDT	0–2000	pg	
PCB	0–1000	pg	

1.6	Standard deviation	See Tables II and III
1.7	Limit of detection	See Table IV
1.8	Sensitivity	Dependent upon determinand and instrument in use.

1.9 Bias

Dependent upon extraction efficiency which will vary with the sample and determinand. In one series of tests the mean % recoveries were:

Insecticide	Primary sewage	Settled sewage	Act sludge	Mud	Fish
Lindane	120	78	127	92	63
Aldrin	81	38	138	105	87
Dieldrin	47	44	49	109	95
pp'-DDE	72	67	179	122	173

		pp'-TDE	85	44	173	110	89
		pp'-DDT	76	36	116	76	68
		PCB	51	23	37	37	23
		In these tests the elapsed time between spiking and extraction was less than one hour. If the contact time were increased the % recoveries would be expected to be lower.					
1.10	Interference	Any electron capturing material which passes through the procedure and has similar gas-chromatographic characteristics to any determinand.					
1.11	Time required for analysis	Total analysis time including preparation of reagents, apparatus etc and confirmation of the identity of the determinand approximately 6 samples per man week.					

2 Principle

Crude sewage is allowed to settle before extraction with hexane and the settled solids extracted separately. Settled sewage is saturated with magnesium sulphate before extraction. Sludges and muds are shaken with propan-2-ol before extraction with hexane. The hexane extract is cleaned up on an alumina-silver nitrate column. 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.

3 Interferences

In principle, any substances 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 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 columns and confirmatory tests. The information given in references 2, 3 and 4 will also be of value here.

4 Hazards

Pesticides are toxic, and care must be taken when weighing solids and handling concentrated solutions; wear impermeable gloves, do not re-use gloves, and avoid inhalation of dust.

The advice given in Warning to uses is especially applicable.

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 neutralized 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 oxidizing 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.

All refrigerators used for storing solvents, solutions or samples must be flameproof.

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 and verified by the running of procedural blanks with each batch of samples analysed.

Reagents may become contaminated by contact with air and with materials, particularly plastics. Storage should be in tightly sealed all-glass containers.

5.1 Propan-2-ol — AR Grade redistilled if required in an all-glass apparatus using a 300mm Dufton spiral fractionating column or equivalent.

5.2 Acetone — Redistilled as under 5.1.

5.3 Diethyl ether — Redistilled from potassium hydroxide using the apparatus described in 5.1.

5.4 Hexane — Redistilled as in 5.3.

5.5 Water — Distilled in an all-glass apparatus and stored under hexane in an all-glass aspirator.

5.6 Cotton wool — washed with hexane and ether.

5.7 Sodium sulphate — Granular, anhydrous AR grade. Heat at 500°C ± 20°C for 4 hours ± 30 minutes. Cool to about 200°C in the muffle furnace and then to ambient temperature in a desiccator containing magnesium perchlorate or equivalent alternative.

5.8 Silver nitrate — AR grade.

5.9 Alumina — Woelm W200 neutral or an equivalent of the same mesh size. Heat at 500°C ± 20°C for 4 hours ± 30 minutes. Cool to about 200°C in the muffle furnace and then to ambient temperature in a dessicator containing magnesium perchlorate. Place in a sealable all-glass container and add 7% w/w ± 0.2% water. Seal and agitate for at least 2 hours to ensure uniformity. Store in a sealed 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.

5.10 Alumina — Silver Nitrate — Dissolve 0.75 g ± 0.1 g silver nitrate in 0.75 ml ± 0.01 ml water (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.

Store in the dark and use within four hours of preparation.

5.11 Magnesium sulphate — LR grade. Anhydrous. Heat at 500°C ± 20°C for 4 hours ± 30 mins.

5.12 Anti-bumping granules — Washed with acetone.

5.13 Silica gel — Merck 7754 or an equivalent of the same mesh size. Heat to 500°C ± 20°C for at least 2 hours. Cool to 200°C in the muffle furnace and then to ambient in a desiccator containing magnesium perchlorate, where it may be stored for up to a fortnight.

5.14 Prepared Silica gel — To a suitable quantity of 5.13 add 3.5%±0.1% w/w water in an all-glass container and seal. Agitate for at least 2 hours to ensure uniformity and store in the sealed glass container. This silica gel partially hydrated in the manner prescribed should be prepared daily.

5.15 Silica gel column test solutions.

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

5.15.2 Prepare a similar solution separately such that 1 ml contains 1 μg of Aroclor 1260.

5.16 Solutions of organochlorine compound derivatives.

These are prepared by the procedures which follow in Section 7, starting with known samples of pesticide, etc.

Additional reagents required for the Appendix

5.17 Alcoholic potassium hydroxide solutions

5.17.1 *Alcoholic potassium hydroxide, 0.5M*

Dissolve 2.8 g ± 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 made up to the mark with ethanol.

5.17.2 *Alcoholic potassium hydroxide, 0.01M*

Dilute 2 ml ± 0.02 ml of alcoholic potassium hydroxide solution 0.5M (see section 5.17.1) 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.

5.18 Glacial acetic acid — redistilled in all-glass apparatus.

5.19 Sodium hydroxide solution, 5M

Dissolve 20 ± 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.

5.20 Chromium trioxide — Analytical Reagent grade

5.21 Hydrobromic acid/glacial acetic acid — 1:1 V/V mixture

5.22 Antimony pentachloride — Reagent Grade

5.23 Hydrochloric acid (d_{20} 1.18) — Analytical Reagent grade

5.23.1 *Hydrochloric Acid 6M.* Take 600 ± 10 ml of Hydrochloric Acid (d_{20} 1.18) and make up to 1 litre in a measuring cylinder with insecticide free water.

5.24 Bleaching powder — commercial grade

5.25 Sodium hypochlorite solution — containing approximately 10% available chlorine in water.

5.26 Chloroform — redistilled.

5.27 Sodium sulphate solution 2% W/V

Dissolve 2.0 g ± 0.2 g sodium sulphate (see Section 5.6) in 100 ml insecticide-free water.

5.28 Toluene — Analytical Reagent grade

5.29 1.2.4 Trichlorobenzene

6 Apparatus

All apparatus should be checked for contamination. Some brands of detergents can give rise to interferences. A suitable cleaning procedure for glassware is as follows:—

Wash with a suitable detergent, rinse with pesticide-free water and dry in an oven (60°C–120°C). Immediately before use rinse with acetone and then hexane and allow to drain.

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

6.1 Glass Columns

130 mm long by 5–6 mm internal diameter (Fig 1).

6.2 Kuderna-Danish Evaporator (Fig 2).

6.3 Micro-Snyder Column (Fig 3).

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

6.5 100 ml glass centrifuge tubes.

6.6 Air or nitrogen line — gases cleaned by passage through molecular sieve type 13X pellets and silica gel (15–40 mesh) terminating in a fine jet of glass or metal and controllable 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).

6.7 Separating funnels pear-shaped 1 litre, 500 ml and 100 ml capacity used with a grease-free glass tap or fitted with a PTFF tap.

6.8 Glass Soxhlet assembly EX5/63 with 250 ml flask and a condenser.

6.9 Glass Soxhlet thimble of medium porosity sinter 100 × 35 mm.

6.10 Alumina/Silver nitrate column

Plug a glass column with cotton wool. Put in 15 ml ± 1 ml n-hexane then add 1.0 g ± 0.2 g alumina/silver nitrate and allow to settle. Then add 2.0 g ± 0.2 g alumina and again allow to settle. Add sufficient sodium sulphate (5.7) to give a layer 5 mm deep on top of the alumina. The whole column should be prepared immediately before use.

6.11 Silica gel column

6.11.1 *Preparation*

Plug a glass column with cotton wool. Add 2.00 g + 0.01 g prepared silica gel (5.14). 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 as in 6.10. A fresh column must be prepared before each determination.

6.11.2 *Checking of activity*

To the prepared silica gel (5.14) add 1.0 ml ± 0.1 ml of solution 4.15 and 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 the column with 10 ml ± 0.1 ml hexane, separately collecting 1.0 ml ± 0.1 ml fractions in marked centrifuge tubes (6.4) until nine fractions have been collected. Change the receiver and allow the rest of the hexane into the same receiver. Concentrate the final eluate to 1.0 ml ± 0.2 ml using a Kuderna-Danish evaporator (6.2) and finally a gentle stream of dry air or nitrogen.

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 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 procedure (Section 9 step 7) 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 5.15.2.

6.12 Gas Chromatography

A gas chromatograph with electron capture detection is required. This should be operated in accordance with manufacturers instructions. On-column or glass lined injection systems should be used. Many different columns have been used for insecticide 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. For Use of Capilliary Columns, which are most effective, see the addition to the Water Method at the end of the booklet. Columns should have an efficiency of better than 1500 theoretical plates and give minimal 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 hours 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 full calibration daily and 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 strengths of the standard solutions and the volume to which the extract is finally made-up.

6.12.1 Slight changes in the Gas Chromatograph may be needed for some of the tests in the Appendix.

6.13 Perchlorination tube — see Figure 4.

6.14 Pasteur pipette.

7 Standards

7.1 Preparation

Individual standards may be made up in hexane (5.4) 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 similar heights. The concentrations needed to achieve this will vary with each instrument but the following procedure has been found to give satisfactory results:—

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

γ-HCH	40 mg
Aldrin	60 mg
Dieldrin	100 mg
pp'-DDE	100 mg
pp'-TDE	200 mg
pp'-DDT	400 mg
Aroclor 1260	2000 mg

7.1.1 *Individual standards*

Individual standards may be made by taking 100 μl of a primary stock solution in a 100 or 250 μl syringe and diluting to 100 ml with hexane (5.4). Then 100 μl of the diluted solution

is further diluted to 10 ml with hexane. A 5 μl aliquot of the final diluted solution contains x/2 pg of the material. Separate syringes should be used for serial dilutions at different concentration levels.

7.1.2 *Composite Standards of Insecticides*

Composite standards of insecticides, which must not contain PCB's, may be made by adding 100 μl of each primary insecticide stock solution to a volumetric flask and diluting to 100 ml with hexane. 100 μl of this solution is then further diluted to 10 ml to give a composite standard containing x/2 pg of each insecticide per 5 μl.

7.1.3 *Further calibration standards* may be diluted from the above solutions using either syringes, pipettes or burettes. Pipettes may give significant drainage errors using solvents. Standards should be diluted to cover the linear range of the detector in use, in steps of 1, 2, 5 and 10 pg/5 μl HCH etc or corresponding quantities of other insecticides.

7.2 Storage

Most organo-chlorine 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:

7.2.1 Store primary stock solution in the dark, in a spark-proof refrigerator.

7.2.2 Hexane and acetone are volatile and on prolonged storage significant leakage past ground glass joints will occur. This may be compensated for in primary stock solution by recording the gross weight of the solution and container. Before withdrawal of an aliquot after prolonged storage the solution and container are made up to the last recorded weight and mixed at room temperature. After withdrawal of the aliquot the new weight is recorded. The problem will be minimized if these solutions are stored in a spark-proof refrigerator.

7.2.3 The working standards should be stored in a spark-proof refrigerator and discarded when their volumes are depleted by 5%.

8 Sample Storage

Sample bottles should be all-glass with glass stoppers, cleaned in the manner described for glass apparatus (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 (ng/l) 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.

Degradation is particularly evident in sewage samples. It is recommended that all samples be extracted as soon as possible after sampling and the solvent extracts stored, if necessary, at 4°C in a refrigerator. Solvent extracts are stable for periods of 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.

Step	Procedure	Notes
9.1	**Settled Sewage**	
9.1.1	Place 500 ml ± 5 ml of sample in a 1 litre separating funnel.	
9.1.2	Cool add 50 ml ± 1 ml of hexane and shake the funnel for 5 minutes and allow to stand.	
9.1.3	Run off any deposited solids into a 100 ml separating funnel. Discard the clear aqueous phase. Collect the interfacial cuff in the same 100 ml separating funnel.	
9.1.4	To the 100 ml separating funnel add a volume of propan-2-ol which is approximately equal to that of the solids and emulsion previously collected. Shake for 5 mins. Add 25 ml of hexane and shake for 5 mins. Allow the layers to separate and discard the lower layer.	
9.1.5	Combine the hexane extracts in the 1 litre separating funnel and proceed to step 9.5.	
9.2	**Crude Sewage**	
9.2.1	Place 500 ml ± 5 ml of sample in a 1 litre separating funnel and allow to settle. Run the solids into a 100 ml separating funnel (a). The remaining sewage is extracted as described under step 9.1.	(a) Most insecticides will be associated with the solid material. Recoveries improve if the solids are extracted separately.
9.2.2	Add water to the solids to bring the volume up to 15 ml ± 1 ml (b). Add 15 ml ± 1 ml propan-2-ol and shake vigorously for 5 mins.	
9.2.3	Add 25 ml ± 1 ml hexane and shake vigorously for 5 minutes. Allow the layers to separate and run the lower layer into a second 100 ml separating funnel. Add a further 25 ml ± 1 ml hexane to the second separating funnel and shake vigorously for 5 minutes. (c).	(c) A wrist action shaker has been found to be convenient.
9.2.4	Add the hexane layer from the first extraction to the second funnel and allow the whole to settle. Run off any aqueous layer which may be present.	
9.2.5	Transfer the extract, emulsion and solids to a 100 ml centrifuge tube and centrifuge for 5 minutes at 1000 revs/minute.	
9.2.6	Combine the hexane extracts in the 1 litre separating funnel containing the hexane extract of the liquid from step 9.2.1 and proceed to step 9.5.	

Step	Procedure	Notes
9.3	**Sewage Sludge and Mud**	
9.3.1	Place a measured 5–10 ml sample of sludge or a 5 ± 0.1 g mud sample in a 100 ml separating funnel. (d)	(d) An estimate of total solids should be carried out on the sample.
9.3.2	Add water to bring the volume up to 15 ± 1 ml (b). Add 15 ml ± 1 ml propan-2-ol and shake vigorously for 5 minutes (c).	
9.3.3	Add 25 ml ± 1 ml hexane and shake vigorously for 5 minutes. (c) Allow separation to take place or centrifuge as in step 9.2.5 if necessary. Carefully pipette off the hexane layer and transfer to the drying column described in step 9.5. (e).	(e) A curved pipette is convenient.
9.3.4	Repeat the extract of the solids with a further 25 ml ± 1 ml of hexane and transfer the second extract to the same drying column. Proceed to step 9.5.	
9.4	**Fish Tissues**	
9.4.1	Weight 10 g ± 0.2 g of homogenized tissue (f) into a 250 ml beaker containing 30 g ± 2 g of granular anhydrous sodium sulphate and mix thoroughly with a metal spatula.	(f) A top drive macerator such as the Ultra Turrax TP 18–10 is satisfactory.
9.4.2	Add sufficient sodium sulphate to a 100 × 35 mm glass Soxhlet thimble to produce a 10–20 mm bed above the centre (g) and pack with the prepared tissue sample from step 9.4.1.	(g) This facilitates subsequent cleaning of the thimble.
9.4.3	Insert the packed thimble into the Soxhlet assembly fitted with a 250 ml flask containing anti-bumping granules and approximately 100 ml hexane. Extract for 2–3 hours.	
9.4.4	After cooling to room temperature, transfer the extract quantitatively to a 100 ml volumetric flask and dilute to the mark with hexane.	
9.4.5	Measure 50 ml ± 1 ml into a tared 100 ml flask and distill off the solvent on a steam bath in a fume cupboard. Cool to room temperature, re-weigh and calculate the total extractable solids. (h).	(h) The total extractable figure determines the appropriate aliquot to be used in the clean up stage and is necessary if residue levels are to be calculated on an 'extractable fat' basis. Allow a maximum of 100 mg of extractable solids onto the alumina column 6.2. For other clean up techniques see ref 1.
9.4.6	Evaporate the appropriate aliquot (h) to 1 ml ± 0.1 ml as in step 9.5 and proceed to step 9.6.	
9.5	**Concentration**	
	Dry the hexane extract by passing it through a column containing a 5 ± 1 g granular anhydrous sodium sulphate supported by a small plug of cotton wool. (i). Collect the dried extract in a Kuderna-Danish 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 ± 2 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). (j).	(i) Larger quantities of drying agent may be needed if water from emulsions or cuffs is included in the extracts. (j) Various methods of solvents evaporation have been used. All need care to avoid loss of the more volatile insecticides and contamination during the procedure.

Step	Procedure	Notes

9.6 Clean-Up Procedure

Prepare an alumina-alumina/silver nitrate column as in Section 6.2. 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 9.5. (k, l and m).

(k) During the additions to the column do not allow the meniscus of the hexane to fall below the surface of the alumina.

(l) If the alumina/silver nitrate column blackens along its entire length prepare a fresh column and repeat step 9.6. Experience in particular cases may indicate the use of different column sizes

(m) The examination of a 5 μl aliquot of the concentrated cleaned-up extract by gas chromatography at this stage will give a guide to the compounds present.

9.7 Separation of Insecticides and PCBs

Prepare a silica gel column as in section 6.3. Add the concentrated sample extract (1.0 ± 0.1 ml) to the column (note n). 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 n). Add 10 ml ± 0.2 ml hexane and collect the first 7 ml eluate (see note o). This eluate (fraction 1) contains PCBs (see note t). 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 (note p) (fraction 2).

(n) The liquid will penetrate the column. Retain the meniscus at the surface of the silica gel. Subsequently do not let the meniscus drop below the surface of the silica gel.

(o) 7 ml or a volume as determined in section 6.11.2.

Concentrate each fraction to 1.0 ± 0.1 ml as in step 9.5.

(p) Fraction 1 should contain, if present, PCBs, pp'-DDE, Heptachlor, Aldrin. Fraction 2 should contain the other organochlorine insecticides and their metabolites.

9.8 Gas Chromatography

Inject 5 μl of each extract on to each column and tentatively identify the components by comparison of their retention times with those obtained by the injection of 5 μl portions of calibration standards (notes (q) and (r)).

(q) 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. (see Appendix).

(r) A complete reagent blank should be carried out.

9.9 Calculation of Concentration

(a) 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:—

9.9.1 Crude and settled sewage

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

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

Step	Procedure	Notes

9.9.2 **Sewage sludge and mud**

$$C = \frac{vA}{5W} \text{ ng/g dry wt}$$

where W = weight of dry solids (in g) taken.

9.9.3 **Fish tissues**

$$C = \frac{vA}{5W_1} \times \frac{V_1}{V_2} \text{ ng/g wet fish}$$

Where W_1 = weight of wet fish taken
V_1 = total volume of extract (step 9.4.4)
V_2 = volume of extract taken (step 9.4.6)

(b) **PCB**

Aroclor 1260 is used as the standard material in the method but other PCB formulations may be more appropriate depending 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 closely resembling the sample or the peak areas can be calculated as DDE. (See Appendix A3.7)

10 Calibration of the Gas Chromatograph

Initially it will be necessary to inject each standard solution separately to obtain the retention times. Subsequently composite standards may be used. 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. Construct calibration graphs of peak height or peak area against the relevant quantity of material.

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
HCB	—	0.24	—	—	—	—	—	—	1.17
Chlorodane	—	—	—	—	—	—	—	—	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)

Table II Means and standard deviations

	Primary sewage ng/l			Settled sewage ng/l			Activated sludge ng/g dry weight			Mud ng/g dry weight			Fish ng/g wet weight		
	Mean	S_w	S_t	Mean	S_w	S_t	Mean	S_w	S_t	Mean	S_w	S_t	Mean	S_w	S_t
Lindane	136.6	20.67	38.48	50.7	5.19	7.22	85.1	24.22	42.84	0.3	0.38	0.36	1.0	0.16	1.13
Aldrin	2.4	5.27	7.08	0.2	0.57	0.53	1.9	0	0	0.3	1.27	1.18	0	0	0
Dieldrin	76.3	7.55	47.04	15.3	4.92	12.52	180.5	26.71	105.92	0	0	0	0.5	0.43	0.61
pp'-DDE	26.8	11.25	18.30	19.6	8.06	10.99	43.7	9.21	29.54	0	0	0	1.6	0.30	0.98
pp'-TDE	3.2	11.24	11.24	4.0	16.37	16.82	7.2	25.52	25.52	0	0	0	0.6	1.24	1.14
pp'-DDT	3.6	12.94	12.94	15.4	19.31	16.65	0	0	0	0.1	0	0	0.2	0.40	0.42
PCB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table III Standard deviation of blanks

Insecticide	Primary sewage		Settled sewage		Act. sludge		Mud		Fish	
	S_w	S_t	S_w	S_t	S_w	S_t	S_w	S_t	S_w	S_t
Lindane	2.5 (5)	4.4 (6)	6.0 (4)	6.0 (7)	6.3 (5)	8.5 (7)	0.1 (5)	0.2 (8)	0.2 (5)	0.3 (9)
Aldrin	0 (5)	0 (9)	1.8 (4)	1.8 (7)	8.1 (5)	8.1 (9)	0 (5)	0 (9)	0 (5)	0 (9)
Dieldrin	0 (5)	0.3 (4)	10.0 (4)	10.3 (7)	10.9 (9)	10.9 (9)	0 (5)	0 (9)	0 (5)	0 (9)
pp'-DDE	0 (5)	0 (9)	0 (4)	0 (7)	0 (5)	0 (9)	0 (5)	0 (5)	0.2 (5)	0.3 (9)
pp'-TDE	0 (5)	0 (9)	0 (4)	0 (7)	26.7 (5)	26.7 (9)	0.5 (5)	0.5 (9)	0.7 (5)	0.7 (9)
pp'-DDT	0 (5)	0 (9)	3.2 (4)	41.9 (7)	0 (5)	0 (9)	1.0 (5)	1.0 (9)	0.4 (5)	0.4 (9)
PCB	0 (5)	0 (9)	0 (4)	0 (7)	0 (5)	0 (9)	0 (5)	0 (9)	0 (5)	0 (9)

Figures in brackets represent the number of degrees of freedom.
The blanks under the conditions used in the tests often produced no discernible peak and hence zero has been entered in the tables.

Table IV Limits of detection

Limits of detection where possible have been calculated from the estimates of the standard deviation of the blanks. When no response was observed detection limits were calculated from the estimates of the standard deviation of a real sample. When no response was observed with these samples an estimation of the detection limit was made by ascertaining the minimum discernible response given by the chromatograph under the conditions of use.

Insecticide	Primary sewage ng/l	Settled sewage ng/l	Act sludge µg/g dry wt	Mud µg/g dry wt	Fish µg/g wet wt
Lindane	14.2	35.9	36.0	0.7	1.1
Aldrin	24.5*	10.6	46.2	5.9*	0.8†
Dieldrin	35.1*	60.2	62.3	0.8†	2.0*
pp'-DDE	52.3*	37.5*	42.8*	0.8†	1.4
pp'-TDE	52.3*	76.1*	152.0	2.8	4.2
pp'-DDT	60.2*	19.4	2.5†	5.8	2.2
PCB	7.2†	7.2†	7.2†	7.2†	7.2†

* Determined from estimates of the standard deviation of real samples.
† Minimum quantity required to give a discernible peak (Criterion of detection).

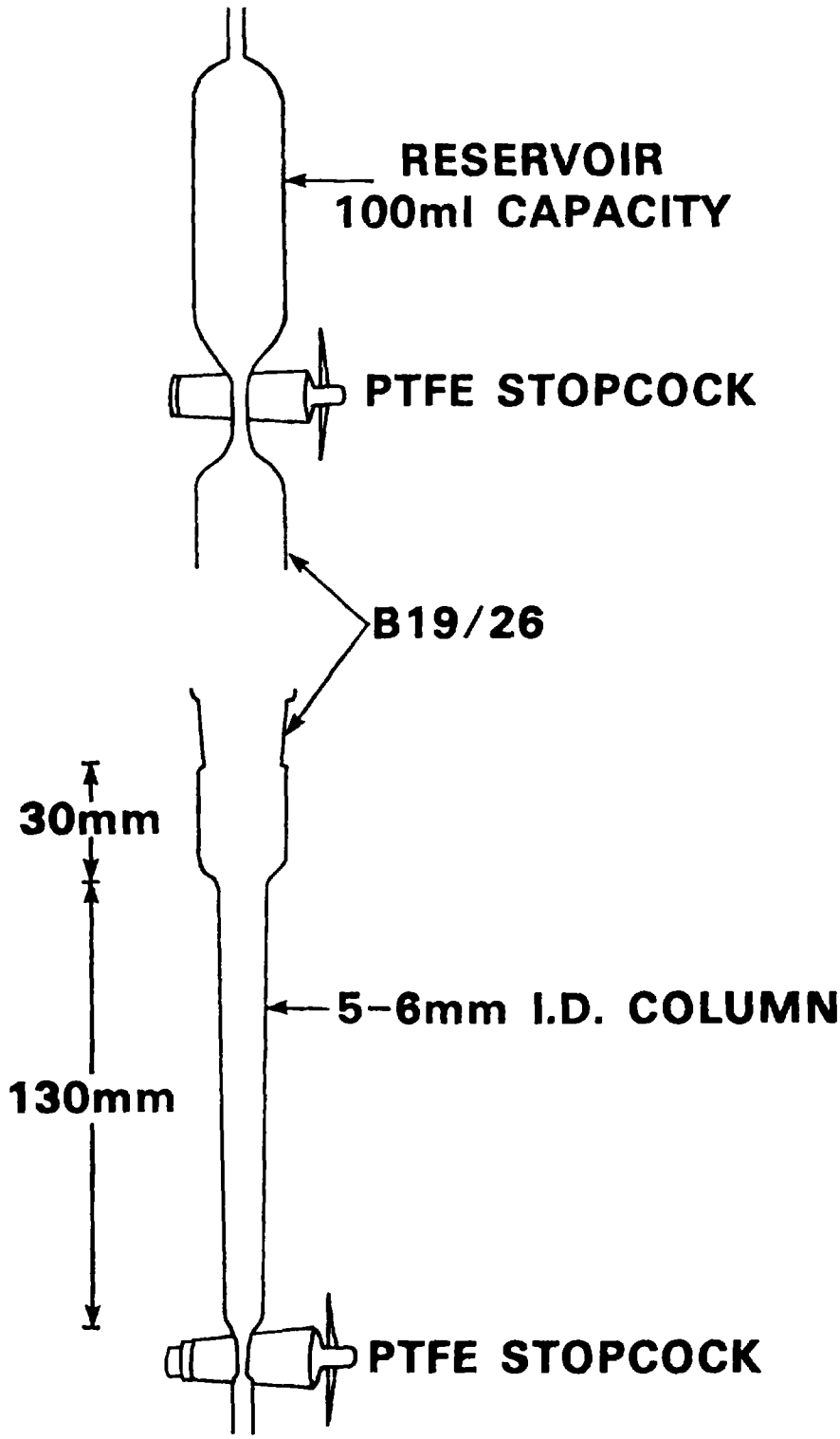

Fig. 1. Adsorption Column

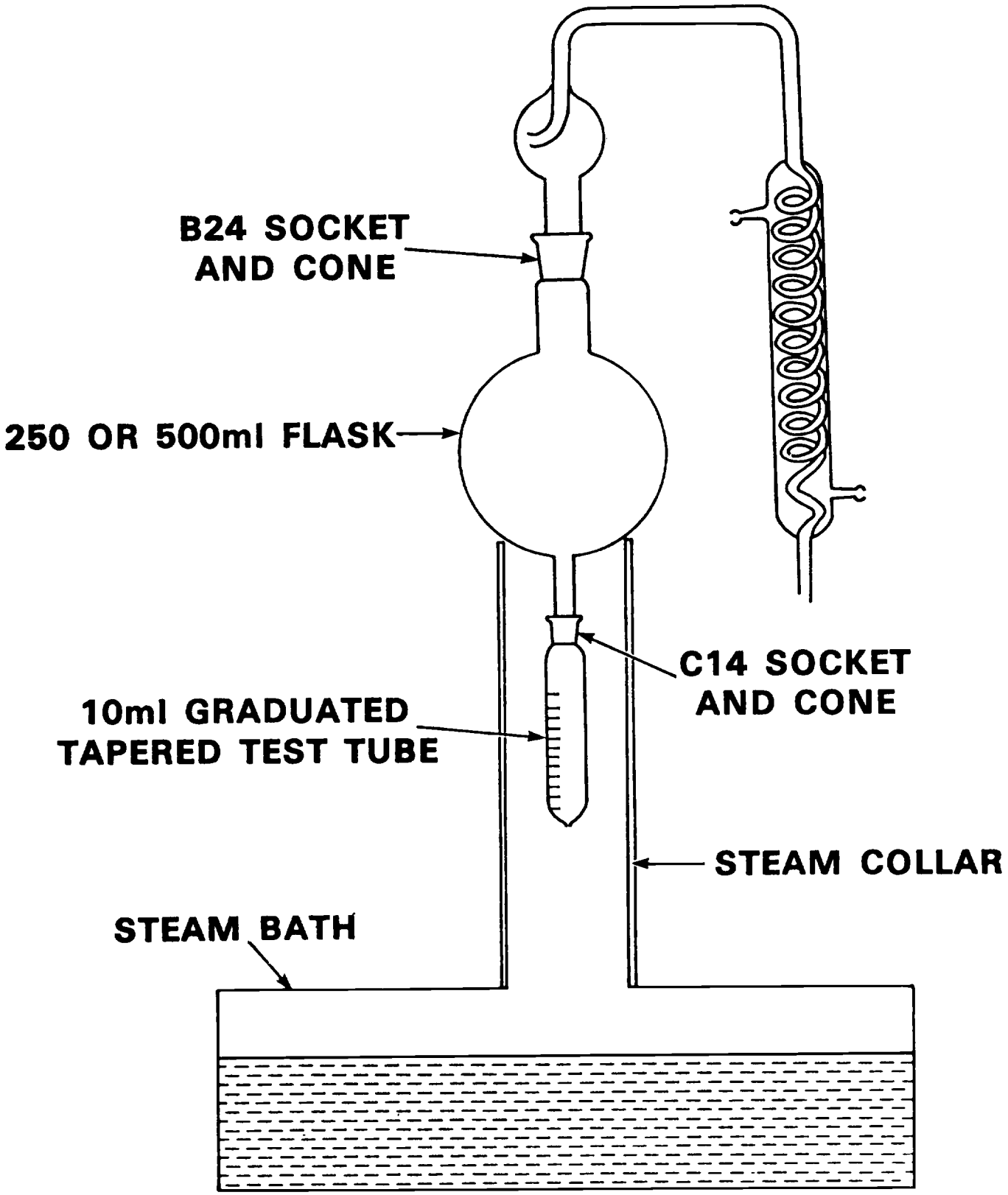

Fig. 2. Kuderna-Danish Evaporator System for Concentration of Pesticide Solutions

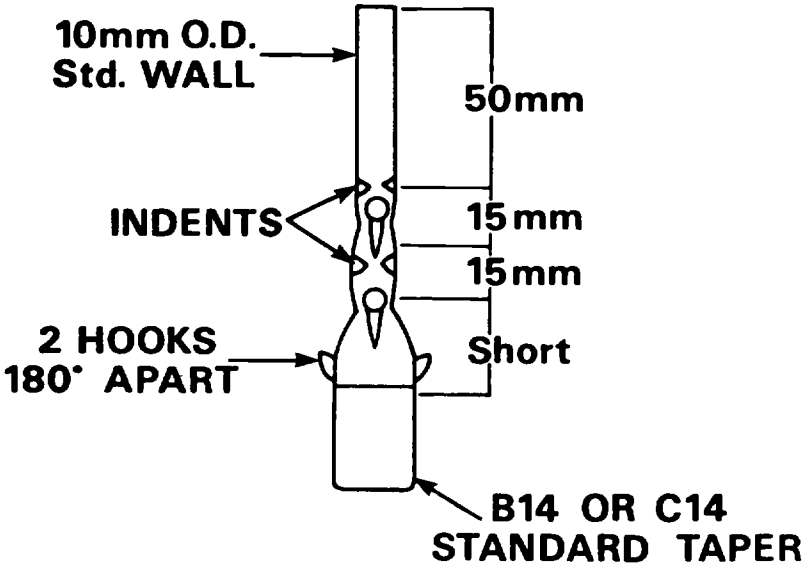

Fig. 3 Micro Snyder Column

Appendix

The Confirmation of the Identity of Chlorinated Insecticide Residues and PCB

A1 Introduction

As normally employed, gas-liquid chromatography does not give an absolute identification of residues found in a sample extract; peaks which appear on the chromatograms at the same retention times as the chlorinated insecticide residues may be due to the presence of other organic compounds with fortuitously similar properties. Confirmation of identity may be obtained at times by the use of other techniques such as infra-red or mass 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 using the sensitivity of electron-capture GLC to determine their presence (see Ref. 5). Proof of identity is then based on the disapperaance of the parent compound from the chromatogram and the appearance of the derivative peak(s) 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 eg 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 V) may be tried, but the combination of residues in samples is so variable that a standard procedure for their separation cannot be given.

A3 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 4 ON HAZARDS BEFORE STARTING THE FOLLOWING PROCEDURES

A3.1 **pp'-DDT** (Ref 6)

Step	Procedure	Notes
A3.1.1	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.	
A3.1.2	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.
A3.1.3	Remove the micro-Snyder column and introduce 1.0 ml ± 0.1 ml of freshly prepared 0.5 M alcoholic potassium hydroxide solution. Replace the micro-Snyder column.	
A3.1.4	Heat the tube for 5 mins in boiling water (note b).	(b) pp'-DDT is converted to pp'-DDE.
A3.1.5	Cool to room temperature, add 1.0 ml ± 0.1 ml of hexane, stopper the tube and shake vigorously for 30 s.	

Step	Procedure	Notes
A3.1.6	Add 10 ml ± 1 ml of 2% w/v sodium sulphate solution, stopper, shake and allow the two layers to separate.	
A3.1.7	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.
A3.1.8	Carry out a complete 'blank' test on all the reagents at the same time as the sample extract is being examined.	
A3.1.9	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.

A3.2 **pp'-TDE** (Ref 7)

Step	Procedure	Notes
A3.2.1–8	Use the procedure as outlined in A3.1 but extend the heating period in Step A3.1.4 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.
A3.2.9	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.

A3.3 **pp'-DDE** (Ref 8)

Step	Procedure	Notes
A3.3.1	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.	
A3.3.2	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.
A3.3.3	Remove the micro-Snyder column and introduce 2.0 ml ± 0.2 ml of glacial acetic acid and 100 mg ± 10 mg of chromium trioxide. (note b)	(b) The acid should be redistilled.
A3.3.4	Replace the micro-Snyder column and heat the tube in boiling water for 15 mins (note c).	(c) The reaction mixture should remain brown — if green, add more chromium trioxide and continue heating.
A3.3.5	Cool the tube to room temperature, add 2.0 ml of hexane, stopper the tube and shake vigorously for about 15 s.	
A3.3.6	Carefully, with cooling and agitation, add enough 5M sodium hydroxide solution to the tube to neutralize the acid (note d). Invert the tube several times and then set it aside until two layers separate.	(d) CAUTION: heat will be generated by the addition of the sodium hydroxide. About 7 ml will be required.

Step	Procedure	Notes
A3.3.7	Inject 5 μl 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.	
A3.3.8	Carry out a complete 'blank' test on all the reagents at the same time as the sample extract is being examined.	
A3.3.9	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 e).	(e) 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.

A3.4 **Dieldrin, Endrin, Heptachlor epoxide** (Refs. 6 and 7).

Step	Procedure	Notes
A3.4.1	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.	
A3.4.2	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.
A3.4.3	Cool the tube and introduce 0.5 ml of a 1 + 1 solution of concentrated hydrobromic acid and glacial acetic acid (note b).	(b) The acid should be redistilled.
A3.4.4	Replace the micro-Snyder column and heat the mixture in boiling water for 10 mins.	
A3.4.5	Cool to room temperature. Add 1.0 ml ± 0.1 ml hexane, stopper the tube and shake it vigorously for 30 s.	
A3.4.6	Dilute the solution with 10 ml of 2% sodium sulphate solution and set the tube aside until the two layers separate.	
A3.4.7	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 c).	(c) Concentrate or dilute the final solution to give a suitable peak for comparison.
A3.4.8	Carry out a complete 'blank' determination on all the reagents at the same time as the sample and standard are being examined.	
A3.4.9	The disappearance of the parent peak from the chromatograms and the appearance of two derivate peaks (note d) affords the proof of identity of the compound being examined.	(d) 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.

A3.5 **γ-HCH** (Ref 9).

Step	Procedure	Notes
A3.5.1	Transfer the solution to be examined to a 10 ml graduated test-tube, add 2 anti-bumping granules and 1 ml ± ml of freshly prepared 0.01 M alcoholic potassium hydroxide solution.	
A3.5.2	Carefully evaporate the hexane layer using a gentle stream of dry air or nitrogen.	
A3.5.3	Fit a micro-Snyder column to the tube and then heat the tube in boiling water for 5 mins.	
A3.5.4	Cool to room temperature, add 1.0 ml ± 0.1 ml of hexane, stopper the tube and shake vigorously for 15 s.	
A3.5.5	Dilute the mixture with about 8 ml of water and set the tube aside until the two layers have separated.	
A3.5.6	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.
A3.5.7	Carry out a complete 'blank' test on all the reagents at the same time as the sample is being examined.	
A3.5.8	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.	

A3.6 **Aldrin** (Ref 6).

Step	Procedure	Notes
A3.6.1	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 ± 0.05 ml of 10% aqueous solution of sodium hypochlorite (Section A4.10) may be used in place of the bleaching powder.
A3.6.2	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.	
A3.6.3	Dissolve the residue in 0.5 ml of chloroform and add 0.1 ml of the reagent prepared in Step A3.6.1.	
A3.6.4	Set the mixture aside for 5 mins at room temperature, and, after evaporating the chloroform to dryness, dissolve the residue in 1.0 ml ± 0.1 ml of hexane.	

Step	Procedure	Notes
A3.6.5	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.
A3.6.6	Carry out a complete 'blank' test on all the reagents at the same time as the sample and standard are being examined.	

A3.7 **Polychlorinated biphenyls (PCB)** (Ref 10).

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 A3.3.3); the polychlorinated biphenyls remain unchanged by this procedure.

Step	Procedure	Notes
A3.7.1	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 4).	
A3.7.2	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.
A3.7.3	Add about 0.01 g of iron powder and 0.2 ml of antimony pentachloride to the tube and close the PTFE tap (note b).	(b) Carry out these operations in a fume cupboard, wear rubber gloves and safety glasses.
A3.7.4	Place the lower end of the tube in an electric heating block which has been pre-heated to 180°C and leave it at this temperature for 10 mins.	
A3.7.5	Remove the tube from the block and allow it to cool. Point the side-arm away from the face and carefully open the tap and remove the stopper.	
A3.7.6	Introduce about 0.5 ml of 6 M 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.
A3.7.7	Add 2.0 ml ± 0.1 ml of hexane to the tube, close the tap and stopper and shake the mixture vigorously for 2 mins.	
A3.7.8	Allow the solutions to separate, then transfer 1.0 ml of the upper hexane layer to an adsorption column containing 2.0 g of alumina (10% moisture content), which has been slurried in hexane and overlayered with a little anhydrous sodium sulphate. Allow the extract to run into the column and, when the meniscus has just entered the surface of the sodium sulphate, add 6 ml of hexane. Collect all the eluate from the column in a graduated test-tube and adjust to a suitable volume for GLC examination.	

Step	Procedure	Notes
A3.7.9	Compare the resulting chromatogram with that produced by the treatment of a known amount of PCB (eg A1260). A reagent blank should always be run at the same time (Notes d, e and f).	(d) Concentrations of ca 1.0 μg/ml PCB are useful for most applications. (e) Some batches of antimony pentachloride contain bromide as an impurity which results in the formation of bromononachloro-biphenyl; 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. (f) See the note on the effect of unsubstituted biphenyl at the head of this section.

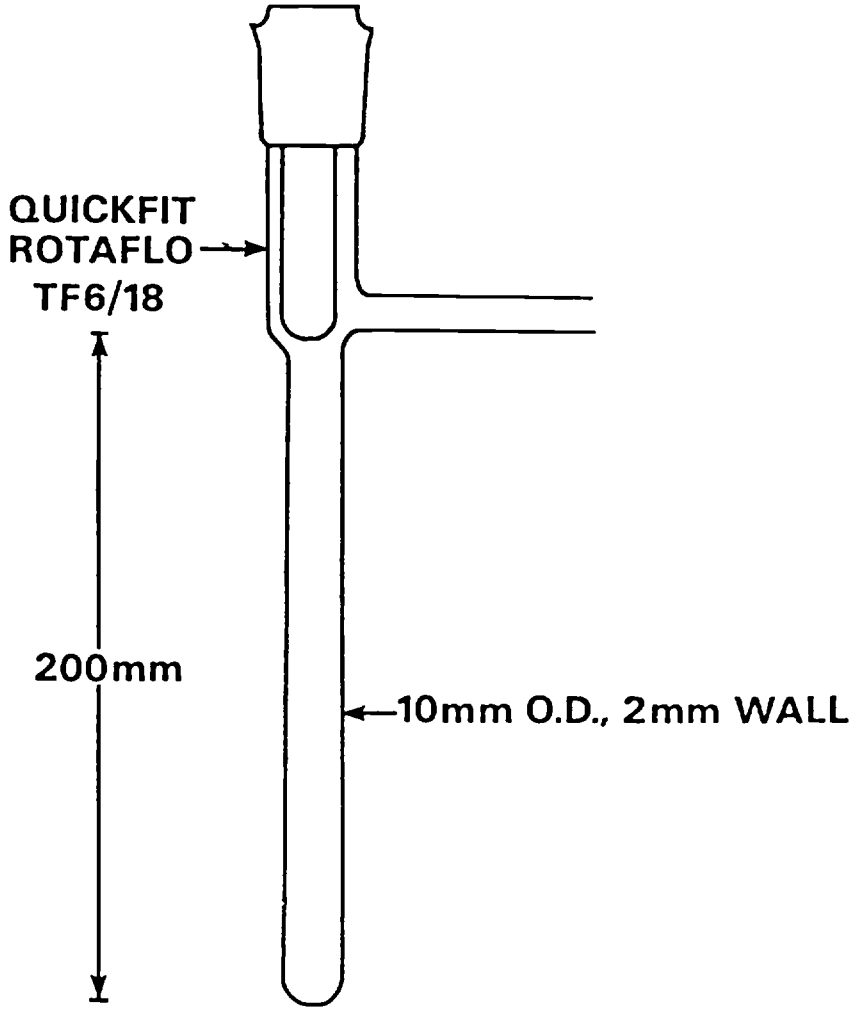

Fig. 4. Perchlorination Tube

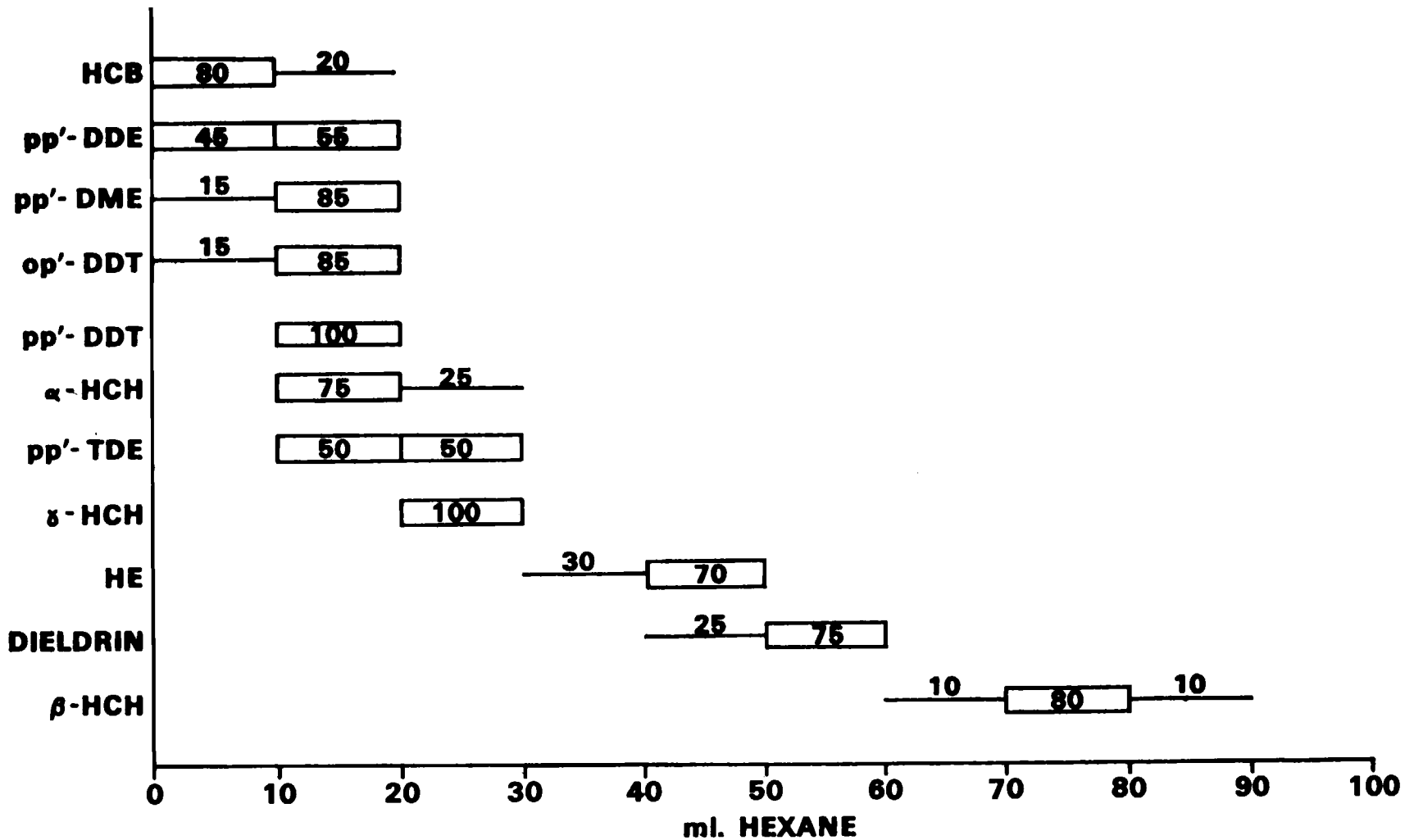

Table V % Recovery of Chlorinated Pesticide resudues from a 10g Alumina Column (7% Moisture)

Organochlorine Insecticides and Polychlorinated Biphenyls in Water, an addition, 1984

Use of capillary columns in PCB analysis

Wall-coated open tubular (WCOT) columns made from glass or fused silica are now in common use and, because of the increased resolution obtainable, are to be preferred for most pesticide residue analyses.

The most useful coating is a methyl silicone phase (eg OV1 or OV101) with a polar phase for confirmatory columns. Many appropriate and equivalent phases are commercially available and are perfectly satisfactory.

Splitless, split and on-column injection systems have been used but when high temperatures isothermal analyses are performed split injections usually give the best results.

In PCB analysis where interfering peaks can and do occur the use of capillary columns is advantageous as merged interfering peaks, which can give high results, are often resolved. Whilst (unlike air samples) this is not usually a serious problem in water samples, the use of capillary columns increases the certainty of identification and confidence in the analysis, especially if used in conjunction with the perchlorination confirmation procedure.

Suggested columns for use in organochlorine analysis are:

(1) 50m 0.3 m.m. ID WCOT glass or fused silica column coated with OV1.
(2) 50m 0.3 m.m. ID WCOT glass or fused silica column coated with Carbowax 20M TPA.

Analysis for Hexachlorobenzene (HCB)

HCB can be determined in water as well as sewage sludge using data in Table 1 of this booklet.

Improved Confirmatory Test for PCBs

The revised confirmatory test given for sludge in this booklet is equally suitable for water samples.

Amendment to 1977 Booklet

p11. Section 6.2.2.2. in the sixth line the elution volume should be 10 ml not 16 ml as given there.

Analytical Quality Control

Once the methods have been put into routine operation, many factors may subsequently adversely affect the accuracy of the analytical results. It is recommended that experimental tests to check sources of inaccuracy should be made regularly. Many tests are possible and they should be used as appropriate. As a minimum, it is suggested that at least one sample of suitable concentration in each batch of samples be analysed at least in duplicate. Inclusion of a quality control standard of concentration unknown to the actual operator is also useful. Plots of the deviation between multiplicate samples, or of the control standard result, will facilitate detection of inadequate precision and allow the standard deviation of routine analytical results to be estimated. For further information see Refs 11 and 12.

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(11) British Standards 5700 to 5703 inclusive.

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

Address for Correspondence

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

The Secretary
The Standing Committee of Analysts
The Department of the Environment
43 Marsham Street
LONDON SW1P 3PY
England

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