ENVIRONMENT AGENCY

The determination of organochlorine pesticides and polychlorinated biphenyls in waters and complex matrices (2000)

Methods for the Examination of Waters and Associated Materials

A The determination of organochlorine pesticides in waters by solvent extraction and gas chromatography with electron capture detection and gas chromatography with mass spectrometric confirmation

A1 Performance characteristics of the method

A1.1	**Substances determined**	See Table A1. Other organochlorine compounds may also be determined.
A1.2	**Type of sample**	Potable, river, saline and waste waters.
A1.3	**Basis of method**	Determinands are extracted from the sample with hexane. The extracts are dried, concentrated and "cleaned-up", if necessary. The extracts are analysed by GC on a single capillary column using ECD. Any positive results are confirmed by GC-MSD.
A1.4	**Calibration curves**	Generally, the method is linear over the range of application.
A1.5	**Standard deviation**	See Table A1.
A1.6	**Limit of detection**	See Table A1.
A1.7	**Bias**	Extraction efficiencies are normally less than 100%. See Table A1.

A2 Principle

The determinands are extracted from a known volume of water with hexane. The hexane extract is dried, evaporated to approximately 1 ml, "cleaned-up" if necessary, and analysed by GC/ECD using a single capillary column. Any positive results are confirmed by GC-MSD.

A3 Interferences

Any co-extracted compound which has a similar GC retention time to any of the compounds of interest and which gives a response to the detector will interfere. In practice, many potentially interfering substances will be removed during the "clean-up" procedure. Co-eluting extracted substances may give rise to false electron capture responses or may hinder the MS identification. "Clean-up" procedures may be necessary for certain samples.

A4 Hazards

Skin contact or inhalation of reagents and their solutions should be avoided. Hexane is harmful, narcotic and flammable; acetone is flammable and organochlorine pesticides are toxic. Ensure adequate ventilation and work in a flame- or spark-proof area. Spark-proof refrigerators should be used to store standard solutions and their extracts. Appropriate safety procedures should be followed.

A5 Reagents

All reagents should be of sufficient purity that they do not give rise to interfering peaks during the determination or confirmation. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Pesticide- or HPLC-grade solvents and analytical grade materials are normally suitable unless otherwise specified and details of preparation are provided, where appropriate.

The water used for blank determinations and preparation of control samples should show negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed containers or other suitable vessels and kept in the dark if necessary.

A5.1 **Water.**

A5.2 **Hexane.**

A5.3 **Acetone.**

A5.4 **Granular anhydrous sodium sulphate.** Heat at 500 ± 20 °C in a muffle furnace for 4.0 ± 0.5 hours. Cool to about 200 °C in the muffle furnace and then to ambient temperature in a desiccator. Store in an all-glass container.

A5.5 **Anti-bumping granules.** Acetone-washed.

A5.6 **Silver nitrate.**

A5.7 **Alumina.** Woelm W200 neutral or an equivalent of the same mesh size. Heat at 500 ± 50 °C in a muffle furnace for 4.0 ± 0.5 hours. Cool to about 200 °C in the muffle furnace and then to ambient temperature in a desiccator.

A5.8 **De-activated alumina.** Add 7.0 ± 0.2% w/w of water (A5.1) to alumina (A5.7) and seal in a jar. Agitate the container for at least 2 hours to ensure the alumina and water are well mixed. Once the container has been opened, the de-activated alumina is normally suitable for use for about one week. After this period, unused amounts may be reprocessed as described in A5.7 and above.

A5.9 **Alumina-silver nitrate.** Dissolve 0.75 ± 0.10 g of silver nitrate (A5.6) in 0.75 ± 0.01 ml of water (A5.1). Add 4.0 ± 0.2 ml of acetone (A5.3) followed by 10.0 ± 0.2 g of dry alumina (A5.7). Mix thoroughly in a conical flask, protected from light. Allow the acetone to evaporate at room temperature. Store in the dark and use within 4 hours of preparation.

A5.10 **Stock standard solutions.** Solutions of individual pesticides are prepared by dissolving accurately weighed amounts of pure or certified pesticides in acetone in separate flasks. Suitable concentrations range between 250 - 500 mgl^{-1}.

A5.11 **Mixed intermediate standard solution.** A mixed intermediate standard solution in acetone is prepared by suitable dilution of the stock standard solutions (A5.10). For example, 2 mgl^{-1}.

A5.12 **Calibration standard solutions.** A series of calibration solutions in hexane are prepared by suitable dilution of the mixed intermediate standard solution (A5.11). For example, in the range up to 0.1 μgl^{-1}.

A6 Apparatus

Apparatus should be free from contamination before use. Glassware should be thoroughly clean, rinsed with hexane before use, and allowed to dry.

A6.1 **Sample bottles.** Glass, 1.2 litre capacity, marked at 1 litre, fitted with glass stoppers or PTFE-lined screw caps.

A6.2 **Separating funnel.** Glass, nominal 2 litre capacity fitted with glass or PTFE tap and stopper.

A6.3 **Kuderna-Danish evaporator.** Other evaporating systems may be used.

A6.4 **Nitrogen.** Oxygen-free, filtered and dry.

A6.5 **Chromatography columns for use in "clean-up" procedures.** Glass, 130 mm long by 5-6 mm internal diameter, fitted with a sintered glass frit and a glass or PTFE tap.

A6.6 **Gas chromatography.** A gas chromatograph fitted with a capillary non-polar or slightly polar column, glass-lined injector with ECD. The operating conditions may vary depending on the sample. The operating conditions used to generate the performance test data in Table A1 are:

Column:	60 m x 0.32 mm internal diameter, 0.25 µm film thickness, 5 % phenyl, 95% methyl siloxane coating.
Injector temperature:	250°C.
Injector:	split.
Column temperature:	185°C.
Detector temperature:	250°C.
Carrier gas:	helium, 1 $mlmin^{-1}$.

Typical chromatograms are shown in Figures A1 and A2.

A7 Sample collection and storage

Samples should be taken in glass bottles with glass stoppers or PTFE-lined screw caps (A6.1). They should be extracted as soon as possible after sampling unless stability data show otherwise. If samples cannot be analysed on the day of collection, solvent should be added to the sample bottle within 24 hours and the bottles shaken and stored between 1 - 8 °C. The sample bottles should be protected from contamination and should not be placed in proximity to standard materials or their concentrated solutions. Hydrolysis of some organochlorine pesticides has been demonstrated under alkaline extraction conditions.

A8 Analytical procedure

Step	Procedure	Notes
A8.1	**Extraction**	
A8.1.1	To 1000 ± 10 ml of the sample in the sample bottle (note a) add 50 ± 5 ml of hexane (A5.2). Stopper and shake vigorously for 120 ± 10 seconds (note b). Transfer the contents of the bottle to a 1 litre separating funnel. Rinse the sample bottle with a further 10 ± 1 ml of hexane and transfer the washings to the separating funnel. Alternatively, the extraction may be undertaken in a separating funnel. Vigorously shake the sample in the sample bottle and transfer 1000 ± 10 ml of the sample to a separating funnel. Rinse the empty bottle with 50 ± 5 ml of hexane (A5.2) and transfer to the separating funnel. Shake vigorously for 120 ± 10 seconds. Occasionally, the funnel should be vented to avoid build-up of pressure.	(a) If the same extract is later to be used for GC-MS confirmation then a surrogate standard should be added before the hexane. (b) A machine for shaking bottles in a horizontal plane may be used. In this case, the shaking period should be extended to at least 5 minutes.
A8.1.2	Allow the phases to separate. When good separation has been achieved, run off and discard the lower aqueous phase (notes c and d).	(c) Vigorous shaking may produce emulsions with some samples. These may be broken by the addition of acids or salts, or by centrifugation. Any solvent-water interfacial cuff should be included with the hexane extract and the water removed at the drying stage.

Step	Procedure	Notes
		(d) Where samples contain particulate material, the efficiency of the extraction will be improved by repeating the extraction.
A8.2	**Drying and concentration**	
A8.2.1	Run the hexane layer into a vessel containing approximately 10 g of sodium sulphate (A5.4) (note e). Rinse the separating funnel with 10 ± 1 ml of hexane and transfer the washings to the vessel. Swirl the vessel and leave to stand for at least 10 minutes, swirling occasionally.	(e) If emulsions are formed, it may be necessary to add more sodium sulphate.
A8.2.2	Transfer the dried extract to a Kuderna-Danish evaporator (note f). Wash the sodium sulphate with 10 ± 1 ml of hexane. Decant the washings into the Kuderna-Danish evaporator, add an anti-bumping granule and evaporate the extract to 3 ± 2 ml.	(f) An alternative evaporating system may be used.
A8.2.3	If "clean-up" is not required, reduce the volume of the extract using a stream of nitrogen (A6.4) to 1.00 ± 0.01 ml (notes g and h). The extract is now ready for GC/ECD determination (A8.4).	(g) For example, reduce the volume to less than 1 ml. Using a 1 ml precision syringe, draw the extract into the syringe and measure the volume. Return the extract to the tube and, using the same syringe, add sufficient hexane to the tube so that the final volume is 1.00 ± 0.01 ml. (h) An internal standard can be added at this stage, if required.
A8.2.4	If "clean-up" is required, reduce the volume of the extract using a stream of nitrogen (A6.4) to approximately 1 ml.	
A8.3	**"Clean-up" procedure**	
A8.3.1	Prepare an alumina/alumina-silver nitrate column. For example, place 15 ± 1 ml of hexane (A5.2) into the column (A6.5) then add 1.0 ± 0.2 g alumina-silver nitrate (A5.9) and allow to settle. Add 4.0 ± 0.4 g of de-activated alumina (A5.8) and again allow to settle. Add sufficient sodium sulphate (A5.4) to give a layer approximately 5 mm deep on top of the alumina (note i). The whole column should be prepared immediately before use.	(i) Alternative techniques are available for the removal of sulphide.

Step	Procedure	Notes
A8.3.2	Run off the surplus hexane. When the hexane level reaches the top of the sodium sulphate layer, add the concentrated sample extract from step A8.2.4. Rinse the sample tube twice, each time with 1 ml of hexane, and add the washings to the column (note j). Elute with 40 ± 4 ml of hexane and collect the eluate in a Kuderna-Danish evaporator, add an anti-bumping granule and evaporate the extract to 3 ± 2 ml (notes k and l). Reduce the volume of the extract using a stream of nitrogen (A6.4) to 1.00 ± 0.01 ml (notes g and h). The extract is now ready for GC/ECD determination (A8.4).	(j) During the additions to the column, do not allow the meniscus of the hexane to fall below the surface of the sodium sulphate. (k) If the alumina-silver nitrate blackens along its entire length, prepare a fresh column and repeat the "clean-up". (l) The use of the "clean-up" column will not quantitatively elute all pesticides.
A8.4	**GC/ECD determination**	
A8.4.1	Set up the instrument according to the manufacturer's instructions using the appropriate conditions given in section A6.6.	
A8.4.2	Inject aliquots of calibration standard solutions, extracts of samples, blank and recovery solutions into the GC (note m).	(m) If an internal standard is added to extracts of samples, the same amount should be added to calibration standard solutions. The same injection volume should also be used.
A8.4.3	Compare the retention times of each of the peaks of interest with those previously obtained from the injection of individual standard solutions. Measure the peak response (ie peak areas or peak heights) of each of the peaks of interest.	
A8.4.4	Construct a calibration graph of peak response (y-axis) versus concentration of calibration standard solutions (x-axis) for each of the compounds of interest.	
A8.4.5	Read the concentration of each compound of interest in sample extracts from its calibration graph (notes n and o). Calculate the concentration present in the original sample (see section A9).	(n) If an internal standard is added to calibration standard solutions and samples, measure the peak response for the internal standard in each analysis. Construct a calibration graph for each compound of interest using peak response ratios relative to the internal standard. (o) In cases where the response ratio for the compound of interest exceeds the calibration range, repeat the analysis using a smaller volume of sample.

Step	Procedure	Notes
A8.5	**Confirmation**	
A8.5.1	Analyse the extract containing the surrogate standard by GC-MSD using the procedures described in Method B.	
A8.6	**Blanks and recoveries**	
A8.6.1	Adequate blank values should be obtained using interference-free water before analysing samples. Adequate recovery values should be obtained using water of a similar nature to the sample being analysed. At least one reagent blank should be analysed with each batch of samples. Check the recovery of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard solutions to separate samples of interference-free water (note p). Process these solutions under conditions identical with those used for the samples under analysis.	(p) Spike the water at an appropriate level with the compounds of interest, for example, 50 µl of mixed intermediate standard solution (A5.11) added to 1000 ± 10 ml of water.
A8.6.2	If the peak responses of extracted calibration standard solutions are used in the calibration graph, an automatic correction is obtained. If not, recovery data of samples obtained from previous tests should be averaged and a mean correction factor determined for correcting recoveries (note q).	(q) Use the surrogate standard to indicate whether the analysis has proceeded satisfactorily for each individual sample.
A8.7	**AQC**	
A8.7.1	Carry out the entire procedure using interference-free water spiked at an appropriate concentration with each of the compounds of interest. Analyse a corresponding interference-free blank water.	

A9 Calculation

The concentration of each organochlorine pesticide is given by:

External standard procedure

$$\text{concentration in original sample} = \frac{\text{peak response of sample}}{\text{slope}} \times \frac{1000}{\text{DF}} \quad \mu g l^{-1}$$

where slope is the slope of the calibration graph of peak response of calibration standard solutions (y-axis) versus concentration of calibration standard solutions (x-axis), and

DF is dilution factor, ie 1000, if 1000 ml of sample is concentrated to 1 ml.

Table A1 Performance data generated using GC/ECD

Compound	Spiked blank water			Spiked river water			LOD
	Mean	S_t	Rec	Mean	S_t	Rec	
Aldrin	0.091	0.003	91	0.088	0.007	88	0.005
Dieldrin	0.095	0.005	95	0.90	0.007	90	0.009
Endrin	0.096	0.004	96	0.099	0.002	99	0.019
o,p'-DDE	0.094	0.005	94	0.093	0.005	93	0.019
p,p'-DDE	0.091	0.005	91	0.089	0.007	89	0.019
o,p'-DDT	0.091	0.003	91	0.083	0.004	83	0.009
p,p'-DDT	0.087	0.012	87	0.084	0.01	84	0.032
α-Endosulphan	0.093	0.003	93	0.088	0.004	88	0.009
β-Endosulphan	0.094	0.004	94	0.097	0.003	97	0.019
α-HCH	0.010	0.001	95	0.009	0.001	92	0.005
β-HCH	0.045	0.003	89	0.045	0.006	90	0.009
γ-HCH (lindane)	0.044	0.002	87	0.044	0.003	89	0.009
Heptachlor	0.091	0.005	91	0.085	0.007	85	0.009
p,p'-TDE	0.088	0.009	88	0.088	0.008	88	0.019
Tecnazene	0.085	0.013	85	0.093	0.014	93	0.037
Trifluralin	0.093	0.011	93	0.085	0.016	85	0.050
Chlorothalonil	0.108	0.018	108	0.108	0.016	108	0.036
Propyzamide	0.098	0.015	98	0.091	0.006	91	0.036
Triademefon	0.087	0.017	87	0.085	0.017	85	0.024

All values expressed in $\mu g l^{-1}$ unless otherwise stated and based on analyses of 11 batches carried out in duplicate.
LOD is calculated from 4.65 x S_w of the spiked blank water which consisted of de-ionised water.
Data provided by Essex & Suffolk Water.

Additionally, hexachlorobutadiene, δ-HCH, ε-HCH, THE, diflufenican, isodrin, HCB, methoxychlor, cis-chlordane and trans-chlordane can be analysed using LLE with pentane as solvent, giving similar performance data (information provided by Southern Science Ltd).

Figure A1 Typical chromatogram of organochlorine pesticides standard mixture using GC/ECD

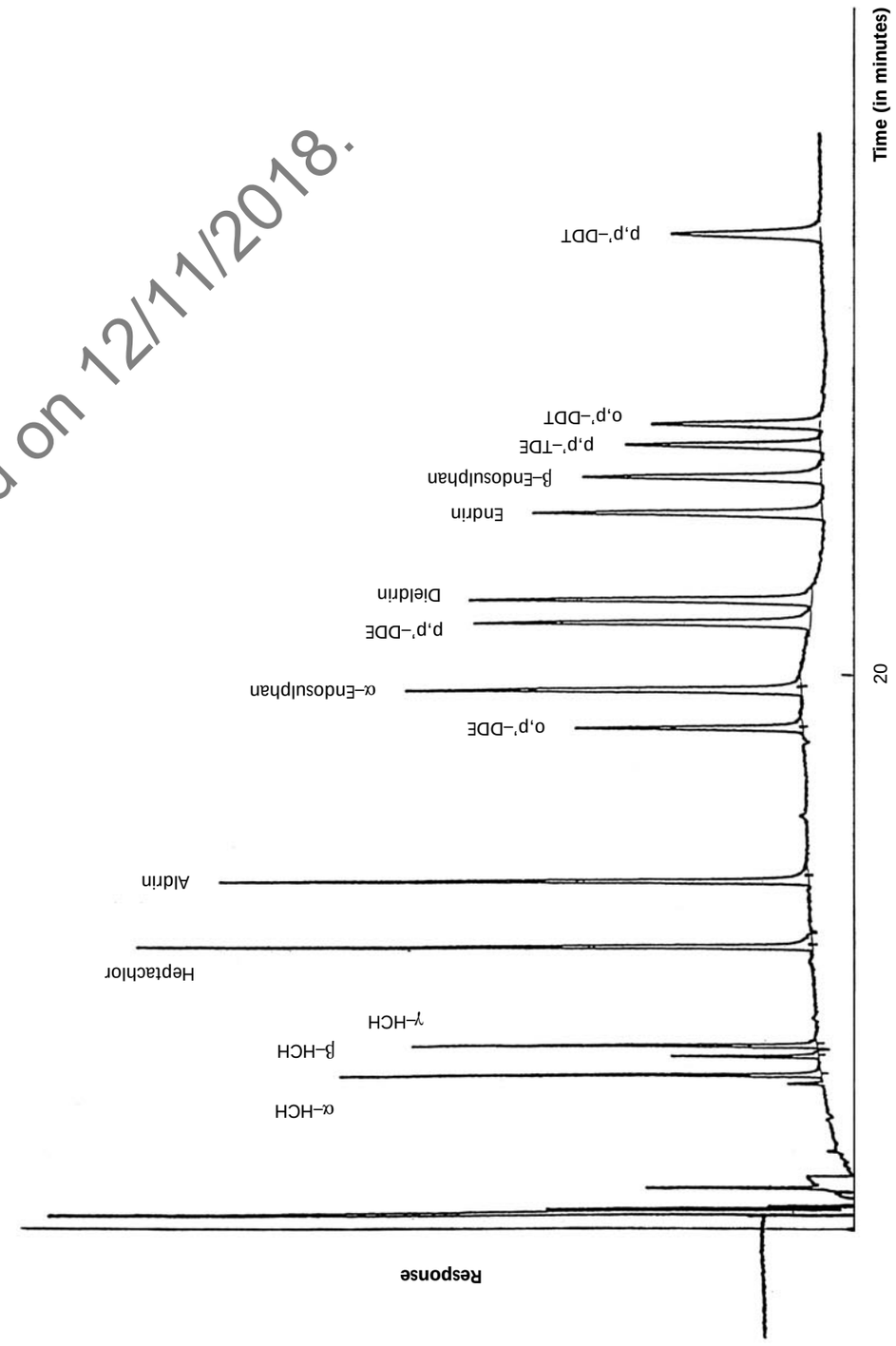
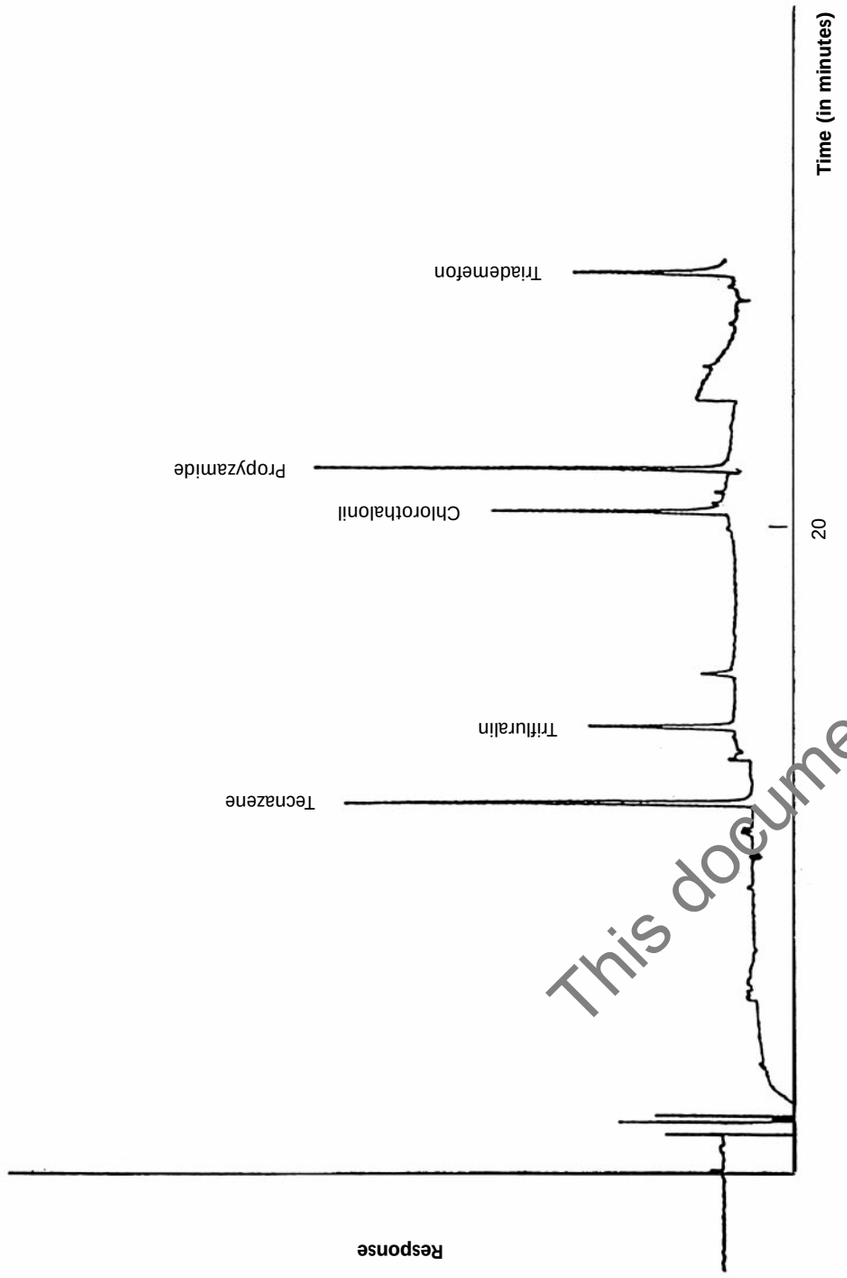


Figure A2 **Typical chromatogram of organochlorine pesticides standard mixture using GC/ECD**



B The determination of organochlorine pesticides and selected polychlorinated biphenyls in waters by solid phase extraction and gas chromatography with electron capture detection and gas chromatography with mass spectrometric confirmation

B1 Performance characteristics of the method

B1.1	**Substances determined**	See Table B1. Other organochlorine pesticides and selected PCBs may also be determined.
B1.2	**Type of sample**	Raw, potable, saline and surface waters. It may not be possible to analyse waters with a high solids content using the procedures described in this method.
B1.3	**Basis of method**	Determinands are extracted from the sample using an SPE cartridge. After elution, the extract is reconstituted in 2,2,4-trimethylpentane before analysis by capillary GC/ECD. Positive results are confirmed by GC-MSD.
B1.4	**Range of application**	Up to 0.2 $\mu g l^{-1}$ for each individual compound. The upper limit may be extended by dilution of the sample extract or by taking a smaller sample volume.
B1.5	**Calibration curves**	Linear over the range of application.
B1.6	**Standard deviation**	See Table B1.
B1.7	**Limit of detection**	Typically, 0.01 $\mu g l^{-1}$ per compound (for 1000 ml of sample concentrated to 1 ml). See Table B1.
B1.8	**Bias**	Extraction efficiencies are normally less than 100%. See Table B1.

B2 Principle

Water samples are prepared for extraction by addition of hydrochloric acid and propan-2-ol. The samples are extracted and concentrated using SPE. Determinands are adsorbed onto the cartridge material and then eluted using solvent. The extracts are reconstituted in 2,2,4-trimethylpentane and further concentrated to a specified volume using a stream of dry nitrogen. The extracts are analysed by capillary GC/ECD and any positive results are confirmed by GC-MSD operating in EI mode with SIM.

B3 Interferences

Any co-extracted compound which has a similar GC retention time to any of the determinands of interest and which gives a response to the detector will interfere. Co-eluting extracted substances may give rise to electron capture responses. Any extract which shows peaks with identical retention times to those of organochlorine pesticides, or PCBs of interest, should be re-analysed by GC-MSD.

B4 Hazards

Skin contact or inhalation of reagents and their solutions should be avoided. Ensure adequate ventilation and work in a flame- or spark-proof area. Spark-proof refrigerators should be used for storing extracts of samples and standard solutions. Appropriate safety procedures should be followed. Hexane, acetone, ethyl acetate and 2,2,4-trimethylpentane are harmful, narcotic and flammable. Propan-2-ol is flammable, methanol is toxic and flammable and dichloromethane is harmful. Hydrochloric acid is corrosive and its vapours are irritating and toxic. Organochlorine pesticides and PCBs are toxic and suspected carcinogens.

B5 Reagents

All reagents should be of sufficient purity that they do not give rise to interfering peaks during the determination and confirmation. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed.

Pesticide- or HPLC-grade solvents and analytical grade materials are normally suitable unless otherwise specified and details of preparation are provided, where appropriate.

The water used for blank determinations and preparation of control samples should show negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all glass containers or other suitable vessels and stored in the dark if necessary.

B5.1 **Water.**

B5.2 **Methanol.**

B5.3 **Propan-2-ol.**

B5.4 **Acetone:ethyl acetate.** A 3:1 v/v mixture.

B5.5 **Propan-2-ol in water.** A 1.0 % v/v mixture.

B5.6 **Acetone:dichloromethane.** A 1:1 v/v mixture.

B5.7 **Hexane.**

B5.8 **2,2,4 trimethylpentane (iso-octane).**

B5.9 **Hydrochloric acid.** Concentrated (d_{20} 1.18).

B5.10 **Hydrochloric acid.** (50% v/v). Slowly, with constant stirring add 500 ± 5 ml of concentrated hydrochloric acid (B5.9) to 500 ± 5 ml of water (B5.1).

B5.11 **Sodium thiosulphate solution.** (5% w/v). Dissolve 12.5 ± 0.5 g of sodium thiosulphate pentahydrate in 250 ± 1ml of water (B5.1) and mix thoroughly.

B5.12 **Sodium sulphate. Granular, anhydrous.** Heat at 500 ± 20 °C in a muffle furnace for 4.0 ± 0.5 hours. Cool to about 200 °C in the muffle furnace and then to ambient temperature in a desiccator. Store in an all-glass container.

B5.13 **Standard stock solutions.** Prepare individual stock solutions of pure or suitably certified standards in acetone at a concentration of 100 mgl^{-1}. Alternatively, suitable certified solutions can be obtained commercially, for example, at a concentration of 10 mgl^{-1}.

B5.14 **Spiking standard solutions.** By dilution of the stock solutions (B5.13) prepare standards in acetone at 1 mgl^{-1} to act as recovery standard solutions.

B5.15 **Working standard solutions.** By dilution of the stock solutions (B5.13) prepare a series of working standards in 2,2,4-trimethylpentane at, for example 200, 100 and 20 μgl^{-1}.

B5.16 **Internal standard.** A non-interfering compound should be chosen that gives a suitable response to the detector and is unlikely to occur in the samples. For example, dichlorobenzylhexyl ether or THE. A solution containing 80 μgl^{-1} in 2,2,4-trimethylpentane should be prepared.

B5.17 **AQC standards.** Prepare a standard solution of the compounds of interest in acetone each at a concentration of 1 mgl^{-1}. Use a different set of stock standards preferably from different sources to those used for the recovery and calibration standards.

B6 Apparatus

Apparatus should be free from contamination before use. Glassware should be thoroughly clean, rinsed immediately before use with hexane, and allowed to dry.

B6.1 **Sample bottles.** Glass, 1.2 litre capacity, marked at 1 litre, fitted with glass stoppers or PTFE-lined screw caps.

B6.2 **C_8 sorbent SPE cartridges (500 mg).** The performance of the method may vary with different batches of cartridge material and their supplier. The performance testing of the method was undertaken using C_8 end-capped Isolute cartridges (500 mg, 3 ml).

B6.3 **Cartridge manifold and pump or automated sample extraction device.** Suitable for use with C_8 sorbent cartridges.

B6.4 **Evaporating system.**

B6.5 **Nitrogen.** Oxygen-free, filtered and dry.

B6.6 **Centrifuge.**

B6.7 **Gas chromatography.** A gas chromatograph fitted with a capillary column, glass-lined injector with ECD. Operating conditions that may be used are:

Column:	60 m x 0.25 mm internal diameter, 0.25 µm film thickness coated with DB-5, or equivalent.
Carrier gas:	hydrogen, 120 kPa.
Injection volume:	1µl, on column.
Temperature programme:	60°C hold 1 minute, ramp at 25°$Cmin^{-1}$ to 180 °C, then 2°$Cmin^{-1}$ to 260 °C.
Injector temperature:	250 °C.
Detector temperature:	310 °C.

Example chromatograms of calibration standard solutions obtained under these conditions are given in Figures B1 and B2. Other capillary columns and conditions can be used provided the performance is equivalent to or better than that reported here.

B6.8 **Gas chromatography.** A gas chromatograph fitted with a capillary column and a detector capable of operating in the EI mode with SIM. This enables different groups of ions to be monitored at selected time intervals during the analysis. The operating conditions used to obtain the test data shown in Table B1 are:

Column:	60 m x 0.25 mm internal diameter, 0.25 µm film thickness coated with DB-5.
Carrier gas:	helium, 1 $mlmin^{-1}$.
Injection volume:	1µl, on column.
Temperature programme	60 °C (hold 1 minute), ramp at 25°$Cmin^{-1}$ to 180 °C, then 3 °$Cmin^{-1}$ to 260 °C (hold 8 minutes).
MS source temperature:	265 °C.

The SIM scheme is given in Table B2 and example chromatograms of calibration standards obtained under these conditions are given in Figures B3 and B4. Other capillary columns and conditions can be used provided the performance is equivalent to or better than that reported here.

B7 Sample collection and storage

Samples should be taken in glass bottles (B6.1). For sample de-chlorination, add 4-5 drops (0.05 ml) of sodium thiosulphate solution (B5.11) at the time of sampling. Samples should be extracted as soon as possible after sampling unless stability data show otherwise. If samples cannot be extracted on the day of collection, they should be stored in a refrigerator between 1-8°C. The sample bottles should be protected from contamination and should not be placed in proximity to standard materials or their concentrated solutions.

B8 Analytical procedure

Step	Procedure	Notes
B8.1	**Preparation**	
B8.1.1	To 1000 ± 10 ml of sample in the sample bottle, add 10.0 ± 0.5 ml of propan-2-ol (B5.3) and 5.0 ± 0.5 ml of hydrochloric acid (B5.10). Shake thoroughly and refrigerate the sample at 1 - 8°C for a minimum of 4 hours prior to the solid phase extraction step (note a).	(a) Reducing the pH and cooling the sample prior to extraction increases determinand retention on the cartridge. For compounds other than those listed in Table B1, it may be necessary to reduce the pH with an alternative acid to avoid precipitation of sulphur where samples have been treated with sodium thiosulphate.
B8.2	**Solid phase extraction**	
B8.2.1	Condition a 500 mg cartridge (B6.2) with 10.0 ± 0.5 ml of propan-2-ol (B5.3) and 10.0 ± 0.5 ml of 1% propan-2-ol (B5.5) (notes b and c).	(b) A flow rate of up to 40 ± 2 $mlmin^{-1}$ can be used. (c) Do not allow the cartridge to dry out at any stage during the conditioning.
B8.2.2	Load the sample onto the cartridge at a flow rate of up to 30 $mlmin^{-1}$.	
B8.2.3	When all of the sample has passed through the cartridge, dry the cartridge with nitrogen gas (B6.5) (note d).	(d) It is important to remove as much water as possible from the cartridge to prevent the presence of water in the final extract.
B8.2.4	Elute the dried cartridge with 3.0 ± 0.1 ml of acetone:ethyl acetate mixture (B5.4) at a flow rate of not more than 20 ± 2 $mlmin^{-1}$ and collect the eluate in a test tube. Elute the cartridge with 3.0 ± 0.1 ml of acetone: dichloro-methane mixture (B5.6) at a flow rate of not more than 20 ± 2 $mlmin^{-1}$ and collect the eluate in the same test tube used to collect the acetone:ethyl acetate eluate.	

Step	Procedure	Notes
B8.3	**Concentration**	
B8.3.1	Add 2.0 ± 0.l ml of 2,2,4-trimethylpentane to the combined eluates and reduce the volume of the extract using a stream of nitrogen (B6.5) to 1.00 ± 0.01 ml (notes e and f).	(e) For example, reduce the volume to less than 1 ml. Using a 1 ml precision syringe, draw the extract into the syringe and measure the volume. Return the extract to the tube and, using the same syringe, add sufficient 2,2,4-trimethylpentane to the tube so that the final volume is 1.00 ± 0.01 ml. (f) An internal standard can be added at this stage, if required.
B8.3.2	Transfer to a vial containing a small amount of sodium sulphate (B5.12).	
B8.3.3	Centrifuge the vial for 5 minutes at 3000 rpm. Transfer the upper layer to a second vial and store between 1 - 8 °C until ready for GC analysis.	
B8.4	**GC/ECD determination**	
B8.4.1	Set up the instrument according to the manufacturer's instructions using the appropriate conditions given in section B6.7.	
B8.4.2	Inject aliquots of calibration standard solutions, extracts of samples, blank and recovery solutions into the GC (note g).	(g) If an internal standard is added to extracts of samples, the same amount should be added to calibration standard solutions. The same injection volume should also be used.
B8.4.3	Compare the retention times of each of the peaks of interest with those previously obtained from the injection of individual standard solutions. Measure the peak response (ie peak areas or peak heights) of each of the peaks of interest.	
B8.4.4	Construct a calibration graph of peak response (y-axis) versus concentration of calibration standard solutions (x-axis) for each of the compounds of interest.	
B8.4.5	Read the concentration of each compound of interest in the sample extracts from its calibration graph (notes h and i). Calculate the concentration present in the original sample (see section B9).	(h) If an internal standard is added to calibration standard solutions and samples, measure the peak response for the internal standard in each analysis. Construct a calibration graph for each compound of interest using peak response ratios relative to the internal standard.

Step	Procedure	Notes
		(i) In cases where the response ratio for the compound of interest exceeds the calibration range, repeat the analysis using a smaller volume of sample.
B8.5	**Confirmation using GC-MSD**	
B8.5.1	Set up the instrument in EI mode with SIM in accordance with the manufacturer's instructions. The conditions and SIM scheme are given in section B6.8.	
B8.6	**Blanks and recoveries**	
B8.6.1	Adequate blank values should be obtained using interference-free water before analysing samples. Adequate recovery values should be obtained using water of a similar nature to the sample being analysed. At least one reagent blank should be analysed with each batch of samples. Check the recovery of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard solutions to separate samples of interference-free water (note j). Process these solutions under conditions identical with those used for the samples under analysis.	(j) Spike the water at an appropriate level with spiking standard (B5.14).
B8.6.2	If the peak responses of extracted calibration standard solutions are used in the calibration graph, an automatic correction is obtained. If not, recovery data of samples from previous tests should be averaged and a mean correction factor determined for correcting recoveries (note k).	(k) Use a surrogate standard to indicate whether the analysis has proceeded satisfactorily for each individual sample.
B8.7	**AQC**	
B8.7.1	Carry out the entire procedure using interference-free water spiked at an appropriate concentration with each of the compounds of interest. Analyse a corresponding interference-free blank water.	

B9 Calculation

B9.1 **Results**

The amount determined, A, for each compound of interest can be calculated using the following equation:

A = peak response x CF / S $\mu g l^{-1}$

where S is the slope of the calibration graph with peak response of calibration standard solutions (y-axis) versus concentration of standard solutions (x-axis);

CF is concentration factor and is given by:

$$CF = \frac{\text{final extract volume (ml)}}{\text{original sample volume (ml)}}$$

B9.2 **Recovery correction**

The recovery correction factor (RCF) can be calculated according to the following formula:

$$RCF = \frac{\text{concentration spiked}}{\text{concentration determined}}$$

The RCF for each compound of interest can be converted to a per cent recovery figure using the following formula:

% recovery = (1/RCF) x 100

The amount determined, A, for each compound of interest is multiplied by the recovery correction factor to give the corrected concentration present in the sample.

Concentration = A x RCF $\mu g l^{-1}$

Table B1 Performance data generated using GC/ECD

Compound	Recovery (%)	LOD (μgl^{-1})	De-ionised water S_t (μgl^{-1})	Tap water S_t (μgl^{-1})
PCB 31	75	0.003	0.006(14)	0.011(12)
PCB 28	71	0.001	0.006(13)	0.010(13)
PCB 52	79	0.001	0.007(6)	0.018(5)
PCB 101	64	0.003	0.009(12)	0.011(13)
PCB 149	55	0.009	0.01(12)	0.012(14)
PCB 118	57	0.001	0.01(12)	0.011(14)
PCB 105	53	0.001	0.009(12)	0.011(14)
PCB 153	57	0.004	0.01(13)	0.015(13)
PCB 138	52	0.001	0.009(17)	0.014(14)
PCB 180	81	0.005	0.012(11)	0.013(9)
Trifluralin	125	0.005	0.006(10)	0.006(11)
α-HCH	87	0.003	0.010(9)	0.01(10)
HCB	93	0.003	0.013(9)	0.017(8)
γ-HCH (lindane)	96	0.007	0.006(12)	0.007(12)
Heptachlor	81	0.007	0.012(9)	0.017(10)
Aldrin	68	0.007	0.012(9)	0.020(6)
o,p'-DDE	83	0.006	0.008(10)	0.010(9)
α-Endosulphan	97	0.004	0.004(15)	0.007(10)
p,p'-DDE	79	0.004	0.008(10)	0.010(9)
Dieldrin	97	0.003	0.005(11)	0.008(8)
o,p'-TDE	105	0.004	0.004(13)	0.008(8)
Endrin	90	0.005	0.007(12)	0.008(8)
β-Endosulphan	101	0.004	0.004(13)	0.029(13)
p,p'-TDE	105	0.005	0.005(12)	0.011(7)
o,p'-DDT	92	0.005	0.009(10)	0.012(10)
p,p'-DDT	83	0.005	0.012(14)	0.016(12)

LOD is calculated as 4.65 x S_w.
De-ionised water and tap water spiked at 0.09 μgl^{-1}.
Figure in brackets represents DF.
Data provided by East of Scotland Water.

Additionally, hexachlorobutadiene, δ-HCH, THE, diflufenican, isodrin, methoxychlor, chlordane, cyfluthrin, cypermethrin, dichlobenil, propiconazole, propyzamide, pendimethalin, cis-permethrin, trans-permethrin, tecnazene, triademefon and triallate can be analysed using SPE with ethyl acetate, giving similar performance data (information provided by Severn Trent Laboratories).

Table B2 Selected ions used for GC-MS quantification

Compound	Time window (minutes.seconds) Start	End	Primary ion m/z	Secondary ion m/z
trifluralin	13.00	14.30	306.1	264.0
α-HCH	14.30	17.30	183.0	181.0
HCB			283.8	285.8
γ-HCH (lindane)			183.0	181.0
PCB 31	17.30	20.20	256.0	258.0
PCB 28			256.0	258.0
heptachlor			272.0	274.0
PCB 52			291.9	289.9
aldrin	20.30	25.30	264.9	262.9
THE (internal standard)			217.0	236.8
o,p'-DDE			246.0	248.0
PCB 101			326.0	327.9
α-endosulphan			241.0	264.9
p,p'-DDE	25.30	27.15	246.0	248.0
dieldrin			260.9	262.9
o,p'-TDE			235.0	237.0
endrin	27.15	29.00	262.9	264.9
PCB 149			359.9	361.8
PCB 118			325.9	327.9
β-endosulphan			241.0	236.9
p,p'-TDE			235.0	237.0
o,p'-DDT			235.0	237.0
PCB 153	29.00	32.00	359.9	361.8
PCB 105			325.9	327.9
p,p'-DDT			235.0	237.0
PCB 138			359.9	361.8
PCB 180	32.00	35.00	393.8	391.8

LOD is calculated as 4.65 x S_w.
De-ionised water and tap water spiked at 0.09 μgl^{-1}.
Figure in brackets represents DF.
Data provided by East of Scotland Water.

Additionally, hexachlorobutadiene, δ-HCH, THE, diflufenican, isodrin, methoxychlor, chlordane, cyfluthrin, cypermethrin, dichlobenil, propiconazole, propyzamide, pendimethalin, cis-permethrin, trans-permethrin, tecnazene, triademefon and triallate can be analysed using SPE with ethyl acetate, giving similar performance data (information provided by Severn Trent Laboratories).

Figure B1 **Typical GC/ECD chromatogram of a standard mixture of PCB**
(PCB concentrations are approximately 200 µgl^{-1})

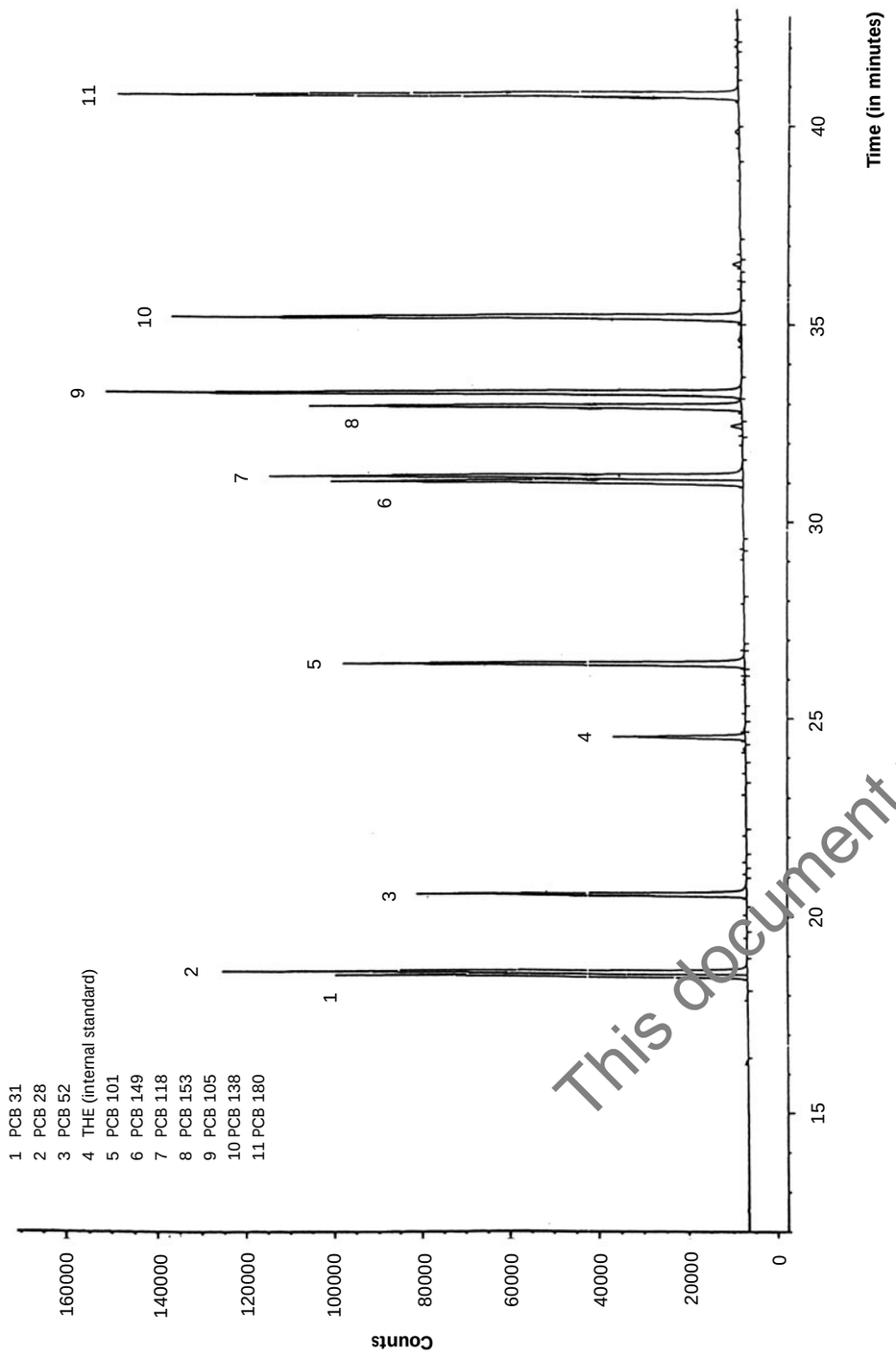
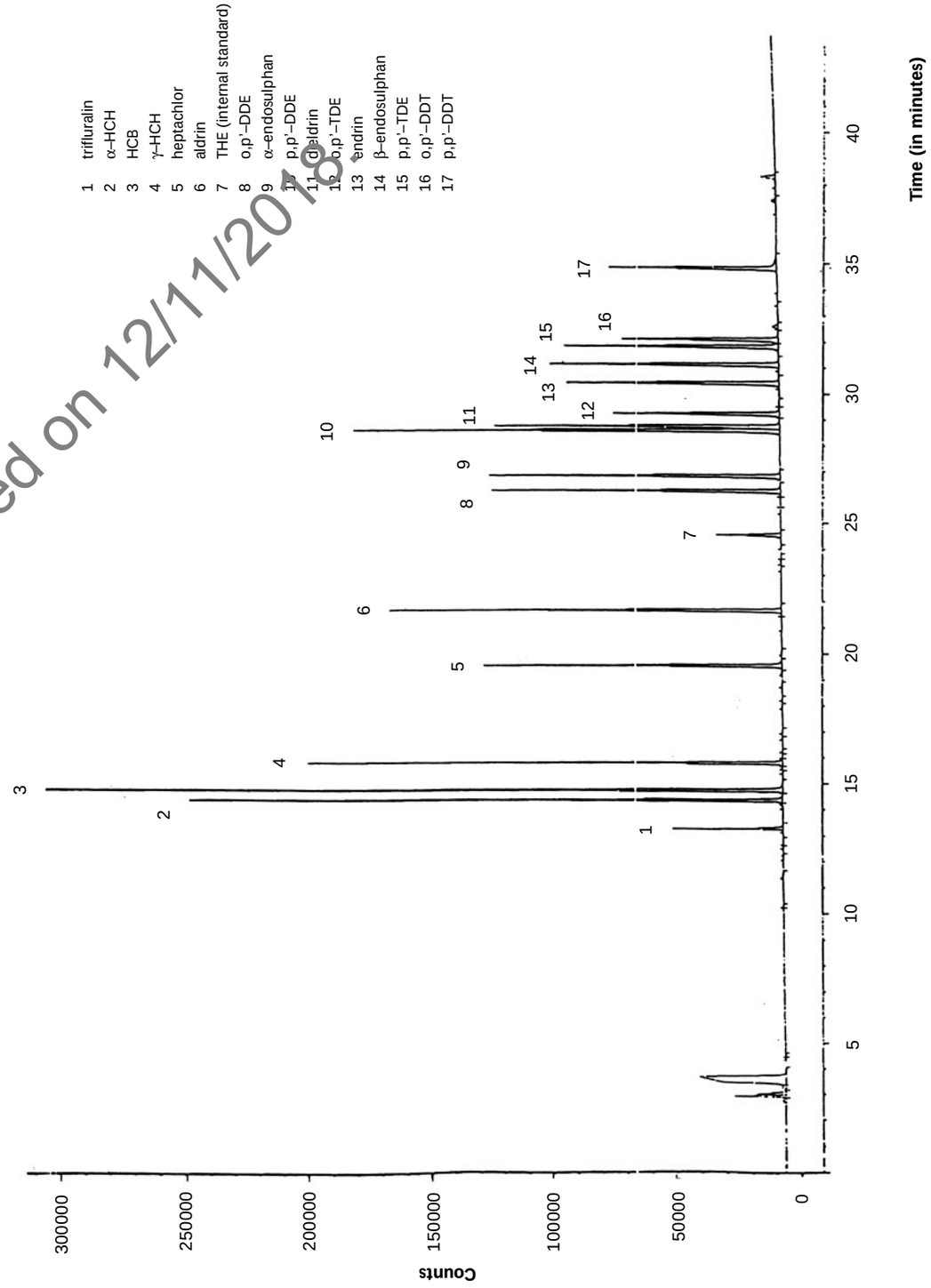


Figure B2 **Typical GC/ECD chromatogram of a standard mixture of organochlorine pesticides**
(concentration 200 µgl^{-1} of each compound)



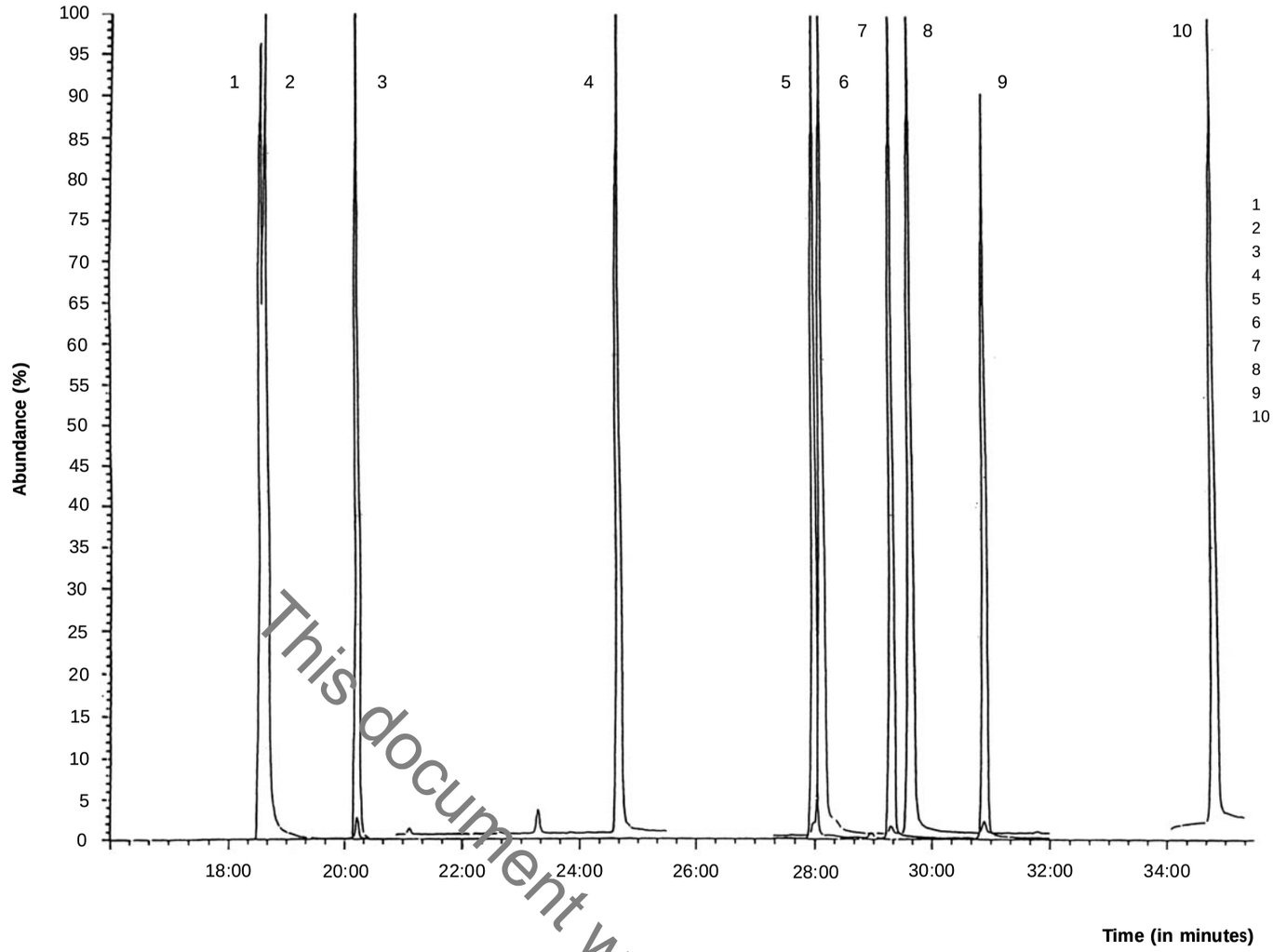


Figure B3 **Typical selected ion mass chromatogram of a standard mixture of PCB**
(PCB concentrations are approximately 200 $\mu g l^{-1}$)

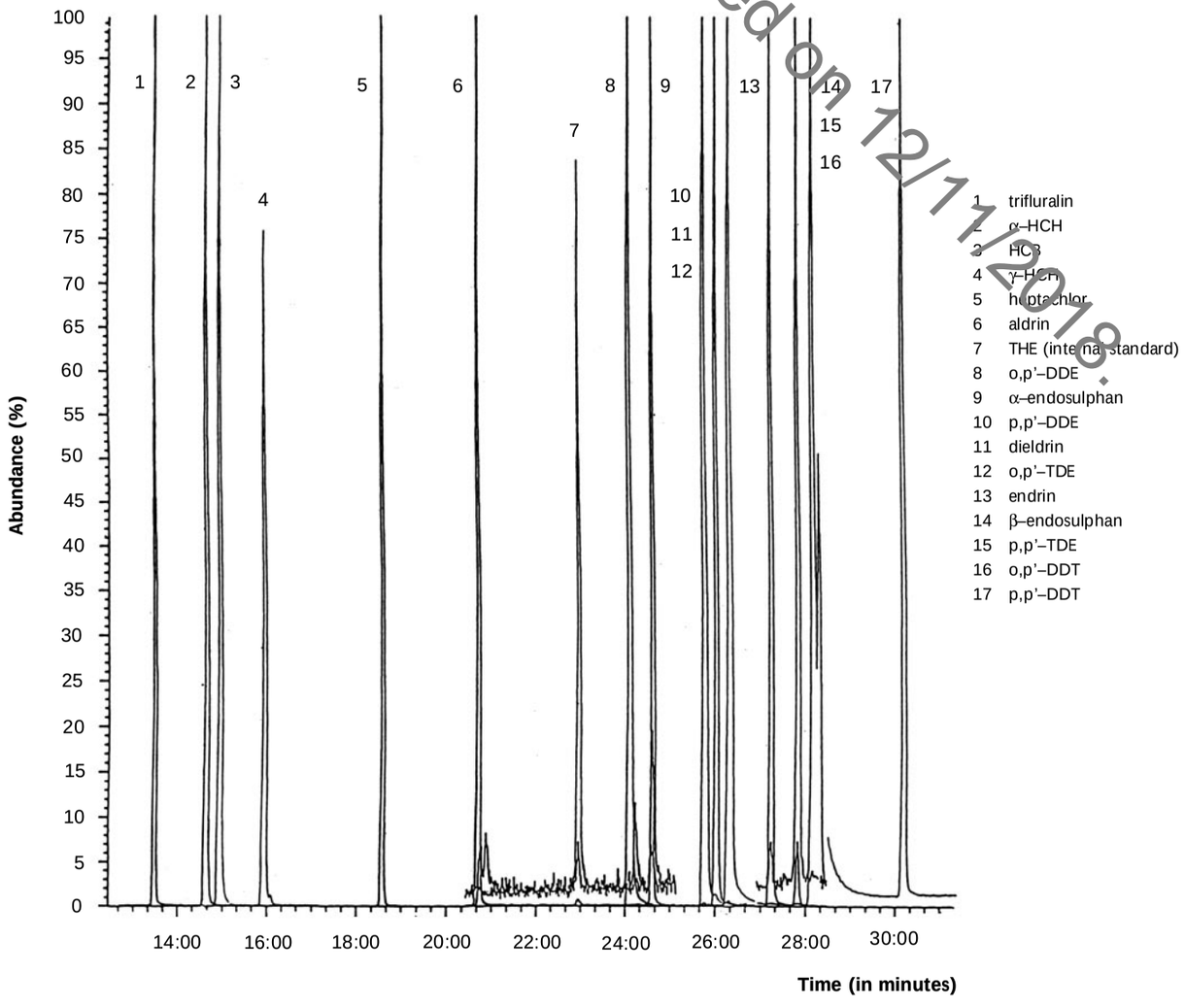


Figure B4 **Typical selected ion mass chromatogram of a standard mixture of organochlorine pesticides**
(concentration 200 $\mu g l^{-1}$ of each compound)

C A note on the determination of organochlorine pesticides in complex matrices by gas chromatography and mass spectrometry

C1 Performance characteristics of the method

C1.1	**Substances determined**	Most organochlorine pesticides and related compounds, for example, see Table A1.
C1.2	**Type of sample**	Settled sewage, crude sewage, sewage sludges and fish tissue. The method may also be extended to soils and muds.
C1.3	**Basis of method**	Sewage samples are extracted with hexane. Soil samples are air dried and extracted with hexane. Fish tissues are macerated and Soxhlet extracted with hexane. The resulting extracts are dried, "cleaned-up" with alumina and concentrated by evaporation before analysis by GC-MSD using SIM.
C1.4	**Range of application**	This varies depending on the compounds determined, but is typically up to 2 $mgkg^{-1}$. The upper limit may be extended by dilution of the extract or by taking a smaller quantity of sample.
C1.5	**Calibration curves**	These vary depending on the compounds determined.
C1.6	**Limit of detection**	Dependent upon the GC characteristics of each determinand and on the MSD response of the fragment ions used for quantification. At some m/z values, the limit of detection will be affected by background levels of interference from the sample. For most pesticides, a limit of detection in the extract of 0.05 $mgkg^{-1}$ is achievable.
C1.7	**Bias**	Extraction efficiencies are normally less than 100%.

C2 Principle

Pesticides (and PCBs) are extracted from the sample with hexane. For fish tissues, including freeze-dried samples, the sample is macerated with sodium sulphate and Soxhlet extracted. For sewage sludges, crude sewage and settled sewage, the determinands are extracted from the sample with propan-2-ol and hexane. For soils, the sample is air-dried and ultra sonicated in the presence of hexane. Extracts are dried, "cleaned-up" on alumina and analysed by GC-MSD.

C3 Interferences

Any compounds extracted under the conditions used and with retention time and mass spectral characteristics close to those of any determinands of interest will interfere. In practice, most interfering compounds will be removed at the "clean-up" stage. The presence of large quantities of interfering materials (for example, oils) may cause a change in the detector response or retention times of the determinands.

C4 Hazards

Skin contact or inhalation of reagents and their solutions should be avoided. Hexane is harmful, narcotic and flammable, acetone is flammable and organochlorine pesticides are toxic. Ensure adequate ventilation and work in a flame- or spark-proof area. Spark-proof refrigerators should be used. Appropriate safety procedures should be followed.

C5 Reagents

All reagents should be of sufficient purity that they do not give rise to interfering peaks during the determination and confirmation. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed.

Pesticide- or HPLC-grade solvents and analytical grade materials are normally suitable unless otherwise specified and details of preparation are provided, where appropriate.

The water used for blank determinations and preparation of control samples should show negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other suitable vessels and stored in the dark if necessary.

C5.1 **Water.**

C5.2 **Hexane.**

C5.3 **Acetone.**

C5.4 **Sodium sulphate.** Anhydrous, granular. Heat at 500 ± 20° C in a muffle furnace for 4.0 ± 0.5 hours. Cool to approximately 200 °C in the muffle furnace and then to ambient temperature in a desiccator. Store in a closed glass container.

C5.5 **Propan-2-ol.**

C5.6 **Anti-bumping granules.** Acetone-washed.

C5.7 **Alumina.** Woelm W200 neutral or an equivalent of the same mesh size. Heat at 500 ± 50 °C in a muffle furnace for 4.0 ± 0.5 hours. Cool to about 200 °C in the muffle furnace and then to ambient temperature in a desiccator.

C5.8 **De-activated alumina.** Add 7.0 ± 0.2% w/w of water (C5.1) to alumina (C5.7) and seal in a jar. Agitate the container for at least 2 hours to ensure the alumina and water are well mixed. Once the container has been opened, the de-activated alumina is normally suitable for use for about one week. After this period, unused amounts may be reprocessed as described in C5.7 and above.

C5.9 **Standard stock solutions.** Prepare individual stock solutions of each of the determinands in acetone. A concentration of 100 mgl^{-1} is suitable.

C5.10 **Mixed working solutions.** Prepare a mixed working solution of all the determinands. Using microlitre syringes, add appropriate volumes of the stock solutions to hexane in a volumetric flask. Dilute to the mark with hexane. The concentration of each determinand in the working standards should be similar to that found in the sample extracts. Typically, a composite intermediate solution containing 10 mgl^{-1} of each determinand may be prepared. From this mixed working solution, standard solutions covering the range 0.01 to 2.0 mgl^{-1} can be prepared.

C5.11 **Surrogate standard.** A spiking solution containing a suitable surrogate standard may be prepared, if required, to monitor the performance of the method. A solution of 100 mgl^{-1} in acetone may be appropriate. The compound selected should be of approximately the same polarity as the determinands, so as to give similar extraction behaviour, should not occur in the samples and should not yield fragment ions identical to those of a co-eluting determinand. Analogues of one or more determinand, suitably labelled with ^{13}C or deuterium, may be suitable.

C5.12 **Recovery spiking standard.** A separate solution containing, for example, 100 mgl^{-1} of each determinand may be prepared in acetone for use for spiking samples for recovery checks.

C5.13 **Internal standard.** If an internal standard is to be added to the final extract, then the compound chosen should not be present in samples to be analysed. A solution containing 10 mgl^{-1} in hexane should be prepared. Chlorodecane, chloroundecane or chlorododecane may be suitable.

C6 Apparatus

C6.1 **Sample containers.** Glass containers. If plastic containers are used, determinand losses from some matrices may occur through adsorption onto the walls of the sample container. Wide-necked jars are suitable for fish and sediment samples.

C6.2 **Muffle furnace.** Capable of operating at 500 °C.

C6.3 **Kuderna-Danish evaporator.** Kuderna-Danish equipment or a suitable alternative evaporating system may be used.

C6.4 **Soxhlet apparatus.** 250 ml flask with thimbles. The thimbles should be checked to ensure that they do not give rise to any interference in the analysis.

C6.5 **Chromatography columns for "clean-up".** Glass, 130 mm long by 5 - 6 mm internal diameter fitted with a sintered glass frit and a glass or PTFE tap.

C6.6 **Nitrogen.** Oxygen-free, filtered and dry.

C6.7 **Gas chromatography.** A gas chromatograph fitted with a capillary column, glass-lined injector with MSD. Operating conditions that may be used are:

Column:	Fused silica capillary, 15 m x 0.54 mm internal diameter, 0.83 µm film thickness, coated with DB-608, or equivalent.
Carrier gas:	helium, 5 $mlmin^{-1}$.
Injection volume:	1 µl.
Column temperature:	Programmed, 180 °C to 260 °C at 10 $°Cmin^{-1}$, hold 8 minutes.
Injector temperature:	240 °C.
Detector temperature:	260 °C.

Other capillary columns and conditions can be used. A longer, narrower column with a similar stationary phase may be used to improve separation efficiency if required.

C7 Sample collection and storage

Aqueous samples should be taken in glass containers (C6.1) and extracted as soon as possible after sampling. If samples cannot be extracted on the day of collection, they should be stored in a refrigerator between 1 - 8 °C. Samples of fish tissue can be frozen (in plastic containers) provided they are thawed to room temperature before analysis. The sample containers should be protected from contamination and should not be placed in proximity to standard materials or their concentrated solutions.

C8 Analytical procedure

Step	Procedure	Notes
C8.1	**Extraction**	
C8.1.1	Sewage sludge and mud	
	Place an accurately measured quantity of sludge, normally 5-10 ml, or 5.00 ± 0.01 g of mud into a 100 ml separating funnel (notes a and b). Add water (C5.1) to bring the volume to 15 ± 1 ml. Add 15 ± 1 ml of propan-2-ol (C5.5) and shake vigorously for 5 minutes. Add 25 ± 1 ml of hexane (C5.2) and shake vigorously for 5 minutes. Allow the layers to separate (note c) and discard the aqueous phase.	(a) If the result is to be reported on a dry weight basis, the total solids content should be determined on a separate portion of the sample. (b) The surrogate standard, if used, should be added at this point. (c) If an emulsion is formed, transfer the contents of the separator to a glass centrifuge tube and separate the layers by centrifugation.
C8.1.2	Soil	
	Air-dry the soil at ambient temperature. Remove any large stones and grind in a mortar with a pestle. Sieve the ground soil through a 2 mm sieve. Place 5.00 ± 0.01 g of dried soil in a 40 ml vial (note b) and add 10 ml of hexane. Seal the vial and ultra sonicate for 10 ± 2 minutes. The supernatant is ready for "clean-up" (notes d and e).	(d) No drying step should be necessary. (e) Some mud samples may be treated as soils.
C8.1.3	Fish tissue	
	Place a sample of tissue in a macerator (note f) and homogenise for 60 ± 10 seconds. Weigh 10.00 ± 0.01 g of homogenised material into a beaker (note b) and add sufficient sodium sulphate (C5.4) to give, after mixing, a free-flowing powder (note g). Transfer the material to the thimble of a Soxhlet extractor and extract for 2.0 ± 0.2 hours with hexane (note h).	(f) A top drive macerator is suitable. (g) Note that the powder can "cake" on standing. (h) An acetone/hexane mixture may produce better extraction efficiencies.
C8.1.4	Settled sewage	
	Place 500 ± 5 ml of sample in a nominal 1 litre separating flask (note b). Add 50 ± 1 ml of hexane, shake the funnel for 5 ± 2 minutes and allow to stand. Run off any deposited solids into a 100 ml separating flask. Discard the clear aqueous phase. Collect the interfacial cuff in the same 100 ml separating flask. To the 100 ml separating flask, add a volume of propan-2-ol which is approximately equal to that of the solids and emulsion previously collected. Shake for 5 ± 2 minutes. Add 25 ± 5 ml of	

Step	Procedure	Notes
	hexane and shake for 5 ± 2 minutes. Allow the layers to separate and discard the lower layer. Combine the hexane extracts in the 1 litre separating flask.	
C8.2	**Drying, concentration and "clean-up"**	
C8.2.1	Drying Run the hexane extract into a flask containing approximately 10 g of sodium sulphate (C5.4). Rinse the container with 10 ± 1 ml of hexane and transfer the washings to the flask. Swirl the flask and leave to stand, swirling occasionally.	
C8.2.2	Concentration Transfer the dried extract to a Kuderna-Danish evaporator (note i). Wash the sodium sulphate with 10 ± 1 ml of hexane. Decant the washings into the Kuderna-Danish evaporator. Add an anti-bumping granule and evaporate to 3 ± 2 ml. Remove the graduated tube from the evaporator and reduce the hexane volume using a stream of nitrogen (C6.6) to approximately 1 ml. Alternatively, depending upon the solids content, reduce the extract to a recorded volume.	(i) An alternative evaporation system may be used.
C8.2.3	"Clean-up" Prepare an alumina bed in the chromatography column (C6.5). For example, (note j), close the tap at the base of the column and add 15 ml of hexane followed by 5.0 ± 0.1 g of de-activated alumina (C5.8). Tap the column gently and allow the alumina to settle. Add a 0.5 cm layer of sodium sulphate (C5.4). Open the tap and drain the hexane to the level of the top of the sodium sulphate. Transfer the sample extract (or a suitable aliquot where the concentrated extract volume exceeds 1 ml) to the top of the column. Place a Kuderna-Danish tube below the base of the column to collect the eluate. Where the sample was contained in a tube, wash the tube with 1.0 ± 0.2 ml of hexane and add the washings to the column. As soon as the sample has reached the level of the sodium sulphate, elute the column with 40 ± 1 ml of hexane. Concentrate	(j) Alternative procedures may be used, for example, see C8.3.1. (k) For example, reduce the volume to less than 1 ml. Using a 1 ml precision syringe, draw the extract into the syringe and measure the volume. Return the extract to the tube and, using the same syringe, add sufficient hexane to the tube so that the final volume is 1.00 ± 0.01 ml. (l) Add the internal standard at this stage if required (C5.13).

Step	Procedure	Notes
	the extract to between 3 and 5 ml. Remove the tube from the Kuderna-Danish evaporator and reduce the volume of hexane using a stream of nitrogen (C6.6) to 1.00 ± 0.01 ml (notes k and l).	
C8.3	**GC-MSD determination**	
C8.3.1	Set up the GC-MS apparatus in accordance with the manufacturer's instructions using conditions given in C6.7.	
C8.3.2	Using a concentrated standard (see C5.10) inject the mixture of determinands in full scan mode. Identify the peaks by their characteristic mass spectra and note the retention time for each determinand (note m).	(m) Depending on the compounds of interest, individual standard solutions may need to be used to enable retention time data to be determined.
C8.3.3	Select suitable m/z values for each determinand (usually the molecular ion) and select qualifier ions for confirmation. Run the calibration standards (C5.10) and plot calibration curves for each compound.	
C8.3.4	Inject the extracts of samples, blanks and spiked samples; record the retention times and responses. Calculate the concentration of determinands present in the original sample. If a dilution of an extract, or an aliquot of the extract, is used, the calculation should reflect this.	
C8.3.5	Check the recovery (note n) of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard PCBs and organochlorine compounds to separate samples. Process these solutions under conditions identical with those to be used for samples.	(n) To determine the recovery, spike a sample matrix expected to be low in PCB and organochlorine compounds (for example silversand, or a non-industrial sludge, etc) at an appropriate concentration.
C8.3.6	Recovery data from previous tests should be averaged and a mean correction factor determined and used for correcting recoveries (note o)	(o) Use a surrogate standard to indicate whether the analysis has proceeded satisfactorily for each individual sample.

D

A screening method for selected polychlorinated biphenyls in waters by solvent extraction and gas chromatography with electron capture detection

Introduction

Method D is intended as a screening procedure, whilst method E is for confirmation and quantification. PCBs were sold under a variety of trade names as PCB-based dielectric fluids, including Aroclor, Pyroclor, Inerteen, Pyranol, Clophen, Apirolio, Kaneclor and Solvol. Other names, which have been used for PCB products intended for different applications, are no longer in current use. Tables D2 and D3 show approximate chlorine percentages in PCBs and approximate percentage composition in a number of Aroclor mixtures. Table D4 shows the structures and numbering of PCBs. The World Health Organisation has recently issued toxic equivalent factors for several PCBs.

D1 Performance characteristics of the method

D1.1	**Substances determined**	PCBs may not be separated or identified as individual compounds. Where no peaks are observed, PCBs may be reported as not being detected; however, when peaks are observed, they should be confirmed using GC-MSD.
D1.2	**Type of sample**	Potable, river and saline waters.
D1.3	**Range of application**	Up to 2 $\mu g l^{-1}$ per standard congener solution (a 1 litre sample concentrated to 1 ml). The upper limit may be extended by taking a smaller sample volume, or diluting the sample extract.
D1.4	**Calibration curves**	Linear over the range of application.
D1.5	**Standard deviation**	See Table D1 for standard solutions.
D1.6	**Limit of detection**	Typically, 0.001 $\mu g l^{-1}$ per standard congener solution for a 1 litre sample.
D1.7	**Sensitivity**	Dependent on the instrument used.
D1.8	**Bias**	Extraction efficiencies are normally less than 100%. See Table D1.

D2 Principle

PCBs are extracted into hexane. The extract is dried with anhydrous sodium sulphate, reduced to a small volume and reconstituted in 2,2,4-trimethylpentane. Reconstituted extracts are analysed by GC/ECD using a single capillary column.

D3 Interferences

Any substances present after extraction which have similar retention times to any of the PCB standards used and which give a detector response will interfere. Interferences which have been identified include organochlorine pesticides and certain phthalate compounds. It is recognised that other PCB congeners may also co-elute with the PCB standards used in the calibration. Any extract which subsequently shows a peak with an identical retention time to that of a reference PCB congener should be re-analysed by the procedures described in Method E.

D4 Hazards

Skin contact or inhalation of all reagents and their solutions should be avoided. Hexane is harmful and flammable and acetone and 2,2,4-trimethylpentane are flammable. PCBs are toxic. Ensure adequate ventilation and work in a flame- or spark-proof area. Spark-proof refrigerators should be used to store standard solutions and extracts. Appropriate safety procedures should be followed.

D5 Reagents

All reagents should be of sufficient purity that they do not give rise to interfering peaks during the GC/ECD analysis. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Pesticide- or HPLC-grade solvents and analytical grade materials in the case of other reagents are normally suitable and details of preparation are provided, where appropriate.

The water used for blank determinations and preparation of control samples should show negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other suitable vessels and stored in the dark if necessary.

D5.1 Water.

D5.2 Hexane.

D5.3 2,2,4-trimethylpentane (iso-octane).

D5.4 Acetone.

D5.5 Sodium sulphate. Granular anhydrous. Heat to 500 ± 20 °C in a muffle furnace for 4.0 ± 0.5 hours. Cool to about 200 °C in the muffle furnace and then to ambient temperature in a desiccator. Store in a closed glass container.

D5.6 Individual standard stock solutions. Prepare individual stock solutions of pure or suitably certified PCBs in 2,2,4-trimethylpentane at a concentration of 100 $mg l^{-1}$. Alternatively, suitably certified solutions can be obtained commercially, for example, at a concentration of 10 $mg l^{-1}$. Stock solutions of, for example, the following PCBs should be available: PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180, see Table D4.

D5.7 Mixed working standard solutions. By dilution of the stock solutions (D5.6), prepare a series of at least five mixed working standard solutions of PCB congeners in 2,2,4-trimethylpentane. For example, a useful working range for each congener is 0.001 to 0.04 $mg l^{-1}$.

D6 Apparatus

Apparatus should be free from contamination before use. Glassware should be thoroughly clean, rinsed with acetone and then hexane before use, and allowed to dry.

D6.1 Sample bottles. Glass. 1.2 litre capacity, marked at 1 litre, fitted with glass stoppers or PTFE-lined screw caps.

D6.2 Separating funnels. Glass, nominal 1 litre capacity, fitted with grease-free glass or PTFE tap and stopper.

D6.3 Volumetric flasks, measuring cylinders, pipettes, syringes and glass vials. These should be of various sizes.

D6.4 Kuderna-Danish evaporator. An alternative evaporating system may also be used.

D.6.5 Nitrogen. Oxygen-free, filtered and dry.

D6.6 Gas chromatography. A gas chromatograph fitted with a capillary column, glass-lined injector with ECD. Other capillary columns and conditions can be used. To separate individual congeners more effectively, a longer, narrower diameter column can be used, for example, 30 m x 0.25 mm internal diameter, coated with methylsilicone.

D7 Sample collection and storage

Samples should be taken in glass bottles with glass stoppers or PTFE-lined screw caps. They should be extracted as soon as possible after sampling. If samples cannot be extracted on the day of collection, they should be stored in a refrigerator between 1 - 8°C. The sample bottles should be protected from contamination and should not be placed in close proximity to standard materials or their concentrated solutions. Consideration should be given to taking duplicate samples. This is especially important if samples are analysed and found to contain peaks in the chromatogram using ECD which then require confirmation and quantification with GC-MSD.

D8 Analytical procedure

Step	Procedure	Notes
D8.1	**Extraction**	
D8.1.1	To 1000 ± 10 ml of sample in the sample bottle, add 40 ± 5 ml of hexane (D5.2). Stopper and shake vigorously for 120 ± 10 seconds (note a). Transfer the contents of the bottle to a 1 litre separating funnel. Rinse the bottle with a further 10 ± 1 ml of hexane and transfer the washings to the separating funnel. Alternatively, the extraction may be undertaken in a separating funnel. Transfer 1000 ± 10 ml of sample to the separating funnel. Rinse the empty bottle thoroughly with 50 ± 5 ml of hexane. Add the hexane to the separating funnel and shake vigorously for 120 ± 10 seconds.	(a) A machine for shaking bottles in a horizontal plane may be used. In this case, the shaking period should be extended to at least 5 minutes.
D8.1.2	Allow the phases to separate. When good separation has been achieved, run off and discard the lower aqueous phase (notes b and c).	(b) Vigorous shaking may produce emulsions with some samples. These may be broken by the addition of inorganic acids or salts, or by centrifugation. If a solvent-water interfacial cuff remains, this should be included with the hexane extract. (c) In the case of samples containing particulate material, the efficiency of the extraction may be improved by repeating the extraction.
D8.2	**Drying and concentration**	
D8.2.1	Run the hexane layer into a flask containing approximately 10 g of sodium sulphate (D5.5). Rinse the separating funnel with 10 ± 1 ml of hexane and transfer the washings to the flask. Swirl the flask and leave to stand. Swirl occasionally.	
D8.2.2	Transfer the dried extract to a Kuderna-Danish evaporator (note d). Wash the sodium sulphate with 10 ± 1 ml of hexane. Decant the washings into the Kuderna-Danish evaporator, add an anti-bumping granule and evaporate the extract to 3 ± 2 ml.	(d) An alternative evaporating system may be used.
D8.2.3	Add 0.50 ± 0.10 ml of 2,2,4-trimethylpentane and evaporate to remove the hexane using a gentle stream of nitrogen (D6.5) to 1.00 ± 0.01 ml (note e). This solution is now ready for GC/ECD determination.	(e) For example, reduce the volume to less than 1 ml. Using a 1 ml precision syringe, draw the extract into the syringe and measure the volume. Return the extract to the tube and, using the same syringe, add sufficient 2,2,4-trimethylpentane to the tube so that the final volume is 1.00 ± 0.01 ml.

Step	Procedure	Notes
D8.3	**GC/ECD determination**	
D8.3.1	Set up the instrument in accordance with the manufacturer's instructions.	
D8.3.2	Inject aliquots of standard solutions and extracts from samples, blank and recovery spikes into the GC (note f).	(f) The same injection volume should also be used.
D8.3.3	Measure the response of each of the PCB congeners in the standard solutions. Compare the retention times of each of the peaks in the sample extracts with those previously obtained from the standard PCB congeners used.	
D8.3.4	Construct calibration graphs of the PCB peak responses versus concentrations for each congener.	
D8.3.5	Read the concentration of each suspected congener, as identified from the retention time, in sample extracts from the calibration graph. Calculate the concentration, expressed as a particular PCB congener, present in the original sample.	
D8.4	**Confirmation**	
D8.4.1	Confirmation and quantification of any positive result should be undertaken on a separate (duplicate) sample containing surrogate and/or internal standards using the procedures described in Method E.	

Table D1 **Recoveries of PCB standard solutions using GC/ECD**

PCB	Added spike	Spiked ultrapure water S_t	Rec (%)	RSD (%)	LOD	Spiked tap water S_t	Rec (%)	RSD (%)	LOD
PCB 28	0.002	0.0002(12)	101	11.7	0.001	0.0002(12)	90	13.6	0.001
	0.005	0.0005(11)	106	9.5		0.0005(12)	96	9.9	
	0.020	0.0020(12)	94	10.1		0.0010(15)	100	4.8	
PCB 52	0.002	0.0004(14)	115	15.5	0.002	0.0003(12)	121	11.8	0.001
	0.005	0.0007(12)	113	13.1		0.0004(15)	106	7.7	
	0.020	0.0026(14)	98	13.4		0.0017(12)	102	8.2	
PCB 101	0.002	0.0002(12)	98	8.4	0.0008	0.0003(12)	100	12.9	0.001
	0.005	0.0005(12)	100	3.6		0.0003(12)	100	5.5	
	0.020	0.0026(12)	89	14.6		0.0007(12)	100	3.5	
PCB 118	0.002	0.0001(12)	95	4.7	0.0004	0.0002(12)	94	9.4	0.0008
	0.005	0.0002(12)	98	3.8		0.0004(15)	94	9.2	
	0.020	0.0026(15)	90	14.4		0.0009(15)	100	4.5	
PCB 138	0.002	0.0003(14)	98	16.2	0.002	0.0002(12)	87	10.2	0.0008
	0.005	0.0005(12)	101	10.9		0.0004(12)	90	4.7	
	0.020	0.0025(12)	85	14.6		0.0010(12)	99	5.3	
PCB 153	0.002	0.0001(12)	100	5.5	0.0005	0.0002(14)	96	10.0	0.0009
	0.005	0.0002(15)	99	4.1		0.0004(12)	90	8.1	
	0.020	0.0026(14)	88	14.9		0.0011(12)	97	5.9	
PCB 180	0.002	0.0002(12)	94	8.1	0.0007	0.0002(12)	98	8.9	0.0008
	0.005	0.0002(14)	101	4.9		0.0003(13)	93	6.9	
	0.020	0.0028(15)	82	17.1		0.0012(12)	100	5.9	

All units expressed as $\mu g l^{-1}$ unless otherwise specified.
LOD is calculated using 4.65 x S_w of the low spike.
Figure in brackets represents DF.
Data provided by Wessex Water.

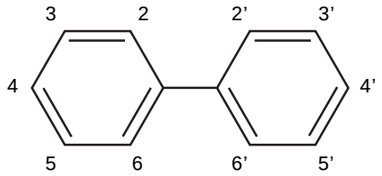
Table D2 **Number of possible isomers and per cent chlorine for the ten PCB groups**

PCB group	Empirical Formula	Molecular weight	Number of possible isomers	Percent chlorine by weight
monochlorobiphenyl	$C_{12}H_9Cl$	188.5	3	18.8
dichlorobiphenyl	$C_{12}H_8Cl_2$	223	12	31.8
trichlorobiphenyl	$C_{12}H_7Cl_3$	257.5	24	41.3
tetrachlorobiphenyl	$C_{12}H_6Cl_4$	292	42	48.6
pentachlorobiphenyl	$C_{12}H_5Cl_5$	326.5	46	54.3
hexachlorobiphenyl	$C_{12}H_4Cl_6$	361	42	58.9
heptachlorobiphenyl	$C_{12}H_3Cl_7$	395.5	24	62.8
octachlorobiphenyl	$C_{12}H_2Cl_8$	430	12	66.0
nonachlorobiphenyl	$C_{12}HCl_9$	464.5	3	68.7
decachlorobiphenyl	$C_{12}Cl_{10}$	499	1	71.2

Table D3 **Approximate per cent congener composition of several Aroclor mixtures**

	Aroclor 1016	1221	1232	1242	1248	1254	1260
monochlorobiphenyl	1	51	26	1			
dichlorobiphenyl	20	32	29	17	1		
trichlorobiphenyl	57	4	24	40	23		
tetrachlorobiphenyl	21	2	15	32	50	16	
pentachlorobiphenyl	1	1	1	10	20	60	12
hexachlorobiphenyl				1	1	23	46
heptachlorobiphenyl						1	36
octachlorobiphenyl						6	
nonachlorobiphenyl							
decachlorobiphenyl							

Table D4 Structure and positional isomers of PCBs



No.	Position	No.	Position	No.	Position	No.	Position	No.	Position
1	2	44	2,2',3,5'	89	2,2',3,4,6'	134	2,2',3,3',5,6	179	2,2',3,3',5,6,6'
2	3	45	2,2',3,6	90	2,2',3,4',5	135	2,2',3,3',5,6'	180	2,2',3,4,4',5,5'
3	4	46	2,2',3,6'	491	2,2',3,4',6	136	2,2',3,3',6,6'	181	2,2',3,4,4',5,6
		47	2,2',4,4'	92	2,2',3,5,5'	137	2,2',3,4,4',5	182	2,2',3,4,4',5,6'
4	2,2'	48	2,2',4,5	93	2,2',3,5,6	138	2,2',3,4,4',5'	183	2,2',3,4,4',5',6
5	2,3	49	2,2',4,5'	94	2,2',3,5,6'	139	2,2',3,4,4',6	184	2,2',3,4,4',6,6'
6	2,3'	50	2,2',4,6	95	2,2',3,5',6	140	2,2',3,4,4',6'	185	2,2',3,4,5,5',6
7	2,4	51	2,2',4,6'	96	2,2',3,6,6'	141	2,2',3,4,5,5'	186	2,2',3,4,5,6,6'
8	2,4'	52	2,2',5,5'	97	2,2',3',4,5	142	2,2',3,4,5,6	187	2,2',3,4',5,5',6
9	2,5	53	2,2',5,6'	98	2,2',3',4,6	143	2,2',3,4,5,6'	188	2,2',3,4',5,6,6'
10	2,5'	54	2,2',6,6'	99	2,2',4,4',5	144	2,2',3,4,5',6	189	2,3,3',4,4',5,5'
11	3,3'	55	2,3,3',4	100	2,2',4,4',6	145	2,2',3,4,6,6'	190	2,3,3',4,4',5,6
12	3,4	56	2,3,3',4'	101	2,2',4,5,5'	146	2,2',3,4',5,5'	191	2,3,3',4,4',5',6
13	3,4'	57	2,3,3',5	102	2,2',4,5,6'	147	2,2',3,4',5,6	192	2,3,3',4,5,5',6
14	3,5	58	2,3,3',5'	103	2,2',4,5',6	148	2,2',3,4',5,6'	193	2,3,3',4',5,5',6
15	4,4'	59	2,3,3',6	104	2,2',4,6,6'	149	2,2',3,4',5',6		
		60	2,3,4,4'	105	2,3,3',4,4'	150	2,2',3,4',6,6'	194	2,2',3,3',4,4',5,5'
16	2,2',3	61	2,3,4,5	106	2,3,3',4,5	151	2,2',3,5,5',6	195	2,2',3,3',4,4',5,6
17	2,2',4	62	2,3,4,6	107	2,3,3',4',5	152	2,2',3,5,6,6'	196	2,2',3,3',4,4',5',6
18	2,2',5	63	2,3,4',5	108	2,3,3',4,5'	153	2,2',4,4',5,5'	197	2,2',3,3',4,4',6,6'
19	2,2',6	64	2,3,4',6	109	2,3,3',4,6	154	2,2',4,4',5,6'	198	2,2',3,3',4,5,5',6
20	2,3,3'	65	2,3,5,6	110	2,3,3',4',6	155	2,2',4,4',6,6'	199	2,2',3,3',4,5,6,6'
21	2,3,4	66	2,3',4,4'	111	2,3,3',5,5'	156	2,3,3',4,4',5	200	2,2',3,3',4,5',6,6'
22	2,3,4'	67	2,3',4,5	112	2,3,3',5,6	157	2,3,3',4,4',5'	201	2,2',3,3',4',5,5',6
23	2,3,5	68	2,3',4,5'	113	2,3,3',5',6	158	2,3,3',4,4',6	202	2,2',3,3',5,5',6,6'
24	2,3,6	69	2,3',4,6	114	2,3,4,4',5	159	2,3,3',4,5,5'	203	2,2',3,4,4',5,5',6
25	2,3',4	70	2,3',4',5	115	2,3,4,4',6	160	2,3,3',4,5,6	204	2,2',3,4,4',5,6,6'
26	2,3',5	71	2,3',4',6	116	2,3,4,5,6	161	2,3,3',4,5',6	205	2,3,3',4,4',5,5',6
27	2,3',6	72	2,3',5,5'	117	2,3,4',5,6	162	2,3,3',4',5,5'		
28	2,4,4'	73	2,3',5',6	118	2,3',4,4',5	163	2,3,3',4',5,6	206	2,2',3,3',4,4',5,5',6
29	2,4,5	74	2,4,4',5	119	2,3',4,4',6	164	2,3,3',4',5',6	207	2,2',3,3',4,4',5,6,6'
30	2,4,6	75	2,4,4',6	120	2,3',4,5,5'	165	2,3,3',5,5',6	208	2,2',3,3',4,5,5',6,6'
31	2,4',5	76	2',3,4,5	121	2,3',4,5',6	166	2,3,4,4',5,6		
32	2,4',6	77	3,3',4,4'	122	2',3,3',4,5	167	2,3',4,4',5,5'	209	2,2',3,3',4,4',5,5',6,6'
33	2',3,4	78	3,3',4,5	123	2',3,4,4',5	168	2,3',4,4',5',6		
34	2',3,5	79	3,3',4,5'	124	2',3,4,5,5'	169	3,3',4,4',5,5'		
35	3,3',4	80	3,3',5,5'	125	2',3,4,5,6'				
36	3,3',5	81	3,4,4',5	126	3,3',4,4',5	170	2,2',3,3',4,4',5		
37	3,4,4'			127	3,3',4,5,5'	171	2,2',3,3',4,4',6		
38	3,4,5	82	2,2',3,3',4			172	2,2',3,3',4,4',5'		
39	3,4',5	83	2,2',3,3',5	128	2,2',3,3',4,4'	173	2,2',3,3',4,5,6		
		84	2,2',3,3',6	129	2,2',3,3',4,5	174	2,2',3,3',4,5,6		
40	2,2',3,3'	85	2,2',3,4,4'	130	2,2',3,3',4,5'	175	2,2',3,3',4,5',6		
41	2,2',3,4	86	2,2',3,4,5	131	2,2',3,3',4,6	176	2,2',3,3',4,6,6'		
42	2,2',3,4'	87	2,2',3,4,5'	132	2,2',3,3',4,6'	177	2,2',3,3',4',5,6		
43	2,2',3,5	88	2,2',3,4,6	133	2,2',3,3',5,5'	178	2,2',3,3',5,5',6		

E A note on the determination of total polychlorinated biphenyls in waters by gas chromatography-mass spectrometry

E1 Introduction

The use of this method (and Method F) enables PCBs in a sample to be identified according to the level of chlorination. Individual congeners are identified by their retention times and their mass to charge ratio. The chlorination level can normally be quantified by reference to two ions. The peak response ratio for the most abundant ion is used to calculate the concentration of PCB at each chlorination level. A value for total PCBs is then obtained by summation of the individual values calculated for each chlorination level.

In order for a peak to be identified as a PCB, the response ratio for the two ions selected should differ by less than 20 % from the standard response ratio for the corresponding level of chlorination. See Table E2. Its retention time should also be within the experimental range established when the method is set up.

The method should be applicable to potable, river and saline waters.

E2 Principle

PCBs are extracted into hexane. The extract is dried with anhydrous sodium sulphate, evaporated to low volume and reconstituted in 2,2,4-trimethylpentane. The reconstituted extract is analysed by GC-MSD operating in EI mode with SIM. Quantification is by internal standard added to the extract after evaporation. A ^{13}C-surrogate standard added before extraction is used to monitor the performance of the analysis for each sample.

E3 Interferences

Any co-extracted compound which has a GC retention time within the ranges established for the determinands and which gives a detector response for both of the ions being monitored within the range in the required ratio will interfere. Organochlorine pesticides need not be removed from the extract before analysis.

E4 Hazards

Skin contact or inhalation of all reagents and their solutions should be avoided. Hexane is harmful and flammable and acetone and 2,2,4-trimethylpentane are flammable. PCBs are toxic. Ensure adequate ventilation and work in a flame- or spark-proof area. Spark-proof refrigerators should be used to store standard solutions and extracts. Appropriate safety procedures should be followed.

E5 Reagents

All reagents should be of sufficient purity that they do not give rise to interfering peaks during the GC-MSD determinattion. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Pesticide- or HPLC-grade solvents and analytical grade materials in the case of other reagents are normally suitable unless otherwise specified and details of preparation are provided, where appropriate.

The water used for blank determinations and preparation of control samples should show negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other suitable vessels and stored in the dark if necessary.

E5.1 Water.

E5.2 Hexane.

E5.3 2,2,4-trimethylpentane (iso-octane).

E5.4 Acetone.

E5.5 Sodium sulphate. Granular anhydrous. Heat to 500 ± 20 °C in a muffle furnace for 4.0 ± 0.5 hours. Cool to about 200 °C in the muffle furnace and then to ambient temperature in a desiccator. Store in a closed glass container.

E5.6 Mixed standard stock solution. Suitably certified solutions of PCBs in 2,2,4-trimethylpentane can be obtained commercially, for example, at concentrations of 10 mgl^{-1}. Stock solutions of congeners representing each chlorination level, for example the following congeners, should be available; PCB 1, PCB 3, PCB 7, PCB 30, PCB 50, PCB 97, PCB 143, PCB 183, PCB 202, PCB 207 and PCB 209. See Table D4.

E5.7 Stock solution of internal standard. Prepare a stock solution of pure or suitably certified internal standard. For example, 4,4'-dibromobiphenyl in 2,2,4-trimethylpentane at a concentration of 100 mgl^{-1}. Alternatively, a suitably certified solution can be obtained commercially.

E5.8 Stock solution of surrogate standard. A suitably certified stock solution of an appropriate surrogate standard (to be added before extraction). For example, ^{13}C-PCB 97 for a Cl_5-PCB, or ^{13}C-PCB 138 for a Cl_6-PCB, in 2,2,4-trimethylpentane at a concentration of 10 mgl^{-1}.

E5.9 Stock solution of Aroclors. For example, prepare a stock solution of certified Aroclor 1242, Aroclor 1254 or Aroclor 1260 (or a mixture of these) in 2,2,4-trimethylpentane at, for example, a concentration of 50 mgl^{-1} of each.

E5.10 Spiking solution of the internal standard. By dilution of the stock solution of internal standard (E5.7), prepare a solution in 2,2,4-trimethylpentane at a concentration of, for example, 0.25 mgl^{-1}.

E5.11 Spiking solution of the surrogate standard. By dilution of the stock solution of surrogate standard (E5.8) prepare a solution in acetone at a concentration of, for example, 0.5 mgl^{-1}.

E5.12 Mixed working standard solutions of PCB with surrogate standard. By dilution of the mixed standard stock solution (E5.6) and stock spiking solution of surrogate standard (E5.8) prepare a series of at least five mixed working standard solutions of PCB and the surrogate standard in 2,2,4-trimethylpentane each containing, for example, 0.25 mgl^{-1} of surrogate standard. For example, a useful working range for each congener is 0.05 to 2 mgl^{-1}.

E6 Apparatus

All reagents should be of sufficient purity that they do not give rise to interfering peaks during the GC-MSD determinattion. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Pesticide or HPLC-grade solvents and analytical grade materials in the case of other reagents are normally suitable unless otherwise specified and details of preparation are provided, where appropriate.

E6.1 Sample bottles. Glass, 1.2 litre capacity, marked at 1 litre, fitted with glass stoppers or PTFE-lined screw caps.

E6.2 Separating funnels. Glass, 1 litre capacity, fitted with grease-free glass or PTFE tap and stopper.

E6.3 Volumetric flasks, measuring cylinders, pipettes, syringes and glass vials. These should be of various sizes.

E6.4 Kuderna-Danish evaporator. An alternative evaporating system may also be used.

E6.5 Nitrogen. Oxygen-free, filtered and dry.

E6.6 Gas chromatography. A gas chromatograph fitted with a capillary column and glass-lined injector. This should be fitted with a detector capable of operating in EI mode with SIM. This will permit different groups of ions to be monitored at selected time intervals during the analysis. Operating conditions that may be used are:

Column:	fused silica WCOT, 30 m x 0.25 mm internal diameter, 0.25 µm film thickness, coated with HP5-MS, or equivalent.
Carrier gas:	helium at 1 $mlmin^{-1}$.
Injection volume:	2 µl.
Injection mode:	on column.
Column temperature:	programmed, 75 °C for 2 min, 30 °$Cmin^{-1}$, to 120 °C, 10 °$Cmin^{-1}$ to 240 °C, hold for 20 min.
Injector temperature:	300 °C.

A chromatogram showing PCB congeners (representing each level of chlorination) and the internal standard obtained under these conditions is given in Figure E1. Figure E8 shows a SIM chromatogram, obtained under conditions similar to those for seven PCB congeners included in Method D. Other capillary columns and conditions can be used.

E7 Sample collection and storage

Samples should be taken in glass bottles with glass stoppers or PTFE-lined screw caps. They should be extracted as soon as possible after sampling. If samples cannot be extracted on the day of collection, they should be stored in a refrigerator between 1 - 8 °C. The sample bottles should be protected from contamination and should not be placed in close proximity to standard materials or their concentrated solutions.

E8 Analytical procedure

Step	Procedure	Notes
E8.1	**Extraction**	
E8.1.1	To 1000 ± 10 ml of sample in the sample bottle, add 100 ± 5 µl of spiking solution of surrogate standard (E5.11). Add 40 ± 5 ml of hexane (E5.2). Stopper and shake vigorously for 120 ± 10 seconds (note a). Transfer the contents of the bottle to a 1 litre separating funnel. Rinse the bottle with a further 10 ± 1 ml of hexane and transfer the washings to the separating funnel. Alternatively, the extraction may be undertaken in a separating funnel. Add 100 ± 5 µl of spiking solution of surrogate standard (E5.11) to 1000 ± 10 ml of sample in the sample bottle. Transfer the spiked sample to a separating funnel. Rinse the empty bottle thoroughly with 50 ± 5 ml of hexane. Add the hexane to the separating funnel. Seal and shake vigorously for 120 ± 10 seconds.	(a) A machine for shaking bottles in a horizontal plane may be used. In this case, the shaking period should be extended to at least 5 minutes.
E8.1.2	Allow the phases to separate. When good separation has been achieved, run off and discard the lower aqueous phase (notes b and c).	(b) Vigorous shaking may produce emulsions with some samples. These may be broken by the addition of inorganic acids or salts, or by centrifugation. If a solvent-water interfacial cuff remains, this should be included with the hexane extract and the water removed at the drying stage.

Step	Procedure	Notes
		(c) The efficiency of the extraction may be improved by repeating the extraction, especially in cases where samples contain particulate matter.
E8.2	**Drying and concentration**	
E8.2.1	Run the hexane layer into a flask containing approximately 10 g of sodium sulphate (E5.5). Rinse the separating funnel with 10 ± 1 ml of hexane and transfer the washings to the flask. Swirl the flask and leave to stand, swirling occasionally.	
E8.2.2	Transfer the dried extract to a Kuderna-Danish evaporator (note d). Wash the sodium sulphate with 10 ± 1 ml of hexane. Decant the washings into the Kuderna-Danish evaporator, add an anti-bumping granule and evaporate the extract to 3 ± 2 ml.	(d) Alternative evaporating systems may be used.
E8.2.3	Reduce the volume of the extract using a stream of nitrogen (E6.5) to 1.00 ± 0.01 ml (notes e and f). The solution is now ready for GC-MSD determination.	(e) For example, reduce the volume to less than 1 ml. Using a precision syringe, draw the extract into the syringe and measure the volume. Return the extract to the tube and, using the same syringe, add sufficient 2,2,4-trimethylpentane to the tube so that the final volume is 1.00 ± 0.01 ml. (f) Add 100 ± 5 µl of internal standard spiking solution (E5.10) if required.
E8.3	**GC-MSD determination**	
E8.3.1	Set up the instrument in accordance with the manufacturer's instructions using the conditions given in section E6.6.	
E8.3.2	Set up the SIM programme by determining the time intervals over which the groups of PCBs at each level of chlorination are eluted from the GC column. For example, inject an aliquot of the mixed Aroclor standard solution (E5.9) with the GC-MS operating in full scan mode (50-550 amu) (note g).	(g) A chromatogram of Aroclor 1242 obtained under these conditions is shown in Figure E2.
E8.3.3	Select two ions for each of the ten chlorination levels (note h) and reprocess the data (from Figure E2) to produce two SIM chromatograms for each chlorination level. Note the retention times of the first and last peaks for each level of chlorination (note i).	(h) Ten pairs of suitable ions are given in Table E1. (i) The time intervals found for the elution of PCBs under the conditions given in E6.6 are also given in Table E1, which lists the SIM programme. Note that since the time windows for

Step	Procedure	Notes
		different levels of chlorination overlap, it is necessary to monitor for two chlorination levels using four ions at any one time over the chromatographic run. Figures E3 - E6 show examples of the SIM chromatograms for the trichlorobiphenyls (Cl_3-PCB) and the tetrachlorobiphenyls (Cl_4-PCB) obtained by reprocessing the data in Figure E2. For each level of chlorination, determine the ratio of this single combined response relative to the internal standard. Using the appropriate calibration graph, determine the concentration of PCBs at each level of chlorination and then calculate the total concentration of PCBs present in the original sample (section E9).
E8.3.4	Inject aliquots of standards and extracts from samples, blanks and recovery spikes into the GC and obtain the chromatographic data for the duration of the SIM programme.	
E8.3.5	Construct calibration graphs for each of the congeners representing the ten chlorination levels, using the primary ions listed in Table E1 (note j) using peak response ratios relative to the internal standard.	(j) In the case of the Cl_4 level, the more abundant secondary ion should be used.
E8.3.6	Identify any peaks in the chromatograms of sample extracts corresponding to PCBs by confirming that the two peaks with identical retention times are present for the two ions monitored at any given chlorination level, and that their abundance (response) ratio is within ± 20% of the value given in Table E2 (note k).	(k) The choice of SIM mode with two ions selected makes the detection of PCBs sufficiently selective that it is generally not necessary to remove organochlorine compounds from the extract before analysis. Even so, not all peaks detected correspond to PCBs. It is thus, important that PCBs are first identified by a visual examination of the chromatograms produced for each chlorination level. The presence of brominated ethers may cause interference. In order for PCBs to be identified, the 20% criterion should be satisfied.
E8.3.7	Where the ratios are within ± 20%, including those for the surrogate standard (E5.8), determine the response for the more abundant primary ion (note j). For each level of chlorination, add together the response values for all the peaks identified as PCBs within a given chlorination level. This gives a single value for each level.	

Step	Procedure	Notes
E8.3.8	For each level of chlorination, determine the ratio of this single combined response relative to the internal standard. Using the appropriate calibration graph, determine the concentration of PCBs at each level of chlorination and then calculate the total concentration of PCBs present in the original sample (section E9).	
E8.4	**Blanks and recoveries**	
E8.4.1	Adequate blank values should be obtained using interference-free water before analysing samples. Adequate recovery values should be obtained using water of a similar nature to the sample being analysed. At least one reagent blank should be analysed with each batch of samples. Check the recovery of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard PCB solutions to separate samples of interference-free water (E5.1) (note l). Process these solutions under conditions identical with those used for samples under analysis.	(l) To determine the recovery, spike the sample at an appropriate level with mixed PCBs (E5.9) ensuring that the volume of (water-miscible) solvent used is between 20 µl and 100 µl per litre of aqueous sample.
E8.4.2	If the peak responses of the extracted calibration solutions are used for comparison with those of the samples, an automatic correction is obtained. If not, recovery data from previous tests should be averaged and a mean correction factor determined for use when correcting recoveries (note m).	(m) Use the surrogate standard to indicate whether the analysis has proceeded satisfactorily for each individual sample.
E8.5	**AQC**	
	Carry out the entire procedure using interference-free water spiked at an appropriate concentration with PCBs. Analyse a corresponding interference-free blank water.	

E9 Calculation

The concentration of PCBs in the original sample at each level of chlorination is given by:

$$C_{Ln} = \frac{R_n \times A}{V} \quad \mu g l^{-1}$$

The total concentration of PCBs in the original sample is given by:

$$C_{Total} = \sum_{n=1}^{n=10} C_{Ln}$$

where

C_{Total} = total concentration of PCBs in the original sample ($\mu g l^{-1}$);

C_{Ln} = concentration of PCBs for each level of chlorination (where n is 1 to 10) in the original sample ($\mu g l^{-1}$);

V = volume of sample extracted (in litres), normally 1 litre;

R_n = mass ratio of PCB to the internal standard for each level of chlorination obtained from each calibration graph; and

A = amount of internal standard added to the sample (µg).

The calculations are more easily performed using a laboratory data system.

E10 References

E10.1 M.D. Ericson, J.S. Stanley, J.K. Turman, J.E. Going, D.P. Redford and D.T. Heggem, Determination of byproduct polychlorinated biphenyls in commercial products and wastes by high-resolution gas chromatography/electron impact mass spectrometry, Environ. Sci. Technol., **22**, (1988) 71 - 76.

E10.2 M.D. Ericson, Analytical Chemistry of Polychlorinated Biphenyls, Butterworth, Boston,(1986) 219.

Table E1 **Example of SIM programme of retention time intervals including ions monitored for the different levels of chlorination**

Chlorination level	Retention time intervals (minutes)		Ions monitored (m/z)		
	Start	End			
$Cl_1 : Cl_2$	3.5	11.0	188.1	190.0 : 222.0	224.0
$Cl_2 : Cl_3$	11.0	11.75	222.0	224.0 : 256.0	258.0
$Cl_3 : Cl_4$	11.75	13.6	256.0	258.0 : 289.8	291.9
Cl_4 : IS	13.6	14.6	289.8	291.9 : 311.9	313.9
$Cl_4 : Cl_5$	14.6	15.7	289.8	291.9 : 325.8	327.9
$Cl_5 : Cl_6$	15.7	16.5	325.8	327.9 : 359.7	361.7
$Cl_6 : Cl_7$	16.5	18.5	359.7	361.7 : 393.8	395.8
$Cl_7 : Cl_8$	18.5	21.4	393.8	395.8 : 429.8	431.9
$Cl_8 : Cl_9$	21.4	24.0	429.8	431.9 : 463.8	465.8
$Cl_9 : Cl_{10}$	24.0	35.5	463.8	465.8 : 497.8	499.8
	Stop run				

IS is the internal standard (4,4'-dibromobiphenyl).

Since the retention time windows for different levels of chlorination overlap, it is necessary to monitor for two chlorination levels using four ions at any one time.

For any particular column and temperature programme used, the retention time intervals will be different from those quoted here. Appropriate retention time intervals should, therefore, be selected for the system in use by inspection of the mass chromatograms of a PCB mixture.

Table E2 **Abundance ratios for ions monitored within the SIM programme**

Chlorination level	Ions monitored (m/z)		Abundance ratio
Cl_1	188.1	190.0	3.03 ± 0.6
Cl_2	222.0	224.0	1.52 ± 0.32
Cl_3	256.0	258.0	1.01 ± 0.2
Cl_4	289.8	291.9	1.32 ± 0.26
Cl_5	325.8	327.9	1.52 ± 0.3
Cl_6	359.7	361.7	1.22 ± 0.24
Cl_7	393.8	395.8	1.02 ± 0.20
Cl_8	429.8	431.9	1.52 ± 0.30
Cl_9	463.8	465.8	1.32 ± 0.26
Cl_{10}	497.8	499.8	1.15 ± 0.23

The abundance ratio is given as the response of the first (primary) ion listed divided by the response of the second (secondary) ion, except in the case of chlorination level four (Cl_4) where the secondary ion is more abundant. These abundance ratios are taken from published values (see E10.2). They can also be calculated from the mass spectra of any individual PCB congener. Figure E7 shows the mass spectrum of a Cl_4-PCB congener with ions m/z 289.9 and 291.9 (labelled as 290 and 292) indicated.

Figure E1 **Selected ion mass chromatogram of PCB standard mixture**

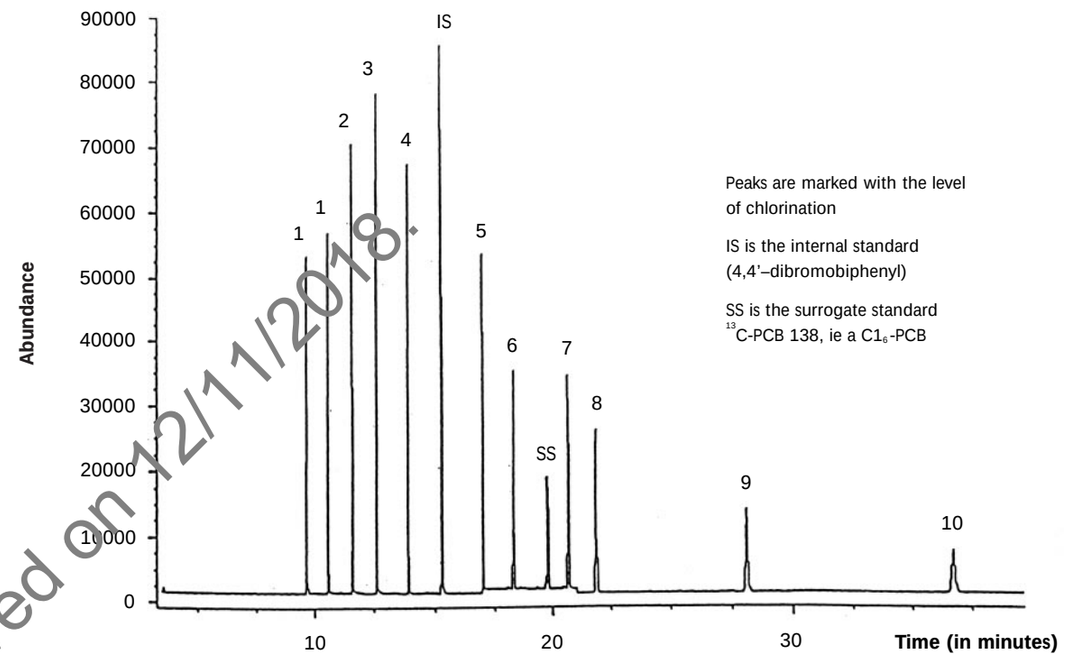


Figure E2 **GC-MS chromatogram of Aroclor 1242 in full scan mode**

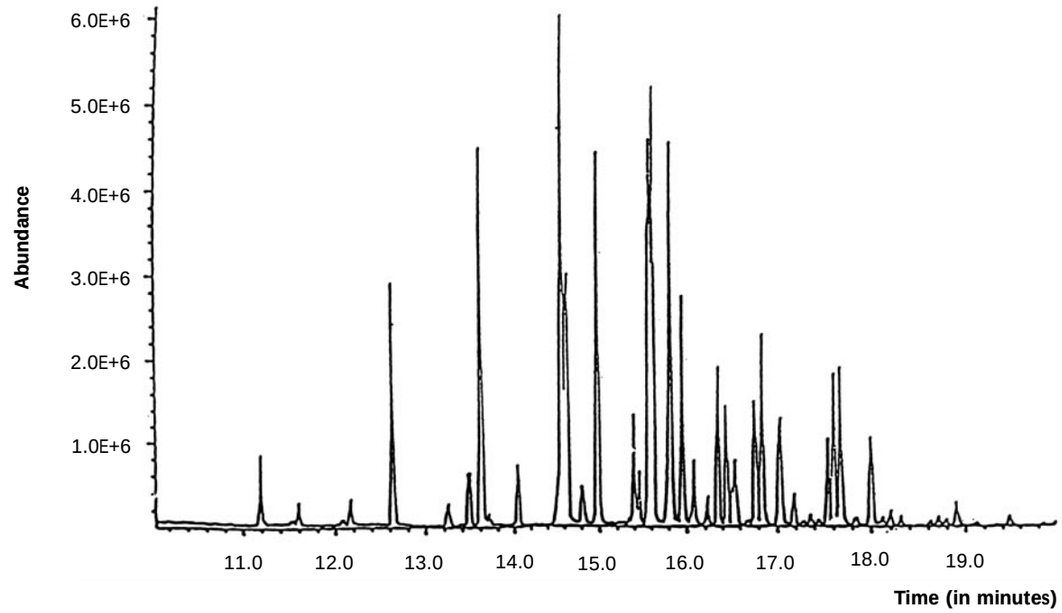


Figure E3 **Mass chromatogram of ion 256 from figure E2** (GC-MS (SIM) of Cl_3-PCB present in Aroclor 1242)

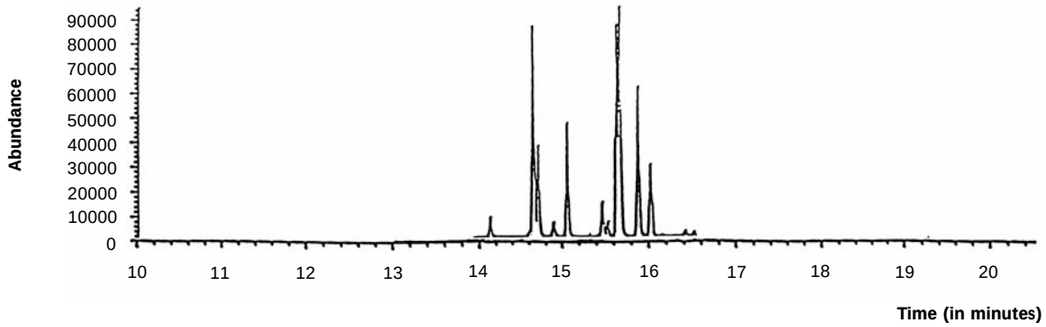


Figure E4 **Mass chromatogram of ion 258 from figure E2** GC-MS (SIM) of Cl_3-PCB present in Aroclor 1242)

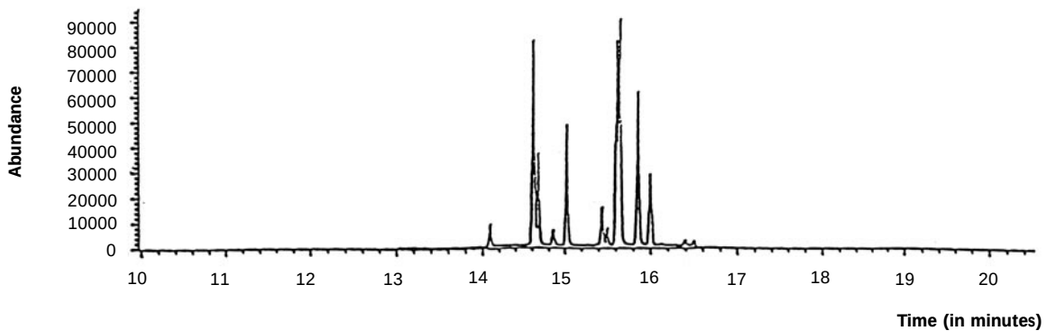


Figure E5 **Mass chromatogram of ion 291.9 from figure E2** (GC-MS (SIM) of Cl_4-PCB present in Aroclor 1242)

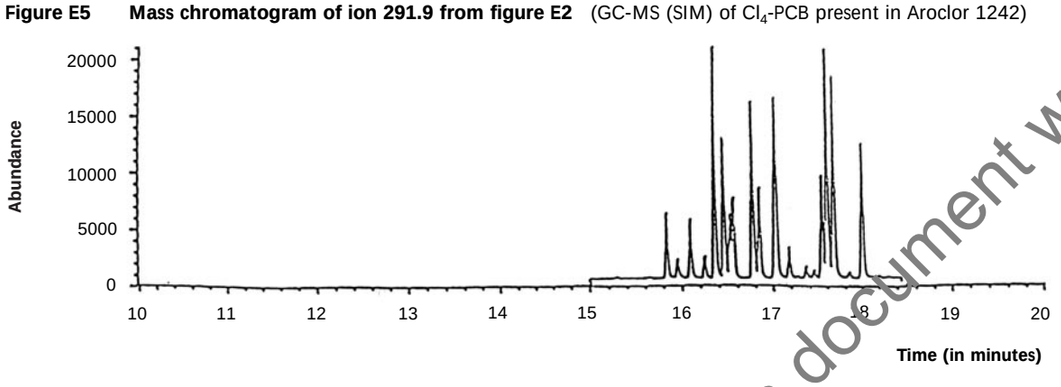


Figure E6 **Mass chromatogram of ion 289.8 from figure E2** (GC-MS (SIM) of Cl_4-PCB present in Aroclor 1242)

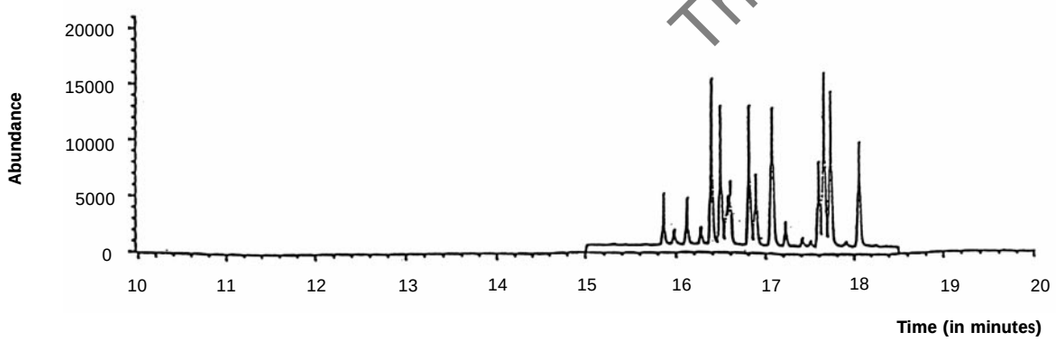


Figure E7 **Example of a mass spectrum from which the m/z abundance ratio can be calculated**
(In this case, the 291.9/289.9 ratio is used to identify Cl_4-PCB)

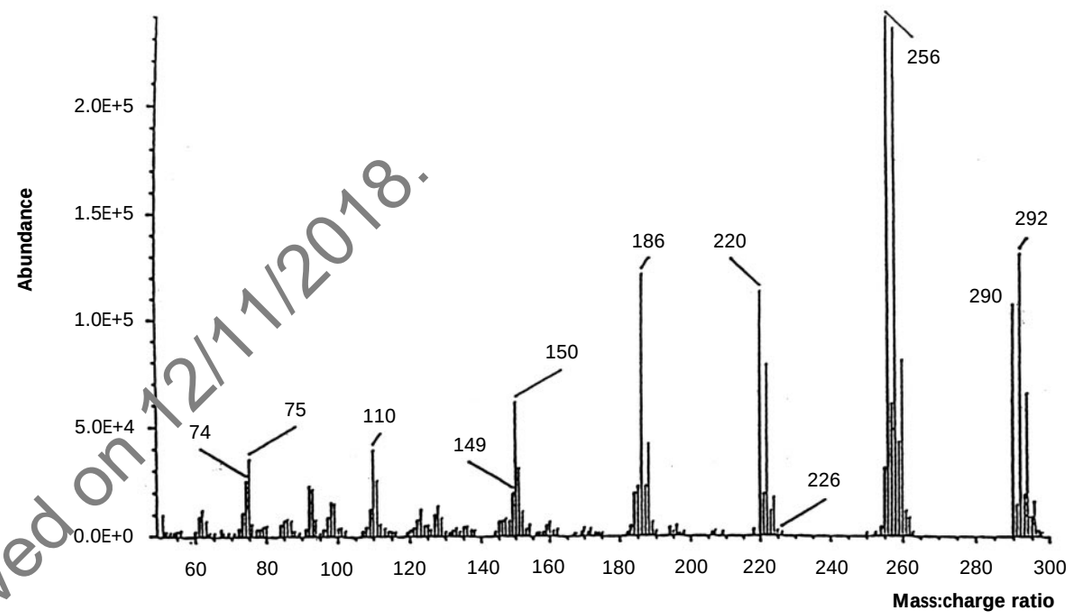
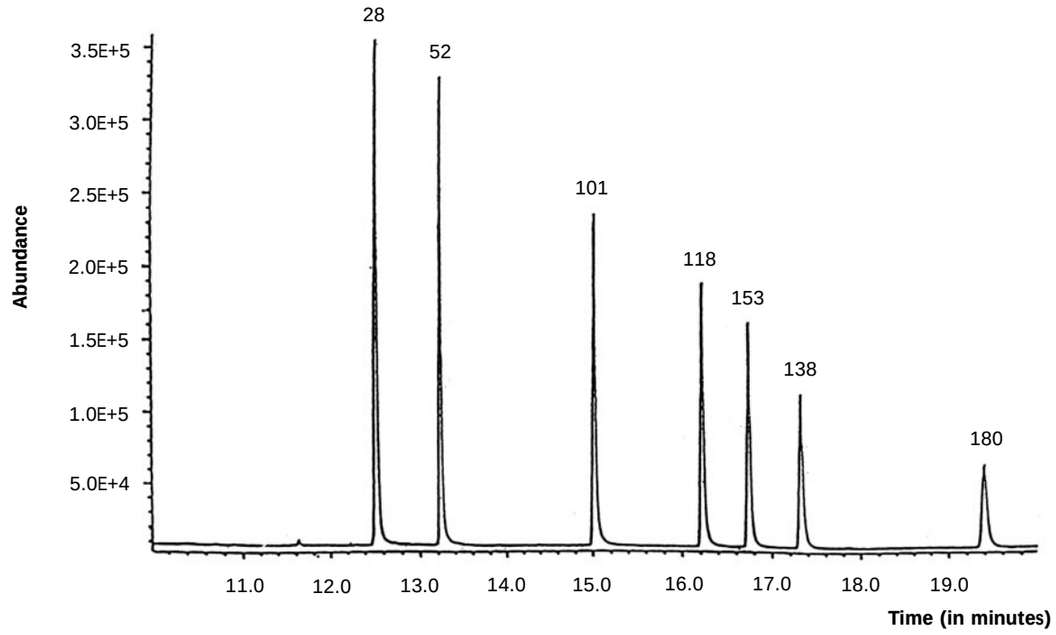


Figure E8 **Typical GC-MS (SIM) chromatogram of seven PCB congeners included in Method D**



F The determination of total polychlorinated biphenyls in complex matrices by gas chromatography – mass spectrometry

Introduction

The use of this method (and method E) enables PCBs in a sample to be identified according to the level of chlorination. Individual congeners are identified by their retention times and their mass to charge ratio. The chlorination level can normally be quantified by reference to two ions. The peak response ratio for the most abundant ion is used to calculate the concentration of PCBs at each chlorination level. A value for total PCBs is then obtained by summation of the individual values calculated for each chlorination level.

F1 Performance characteristics of the method

F1.1	**Substances determined**	Compounds identified as PCBs. In order for a peak to be identified as a PCB, the response ratio for the ions selected should differ by less than 20% from the standard response ratio for the corresponding level of chlorination. See Table E2. Its retention time should also be within the experimental range established.
F1.2	**Type of sample**	Sludges, sediments, fish tissues and soils.
F1.3	**Basis of method**	Determinands are extracted from the sample with hexane and the extracts "cleaned-up", concentrated and analysed by GC-MSD. The amounts of PCBs determined at each chlorination level are summed to give a figure for total PCBs.
F1.4	**Range of application**	The method has been tested up to 2000 $mgkg^{-1}$ (dry weight) for sludges and sediments, and up to 400 $mgkg^{-1}$ for fish tissue.
F1.5	**Linear range**	Linear over range of application.
F1.6	**Standard deviation**	See Table F1.
F1.7	**Limit of detection**	Typical values for total PCBs: 24 μgkg^{-1} for sludges; 18 μgkg^{-1} for sediments; 13 μgkg^{-1} for fish tissue; and 12 μgkg^{-1} for mollusc tissue. See also Table F1.
F1.8	**Sensitivity**	Dependent on the instrument used.
F1.9	**Bias**	Extraction efficiencies are normally less than 100 %. See Table F1.
F1.10	**Time required for analysis**	Ignoring drying times and assuming parallel extraction, typically 6 samples may be analysed per day.

F2 Principle

Sludges and sediments are air-dried and ultra sonically extracted with hexane. Fish tissues, including freeze dried tissues, are ground with sodium sulphate, Soxhlet extracted with hexane and concentrated to about 1 ml. The extracts are dried if necessary with sodium sulphate, evaporated, and "cleaned-up" using alumina. The determination is carried out using GC-MSD operating in EI mode with SIM. Quantification is by internal standard added to the extract after evaporation. A ^{13}C-surrogate standard (added before extraction) is used to monitor the performance of the analysis for each sample.

F3 Interferences

Any co-extracted material which has a GC retention time within the ranges established for the determinands and which gives a detector response for the ions being monitored in the required ratio will interfere. Organochlorine pesticides need not be removed from the extract before analysis.

F4 Hazards

Skin contact or inhalation of all reagents and their solutions should be avoided. Hexane is harmful and flammable and acetone and 2,2,4-trimethylpentane are flammable. PCBs are toxic. Care should be taken with sludges which may be biologically hazardous. Ensure adequate ventilation and work in a flame- or spark-proof area. Spark-proof refrigerators should be used. Appropriate safety procedures should be followed.

F5 Reagents

All reagents should be of sufficient purity that they do not give rise to interfering peaks during the GC-MSD determination. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Pesticide- or HPLC-grade solvents and analytical grade materials in the case of other reagents are normally suitable and details of preparation are provided, where appropriate.

The water used for blank determinations and preparation of control samples should show negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light. Reagents should be stored in tightly sealed all-glass containers or other suitable vessels and stored in the dark if necessary.

F5.1 **Water.**

F5.2 **Hexane.**

F5.3 **2,2,4-trimethylpentane (iso-octane).**

F5.4 **Acetone.**

F5.5 **Sodium sulphate.** Granular, anhydrous. Heat to 500 ± 20°C in a muffle furnace for 4.0 ± 0.5 hours. Cool to about 200°C in the muffle furnace and then to ambient temperature in a desiccator. Store in a closed glass container.

F5.6 **Florisil 60 - 100 mesh.**

F5.7 **Mixed standard stock solution of PCBs.** Suitably certified solutions of PCBs in 2,2,4-trimethylpentane can be obtained commercially, for example, at concentrations of 10 mgl^{-1}. Stock solutions of congeners representing each chlorination level, for example the following congeners should be available; PCB 1, PCB 3, PCB 7, PCB 30, PCB 50, PCB 97, PCB 143, PCB 183, PCB 202, PCB 207 and PCB 209. See Table D4.

F5.8 **Spiking solution of internal standard.** Prepare a stock solution of pure or certified internal standard. For example, 4,4'-dibromobiphenyl in 2,2,4-trimethylpentane at a suitable concentration of, for example, 100 mgl^{-1}. Alternatively, certified solutions can be obtained commercially.

F5.9 **Stock spiking solution of surrogate standard.** A suitably certified solution of an appropriate surrogate standard (to be added before extraction). For example, ^{13}C-PCB 97 for a Cl_5-PCB, or ^{13}C-PCB 138 for a Cl_6-PCB, in 2,2,4-trimethylpentane at a concentration of 10 mgl^{-1}.

F5.10 **Stock solution of Aroclors.** Prepare a mixed stock solution of certified Aroclor 1242 and Aroclor 1260 in 2,2,4-trimethylpentane at, for example, a concentration of 50 mgl^{-1} of each.

F5.11 **Mixed working standard solutions of PCBs with surrogate standard.** By dilution of the mixed standard stock solution (F5.7) and stock spiking solution of surrogate standard (F5.9) prepare a series of at least five mixed working standard solutions of PCBs and the surrogate standard in 2,2,4-trimethylpentane each containing, for example 0.25 mgl^{-1} of surrogate standard. For example, a useful working range for each congener is 0.05 to 2 mgl^{-1}.

F6 Apparatus

Apparatus should be free from contamination before use. Glassware should be rinsed with acetone and then hexane before use and allowed to drain.

F6.1 **Sample containers.** Glass containers. Determinand losses may occur through adsorption onto the walls of the sample container from some sample matrices if plastic containers are used. Wide-necked jars are suitable for fish and sediment samples.

F6.2 **Vials**. Glass, with PTFE-lined cap, capacity 40 ml.

F6.3 **Soxhlet apparatus.** 250 ml flasks with disposable extraction thimbles 33 x 100 mm.

F6.4 **Evaporating dishes**. 400 ml capacity.

F6.5 **Volumetric flasks, measuring cylinders, pipettes, syringes and glass vials**. Various sizes.

F6.6 **Kuderna-Danish evaporator**. An alternative evaporating system may also be used.

F6.7 **"Clean-up" columns.** 250 mm x 6 mm internal diameter.

F6.8 **Nitrogen.** Oxygen-free, filtered and dry.

F6.9 **Gas chromatography.** A gas chromatograph fitted with a capillary column and glass-lined injector. This should be fitted with a detector capable of operating in EI mode with SIM which permits different groups of ions to be monitored at selected time intervals during the analysis. Operating conditions used to obtain the test data are as follows:

Column:	fused silica WCOT, 30 m x 0.25 mm internal diameter, coated with HP5-MS, 0.25 µm film thickness.
Carrier gas:	helium, flowrate 1 $mlmin^{-1}$.
Injection volume:	1 µl.
Injection mode:	on column.
Column temperature:	programmed, 75 °C for 2 minutes, 30 $°Cmin^{-1}$ to 120 °C, then 10 $°Cmin^{-1}$ to 240 °C, hold for 20 minutes.
Injector temperature:	300 °C.

A chromatogram showing PCB congeners (representing each level of chlorination) and the internal and surrogate standards obtained under these conditions is given in figure E1. Other columns and conditions can be used provided that an equivalent or better performance is obtained compared to that reported in this method.

F7 Sample collection and storage

Samples should be taken in glass containers (F6.1) and extracted as soon as possible after sampling. If samples cannot be extracted on the day of collection, they should be stored in a refrigerator between 1- 8 °C. Fish samples may be frozen. The sample containers should be protected from contamination and should not be placed in proximity to standard materials or their concentrated solutions.

F8 Analytical procedure

Step	Procedure	Notes
F8.1	**Extraction of sludges and sediments**	
	Sludges	
F8.1.1	Transfer sufficient sludge to an evaporating basin (F6.4) to give 4-5 g of dry solids after evaporation at 20 - 30 °C (note a).	(a) A determination of the total solids content should be carried out on a portion of the sludge to enable expression of results on a dry basis, if required.
F8.1.2	Transfer a known weight of the dried sludge, normally 3 - 5 g, to a glass vial (F6.2). Spike the sample with 0.50 ± 0.01 ml of ^{13}C-PCB standard (F5.9), (note b). Proceed to step F8.1.5.	(b) The ^{13}C-PCB standard used in the performance testing was ^{13}C-PCB 97.
	Sediments	
F8.1.3	Transfer approximately 100 g of sediment to an evaporating basin (F6.4) and air-dry at 20 - 30 °C (note c). After drying, transfer the sediment to a 2 mm sieve and collect the material passing through the sieve. Mix thoroughly.	(c) The use of a large sample allows sub-sampling to be more representative.
F8.1.4	Transfer a known weight of the dried sieved sediment, normally 3 - 5 g, to a glass vial (F6.2). Spike the sample with 0.50 ± 0.01 ml of ^{13}C-PCB standard (note b).	
F8.1.5	The samples of sludge or sediment are now extracted. To the vial, add 10.0 ± 0.1 ml of hexane and cap. Transfer to an ultrasonic tank and ultra sonicate for 10 minutes. Leave the contents of the vials to settle (note d).	(d) Alternative extraction procedures may be used provided they can be shown to give an equivalent or better performance.
F8.1.6	Transfer 5.0 ± 0.1 ml of the clear hexane extract into a tared flask and evaporate to dryness on a steam bath at 100°C in a fume cupboard.	
F8.1.7	Dry the flask on the outside and allow to cool to room temperature. Re-weigh and calculate the total extractable solids.	
F8.1.8	Transfer a known volume of the remaining extract into a clean flask, such that not more than 200 mg of total extractable solids are taken (note e). Place the flask on the waterbath and allow the solution to concentrate by gentle evaporation to 1 - 2 ml (note d).	(e) If more than 200 mg of total extractable solids are taken, it may be necessary to use more than one "clean-up" column to effect an efficient "clean-up". In these cases, the combined extracts from several columns should be combined and evaporated to 1-2 ml.

Step	Procedure	Notes
F8.1.9	Quantitatively transfer the extract to a disposable glass tube and concentrate to 1 ml at 40°C using a stream of nitrogen (F6.8). Proceed to step F8.3.	
F8.2	**Extraction of fish tissues**	
F8.2.1	Weigh 10.00 ± 0.05 g of macerated tissue into a mortar dish. Add 90 ± 5 g of granular anhydrous sodium sulphate (F5.5) and grind with a pestle to mix thoroughly with the fish tissue.	
F8.2.2	Transfer the mixture to a disposable extraction thimble. Place some glass wool or similar material into the top of the thimble sufficient to cover the surface of the mixture.	
F8.2.3	Insert the packed thimble into the Soxhlet assembly fitted with a 250 ml flask containing approximately 120 ml of hexane and anti-bumping granules. Extract for 3 hours.	
F8.2.4	After the extract has cooled to room temperature, transfer quantitatively to a 100 ml volumetric flask and dilute to the mark with hexane.	
F8.2.5	Measure 50 ± 1 ml of the hexane extract into a tared flask and evaporate to dryness on a steam bath at 100 °C in a fume cupboard.	
F8.2.6	Dry the flask on the outside and allow to cool to room temperature. Re-weigh and calculate the total extractable solids.	
F8.2.7	Transfer a known volume of the remaining extract into a clean flask, such that not more than 200 mg of total extractable solids are taken (note f). Place the flask on the waterbath and allow the solution to concentrate by gentle evaporation to 5 -10 ml (note d).	(f) See note e, but combined extracts are evaporated to 5 -10 ml.
F8.2.8	Transfer the extract to a disposable glass tube and concentrate to 1 ml at 40°C using a stream of nitrogen (F6.8).	
F8.3	**“Clean-up“**	
F8.3.1	Insert a small plug of glass wool into the bottom of a column (F6.7). Add approximately 10 ml of hexane followed by 2.00 ± 0.05 g of florisil	

Step	Procedure	Notes
	(F5.6). Add sufficient sodium sulphate to give a layer, 1 cm deep.	
F8.3.2	Allow the hexane to drip through to waste until the meniscus reaches the sodium sulphate layer (note g). Using a Pasteur pipette, transfer the extract from steps F8.1.9 or F8.2.8 to the top of the column.	(g) Alternative “clean-up“ procedures may be used, see for example A8.3.1 and C8.2.3.
F8.3.3	Place a collection vessel at the base of the column. Allow the extract to drip through until it reaches the top of the sodium sulphate layer.	
F8.3.4	Wash out the tube containing the original extract with approximately 1 ml of hexane and transfer the washings to the column. When the meniscus has reached the top of the column add 22 ± 1 ml of hexane.	
F8.3.5	Collect all the eluate and allow the column to drain.	
F8.3.6	Concentrate the eluate at 40°C using a stream of nitrogen (F6.8) to 1.00 ± 0.05 ml. Add 25 µl of the spiking solution of internal standard (F5.8) and transfer to a suitable vial. The extract is now ready for GC-MS analysis.	
F8.4	**GC-MSD determination**	
F8.4.1	Set up the instrument in accordance with the manufacturer's instructions using the conditions given in section F6.9.	
F8.4.2	Set up the SIM programme by determining the time intervals over which the groups of PCBs at each chlorination level are eluted from the GC column. For example, inject an aliquot of the mixed Aroclor standard solution (F5.10) with the GC-MSD operating in full scan mode (50-550 amu) (note h).	(h) A chromatogram of Aroclor 1242 obtained under these conditions is shown in Figure E2.
F8.4.3	Select two ions for each of the ten chlorination levels (note i) and reprocess the data (from Figure E2) to produce two SIM chromatograms for each chlorination level. Note the retention times of the first and last peaks for each level of chlorination (note j).	(i) Ten pairs of suitable ions are given in Table E2. (j) The time intervals found for elution of PCBs under the conditions given in F6.7 are shown in Table E1 which lists the SIM programme used. Note that since the time windows for different levels of chlorination overlap, it is

Step	Procedure	Notes
		necessary to monitor two ions for two chlorination levels, ie using four ions at any one time over the chromatographic run. Figures E3 - E6 show examples of the SIM chromatograms for trichlorobiphenyls (Cl_3-PCB) and tetrachlorobiphenyls (Cl_4-PCB) obtained by reprocessing the data in Figure E2.
F8.4.4	Inject aliquots of standards and extracts from samples, blanks and recovery spikes into the GC and obtain the chromatographic data for the duration of the SIM programme.	
F8.4.5	Construct calibration graphs for each of the ten PCB standards representing the ten chlorination levels using the primary ion listed in Table E1 (note k) and using peak response ratios relative to the internal standard.	(k) In the case of the Cl_4 level, the more abundant secondary ion should be used.
F8.4.6	Identify any peaks in the chromatograms of sample extracts corresponding to PCBs by confirming that two peaks with identical retention times are present for the two ions monitored at any given chlorination level, and that their abundance (response) ratio is within ± 20% of the value given in Table E2 (note l).	(l) The choice of SIM mode with two ions selected makes the detection of PCB sufficiently selective that it is generally not necessary to remove organochlorine compounds from the extract before analysis. Even so, not all peaks detected correspond to PCBs and it is important that PCBs are first identified by a visual examination of the chromatograms produced for each chlorination level. The presence of brominated ethers may cause interference. In order for PCBs to be identified, the 20% criterion should be satisfied.
F8.4.7	Where the ratios are within 20%, including those for the surrogate standard, determine the response for the primary ion (note j). For each chlorination level, add together the response values for all the peaks identified as PCBs within a given level. This gives a single value for each level.	
F8.4.8	For each chlorination level, determine the ratio of this single combined response relative to the internal standard. Using the appropriate calibration graph, read off the corresponding concentration of PCBs at each level of chlorination, and then calculate the total concentration of PCBs present in the original sample.	

Step	Procedure	Notes
F8.5	**Blanks and recoveries**	
F8.5.1	Adequate blank values should be obtained using interference-free water before analysing samples. Adequate recovery values should be obtained using spiked additions to samples of a similar nature to those being analysed. At least one reagent blank should be analysed with each batch of samples. Check the recovery of the analytical procedure for each batch of samples analysed by adding suitable amounts of standard PCBs to separate samples (note m). Process these solutions under conditions identical with those to be used for samples under analysis.	(m) To determine the recovery, spike a sample matrix expected to be low in PCBs (for example silversand, or a non-industrial sludge, etc) at an appropriate level with the mixed PCB congeners.
F8.5.2	Recovery data from previous tests should be averaged and a mean correction factor determinand used for correcting for recovery (note n).	(n) Use a surrogate standard to indicate whether the analysis has proceeded satisfactorily for each individual sample.
F8.6	**AQC**	
F8.6.1	Carry out the entire procedure using a substance similar in nature to the sample being analysed, spiked at an appropriate concentration with PCBs.	

F9 Calculation

Using equations similar to those in E9 calculate the concentration of total PCBs in the original sample. Results are expressed on a dry weight or wet weight basis as appropriate.

Table F1 Performance data

Compound	Sludge Recovery (%)	LOD	RSD (%)	Sediment Recovery (%)	LOD	RSD (%)
PCB 1	49	9.2(19)	30.2	63	2.4(14)	17.9
PCB 7	77	5.9(13)	25.0	76	4.2(14)	16.8
PCB 30	50	7.2(17)	30.8	81	6.5(10)	15.1
PCB 50	54	5.9(14)	21.8	79	5.4(15)	14.3
PCB 97	57	6.1(16)	15.3	69	6.9(19)	13.4
PCB 143	80	7.5(16)	16.2	67	5.6(13)	11.0
PCB 183	73	8.9(17)	6.5	67	4.6(8)	22.0
PCB 202	91	9.4(12)	21.2	73	9.3(14)	13.8
PCB 207	69	7.9(16)	16.1	70	4.1(14)	14.6
PCB 209	59	7.7(14)	27.0	73	4.6(17)	19.5

Compound	Fish tissue Recovery (%)	LOD	RSD (%)	Mollusc tissue Recovery (%)	LOD	RSD (%)
PCB 1	63	3.5(9)	5.7	44	3.69(9)	22.2
PCB 3	66	3.76(8)	5.0	53	3.14(6)	16.8
PCB 7	59	3.32(8)	3.9	49	2.20(8)	12.6
PCB 30	64	4.42(9)	5.8	54	1.75(7)	9.7
PCB 50	70	3.59(6)	5.3	59	1.32(5)	10.7
PCB 97	79	3.89(5)	9.8	66	1.33(4)	9.8
PCB 143	86	3.89(4)	26.9	70	3.81(4)	29.3
PCB 183	94	3.98(4)	20.9	74	2.77(4)	24.5
PCB 202	101	4.15(4)	18.3	79	3.15(4)	21.2
PCB 207	97	4.08(4)	22.7	75	3.43(5)	23.1
PCB 209	100	4.31(6)	23.3	78	8.27(8)	24.3

Units expressed in units of $\mu g kg^{-1}$ unless otherwise stated.
Figure in brackets represents DF
LOD is calculated for individual PCBs, see F1.7 for total PCBs.

G The determination of organochlorine pesticides and selected polychlorinated biphenyls in waters by solid phase extraction and large-volume injection gas chromatography with electron capture detection

G1 Performance characteristics of the method

G1.1	**Substances determined**	See Table G1. Other organochlorine compounds and PCBs may also be determined.
G1.2	**Type of sample**	Raw and treated waters containing low particulate matter.
G1.3	**Basis of method**	Samples are buffered to a pH value of 7 and the determinands extracted using SPE cartridges. The cartridge is eluted with ethyl acetate and dichloromethane. The extracts are dried, if necessary and analysed by large-volume injection GC on a single capillary column using ECD.
G1.4	**Range of application**	Varies depending on the compound determined. See Table G1 for compounds tested but typically up to 0.5 $\mu g1^{-1}$ (when 1 litre of sample is concentrated to 10 ml of final extract). The upper limit may be extended by dilution of the sample extract or by taking a smaller sample volume.
G1.5	**Calibration curves**	Typically, up to 0.1 $\mu g1^{-1}$.
G1.6	**Standard deviation**	See Table G1.
G1.7	**Limit of detection**	See Table G1.
G1.8	**Bias**	Extraction efficiencies are normally less than 100%. See Table G1.
G1.9	**Interferences**	Any compound extracted under the conditions used and giving a response on the electron capture detector at a retention time indistinguishable from any of the determinands of interest will interfere.

G2 Principle

The sample is buffered to a pH value of 7 and extracted onto a C_{18} reverse phase SPE cartridge. The cartridge is eluted with ethyl acetate and dichloromethane and dried if necessary. The extract is then analysed using large-volume injection GC with ECD. The compounds of interest are identified and quantified by their respective retention time and peak response (peak area or peak height).

G3 Hazards

Skin contact or inhalation of reagents and their solutions should be avoided. Ethyl acetate and methanol are toxic by inhalation and ingestion, and are flammable. Dichloromethane is toxic by inhalation and ingestion. Organochlorine pesticides are toxic. Ensure adequate ventilation and work in a flame- or spark-proof area. Spark-proof refrigerators should be used. Appropriate safety procedures should be followed.

G4 Reagents

All reagents should be of sufficient purity that they do not give rise to interfering peaks during the determination. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Pesticide- or HPLC-grade solvents and analytical grade materials are normally suitable unless otherwise specified and details of preparation are provided, where appropriate.

The water used for blank determinations and preparation of control samples should show negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by the action of light.

G4.1 **Water.**

G4.2 **Ethyl acetate.**

G4.3 **De-ionised water.**

G4.4 **Dichloromethane.**

G4.5 **Methanol.**

G4.6 **Buffer solution.** (pH 7). Dissolve 2.710 ± 0.001 g of potassium dihydrogen phosphate and 3.886 ± 0.001 g of disodium hydrogen phosphate in approximately 800 ml of water (G4.1) in a volumetric flask. Dilute to 1000 ml with water.

G4.7 **Individual standard stock solutions.** (200 mgl^{-1}). Add 50 ± 1 mg of the compounds of interest to individual 250 ml volumetric flasks. Dissolve in approximately 200 ml of ethyl acetate (G4.2) and dilute to the mark with ethyl acetate (G4.2). These solutions are stable for 12 months if refrigerated at 1 – 8°C and stored in the dark.

G4.8 **Mixed intermediate standard stock solution.** (2 mgl^{-1}). Add 1000 ± 10 µl of each stock solution (G4.7) to approximately 50 ml of ethyl acetate (G4.2) in a 100 ml volumetric flask. Dilute to volume with ethyl acetate (G4.2). The solution is stable for 6 months if refrigerated between 1-8°C and stored in the dark.

G4.9 **Mixed working standard solution.** (200 μgl^{-1}). Add 1000 ± 10 µl of mixed intermediate standard (G4.8) to approximately 5 ml of ethyl acetate (G4.2) in a 10 ml volumetric flask. Dilute to volume with ethyl acetate (G4.2). The working standard solution should be freshly prepared on the day of use.

G4.10 **Mixed calibration standard solutions.** Add, for example, 1000 ± 10 µl, 750 ± 10 µl, 500 ± 5 µl, 250 ± 5 µl and 100 ± 1 µl of the mixed working standard solution (G4.9) to 1 litre of water (G4.1) to produce calibration standard solutions of 0.2, 0.15, 0.1, 0.05 and 0.02 μgl^{-1} respectively. These calibration standard solutions are taken through the whole procedure and used to produce a multi-point calibration curve.

G4.11 **Sodium thiosulphate solution. (5% w/v).** Dissolve 12.5 ± 0.5 g of sodium thiosulphate pentahydrate in 250 ± 1 ml of water and mix thoroughly.

G4.12 **Sodium sulphate.** Granular anhydrous. Heat to 300 ± 20°C in a muffle furnace for 4.0 ± 0.5 hours. Cool to about 200°C in the muffle furnace and then to ambient temperature in a desiccator. Store in a closed glass container.

G5 Apparatus

Apparatus should be free from contamination before use.

G5.1 **Sample bottles.** Glass, 1.2 litre capacity, marked at 1 litre, fitted with glass stoppers or PTFE-lined screw caps.

G5.2 **C_{18} reverse phase SPE cartridges.** 500 mg, 3 ml.

G5.3 **Microsyringes.** Various sizes.

G5.4 **Gas chromatography.** A gas chromatograph fitted with a large-volume injector, capillary column, glass-lined injector with ECD. The operating conditions may vary. The actual operating conditions used to obtain the test data are:

Column:	30 m x 0.25 mm internal diameter DB5.
Injection temperature programme:	50°C (hold for 1 minute), ramp at 16°C sec^{-1} to 250°C.
Injection volume:	80 µl.
Injection mode:	split for 1 minute, split-less for 1 minute, then kept split for the remainder of the run.
Oven temperature programme:	initial temperature 60°C (hold for 0.5 minute), increased to 140°C at 20°Cmin^{-1}, increased to 280 °C at 4°Cmin^{-1}.
Detector temperature:	300°C.

G6 Sample collection and storage

Samples should be taken in glass 1 litre bottles with glass stoppers or PTFE-lined screw caps. For sample de-chlorination add 4-5 drops (0.05ml) of sodium thiosulphate solution (G4.11) at the time of sampling. Samples should be extracted as soon as possible after sampling unless stability data show otherwise. If samples cannot be extracted on the day of collection, they should be stored in a refrigerator between 1-8°C. The sample bottles should be protected from contamination and should not be placed in proximity to standard materials or their concentrated solutions.

G7 Analytical procedure

Step	Procedure	Notes
G7.1	**Sample preparation**	
G7.1.1	To 1000 ± 10 ml of sample (note a) add 5.0 ± 0.2 ml of methanol (G4.5) and 25.0 ± 0.5 ml of buffer solution (G4.6). Shake for approximately 1 minute.	(a) A smaller volume of sample may be used if the final extract volume is less than 10 ml (see G7.1.4).
G7.1.2	Condition a C_{18} reverse phase solid phase cartridge (G5.2) with 5 ml of methanol (G4.5) at a flow rate of 10 $mlmin^{-1}$. Repeat with a further 5 ml of solvent. Condition the cartridge again with 5 ml of water (G4.1) at a flow rate of 10 $mlmin^{-1}$. Repeat with a further 5 ml of water (note b).	(b) Do not allow the cartridge to dry out at any stage during the conditioning.
G7.1.3	Pass the buffered sample through the cartridge at a flow rate of 10 $mlmin^{-1}$. When all the sample has been extracted, dry the cartridge by passing air through the cartridge for approximately 1 hour.	
G7.1.4	Elute the cartridge with 5 ml of ethyl acetate (G4.2). Collect the eluate in a suitable container, for example a 15 ml test tube. Elute the cartridge with 5 ml of dichloromethane (G4.4). Collect the eluate in the same container used to collect the ethyl acetate (note c). Make the volume to 10.00 ± 0.05 ml with dichloromethane (note d). Transfer approximately 1 ml of the combined eluate solutions to an auto-sampler vial. The extract is now ready for GC analysis.	(c) If the solution appears cloudy, add sodium sulphate (G4.12) to remove traces of water. (d) An internal standard can now be added if required.

G7.2 GC/ECD determination

G7.2.1 Set up the gas chromatograph in accordance with the manufacturer's instructions.

G7.2.2 Inject aliquots of extracts from blanks, samples, calibration standard solutions and spiked control samples into the GC.

G7.2.3 Compare the retention times of each of the peaks of interest with those previously obtained by injection of calibration standard solutions. Measure the peak response (ie peak area or peak height) of each of the peaks corresponding to the compounds of interest (note e).

(e) If an internal standard has been added, measure the peak response for the internal standard in each analysis. Construct a calibration graph for each compound of interest, using peak response ratios relative to the internal standard.

G7.2.4 Construct a calibration graph of peak response of calibration standard solutions versus concentrations for each of the compounds of interest.

G7.2.5 Read the concentration of each compound of interest in the sample extracts from its calibration graph (note f). Calculate the concentration present in the original sample (see section G8).

(f) In cases where the response ratio for the compound of interest exceeds the calibration range, repeat the analysis using a smaller volume of sample.

G7.3 Blanks and recoveries

G7.3.1 Check the recovery of the analytical procedure for each batch of samples analysed by adding suitable amounts of spiking standard (G4.8) to a litre of interference-free water immediately before extraction. A process blank of interference-free water should also be extracted and analysed with every batch of samples. Process these solutions under conditions identical with those used for the samples. If the peak responses of extracted calibration standard solutions are used for comparison with those of samples, an automatic recovery correction is obtained.

G7.4 AQC

G7.4.1 Carry out the entire procedure using interference-free water spiked at appropriate concentrations with AQC standard solutions.

G8 Calculation

The concentration of each compound of interest is given by:

External standard procedure

$$C = \frac{c \times v}{V} \quad \mu g l^{-1}$$

Internal standard procedure

$$C = \frac{R \times A}{V} \quad \mu g l^{-1}$$

where:

C = concentration of compound of interest in the original sample ($\mu g l^{-1}$)
c = concentration determined in the extract from the calibration graph ($\mu g ml^{-1}$)
V = volume of sample (in litres) extracted, normally 1 litre.
v = volume of the extract (in ml) used for the determination.
R = mass ratio of each compound of interest to the internal standard from the calibration graph, and
A = amount of internal standard added to the sample (µg).

Alternative methods of calculation may be used provided they give equivalent results. The calculations are more easily performed using a laboratory data system.

Table G1 Means, standard deviations, recoveries and limits of detection

	Blank	Low standard	S_t	High standard	S_t	Recovery (%)	S_t	LOD
o,p'-TDE	0.0004	0.020(17)	0.002	0.103(16)	0.007	97(21)	0.004	0.006
trifluralin	0.0006	0.021(16)	0.002	0.104(17)	0.006	99(18)	0.008	0.005
p,p'-DDT	0.0007	0.021(10)	0.006	0.103(16)	0.007	105(18)	0.007	0.005
o,p'-DDT	0.0002	0.020(18)	0.002	0.105(16)	0.006	104(19)	0.006	0.008
β-endosulphan	0.0002	0.020(19)	0.001	0.102(16)	0.005	99(21)	0.005	0.006
p,p'-TDE	0.0009	0.020(21)	0.002	0.102(15)	0.006	98(20)	0.005	0.007
endrin	0.0004	0.019(17)	0.002	0.101(19)	0.006	104(18)	0.009	0.006
dieldrin	0.0003	0.019(19)	0.001	0.101(15)	0.006	97(21)	0.005	0.005
p,p'-DDE	0.0012	0.020(14)	0.002	0.104(12)	0.009	96(15)	0.008	0.005
α-endosulphan	0.0001	0.019(17)	0.001	0.101(14)	0.006	98(21)	0.004	0.005
heptachlor epoxide	0.0004	0.019(19)	0.001	0.101(18)	0.005	97(21)	0.004	0.006
aldrin	0.0002	0.020(17)	0.002	0.104(15)	0.007	92(13)	0.011	0.007
heptachlor	0.0021	0.020(21)	0.002	0.102(15)	0.008	98(14)	0.009	0.009
γ-HCH (lindane)	0.0002	0.018(20)	0.002	0.099(16)	0.006	96(13)	0.010	0.007
β-HCH	0.0001	0.019(19)	0.001	0.101(17)	0.007	98(21)	0.004	0.005
α-HCH	0.0004	0.019(21)	0.002	0.102(18)	0.006	99(16)	0.009	0.009
tecnazene	-	0.041(19)	0.003	0.103(16)	0.004	103(15)	0.004	0.012
hexachlorobenzene	0.0013	0.043(17)	0.002	0.107(19)	0.004	106(17)	0.004	0.008
isodrin	-	0.041(11)	0.004	0.104(15)	0.005	104(14)	0.003	0.006
PCB 153	0.0002	0.043(15)	0.002	0.106(11)	0.007	103(14)	0.006	0.007
PCB 180	-	0.043(17)	0.002	0.109(11)	0.006	107(12)	0.006	0.007
PCB 28	0.0004	0.042(11)	0.003	0.105(17)	0.003	104(20)	0.004	0.005
PCB 118	-	0.042(20)	0.002	0.104(13)	0.005	104(19)	0.004	0.007
PCB 52	-	0.042(20)	0.003	0.103(17)	0.006	104(13)	0.005	0.012
PCB 138	-	0.043(14)	0.003	0.107(13)	0.005	106(14)	0.007	0.009
PCB 101	0.0003	0.044(12)	0.003	0.107(12)	0.005	105(18)	0.004	0.005

Units expressed as $\mu g l^{-1}$ unless otherwise specified.
Figure in brackets represents DF.
LOD calculated as 4.65 x S_w of the low standard solution.
Standards consisted of blank water (ultra-pure water) spiked at 0.02 or 0.04 $\mu g l^{-1}$, or 0.1 $\mu g l^{-1}$.
Recoveries were based on a final treated water spiked at 0.1 $\mu g l^{-1}$.
Data provided by AES Ltd.

Figure G1 **Typical large-volume injection GC/ECD chromatogram of a standard PCB-organochlorine pesticide mixture**

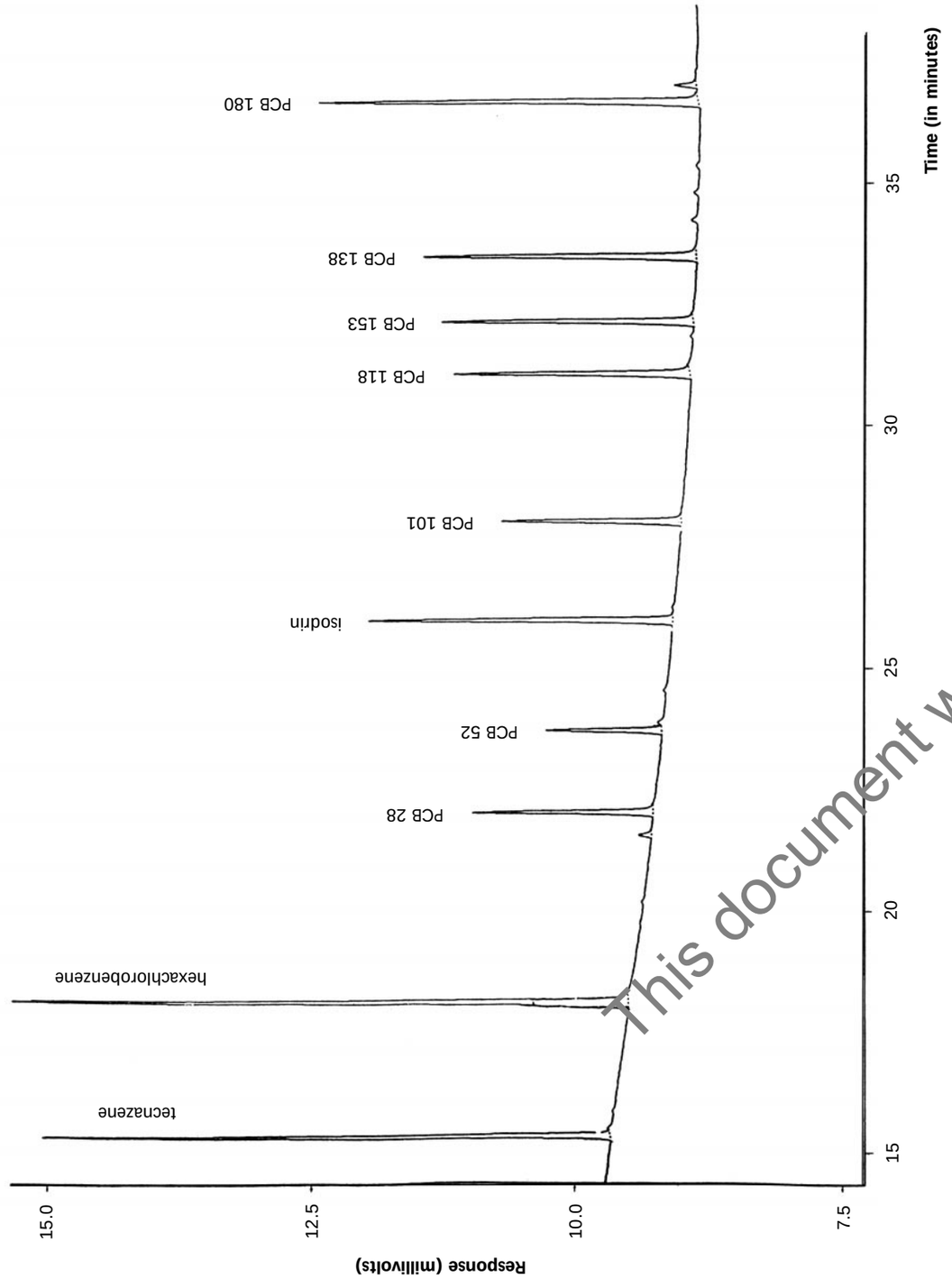
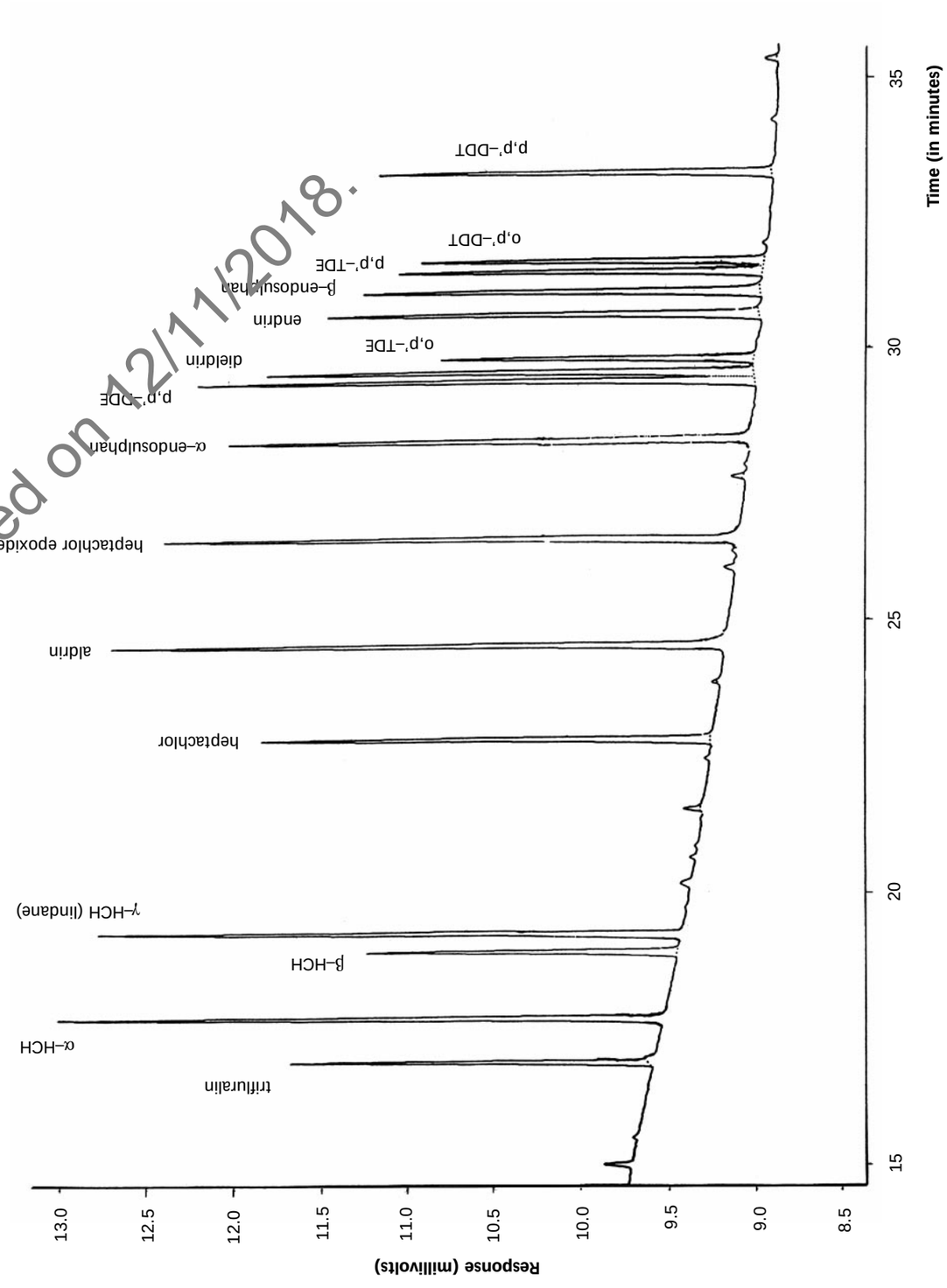


Figure G2 **Typical large-volume injection GC/ECD chromatogram of a standard organochlorine pesticide mixture**



Analytical Quality Control

1 Routine control

Once a method has been selected for routine use, a system of analytical quality control should be adopted in order to validate the analysis. At least one control standard should be analysed with each batch of samples and the results plotted on a control chart. Corrective action should be taken if one value falls outside of the action limit (at ± 3s) or two consecutive values exceed the warning limit (at ± 2s). As more data are acquired, the standard deviation, s, should be updated and the control chart limits re-calculated.

2 Estimation of the accuracy of analytical results using these procedures

None of the procedures given in this booklet has been thoroughly investigated for all types of samples and before general use, the accuracy achievable should be known. It would be of great value if analysts using these procedures would estimate the accuracy of their own analytical results and report their findings to the Secretary of the Standing Committee of Analysts.

Address for correspondence

However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts at the address given below.

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