Chlorobenzenes in Water, Organochlorine Pesticides and PCBs in Turbid Waters, Halogenated Solvents and Related Compounds in Sewage Sludge and Waters, 1985

Methods for the Examination of Waters and Associated Materials

London Her Majesty's Stationery Office

Chlorobenzenes in Water

Organochlorine Pesticides and PCBs in Turbid Waters

Halogenated Solvents and Related Compounds in Sludge and Waters 1985

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Contents

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About this Booklet

This booklet contains 3 methods related to the Organochlorine Insecticide and PCBs in Water 1978 booklet, and a method for the determination Halogenated Solvents and Related Compounds in Sludges and Waters. These methods are:

A. Chlorobenzenes in Waters.

B. Hexachlorobenzene in Water.

C. A more complex variant of the 1978 booklet, using ethyl acetate for improved extraction efficiency, but requiring more time.

D. Halogenated Solvents and Related Compounds in Sludge and Waters.

All these methods are tentative and users are reminded to check them before use, using their own equipment, and samples.

Also given are two errata to the 1978 Sludge/1984 Water booklet.

All test data are from Stevenage Analytical Consultants under:

DOE Contracts PECD 7/7/100-142/83
PECD 7/7/101/143/83
PECD 7/7/108
PECD 7/7/149/85

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.

About this Series

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

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 These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print.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

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

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

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

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

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

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

L R PITTWELL
Secretary

1 July 1986

Warning to Users

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

Local Safety Regulations must be observed.

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

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

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

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

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

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

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

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A. Determination of Chlorobenzenes in Waters

A1 Performance Characteristics of the Method

A1.1	Substances determined	Chlorobenzene, o- and p-dichlorobenzene and other chlorinated benzenes. If Hexachlorobenzene is to be determined method B which follows is preferred.
A1.2	Types of sample	Waters and sewage effluent.
A1.3	Basis of method	The sample is extracted into hexane, dried, concentrated and examined by capillary column GC.
A1.4	Range of application	Chlorobenzene 0–40 μg/l, dichlorobenzenes 0–5 μg/l. The upper limit of the range can be extended by diluting the sample.
A1.5	Calibration curve	The range of linearity depends upon the detectors used. The instruments used in these tests gave a linear response over the ranges: chlorobenzene 0–40 μg/l dichlorobenzenes 0–5 μg/l.
A1.6	Standard deviation	See tables 1 and 2.
A1.7	Limit of detection	See table 3.
A1.8	Sensitivity	Dependent upon the instrument in use. The instruments used gave a peak of approximately 50% FSD, with a baseline noise level of 0.5% for 2.5 ng chlorobenzene, 0.1 ng o-dichlorobenzene, 0.2 ng p-dichlorobenzene.
A1.9	Bias	The extraction efficiency is normally slightly lower than 100%. The p-DCB results from the sewage effluent were systematically high.
A1.10	Interference	Co-extracted material which has a similar retention time to any of the determinands will interfere. In these tests only the sewage effluents contained any significant interferences.
A1.11	Time required for analysis	2 h for 1 sample if no clean-up required. 3 h for 1 sample if clean-up is necessary. 10 samples per day can be run on a routine basis.

Table 1 Means and standard deviations
Low spikes (CB = 1.0 μg/l, DCB = 0.1 μg/l)

Sample	Determinand	Mean (μg/l)	S_w	S_b	S_t
Distilled	CB	0.949	0.035 (10)	ns	0.0035(19)
Water	o-DCB	0.0993	0.0027(10)	ns	0.0034(16)
	p-DCB	0.0996	0.0024(10)	ns	0.0032(15)
Tap	CB	0.947	0.027 (10)	0.199(9)	0.201 (12)
Water	o-DCB	0.0972	0.0026(10)	ns	0.0035(15)
	p-DCB	0.0956	0.0034(10)	ns	0.0039(18)
River	CB	0.931	0.028 (10)	0.230(9)	0.232 (11)
Water	o-DCB	0.097	0.0047(10)	ns	0.0048(19)
	p-DCB	0.097	0.0020(10)	0.054(9)	0.054 (12)
Sewage	CB	0.864	0.041 (10)	0.239(9)	0.242 (12)
Effluent	o-DCB	0.090	0.0055(10)	0.078(9)	0.078 (14)
	p-DCB	0.091	0.0299(10)	0	0.029 (19)

Figures in brackets indicate Degrees of Freedom.
ns denotes not significant.
For DCB in the effluent the results are blank corrected.

Table 2 Means and standard deviations
Low spikes (CB = 10 μg/l, DCB = 1 μg/l)

Sample	Determinand	Mean (μg/l)	S_w	S_b	S_t
Distilled	CB	9.59	0.296(10)	ns	0.367(16)
Water	o-DCB	0.976	0.053(10)	0(9)	0.053(19)
	p-DCB	0.986	0.046(10)	0(9)	0.046(17)
Tap	CB	9.68	0.292(10)	0.625(9)	0.690(13)
Water	o-DCB	0.986	0.038(10)	0.201(9)	0.205(14)
	p-DCB	0.987	0.039(10)	ns	0.051(16)
River	CB	9.44	0.531(10)	ns	0.542(19)
Water	o-DCB	0.979	0.078(10)	0(9)	0.078(17)
	p-DCB	0.983	0.060(10)	0(9)	0.060(19)
Sewage	CB	8.64	0.466(10)	0.929(9)	1.04 (11)
Effluent	o-DCB	0.994	0.045(10)	0.256(9)	0.260(12)
	p-DCB	1.10	0.042(10)	0.418(9)	0.420(10)

Figures in brackets indicate Degrees of Freedom.
ns denotes not significant.
For DCB in the effluent the results are blank corrected.

Table 3 Limits of Detection (μg/l)

Sample	CB	o-DCB	p-DCB
Distilled	0.16	0.012	0.011
Tap	0.13	0.012	0.016
River	0.13	0.022	0.009
Effluent	0.19	0.42	0.42

These limits were calculated from the estimate of the standard deviation of the low spiked sample, ($4.65 \times S_w$).

A2 Principle

The sample is extracted into hexane, dried out and concentrated by evaporation. Sewage effluent samples are cleaned up using an alumina column (with 7% moisture). Samples are finally examined by capillary column isothermal gas chromatography using either an electron capture or a flame ionization detector. The oven temperature may be different for each chlorobenzenes.

A3 Interferences

Any substance, soluble in hexane, not retained by alumina (if used), having similar retention times for the columns under the conditions used will interfere.

A4 Hazards

Organochlorine compounds can be toxic, some more than others. Avoid ingestion. Care must be taken when handling the pure compounds and concentrated solutions. Wear disposable 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.

A5 Reagents

A5.1 Sodium sulphate—anhydrous, heated at 500°C for 4 h.

A5.2 Alumina—Woelm W200 neutral, deactivated by agitation with 7% water.

A5.3 Hexane—fraction from petroleum, redistilled from a glass still with a 1.5 m fractionating column and a variable take-off still head.

A5.4 Acetone—MFC grade.

A5.5 Standard Solutions

A5.5.1 Stock Acetone Solution: 0.4 g/l chlorobenzene, 0.04 g/l dichlorobenzenes, 0.004 g/l other chlorobenzenes. To 200 ± 10 ml of Acetone (A5.4) add the following amounts of the required chlorobenzenes: chlorobenzene 0.1 ± 0.0001 g, dichlorobenzenes 0.01 ± 0.0001 g, higher chlorobenzenes 0.001 ± 0.0001 g, dissolve, transfer quantitativity to a 250 ml glass-stoppered calibrated flask, using small portions of acetone, and make up to the mark with acetone, stopper and mix thoroughly. Store the flask inside a closed polyethylene bag in a spark proof refrigerator. Always shake before use. Discard when a quality control standard prepared from this solution shows a noticeable reduction in peak height for any substance in the standard. Prepare a fresh standard and recheck the peak height as a precaution against other variables.

A5.5.2 Working Standards—1 μg/l chlorobenzene, 0.1 μg/l dichlorobenzenes, 10 ng/l other chlorobenzenes, in water or higher concentrations as required. For the above concentrations add 5 μl of the Stock Solution A5.5.1 to 2000 ± 5 ml water in a glass stoppered bottle, stopper and mix well.

For other concentrations change the volumes of master solution and water proportionally.

A6 Apparatus

A6.1 Bottles. All glass 2L capacity cleaned with chromic cleaner or equivalent.

A6.2 Shaking machine. Laboratory type with tray, in which bottles can be shaken in a horizontal plane at 1 cycle per second.

A6.3 Solvent extract removal device. This is made from a Dreschel bottle top which allows tap water to be forced down the long arm into the bottle while the solvent rises and spills over into a receiver. The tap water used must contain no detectable quantities of the chlorobenzenes. (See Fig. 1).

A6.4 Glass columns. 130 mm long, 5–6 mm I.D. with reservoir. (Fig. 2).

A6.5 Kuderna-Danish evaporators (Fig. 3).

A6.6 Graduated centrifuge tubes, 10 ml, tapered, glass stoppered.

A6.7 Nitrogen line. Silica gel filtered, with glass jet.

A6.8 Gas chromatograph. A machine with interchangeable detectors, or a machine with an electron capture detector and a flame ionization detector is required.

A6.8.1 Chromatographic conditions.

For chlorobenzene:

Injection	—Split 5:1 operated at 250°C.
Column	—50 m OV1 WCOT fused silica (thick film).
Detector	—FID operated at 250°C.
Carrier gas	—Hydrogen, flow rate 1.5 ml/min.
Oven temperature	—65°C.
Volume injected	—5 μl.

For dichlorobenzenes:

Injection	—Split 5:1 operated at 250°C.

Column	—50 OV1 WCOT fused silica.
Detector	—EC operated at 250°C.
Carrier gas	—Hydrogen, flow rate 1.5 ml/min.
Oven temperature	—90°C.
Volume injected	—5 μl.

A7 Sample Storage and Preservation

Sample bottles should be all-glass with glass stoppers, cleaned in the manner described for glass apparatus (see Section A5). 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 chlorobenzene, 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.

A8 Analytical Procedure

For Higher Chlorobenzenes see Section A9. The conditions will vary depending on the column and instrument used.

Step	Procedure	Notes
A8.1	To 2 litres of sample in a calibrated bottle add 20 ml hexane and shake for 10 min in a shaking machine.	
A8.2	Replace the glass stopper with solvent removal device shown in Fig. 1 and force the solvent into a 100 ml separating funnel with tap water. (Notes (a) and (b)).	(a) Some interfacial cuff was present in the effluent samples. This was also transferred. (b) Addition of sodium sulphate to the separator assists with effluent samples when emulsions may have formed.
A8.3	Discard any aqueous layer which has been carried over with the solvent and transfer the remaining solvent layer to a drying tube packed with sodium sulphate. Allow the eluate from the drying tube to drip into a Kuderna-Danish evaporator fitted with a centrifuge tube.	
A8.4	Evaporate the solvent to about 5 ml in the Kuderna on a steam bath and then adjust the volume to 5.0 ± 0.05 ml using a gentle steam of nitrogen.	
A8.5	If no clean-up is required proceed directly to the chromatrographic analysis (Step A10) otherwise proceed to A8.6 (note (c)).	(c) In most instances, only the sewage effluent samples need be cleaned-up.
A8.6	Further evaporate the extract to 1 ml ± 0.01 ml on the nitrogen line.	
A8.7	Plug a glass column with defatted cotton wool and slurry in 2.0 g of 7% alumina in hexane. Cap the column with a 5 mm layer of sodium	

Step	Procedure	Notes
	sulphate and allow the hexane to drain to the level of the top of the packing. Add the sample extract and wash in with 2×0.5 ml portions of hexane.	
A8.8	Elute the column with 25 ml hexane and collect the eluate in a Kuderna-Danish evaporator fitted with a 10 ml centrifuge tube.	
A8.9	Evaporate the sample extract to about 5 ml in the Kuderna on a steam bath and then adjust the volume to 5.0±0.05 ml using, if necessary, a gentle stream of nitrogen.	
Chromatography		
A8.10	Inject 5 µl of samples and standards into the chromatograph operated in the manner described in A6.8.1 (note (d)).	(d) At the temperatures stated and with the capillary columns and flow rates used the retention times for the three compounds were: chlorobenzene — 9.4 min o-dichlorobenzene — 14.3 min p-dichlorobenzene — 13.8 min Retention times were measured from the point of injection with no correction made for an unretained peak. At the temperatures used the higher chlorinated benzenes do not emerge in any reasonable time or with a satisfactory shape. They can, however, be determined by the same method when the oven temperature is increased.
Calculation		
A8.11	Compare the sample peak heights obtained on the chart recorder with those of the standards and calculate the concentration of each chlorobenzene in the original sample (note (e)).	(e) Integrated peak areas are satisfactory.

A9 Determination of Higher Chlorinated Benzenes

The equipment used is that described for the chlorobenzene determination except that the oven is programmed from 35°C to 260°C per minute. A 5 minute initial period at 35°C was allowed. Comparisons are made with standards, but typical retention times are given in Table 4.

Compound	Retention time	Retention Index
chlorobenzene	17.39	825
p-dichlorobenzene	25.48	993
o-dichlorobenzene	26.55	1017
1,3,5-trichlorobenzene	31.29	1122
1,2,4-trichlorobenzene	33.06	1163
1,2,5-trichlorobenzene	34.50	1198
1,2,3,5-tetrachlorobenzene 1,2,4,5-tetrachlorobenzene	39.42	1320
1,2,3,4-tetrachlorobenzene	41.14	1365
pentachlorobenzene	46.58	1516

When run singly 1,2,3,5-tetrachlorobenzene emerges 3 seconds before 1,2,4,5 but in mixture the peaks merge.

The linear temperature programmed retention indices were calculated, using a curve fitting computer program, by comparison with a series of n-alkanes added to the mixed standard. The index is 100×n-alkane carbon number (e.g. C8 = 800, C15 = 1500) with the sample values interpolated.

B. Determination of Hexachlorobenzene in Waters

B1 Performance Characteristics of the Method

B1.1	Substance determined	Hexachlorobenzene.
B1.2	Type of sample	Waters.
B1.3	Basis of method	Extraction into hexane and removal of extraneous materials using a column of alumina-silver nitrate. Separation of HCB by column chromatography on silica-gel followed by gas liquid chromatography using an electron capture detector.
B1.4	Range of application	0–50 ng per litre. The upper limit of the range can be extended by diluting the sample.
B1.5	Calibration curve	The range of linearity depends on the detector in use. The instrument used in the performance tests (Pye 104) gave a linear response over the range 0–100 pg HCB injected.
B1.6	Standard deviation	

Sample	Mean (ng/l)	S_t
Distilled water	0.1	0.1(12)
Distilled water + 5 ng/l	3.7	0.8 (8)
Distilled water + 50 ng/l	38.8	3.6 (8)
River water	0.1	0.1(12)
River water + 5 ng/l	3.9	0.2 (8)
River water + 50 ng/l	38.7	2.3 (6)

B1.7	Limit of detection	0.6 ng/l.
B1.8	Sensitivity	Dependent upon the instrument in use. The instrument used for the performance tests gave a peak of approximately 50% full-scale deflection for 25 pg of HCB when the baseline noise level was 0.5%.
B1.9	Bias	The extraction efficiency is less than 100%. The mean recovery of HCB from distilled water was 75.4% and from river water 77.2%. The presence of up to 20 mg/l of suspended solids does not affect the recovery.
B1.10	Interference	Any electron capturing material which passes through the procedure and has a similar retention time to HCB will interfere. Failure to properly roast the silica-gel may result in gross interference.
B1.11	Time required for analysis	20 samples per week.

B2 Principle

Hexachlorobenzene is extracted into hexane. The extract is cleaned up on an alumina-silver nitrate column (1). Separation is achieved by column chromatography on silica gel prior to gas-liquid chromatography.

B3 Interferences

In principle, any substance soluble in hexane capable of producing a response on the electron capture detector at a retention time indistinguishable from hexachlorobenzene will interfere. The use of capillary columns will minimize the risk of interference.

B4 Hazards

Organochlorine compounds 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.

Diethyl ether may contain peroxides and there is risk of explosion. Old stock should not be used. The bottles used should contain copper gauze to prevent formation of peroxides.

For more detailed information on ether peroxides, detection and safe disposal or recovery of contaminated material see Ref 5. Ether vapour is heavy and may creep along floors and drains. Care should also be taken to prevent flash backs from sources of ignition well removed from the place of use.

B5 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.

B5.1 Acetone—MFC

B5.2 Diethyl ether—redistilled from potassium hydroxide and quinol using a 1.5 m factionating column and a variable take-off stillhead. Only sufficient ether for immediate use should be prepared.

B5.3 Hexane—fraction from petroleum. Boiling range not less than 95% between 67°C and 70°C. Redistilled from potassium hydroxide using a 1.5 m fractionating column and a variable take-off stillhead.

B5.4 Water—distilled in an all-glass apparatus and stored under hexane in an all-glass aspirator.

B5.5 Cotton wool—washed with hexane and ether before use.

B5.6 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.

B5.7 Silver nitrate—analytical reagent grade.

B5.8 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 B5.6). 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.

B5.9 Alumina-silver nitrate—Dissolve 0.75 g ± 0.01 g 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.

B5.10 Anti-bumping granules—wash with acetone before use.

B5.11 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.

B5.12 Prepared silica gel—To a suitable weight of dry silica gel (section B5.11) 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. If there is a need to test activity of column, see Ref. 1 section C6.2.2.2.

B5.13 Standard Solutions:

B5.13.1 Stock solution: Dissolve 100 mg of hexachlorobenzene in 10 ml acetone.

B5.13.2 Spiking solution: Using a microlitre syringe add 10 µl of the stock solution to 10 ml of acetone. The strength of this solution is 10 ng per µl.

B5.13.3 Preparation of spiked samples for testing the complete procedure: Spike five 2 L samples of water with:

1 µl	spiking solution	(= 5 ng/l)
2 µl	" "	(= 10 ng/l)
5 µl	" "	(= 25 ng/l)
10 µl	" "	(= 50 ng/l)
20 µl	" "	(= 100 ng/l)

B5.13.4 Direct standards:

Suitable standards are:

2 µg/l	(2 µl	spiking solution	in	10 ml	hexane)
4 µg/l	(4 µl	"	"	"	"	"
10 µg/l	(10 µl	"	"	"	"	"
20 µg/l	(20 µl	"	"	"	"	"
40 µg/l	(40 µl	"	"	"	"	"

These standards give a range equivalent to 5–100 ng/l in the original sample.

B6 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.

B6.1 General apparatus.

B6.1.1 Glass Columns—130 mm long by 5–6 mm internal diameter (Fig. 2).

B6.1.2 Kuderna-Danish evaporator (see Fig. 3).

B6.1.3 Micro-Synder column (see Fig. 4).

B6.1.4 Graduated centrifuge tubes (10 ml, 0.1 ml graduations) tapered, glass stoppered.

B6.1.5 Air or nitrogen line—the supply of gas must be cleaned by passage through a column of 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 0.55 mm at a distance of 20 mm from the liquid).

B6.1.6 Separating funnel 3-1 capacity with a glass tap (without grease) or a PTFE* tap.

B6.1.7 Bottle rolling machine suitable for rolling bottles at 60 rpm or laboratory shaking machine with tray in which bottles can be shaken in a horizontal plane at one cycle per second.

B6.2 Special apparatus

B6.2.1 Alumina—alumina/silver nitrate column.

Plug a glass column (B6.1.1) with cotton wool. Put in 15 ± 1 ml hexane then add 1.0 g ± 0.2 g alumina/silver nitrate (see Section B5.9) and allow to settle. Then add 2.0 g ± 0.2 g alumina (7% moisture) (see Section B5.8) 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.

B6.2.2 Silica Gel column.

B6.2.2.1 Preparation.

Plug a glass column (B6.1.1) with cotton wool. Add 2.00 g ± 0.01 g prepared silica gel (see Section B5.12). 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.

The activity of the column should be checked at least prior to use of each fresh batch of silica gel, more frequently if deemed necessary, Section C6.2.2.

B6.3 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. Columns should have an efficiency of better than 1,000 theoretical plates. Many different columns have been used. Four 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% OF-1 by weight. Operated at 190°C and 50 ml/min of nitrogen.

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 of nitrogen.

iii. 50 m 0.3 mm ID WCOT film thickness 1 um glass or fused silica column coated with OV1.

iv. 50 m 0.3 mm ID WCOT film thickness 1 um glass or fused silica column coated with Carbowax 20 M.

B7 Sample Storage and Preservation

Sample bottles should be all-glass with glass stoppers, cleaned in the manner described for glass apparatus (see Section B6). 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

*PTFE: Polytetrafluorethylene.

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 chlorinated benzene 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 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.

B8 Analytical Procedure

Step	Procedure	Notes
B8.1	**Extraction**	
B8.1.1	To approximately 2l 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) 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.
B8.1.2	Alternative procedures for waters not containing sediment: To 2l 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 B8.1.2 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 B8.1.2 stoppers must be held into the bottles in some suitable manner (e.g. Ref. 3). (f) Alternative methods of separating the phases have been found suitable which avoid transference to a second vessel (Refs 3 and 4).
B8.2	**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	(g) Larger quantities of drying agent may be needed if water from emulsions or cuffs is included in the extract.

Step	Procedure	Notes
	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 steam of dry air or nitrogen with the tube placed in a warm bath (not exceeding 40°C) (notes (g) and (h)).	(h) Various methods of solvent evaporation have been used. All need care to avoid loss of the more volatile substances and contamination during the procedure.
B8.3	**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 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 B8.3. (k) The examination of a 5 µl aliquot of the concentrated clean-up extract by gas chromatography at this stage will give a qualitative guide to the compounds present.
B8.4	**Separation of HCB from other insecticides**	
	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 (l)). 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 (l)). Add 10 ml ± 0.2 ml hexane and collect the first 7 ml eluate (fraction 1) (note (m)). If pesticides are also present (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 B8.2. If HCB only is to be determined, only fraction 1 is collected. All the HCB should be present in this fraction but this should be checked previously with a standard.	(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 B6.2.2.2. (n) Fraction 1 should contain (if present) HCB plus PCB, pp'DDE, Heptachlor, Aldrin and part of any op'DDT present. Fraction 2 should contain the other organochlorine insecticides and their metabolites.
B8.5	**Gas chromatography**	
B8.5.1	Calibration of gas chromatography. 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 HCB injected (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. Other injection volumes may be necessary if capillary columns are used.

Step	Procedure	Notes
B8.5.2	**Examination of extracts**	
	Inject 5 μl of the extract on to each column and compare the retention time of the HCB peak with that of the calibration standard (notes (p) and (q)).	(p) It is essential that the identity of the peak 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. 2). (q) A complete reagent blank should be carried out.
D8.6	**Calculation of Concentration**	
	Measure peak areas or heights and read off the quantity of HCB (A pg) from the calibration graph. Calculate the concentration (C ng/l) of HCB as follows: $C = \frac{200\ vA}{V}$ 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.	

C. Determination Organochlorine Insecticides and Polychlorinated Biphenyls in Turbid Waters

This method supplements but does not replace the 1977 and 1984 versions. Apart from Aldrin and ppDDT, the testing has not been as thorough as for the earlier versions on which it is based. It offers an improvement in recovery when the samples are turbid.

C1 Performance Characteristics of the Method

C1.1	Substances determined	Organochlorine insecticides and polychlorinated biphenyls (PCB).
C1.2	Type of sample	Natural waters, drinking water and sewage effluents.
C1.3	Basis of method	Double extraction into ethyl acetate, evaporation and re-extraction into hexane then 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.
C1.4	Range of application	Typically up to 250 ng/l.
C1.5	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
C1.6	Standard Deviation	Similar to Tables II and III in Ref. 1.
C1.7	Limit of Detection	At least as low as Table II in Ref. 1.
C1.8	Sensitivity	Dependent on determinand and instrument in use.
C1.9	Bias	The recoveries of insecticides are generally better than those given in Ref. 1 Table III, for DDT being between 85 and 106%, while for Aldrin the recovery is at least between 69 and 93%. If extraction is poor and the standards used are made up in the extraction solvent there

* 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); Pryoclor (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.

		will be a negative bias varying with sample and determinand. For practical reasons, the convention is then to report the biased results thus obtained without correction for recovery. (It may be wise to state this with the result).
C1.10	Interference	Any electron-capturing material which passes through the procedure and has similar gas chromatographic characteristics to the determinand.
C1.11	Time required for analysis	Assuming all reagents prepared and the instrument already calibrated, extraction and clean-up 3 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.

C2 Principle

The insecticides and PCB are doubly extracted into ethyl acetate. A keeper (paraffin) is added, the ethyl acetate evaporated off, the extract taken up in hexane and then 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.

C3 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 Ref. 1 (especially Table 1, the Appendix and Refs. 2, 3, and 5 therein) will provide a further choice of column and identification techniques.

C4 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.

Diethyl ether may contain peroxides and there is risk of explosion. See Section B4 and Ref. 5.

C5 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.

C5.1 Acetone—redistilled using a 300 mm Dufton spiral fractionation column or equivalent. Some batches of solvent may be acceptable without redistillation.

C5.2 Diethyl ether—redistilled from potassium hydroxide and quinol, see B5.2 or Ref. 5.

C5.3 Ethyl acetate—redistilled.

C5.4 Hexane—fraction from petroleum. Boil range not less than 95% between 67°C and 70°C. Redistilled as in B5.2 (hereafter called hexane).

C5.5 Water—distilled in an all-glass apparatus and stored under hexane in an all-glass aspirator.

C5.6 Cotton wool—washed with hexane and ether before use.

C5.7 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.

C5.8 Silver nitrate—analytical reagent grade.

C5.9 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 C5.7). 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.

C5.10 Alumina-silver nitrate—Dissolve 0.75 g ± 0.01 g silver nitrate in 0.75 ml ± 0.01 ml water (use as 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.

C5.11 Anti-bumping granules—wash with acetone before use.

C5.12 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.

C5.13 Prepared silica gel—To a suitable weight of dry silica gel (section C5.12) 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.

C5.14 Standard solutions

HAZARD—See Section C4.

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:

C5.14.1 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

C5.14.2 Individual standards

Take 100 μl of a primary stock solution (C5.14.1) 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 100 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.

C5.14.3 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 per 5 μl γ-HCH or corresponding quantities of other insecticides.

C5.14.4 Silica gel test solutions

C5.14.4.1 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.

C5.14.4.2 Prepare a solution in hexane such that 1 ml contains 1 μg Aroclor 1260.

C5.15 Keeper

either (i) Medicinal Liquid Paraffin BP,
(ii) Toluene—redistilled, or
(iii) n-decane

C5.16 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:

C5.16.1 Primary stock solutions should be stored in the dark in a spark-proof refrigerator.

C5.16.2 Hexane and Acetone are volatile. Significant loss may occur on prolonged storage due to diffusion at ground glass joints. This may be minimized 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.

C5.16.3 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.

C5.17 Alternative Standard Solutions

If extraction recovery is to be included, primary standard and solutions in acetone (C5.14.1) may be converted to secondary standards in water by a similar procedure to that given in A5.5.2 adding known amounts of the acetone solution to a large volume of water, being careful not to exceed the solubility of the pesticide concerned.

C5.18 Sulphuric acid (approx. 2 M) carefully pour 55 ± 5 ml of d_{20} 1.84 sulphuric acid into about 700 ml of water, transfer to a measure and make up to 1 litre with water.

C6 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.

C6.1 General apparatus

C6.1.1 Glass Columns—130 mm long by 5–6 mm internal diameter (Fig. 2).

C6.1.2 Kuderna-Danish evaporator (see Fig. 3).

C6.1.3 Micro-Synder column (see Fig. 4).

C6.1.4 Graduated centrifuge tubes 10 ml, (0.1 ml graduations) tapered, glass stoppered.

C6.1.5 Air or nitrogen line—the supply of gas must be cleaned by passage through a column of 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 0.55 mm at a distance of 20 mm from the liquid).

C6.1.6 Separating funnel 3-1 capacity with a glass tap (without grease) or a PTFE* tap.

C6.1.7 Bottle rolling machine suitable for rolling bottles at 60 rpm.

C6.2 Special apparatus

C6.2.1 Alumina—alumina/silver nitrate column.

Plug a glass column (C5.10) with cotton wool. Put in 15 ± 1 ml hexane then add $1.0\,g \pm 0.2\,g$ alumina/silver nitrate (see Section B5.9) and allow to settle. Then add $2.0\,g \pm 0.2\,g$ alumina (7% moisture) (see Section C5.9) 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.

C6.2.2 Silica Gel column

C6.2.2.1 Preparation.

Plug a glass column (C6.1.1) with cotton wool. Add $2.00\,g \pm 0.01\,g$ prepared silica gel (see Section C5.1). Weigh using a rapid weighing top pan balance in order to avoid

* PTFE: Polytetrafluoroethylene.

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.

C6.2.2.2 Checking of activity

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

Prepare a silica gel column as in Section C6.2.2.1. Add 1.0 ml ± 0.1 ml of insecticide solution (see Section C5.14.4.1), 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 steam of dry air of nitrogen (see Section C6.1.5).

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 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 C8 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 C5.14.4.2).

C6.3 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. Columns should have an efficiency of better than 1,000 theoretical plates. Many different columns have been used. Four 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% OF-1 by weight. Operated at 190°C and 50 ml/min of nitrogen.

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 of nitrogen.

iii. 50 m 0.3 mm ID WCOT glass or fused silica column coated with OV1.

iv. 50 m 0.3 mm ID WCOT glass or fused silica column coated with Carbowax 20 M TPA.

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 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 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.

C6.4 pH meter (or pH test papers for the range pH 1–3).

C7 Sample Storage and Preservation

Sample bottles should be all-glass with glass stoppers, cleaned in the manner described for glass apparatus (see Section C6). 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 chlorinated benzene 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 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.

C8 Analytical Procedure

Read Section C4 on Hazards before starting this procedure.

Step	Procedure	Notes
C8.1	**Extraction of Insecticides and PCB**	
C8.1.1	A 2 litre sample in a 2.5 litre glass bottle is homogenized by shaking. Then using a pH meter or pH paper add dilute sulphuric acid (C5.18) to bring the pH to between 2 and 3. Add 100 ± 2 ml of ethyl acetate. Stopper and shake vigorously for at least 5 mins. Transfer sample and extract to a 3 1 separating funnel. Allow the phases to separate for at least 5 mins. When good separation has been achieved run off the lower aqueous phase back into the 2.5 1 bottle; run the ethyl acetate into a suitable small glass-stoppered vessel (notes (a), (b), (c), (d), (e), (f), and (g)).	(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. Tests show that pesticide are often absorbed by suspended solids, but the degree is dependent on pesticide and solid type. In general, organic matter, silt, clay and chalk absorb most, and clean sand least.
C8.1.2	Add a further 100 ± 2 ml ethyl acetate to the sample in the 2.5 1 bottle, stopper, shake vigorously for 5 mins. Repeat the separation as in step C8.1.1 except that the aqueous layer is now discarded and the two ethyl acetate extracts are combined.	(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 ethyl acetate and the rinsings added to the main extract.
C8.1.3	Fill the 2.5 1 bottle used in steps C8.1.1 and C8.1.2 exactly to the mark made in C8.1.1, measuring the volume of water required as exactly as possible (V ml).	(d) Addition of sodium chloride to the sample (salting out) does not improve extraction efficiency. (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 (e.g. Ref. 3). (f) Alternative methods of separating the phases have been found suitable which avoid transference to a second vessel (Refs 3 and 4).

Step	Procedure	Notes
		(g) Multiple extraction usually improves recovery, but involves additional expense and time and often lowers precision even if accuracy is improved. Assuming no loss, the theoretical extraction efficiencies for repeated extraction of true solutions at the initial efficiencies given below are as follows:

	%extracted (approx.)				
1st	90	80	60	50	40
2nd	99	96	84	75	64
3rd	99.9	99.2	93.6	87.5	78.4
4th		99.8	96.1	93.7	87.0
5th			98.9	96.9	92.2
7th				99.2	97.2
9th					99.0

For extraction of material absorbed into suspended matter (deeper than surface adsorption) gain in efficiency falls off very rapidly.

C8.2 Concentration

Dry the ethyl acetate 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 ethyl acetate and collect in the evaporator. Add two anti-bumping granules and one drop of a Keeper (C5.15) and evaporate to 5 ml ± 1 ml on a steam bath. Further concentrate the extract down to the 1 drop of Keeper added, 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)). Add 1 ± 0.1 ml of hexane and swirl to dissolve the Keeper and extracted substances.

(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.

C8.3 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 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.

If total blackening is a common occurrence, larger columns may be used but additional hexane will be required to elute dieldrin (see Ref. 1, 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.

C8.4 Separation of Insecticides and PCB

Prepare a silica gel column as in section C6.2.2.1. Add the concentrated sample extract (1.0 ml ± 0.1 ml) to the column (note (l)). Wash the container with 1.0 ml ± 0.1 ml hexane, add this to the column retaining the meniscus just

(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.

Step	Procedure	Notes
	below the top of the silica gel (note (l)). Add 10 ml ± 0.2 ml hexane and collect the first 7 ml eluate (fraction 1) (note (m)). If pesticides are also present (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 C8.2.	(m) 7 ml or a volume as determined in section C6.2.2.2. (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.
C8.5	**Gas chromatography**	
C8.5.1	**Calibration of gas chromatography** 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 (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.
C8.5.2	**Examination of extracts** Inject 5 µl of the extract on to 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.
C8.5.3	**Correction for poor extraction efficiency** If it is desired to make this correction run standards prepared as in Section C5.17 through the procedure.	
C8.6	**Calculation of Concentration**	
C8.6.1	**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: $C = \frac{200\ vA}{V}$ 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.	
C8.6.2	**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 Ref. 1 Appendix, Section A5.7).	

Step	Procedure	Notes
C8.6.3	**Correction for Poor Extraction Efficiency** If necessary proportionate the result by multiplying by $\frac{x}{y}$ where x is the C5.17 type standard concentration taken and y is the concentration found for this standard. The mean of multiplicate analyses is essential if this type of correction is to be made.	

D. Determination of Halogenated Hydrocarbon Solvents and Related Compounds in Sewage Sludge and Water by Steam Distillation, Solvent Extraction and Gas Chromatography

D.0 About this Method

D.0.1 Chlorinated hydrocarbon solvents are toxic to bacteria in anaerobic digestion. Where chlorinated hydrocarbon solvents have caused reductions in gas yields in digestion or even complete failure of that process, chloroform, carbon tetrachloride, 1,1,1-trichloroethane, 1, 1, 2-trichloroethane, 1,1,2-triflouroethane, trichloroethylene and tetrachloroethylene have been identified as the compounds most frequently present. The threshold concentrations vary from compound to compound. Problems are also experienced in sewers where excessive amounts of the solvents accumulate in the detritus, which, when disturbed, present a hazard to sewermen.

D.0.2 The recommended method for quantification of the solvents is based on electron capture gas chromatography. Other volatile halogenated hydrocarbons are also determined by this method; but dichloromethane which can cause problems in digestion and sewer management is better analysed using GC with flame ionization detection even though the limit of detection is higher than that of other chlorinated solvents.

D.0.3 This analysis is commonly performed on sludge in case of incipient or total digester failure. The threshold concentrations which are likely to cause problems are:

	mg/l
Arklone	3.4
Chloroform	0.17
1,1,1-trichloroethane	0.17
Carbon tetrachloride	3.4
Trichloroethylene	10
Tetrachloroethylene	60

D.0.4 An alternative method for clean waters is that for trihalomethanes in waters (6) which also can be used for most of these haloalkanes, standards being prepared as detailed here.

D.0.5 For qualitative detection in the atmosphere, Beilstein Lamps may be used, or in inflammable atmospheres other portable devices such as Draeger tubes may be used.

D1 Performance Characteristics of the Method

D1.1	Substances determined	Arklone (1,1,2-trichlorotrifluroethane), carbon tetrachloride, chloroform, dichloromethane, ethylene dibromide (1,2-dibromoethane), Genklene (1,1,1-trichloroethane), tetrachloroethylene, trichloroethylene and other compounds which may be steam distilled from sludge such as methylbromide.
D1.2	Type of sample	Sewage sludges and waters.
D1.3	Basis of method	Steam distillation of the sample, extraction of the distillate with pentane and capillary chromatography of the extract.
D1.4	Range of Application	Arklone: 0–7 mg/l; chloroform: 0–4.5 mg/l; Genklene: 0–2.5 mg/l; carbon tetrachloride: 0–1 mg/l; trichloroethylene: 0–5 mg/l; dibromoethane: 0–5 mg/l; tetrachloroethylene: 0–3 mg/l;

		dichloromethane: 0–35 g/l. The upper limit of the range can be extended by diluting the sample.
D1.5	Calibration curve	The range of linearity depends on the detector and instrument in use. The instrumentation used gave a linear response over the ranges given in Table 1.
D1.6	Standard deviation	See Tables 2, 3 and 4.
D1.7	Limit of detection	See Tables 5 and 6.
D1.8	Sensitivty	Dependent on the instrumentation in use. See Table 7.
D1.9	Bias	The extraction efficiencies are less than 100% (See Table 2). Contamination by the commonly used laboratory solvents will lead to systematically high results.
D1.10	Interferences	Co-distilling compounds which have similar retention times to any of the determinands will interfere. No interferences were observed during the present work.
D1.11	Time required for analysis	4 h per single sample including chromatography 4–5 samples per day may be run on a routine basis.

D2 Principle

The sample is steam distilled and the codensate collected in pentane in a cooled receiver. The pentane extract is analysed by capillary-column gas-chromatography. Electron-capture detection (EC-GC) is used for all the chlorinated solvents except dichloromethane when a better response is obtained by flame-ionization detection. Typical Chromatograms are shown in Fig. 6.

D3 Interferences

Any substance which is extracted by the procedure, which has a similar retention time to any of the determinands and which gives a detector response will interfere. The use of two columns increases the certainty of identification. The use of chlorinated solvents and freons in the laboratory during the course of analysis may lead to gross interference.

D4 Hazards

Pentane is flammable. Chlorinated solvents are toxic and narcotic.

D5 Reagents

D5.1 Pentane: Fraction from petroleum; redistil from sodium in an all glass still fitted with a 1.5 m factionating column and a variable take-off still-head.

D5.2 Chlorinated solvents: AR grade.

D5.3 Sodium sulphate: Anhydrous, roasted at 500°C for 4 h and cooled in a desiccator.

D5.4 Ice/salt for cooling the receiver.

D6 Apparatus

D6.1 Apparatus for steam distillation, (Fig. 5).

D6.2 Gas chromatography fitted with capillary columns and electron capture and flame-ionization detectors.

D6.3 Chromatographic columns: some suitable columns are:

a) 50 m WCOT fused silica column 0.3 mm ID coated with PS 255 or OV1 (thick film 1.0 m).
b) A similar column coated with Carbowax 20M (0.2 m film thickness).

In order to separate dichloromethane from Arklone and the solvent on the non-polar column the over temperature should be 30°C. If the instrument is frequently calibrated the oven door may be left open to reach this temperature but a subambient unit is preferable. On the Carbowax column, carbon tetrachloride and 1,1,1-trichloroethane do not separate. The order to elution is given in Table 8.

D7 Standards

D7.1 Stock solutions: Dissolve an appropriate volume of each chlorinated solvent in a 100 ml pentane using clean microlitre syringes. Appropriate concentrations are:

	mg/l
Arklone	250
carbon tetrachloride	40
chloroform	200
dichloromethane	100,000
ethylene dibromide	200
1,1,1-trichloroethane	100
tetrachloroethylene	100
trichloroethylene	200

D7.2 Working solutions: If the method is to be used to assess digester failure a useful range of solutions is:

	mg/l
Arklone	0–5
Carbon tetrachloride	0–1
Chloroform	0–5.5
Dichloromethane	0–10
1,1,1-trichloroethane	0–0.5
Tetrachloroethylene	0–10
Trichloroethylene	0–2

For low concentrations in water suggested appropriate standards are:

	μg/l
Arklone	0–1000
Carbon tetrachloride	0–200
Chloroform	0–200
Dichloromethane	0–10,000
Ethylene dibromide	0–200
1,1,1-trichloroethane	0–200
Tetrachloroethylene	0–200
Trichloroethylene	0–200

Prepare these standards on the day of use.

D8 Sample Storage and Preservations

Atmospheric contamination of both sample and standards is a common problem especially in laboratories where chlorinated solvents are used. Samples should be sealed in polythene bags and the analysis performed as soon as possible after receipt. The solvent should be checked regularly for contamination. Glass bottles with a Teflon-lined screw cap are effective containers but bottles with ground-glass stoppers are adequate if the samples are analysed immediately.

D9 Analytical Procedure

Step	Procedure	Notes
D9.1	**Extraction Step**	
	For Sludge Samples	
D9.1.1	Set up the apparatus as shown in Fig. 5. Introduce 200 ml sludge into 1 l flask. Introduce 20 ml pentane into separating funnel. (Notes (a) and (b)).	(a) Use small apparatus to minimize dead volume effects. (b) The temperature should be kept below −5°C with salt/ice bath.

Step	Procedure	Notes
D9.1.2	Steam distil until about 100 ml distillate has collected in the separating funnel.	
D9.1.3	Stopper the separating funnel, shake thoroughly for one minute. Allow the layers to separate and discard the lower, aqueous layer. (Note (c)).	(c) The extraction should be performed whilst the mixture is still cold in order to reduce losses.
D9.1.4	Place 2 g anhydrous sodium sulphate into a small funnel plugged with cotton wool. Wash with pentane. Place 25 ml graduated flask under the funnel. Pass the pentane extract through the sodium sulphate and wash through with pentane. Add washings to the extract and dilute to 25 ml. (Note (d)).	(d) If care is taken the pentane layer may be transferred into a 25 ml graduated flask virtually water-free. Errors caused by the presence of the small amount of water dissolved in the pentane are negligible.
	For Water Samples	
D9.1.5	If the apparatus shown in Fig. 5 is used as illustrated, use 200 ml of water, but use of a larger flask will allow larger samples such as 1 or 2 litres to be used. Proceed as above.	
D9.2	**Preparation of chromatograph**	
D9.2.1	Set up the instrument with the appropriate detector according to the manufacturer's instructions and with conditions determined to be suitable for the analysis, see D6.2. (Notes (e) and (f)).	(e) See the GC Review in the series. (f) FID for dichloromethane determination. ECD for the other compounds.
D9.2.2	Allow equipment to stabilize and baseline to become steady.	
D9.3	**Gas chromatographic procedure**	
D9.3.1	Inject the pentane blank. The peaks for chlorinated solvents should be negligible. (Note (g)).	(g) With volatile solvents such as pentane it is easier to eliminate bubbles from the syringe and hence inject reproducibly if the syringe is kept in a refrigerator between injections.
D9.3.2	Inject a series of standards and plot a calibration graph of peak heights vs concentration of determinand injected (μg/l).	
D9.3.3	Inject sample extract. Measure peak heights of individual compounds. (Notes (h) and (i)).	(h) If peaks other than those of standards are present, solutions of other halocarbons may be prepared and tested. (i) Peak areas could also be used.
D9.3.4	Those compounds identified may be quantified by comparison of their peak heights with those given by the standards. (Note (j)).	(j) Typical Chromatograms are shown in Fig. 6.
D9.3.5	Confirmation of identity may be made on a second column.	

D10 Calculation

D10.1 Read off the concentration Aμg/l) of each determinand in the extract from the calibration graphs.

D10.2 Then if the concentration of the determinand in the original sludge or water sample was Cμg/l,

the volume of sludge or water taken at steps D9.1.1 or D9.1.5 respectively was V ml (usually 200 ml for sludge), the volume of final extract was v ml (usually 25 ml), and A is as defined in D10.1, for each determinand.

$$C = \frac{v}{V} A \ \mu g/l \text{ for sludge or water.}$$

or $C = \frac{A}{8}$ $\mu g/l$ for 200 ml samples and 25 ml extract as given in the sludge method.

D10.3 On a dry weight basis for sludge, if S% is the percentage dry solids in the sludge, and the method is adhered to as written:

$$C = \frac{100A}{8S} \ \mu g/kg.$$

Table 1. Ranges linear up to ($\mu g/l$)

Compound	Concentration
Arklone	7000
Chloroform	4500
Genklene	2500
Carbon tetrachloride	1000
Trichloroethylene	5000
Dibromeothane	5000
Tetrachloroethylene	3000
Dichloromethane	35000000

Table 2. Recoveries obtained from high and low level spike extractions

Compound	Spike level ($\mu g/l$)	Standard deviation ($\mu g/l$)	Mean recovery (%)	Spike level ($\mu g/l$)	Standard deviation ($\mu g/l$)	Mean recovery (%)
Arklone	31.6	2.7	44	608.0	38.6	78
Chloroform	24.4	1.8	67	468.1	21.1	77
Genklene	11.6	0.8	72	223.2	25.0	81
Carbon tetrachloride	4.6	0.5	97	88.0	6.5	57
Trichloroethylene	24.2	1.7	77	465.3	28.9	85
Dibromoethane	23.8	1.1	76	456.6	24.7	80
Tetrachloroethylene	14.4	1.1	79	276.6	21.4	86
Dichloromethane	16800	1000	22	323000	60600	49

Table 3. Means and standard deviations of halocarbons in sludge ($\mu g/l$)

Compound	Mean	s_w	s_b	s_t
Arklone	11.2	2.2 (5)	6.5 (4)	6.8 (4)
Chloroform	2.8	1.9 (5)	0.0 (4)	1.9 (8)
Genklene	0.8	0.3 (5)	NS (4)	0.3 (9)
Carbon Tetrachloride	22.9	5.8 (5)	25.3 (4)	25.9 (4)
Trichloroethylene	0.8	1.4 (5)	0.0 (4)	1.4 (9)
Dibromoethane	0.2	0.6 (5)	0.0 (4)	0.6 (9)
Tetrachloroethylene	1.0	0.3 (5)	NS (4)	0.4 (8)
Dichloromethane	2055	776 (5)	0.0 (4)	776 (9)

NS = not significant

Table 4. Means and standard deviations of halocarbons in water blank ($\mu g/l$)

Compound	Mean	s_w	s_b	s_t
Arklone	9.9	3.9 (5)	0 (4)	3.9 (9)
Chloroform	0.6	0.3 (5)	NS (4)	0.4 (7)
Genklene	0.9	0.6 (5)	NS (4)	0.6 (9)
Carbon tetrachloride	9.1	5.5 (5)	NS (4)	8.1 (6)
Trichloroethylene	0.2	0.3 (5)	0 (4)	0.3 (9)
Dibromoethane	0.3	0.1 (5)	0 (4)	0.1 (7)
Tetrachloroethylene	0.1	0.1 (5)	0 (4)	0.1 (7)
Dichloromethane	2300	175 (5)	265 (4)	317 (5)

Table 5. Limits of detection of halocarbons in water (μg/l)

Compound	Detection limit
Arklone	18.1
Chloroform	1.4
Genklene	2.8
Carbon tetrachloride	25.6
Trichloroethylene	1.4
Dibromethane	1.4
Tetrachloroethylene	0.5
Dichloromethane	858

N.B. The detection limit for carbon tetrachloride is higher than normal owing to contamination problems during the course of the work.

Table 6. Limits of detection of halocarbons in sludge (μg/l)

Compound	Detection limit
Arklone	10.2
Chloroform	8.8
Genklene	1.4
Carbon tetrachloride	2.3
Trichloroethylene	6.5
Dibromeoethane	0.7
Tetrachloroethylene	1.4
Dichloromethane	3610

Table 7. Sensitivities obtained during halcarbon analysis (pg/mV)

Compound	Sensitivity
Arklone	2.6
Carbon tetrachloride	0.4
Chloroform	2.0
Dichloromethane	549100
Dibromoethane	4.3
Genklene	1.0
Tetrachloroethylene	1.4
Trichloroethylene	2.5

Table 8. Elution order and retention time of halocarbons on PS 255 (1.0μm film)

Compounds in order of elution	Retention time (secs)
Dichloromethane	340 (approx)
Arklone	364
Chloroform	522
Genklene	639
Carbon tetrachloride	726
Trichloroethylene	893
Dibromoethane	1730
Tetrachloroethylene	2029

Table 9. Elution order and retention times of halocarbons on Carbowax 20M (0.2 μm film)

Compounds in order of elution	Retention time (secs)
Arklone	158
Genklene	182
Carbon tetrachloride	182
Trichloroethylene	208
Tetrachloroethylene	225
Chloroform	248
Dibromethane	451

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. 10 and 11.

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

Figure 1 Device for removal of solvent from extracted samples (after Croll)

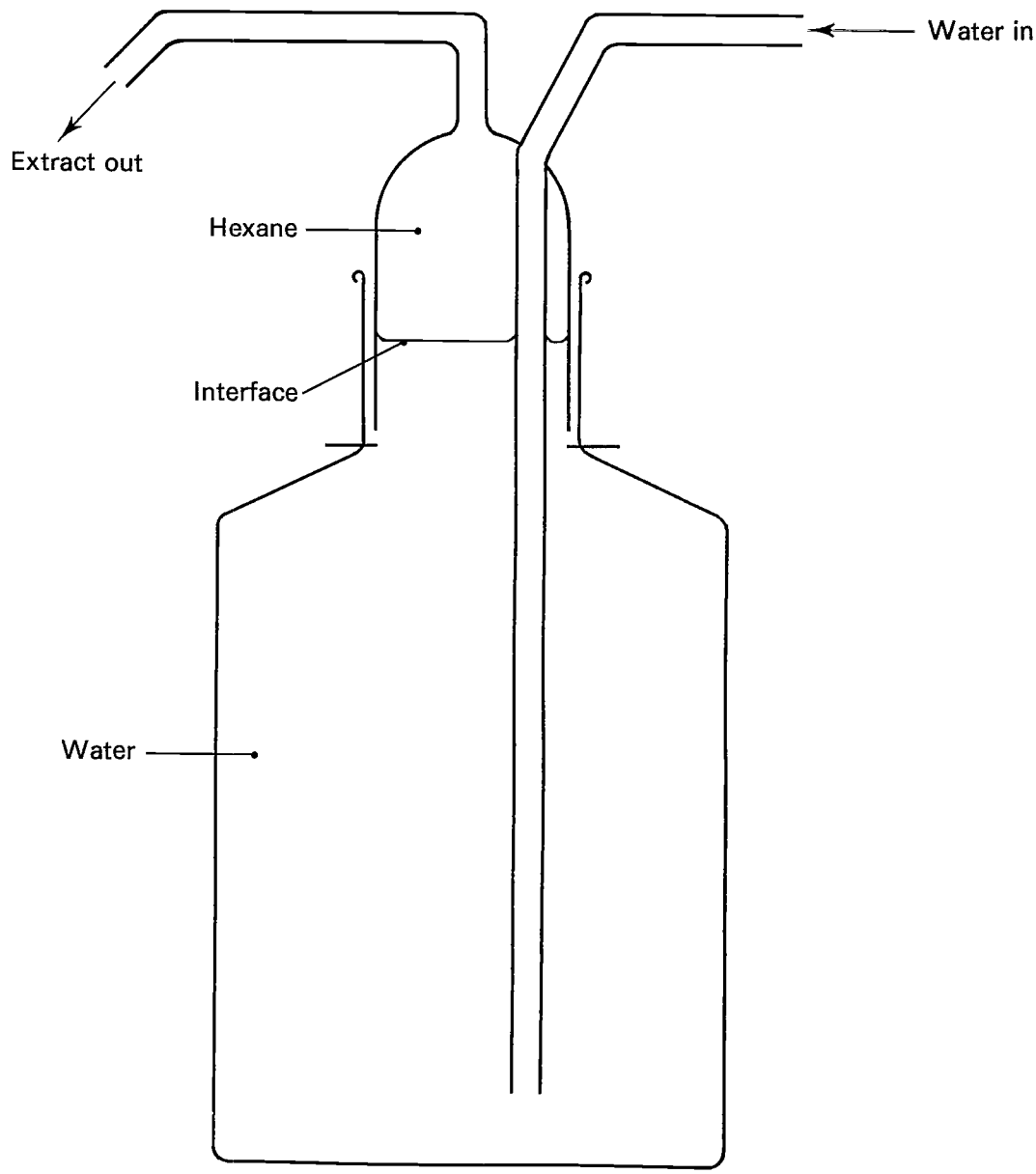


Figure 2 Adsorption column

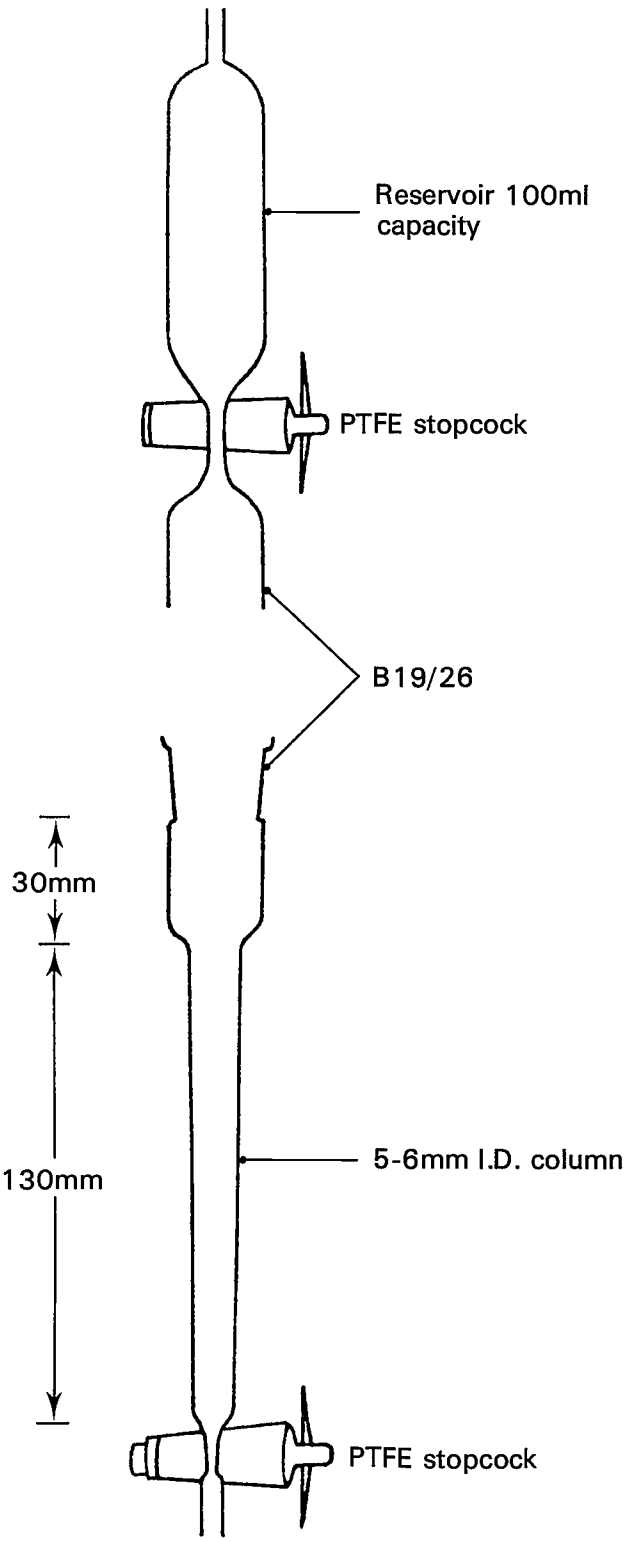


Figure 3 Kuderna-Danish evaporator system for concentration of pesticide solutions

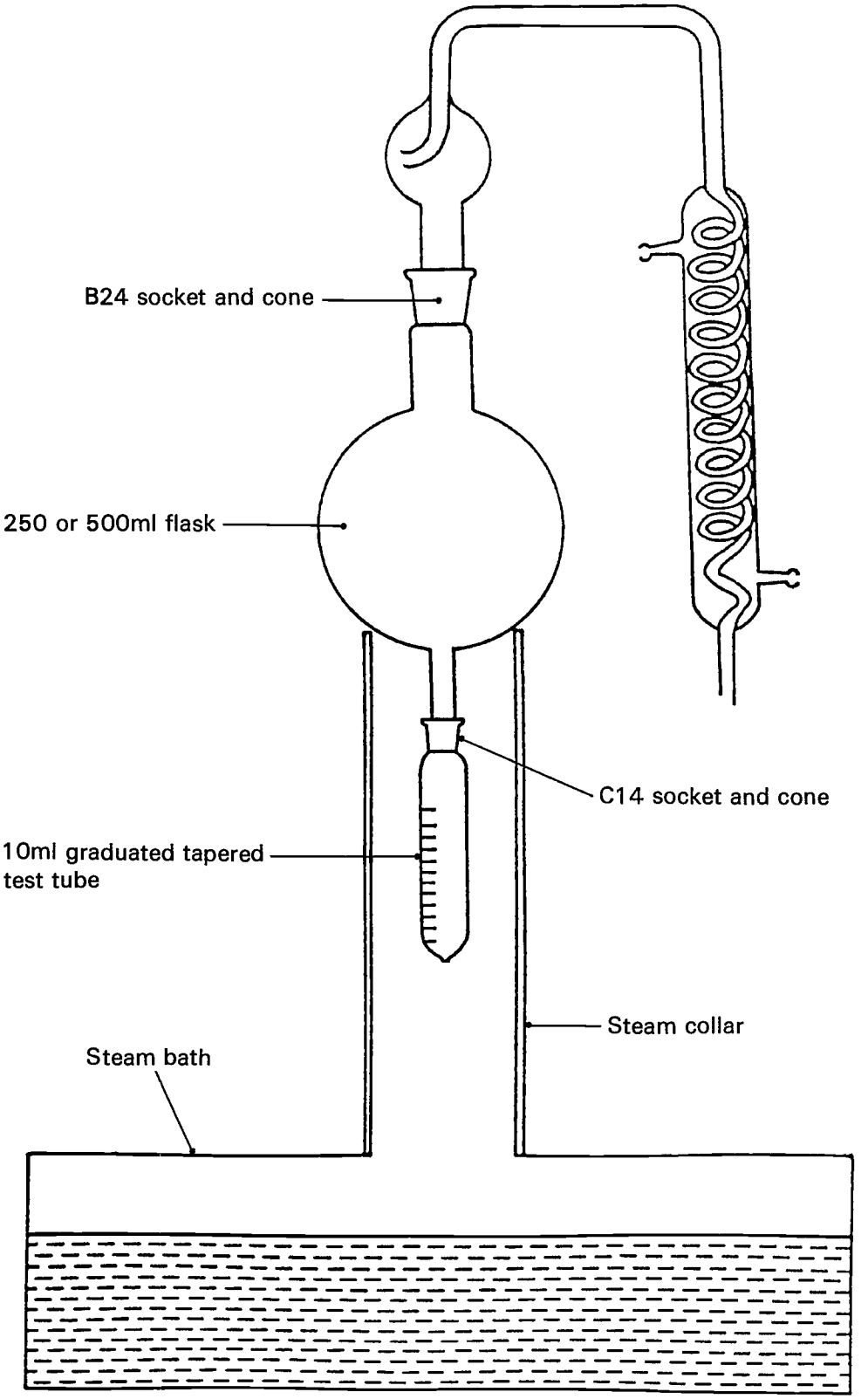
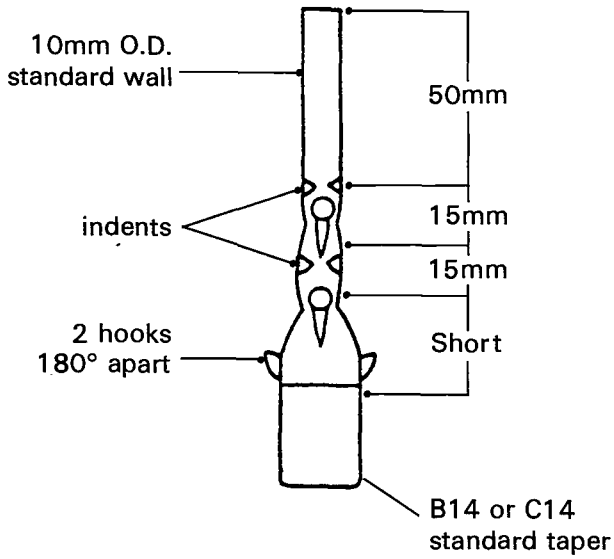


Figure 4 Micro Snyder column



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

Figure 5 Apparatus for steam distillation and collection of volatile chlorinated hydrocarbons

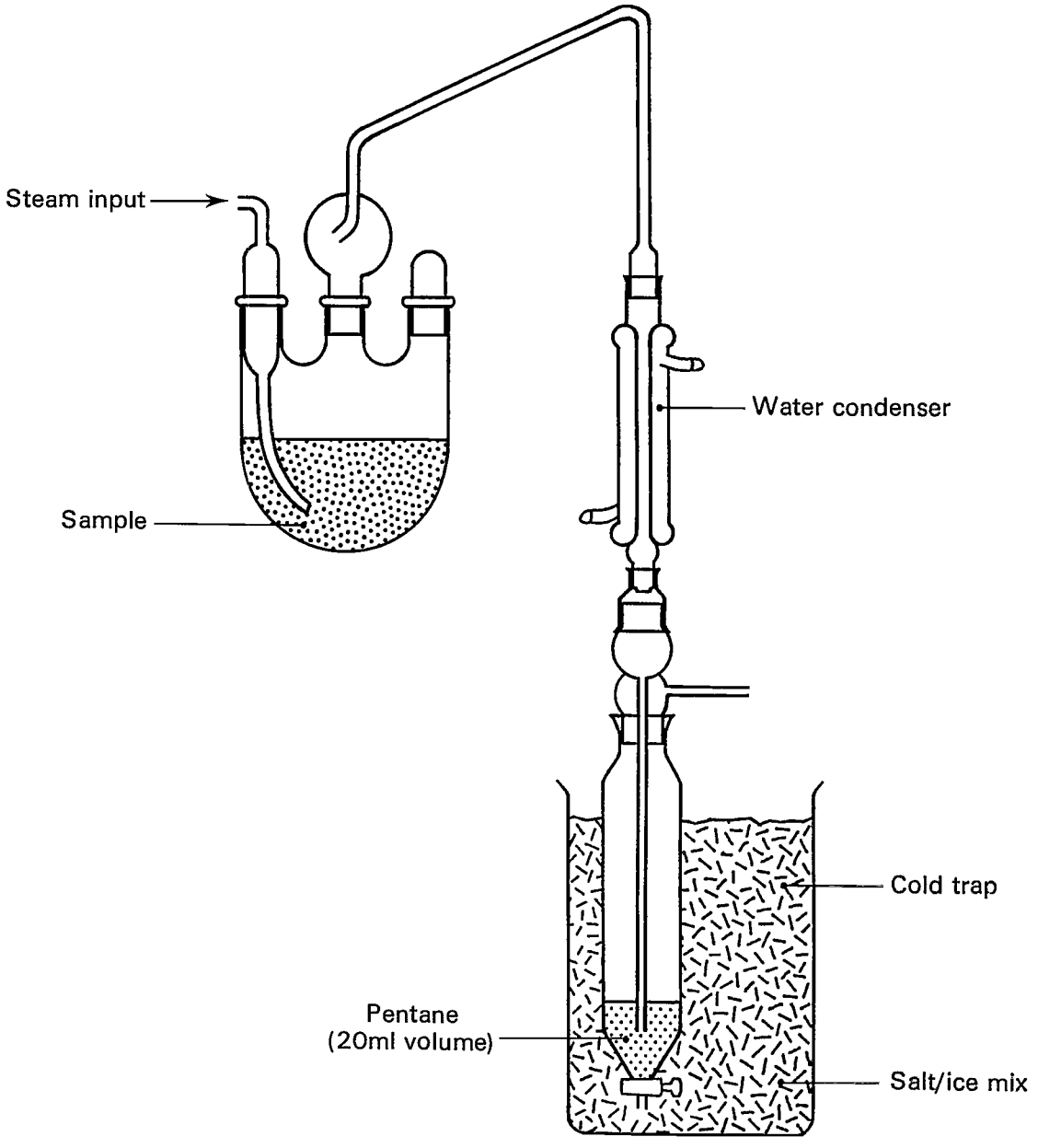
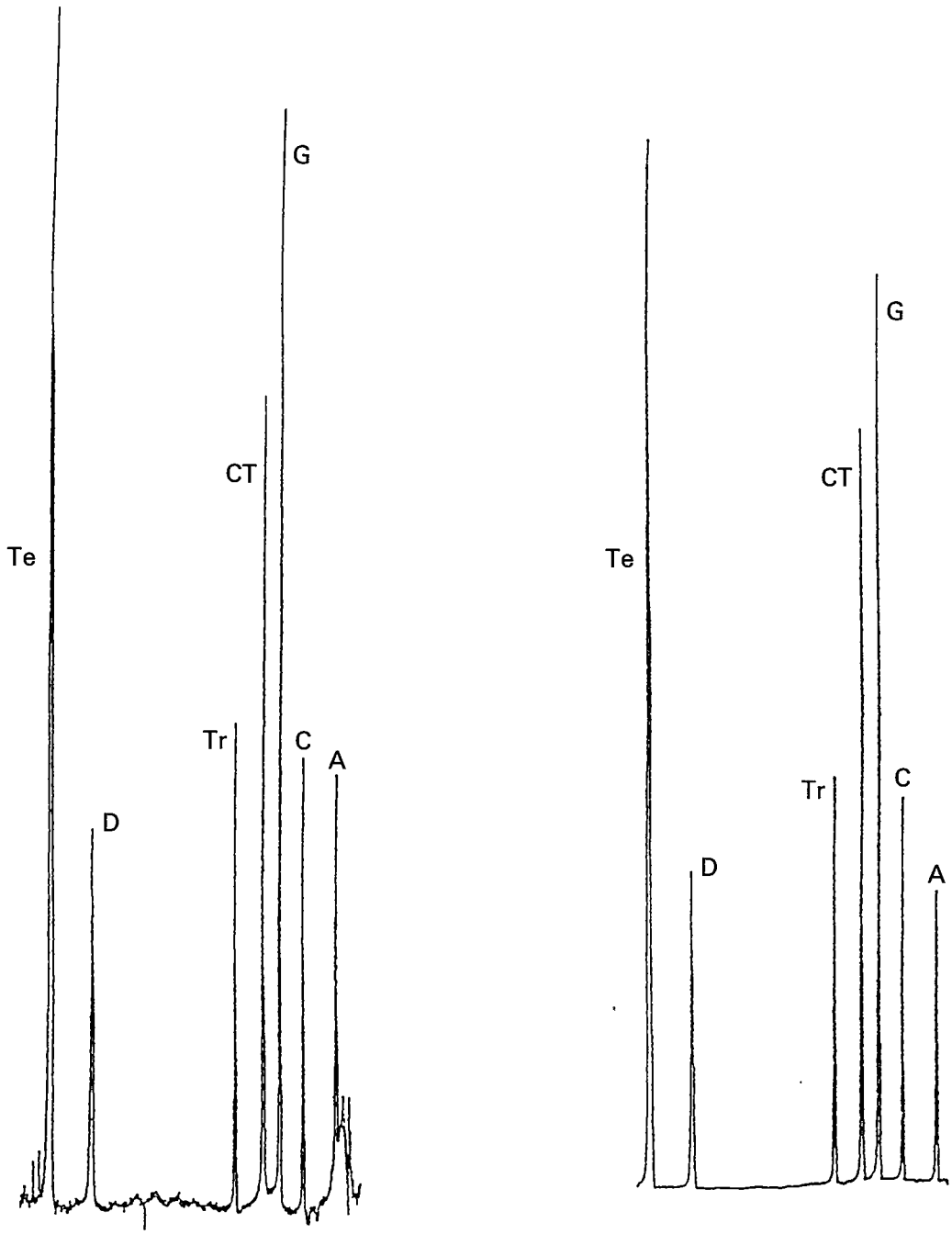


Figure 6 Typical chromatograms



Dilute standard

Concentrated standard

CT = Carbon tetrachloride G = Genklene C = Chloroform A = Arklone
Te = Tetrachloroethylene D = Dibromoethane Tr = Trichloroethylene

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Errata to

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

1. The errata at the foot of p. 29 should be deleted.

2. A line is missing in Section 6.11.2 on p. 9. This section should read exactly as Section C6.2.2.2 in this booklet or Section G2.2.2 in the 1978 water booklet.

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