



ENVIRONMENT AGENCY

The determination of polychlorinated biphenyls
by gas chromatography using mass spectrometric detection (2003)

Methods for the Examination of Waters and Associated Materials

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Methods for the Examination of Waters and Associated Materials

This booklet contains three methods:

- A The determination of polychlorinated biphenyls in soils by solvent extraction using gas chromatography with high-resolution mass spectrometric detection
- B The determination of polychlorinated biphenyls in complex matrices by solvent extraction using gas chromatography with mass spectrometric detection
- C A note on the determination of polychlorinated biphenyls in waters by solvent extraction using gas chromatography with mass spectrometric detection

Chromatographic methods are very sensitive to small physical and chemical variations in the quality of materials and apparatus used. These methods report the use of products actually used, but this does not constitute an endorsement of these products. Equivalent products are available and acceptable, and it should be understood that the performance characteristics may differ with other materials used. It is left to users to evaluate these methods in their own laboratories. Only limited performance data are presented.

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Glossary

Chlorination level	The degree of chlorination (of which there are ten) associated with each PCB (see Tables 1 and 2).
EI	Electron impact.
FC43	Perfluorotributylamine, $[\text{CF}_3(\text{CF}_2)_3]_3\text{N}$, molecular weight 671.1, a compound commonly used for the calibration of the mass scale of the mass spectrometer.
ICES-7	Seven selected prominent and persistent congeners (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180) that were chosen during the International Conference of the Environment of the Sea (ICES) meeting as being appropriate for the monitoring of PCBs in the aquatic environment. Subsequently adopted in some EU legislation.
Internal standard	Compound used for quantification of PCBs. The compound may be added to the sample prior to extraction and possible clean-up (in which case an automatic recovery is incorporated within the reported result). In other cases, it may be added to the sample extract after extraction and possible clean-up (in which case a recovery correction to the reported result would need to be considered to account for extraction and clean-up losses).
Lock mass	A mass spectral peak that, either via bleed from the gas chromatographic column, or originating from an introduced reference material (see FC43) is present in the mass spectrometer for the entire gas chromatographic determination. This peak is used to correct for any drift in the instrument's mass scale during the gas chromatographic determination.
Lock mass check	The lock mass is monitored as if from a sample, and is used in quality control. The chromatogram generated is used to monitor for suppression or other disturbances during the gas chromatographic determination.
LOD	Limit of detection.
m/z	Mass to charge ratio (Dalton).
PCB	Polychlorinated biphenyl.
PFK	Perfluorokerosene, a mixture commonly used to calibrate the mass scale of the mass spectrometer. A "high boiling" fraction is normally used.
Recovery standard	Compound used for evaluating recovery of PCBs and internal standards. Depending on the addition of the internal standard, the compound may be added to the sample prior to extraction and possible clean-up (and in these instances, may also serve as a surrogate standard). In other cases, it may be added to the sample extract after extraction and possible clean-up.
RRF	Relative response factor.
RSD	Relative standard deviation.
SIM	Selected ion monitoring.
TEF	Toxic equivalent factor.

About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments, soil (including contaminated land) and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results encompassing at least ten degrees of freedom from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available. An indication of the status of the method is shown at the front of this publication on whether the method has undergone full performance testing.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 1999 (SI 1999/437). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Standing Committee of Analysts

The preparation of booklets within the series "Methods for the Examination of Waters and Associated Materials" and their continuing revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is now managed by the Environment Agency. At present, there are nine working groups, each responsible for one section or aspect of water quality analysis. They are

- 1 General principles of sampling and accuracy of results
- 2 Microbiological methods
- 3 Empirical and physical methods
- 4 Metals and metalloids
- 5 General non-metallic substances
- 6 Organic impurities
- 7 Biological methods
- 8 Biodegradability and inhibition methods
- 9 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with these methods are listed at the back of this booklet.

Publication of new or revised methods will be notified to the technical press. An index of methods and details on how to obtain copies are available from the Agency's internet web-page (www.environment-agency.gov.uk/nls) or from the Secretary.

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary.

Dr D Westwood
Secretary

January 2003

Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such publications are; "Safe Practices in Chemical Laboratories" and "Hazards in the Chemical Laboratory", 1992, produced by the Royal Society of Chemistry; "Guidelines for Microbiological Safety", 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and "Safety Precautions, Notes for Guidance" produced by the Public Health Laboratory Service. Another useful publication is "Good Laboratory Practice" produced by the Department of Health.

The determination of polychlorinated biphenyls by gas chromatography using mass spectrometric detection

1 Introduction

These methods are capable of analysing polychlorinated biphenyls in soils (Method A), similar complex matrices (Method B) and associated waters (Method C). Methods B and C are, essentially, those described in “The determination of organochlorine pesticides and polychlorinated biphenyls in waters and complex matrices (2000)”⁽¹⁾. In Method A, quantification is based on an internal standard added to the sample before extraction and clean-up. Consequently, an automatic recovery is incorporated within the reported result. The recovery of the internal standard (and extracted PCBs) is determined using a recovery standard added after extraction and clean-up, but just prior to the gas chromatographic determination. However, in Methods B and C, quantification is based on an internal standard added to the sample extract after extraction and clean-up. Hence, in these cases, recoveries are determined using a recovery standard added to the sample prior to extraction and clean-up. Consequently, extraction and clean-up losses may need to be accounted for in the reported result and a recovery correction considered. The analyst should ensure that the method used is appropriate, given the chemical and physical characteristics of the matrix under investigation.

Polychlorinated biphenyls (PCBs) were produced, until recently, as compounds having excellent dielectric strengths and stabilities, making them particularly suitable as transformer oils, dielectric fluids, hydraulic fluids and flame retardants. Production of PCBs was banned after the observation of mammalian toxicity and environmental persistence. Initially, PCBs were produced as crude commercial mixtures manufactured by perchlorination of biphenyl (see Table 1). Each mixture typically contains a range of congener chlorination levels (see Table 2) and, usually, approximately 50 of the 209 possible congeners (see Table 3 using the naming system devised by Ballschmitter and Zelle⁽²⁾). Trade names, such as ‘Aroclor’ and ‘Clophen’ were used together with numbers designating the percentage by weight of chlorine. For example, Aroclor 1254 contains 54 % by weight of chlorine.

In practice, the determination of the PCB composition is difficult in environmental samples where mixtures of Aroclors are present, and where weathering of the individual PCBs has occurred. The weathering process is likely to change the original congener profile of the mixture. Guidance has been reported⁽³⁾ on how Aroclor distribution patterns can be estimated and various procedures have been developed to assist analysts with multivariate analysis of degree of chlorination data.

Recently⁽⁴⁾, certain individual congeners have been assigned a toxic equivalent factor (TEF) relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). See Table 4. Four co-planar PCBs and eight mono-ortho PCB congeners have been characterised in this way. On a like-for-like basis, the concentration of PCB 126 would need to be ten times the concentration of 2,3,7,8-TCDD to exhibit the same toxic effect.

These PCBs may be determined using these methods, utilising calibrations with standards of each target PCB. However, the four co-planar congeners are usually present at very low concentrations and their determination requires further clean-up or separation procedures and enhanced instrumental limits of detection to obtain data that are fit for purpose. Isotopically labelled PCB congeners should be used for the determination of these compounds.

Other methods for the determination of PCBs by gas chromatography involve electron capture detection. However, with these methods, the complexity of the matrix often leads to false positive results or false negative results caused by the presence of co-extractable material interfering with

the analysis. The selectivity of mass spectrometric techniques, generally, overcomes these difficulties.

Two methods are described in this booklet for the determination of PCBs in soils and complex matrices. The first method utilises a sophisticated high-resolution mass spectrometric technique, and the second method utilises a more generally available low-resolution mass spectrometric technique. The choice of method to use depends upon instrument availability, the expected PCB concentration present, and the use for which the data are generated.

The high-resolution approach allows a greater degree of selectivity and sensitivity than the low-resolution method, and should be used when low concentrations of PCBs are being determined. For example, typical background levels of “total” PCBs in UK soils⁽⁵⁾ are approximately $10 \mu\text{g kg}^{-1}$. This implies concentrations for individual PCBs significantly less than $1 \mu\text{g kg}^{-1}$. The limit of detection (LOD) for the low-resolution method is about $10 \mu\text{g kg}^{-1}$ per PCB. Hence, the low-resolution method would be inappropriate for establishing background soil concentrations.

Generally, not all of the 209 PCBs are determined. In practice, a limited range of chlorobiphenyls is usually determined. For example, the chlorobiphenyls in the tri- to hepta- chlorination levels, i.e. the trichlorobiphenyls to the heptachlorobiphenyls. This range accounts for 178 of the 209 possible congeners, and is manageable within a single gas chromatographic-mass spectrometric determination. In such cases, calibration is performed with at least one PCB per chlorination level, implying the assumption that all PCBs in the same chlorination level produce the same response. Where specific PCBs are to be determined, care should be taken to ensure that the gas chromatographic column used adequately separates the PCBs of interest from others within the same chlorination level as no single gas chromatographic column uniquely separates all PCBs. Thus, in the case of the three monochlorobiphenyls, each congener should be separately identified.

Retention times have been reported⁽⁶⁾ for all 209 congeners using a polar SPB-octyl and a non-polar DB-1 column and there are many examples where co-elution occurs. For example, when the ICES-7 congeners are determined using a DB5-ms type column, PCB 28 and PCB 138 are often subject to co-elution with PCB 31 and PCB 158 respectively. If the determination of all 209 PCBs is required, two gas chromatographic-mass spectrometric determinations need to be carried out, usually monitoring the mono-, di-, octa- and nonachlorobiphenyls and decachlorobiphenyl in one determination, and the tri-, tetra-, penta-, hexa- and heptachlorobiphenyls in another. If the monochlorobiphenyls and other low chlorinated biphenyls are to be determined, the sample may need to be extracted on an “as received” basis, since these PCBs, and possibly others, may be lost in the drying process used to prepare an air-dried sample. In addition, clean-up procedures⁽⁷⁾ utilising alumina or Florisil column chromatography can help to separate the non-ortho PCBs from other PCBs.

The PCBs for which TEFs have been assigned, except PCB 118, are usually present in soils and related materials at levels 1000-fold lower than the predominant ones. Hence, LODs in the range of 1 ng kg^{-1} (or lower) may be required for quantification. Such LODs are achievable by the high-resolution mass spectral technique.

These methods have been used on air-dried samples, and as such, may not be suitable for samples containing significant quantities of moisture. Whilst clean-up procedures have been described, it may not be necessary to carry out these procedures on all types of samples. In addition, great care should be taken to ensure that the sub-sample analysed is homogeneous, and representative of the bulk material sampled. Since the efficiency of the extraction process may be a function of the total extractable organic matter present in the sample and incomplete extraction may occur with samples that contain large amounts of PCBs, dilution of the sample extract with additional solvent is not

recommended. Sample containers should be protected from contamination and should not be placed in proximity to standards or their concentrated solutions.

2 Hazards

Since certain PCBs have been classified⁽⁴⁾ as known or suspected human carcinogens, these methods should be carried out with caution whilst wearing appropriate personal protective equipment. Concentrated standard solutions should only be opened in a well-ventilated fume hood. Skin contact or inhalation of reagents and their solutions specified in these methods should be avoided. Most of the solvents used are flammable and 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.

3 Reagents

All reagents should be of sufficient purity so that they do not give rise to significant interfering peaks in the chromatographic analysis. This should be checked for each batch of chemicals and reagents and verified by running procedural blanks with each batch of samples analysed. Solvents suitable for high performance liquid chromatography or pesticide use and analytical grade materials are normally suitable unless otherwise stated and details of preparation are given, where appropriate. 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.

To avoid excessive evaporation of solvent, standard solutions may be stored in a refrigerator or freezer. However, prior to use, all solutions and solvents should be allowed to reach ambient room temperature before volumetric measurements are made. When a standard solution is required for use, the flask and contents can be weighed, the stopper removed and a portion of the solution transferred to a clean, dry container of low, but sufficient volume. The stopper is then replaced and the flask and its (reduced) contents weighed again. If after appropriate storage, any significant difference occurs in the weight of the flask and its contents since it was last used, then this might indicate a possible change in the concentration of the standard solution. Standard solutions of PCBs are available from a number of suppliers.

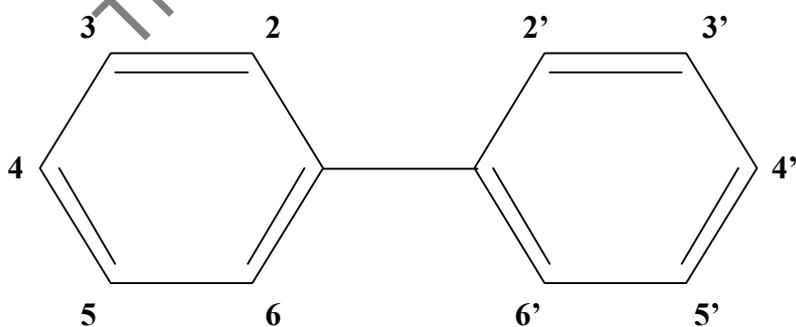
The materials used either for blank determinations or the preparation of control samples should show negligible amounts of the PCBs of interest compared with the smallest concentration to be determined.

Table 1 Number of possible isomers and per cent chlorine for the ten chlorination levels

PCB	PCB chlorination level	Empirical Formula	Nominal molecular weight	Number of possible isomers	Per cent chlorine by weight
	biphenyl	C ₁₂ H ₁₀	154	1	
1-3	monochlorobiphenyls	C ₁₂ H ₉ Cl	188.5	3	18.8
4-15	dichlorobiphenyls	C ₁₂ H ₈ Cl ₂	223	12	31.8
16-39	trichlorobiphenyls	C ₁₂ H ₇ Cl ₃	257.5	24	41.3
40-81	tetrachlorobiphenyls	C ₁₂ H ₆ Cl ₄	292	42	48.6
82-127	pentachlorobiphenyls	C ₁₂ H ₅ Cl ₅	326.5	46	54.3
128-169	hexachlorobiphenyls	C ₁₂ H ₄ Cl ₆	361	42	58.9
170-193	heptachlorobiphenyls	C ₁₂ H ₃ Cl ₇	395.5	24	62.8
194-205	octachlorobiphenyls	C ₁₂ H ₂ Cl ₈	430	12	66.6
206-208	nonachlorobiphenyls	C ₁₂ HCl ₉	464.5	3	68.7
209	decachlorobiphenyl	C ₁₂ Cl ₁₀	499	1	71.2

Table 2 Approximate congener composition (as a %) of several Aroclor mixtures

Aroclor	1016	1221	1232	1242	1248	1254	1260
biphenyl		11	6				
monochlorobiphenyls	1	51	26	1			
dichlorobiphenyls	20	32	29	17	1		
trichlorobiphenyls	57	4	24	40	23		
tetrachlorobiphenyls	21	2	14	2	50	11	
pentachlorobiphenyls	1	1	1	10	20	49	12
hexachlorobiphenyls					1	34	46
heptachlorobiphenyls						6	36
octachlorobiphenyls							6
nonachlorobiphenyls							
decachlorobiphenyl							

Table 3 Structure and positional isomers of PCB

PCB Position	PCB Position	PCB Position	PCB Position
1 2	54 2,2',6,6'	109 2,3,3',4,6	164 2,3,3',4',5',6
2 3	55 2,3,3',4	110 2,3,3',4',6	165 2,3,3',5,5',6
3 4	56 2,3,3',4'	111 2,3,3',5,5'	166 2,3,4,4',5,6
	57 2,3,3',5	112 2,3,3',5,6	167* 2,3',4,4',5,5'
4 2,2'	58 2,3,3',5'	113 2,3,3',5',6	168 2,3',4,4',5',6
5 2,3	59 2,3,3',6	114* 2,3,4,4',5	169* 3,3',4,4',5,5'
6 2,3'	60 2,3,4,4'	115 2,3,4,4',6	
7 2,4	61 2,3,4,5	116 2,3,4,5,6	170 2,2',3,3',4,4',5
8 2,4'	62 2,3,4,6	117 2,3,4',5,6	171 2,2',3,3',4,4',6
9 2,5	63 2,3,4',5	118*\$ 2,3',4,4',5	172 2,2',3,3',4,5,5'
10 2,6	64 2,3,4',6	119 2,3',4,4',6	173 2,2',3,3',4,5,6
11 3,3'	65 2,3,5,6	120 2,3',4,5,5'	174 2,2',3,3',4,5,6'
12 3,4	66 2,3',4,4'	121 2,3',4,5',6	175 2,2',3,3',4,5',6
13 3,4'	67 2,3',4,5	122 2',3,3',4,5	176 2,2',3,3',4,6,6'
14 3,5	68 2,3',4,5'	123* 2',3,4,4',5	177 2,2',3,3',4',5,6
15 4,4'	69 2,3',4,6	124 2',3,4,5,5'	178 2,2',3,3',5,5',6
	70 2,3',4',5	125 2',3,4,5,6'	179 2,2',3,3',5,6,6'
16 2,2',3	71 2,3',4',6	126* 3,3',4,4',5	180\$ 2,2',3,4,4',5,5'
17 2,2',4	72 2,3',5,5'	127 3,3',4,5,5'	181 2,2',3,4,4',5,6
18 2,2',5	73 2,3',5',6		182 2,2',3,4,4',5,6'
19 2,2',6	74 2,4,4',5	128 2,2',3,3',4,4'	183 2,2',3,4,4',5',6
20 2,3,3'	75 2,4,4',6	129 2,2',3,3',4,5	184 2,2',3,4,4',6,6'
21 2,3,4	76 2',3,4,5	130 2,2',3,3',4,5'	185 2,2',3,4,5,5',6
22 2,3,4'	77* 3,3',4,4'	131 2,2',3,3',4,6	186 2,2',3,4,5,6,6'
23 2,3,5	78 3,3',4,5	132 2,2',3,3',4,6'	187 2,2',3,4',5,5',6
24 2,3,6	79 3,3',4,5'	133 2,2',3,3',5,5'	188 2,2',3,4',5,6,6'
25 2,3',4	80 3,3',5,5'	134 2,2',3,3',5,6	189* 2,3,3',4,4',5,5'
26 2,3',5	81* 3,4,4',5	135 2,2',3,3',5,6'	190 2,3,3',4,4',5,6
27 2,3',6		136 2,2',3,3',6,6'	191 2,3,3',4,4',5',6
28\$ 2,4,4'	82 2,2',3,3',4	137 2,2',3,4,4',5	192 2,3,3',4,5,5',6
29 2,4,5	83 2,2',3,3',5	138\$ 2,2',3,3',4',5'	193 2,3,3',4',5,5',6
30 2,4,6	84 2,2',3,3',6	139 2,2',3,4,4',6	
31 2,4',5	85 2,2',3,4,4'	140 2,2',3,4,4',6'	194 2,2',3,3',4,4',5,5'
32 2,4',6	86 2,2',3,4,5	141 2,2',3,4,5,5'	195 2,2',3,3',4,4',5,6
33 2',3,4	87 2,2',3,4,5'	142 2,2',3,4,5,6	196 2,2',3,3',4,4',5',6
34 2',3,5	88 2,2',3,4,6	143 2,2',3,4,5,6'	197 2,2',3,3',4,4',6,6'
35 3,3',4	89 2,2',3,4,6'	144 2,2',3,4,5',6	198 2,2',3,3',4,5,5',6
36 3,3',5	90 2,2',3,4',5	145 2,2',3,4,6,6'	199 2,2',3,3',4,5,6,6'
37 3,4,4'	91 2,2',3,4',6	146 2,2',3,4',5,5'	200 2,2',3,3',4,5',6,6'
38 3,4,5	92 2,2',3,5,5'	147 2,2',3,4',5,6	201 2,2',3,3',4',5,5',6
39 3,4',5	93 2,2',3,5,6'	148 2,2',3,4',5,6'	202 2,2',3,3',5,5',6,6'
	94 2,2',3,3',6'	149 2,2',3,4',5',6	203 2,2',3,4,4',5,5',6
40 2,2',3,3'	95 2,2',3,5',6	150 2,2',3,4',6,6'	204 2,2',3,4,4',5,6,6'
41 2,2',3,4	96 2,2',3,6,6'	151 2,2',3,5,5',6	205 2,3,3',4,4',5,5',6
42 2,2',3,4'	97 2,2',3',4,5	152 2,2',3,5,6,6'	
43 2,2',3,5	98 2,2',3',4,6	153\$ 2,2',4,4',5,5'	206 2,2',3,3',4,4',5,5',6
44 2,2',3,5'	99 2,2',4,4',5	154 2,2',4,4',5,6'	207 2,2',3,3',4,4',5,6,6'
45 2,2',3,6	100 2,2',4,4',6	155 2,2',4,4',6,6'	208 2,2',3,3',4,5,5',6,6'
46 2,2',3,6'	101\$ 2,2',4,5,5'	156* 2,3,3',4,4',5	
47 2,2',4,4'	102 2,2',4,5,6'	157* 2,3,3',4,4',5'	209 2,2',3,3',4,4',5,5',6,6'
48 2,2',4,5	103 2,2',4,5',6	158 2,3,3',4,4',6	
49 2,2',4,5'	104 2,2',4,6,6'	159 2,3,3',4,5,5'	
50 2,2',4,6	105* 2,3,3',4,4'	160 2,3,3',4,5,6	
51 2,2',4,6'	106 2,3,3',4,5	161 2,3,3',4,5',6	
52\$ 2,2',5,5'	107 2,3,3',4',5	162 2,3,3',4',5,5'	
53 2,2',5,6'	108 2,3,3',4,5'	163 2,3,3',4',5,6	

* PCBs assigned with TEFs
 \$ ICES-7 PCBs

Table 4 Toxic Equivalent Factors

Congener	TEF (based on 2,3,7,8-TCDD value of 1)
PCB 77	0.0001
PCB 81	0.0001
PCB 126	0.1
PCB 169	0.01
PCB 105	0.0001
PCB 114	0.0005
PCB 118	0.0001
PCB 123	0.0001
PCB 156	0.0005
PCB 157	0.0005
PCB 167	0.00001
PCB 189	0.0001

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A The determination of polychlorinated biphenyls in soils by solvent extraction using gas chromatography with high-resolution mass spectrometric detection

A1 Performance characteristics of method

A1.1 Substances determined	Potentially, all 209 PCBs can be determined, see Table 3.
A1.2 Type of sample	Soil, including contaminated land.
A1.3 Basis of the method	Known amounts of stable isotopically labelled PCB internal standards are added to a sample of air-dried soil, which is then extracted with a 50:50 mixture of dichloromethane and acetone. The extract is dried, “cleaned-up” if necessary, and analysed using gas chromatography with high-resolution mass spectrometric detection using selected ion monitoring (SIM).
A1.4 Range of application	This varies depending on the PCBs being determined, but is typically, up to 100 µg kg ⁻¹ when expressed as all PCBs determined. The upper limit may be increased if a smaller quantity of sample is taken for analysis, see section 1.
A1.5 Calibration curve	Linear for each congener over the range of application.
A1.6 Limit of detection	This is dependent upon the gas chromatographic characteristics of each PCB and the mass spectrometric response of the mass spectral fragmentation ions used for quantification. At certain m/z values, the limit of detection may be adversely affected by background interference levels. For most congeners, a limit of detection of 0.01 µg kg ⁻¹ is achievable. This is subject to acceptable blank determinations of less than this value.
A1.7 Bias	Extraction efficiencies are normally less than 100%. See Table A3.

A2 Principle

This method involves ultrasonic extraction of an air-dried soil sample with a 50:50 v/v mixture of dichloromethane and acetone, after the addition of known amounts of stable isotopically labelled PCB internal standards. The extract is dried and subjected to “clean-up” if required, and concentrated prior to analysis using gas chromatography with high-resolution mass spectrometric detection using SIM. A resolution of 5000 (10% valley definition) minimises the effects of interferences.

Quantification is achieved via an internal standard calibration technique following a multi-point calibration of the gas chromatographic-mass spectrometric response with standard solutions containing native PCBs and internal standards.

A3 Interferences

Any compounds that are extracted under the conditions used and which exhibit retention time and mass spectral characteristics similar to those of any of the PCBs of interest will interfere. In addition, PCBs produce mass spectral fragmentation ions that may also cause interference. Some molecular ions lose two chlorine atoms to produce mass fragment ions of similar mass to those PCBs with two levels of chlorination lower. For example, the hexachlorobiphenyls may lose two chlorine atoms and cause interference with the tetrachlorobiphenyl determinations. In these cases, the isotopic ratios for these fragments will be different enabling them to be differentiated and hence, eliminated. Furthermore, late-eluting isomers in each chlorination level may be masked by these interferences. This is particularly so with the non-ortho PCBs that elute last (using a non-polar column) in each congener group.

The presence of large quantities of interfering materials in the extract (for example, mineral oils) may cause changes in the detector responses or retention times of the PCBs. The use of a “lock mass check” ion is used to monitor for suppression during the gas chromatographic determination.

A4 Reagents

See section 3.

A4.1 Water.

A4.2 Hexane.

A4.3 Acetone.

A4.4 Dichloromethane.

A4.5 Nonane.

A4.6 Methanol.

A4.7 Acetone:dichloromethane mixture. Thoroughly mix equal volumes of acetone and dichloromethane.

A4.8 Sodium sulphate. To remove traces of organic material, the anhydrous and granular sodium sulphate may need to be washed with dichloromethane and allowed to dry, prior to heating at 500 ± 50 °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 sealed glass container and use within 3 months.

A4.9 Anti-bumping granules. These may need to be pre-washed with acetone and allowed to dry prior to use.

A4.10 Silica gel 60. To remove traces of organic material, the silica gel may need to be washed with dichloromethane and allowed to dry, prior to heating to 200 ± 20 °C in a muffle furnace for 24 hours. Cool to ambient temperature in a desiccator.

A4.11 Glass wool. This may need to be pre-washed with dichloromethane and allowed to dry prior to use.

A4.12 Concentrated sulphuric acid. Specific gravity of 1.84.

A4.13 Potassium hydroxide.

A4.14 Silica impregnated with concentrated sulphuric acid. Concentrated sulphuric-impregnated silica is prepared by mixing 120 ± 10 g silica gel (A4.10) with 80 ± 5 g of sulphuric acid (A4.12) in a 300 ml beaker. The mixture is stirred, for example with a clean glass rod, until a free-flowing powder is obtained which is devoid of lumps.

A4.15 Potassium silicate. Dissolve 84 ± 1 g of potassium hydroxide (A4.13) in 350 ± 20 ml of methanol (A4.6) in a 1 litre flask. Allow the solution to cool. Add 150 ± 10 g of silica gel (A4.10) whilst stirring continuously. The mixture is rotated, for example on a rotary evaporating system (without vacuum) for 4 hours, with the water bath set at 55 ± 5 °C. After four hours, the methanol is drained off and discarded and the "slurry" filtered through glass wool (A4.11) into a 2 litre separating funnel. The slurry is then washed with 500 ± 30 ml of methanol (A4.6) after which the solvent is drained off and discarded. The washing and draining process is repeated using 500 ml of dichloromethane (A4.4). The slurry is then transferred to a 1 litre beaker and activated by heating to 130 ± 20 °C for 72 hours, then transferred to a desiccator.

A4.16 Air. Filtered and dry.

A4.17 Standard PCB stock solutions. Prepare stock solutions (in nonane) of all of the PCBs to be determined. These may be obtained commercially. The solutions may comprise individual PCBs or be mixtures of PCBs. Typical concentrations range between 1 - 10 mg l⁻¹. A single solution (in nonane) containing 1 mg l⁻¹ of each of the PCBs of interest is also prepared.

A4.18 Mixed internal standard ¹³C₁₂-PCB solutions. Prepare a mixed internal standard solution (in nonane) of isotopically labelled PCBs, typical of the PCBs being determined, for example, ¹³C₁₂-PCB 52 and ¹³C₁₂-PCB 153, each at a nominal (but accurately known) concentration of about 2 mg l⁻¹.

A4.19 Recovery standard ¹³C₁₂-PCB solution. Prepare a recovery standard solution (in nonane) of an isotopically labelled PCB, for example ¹³C₁₂-PCB 118 at a nominal (but accurately known) concentration of about 2 mg l⁻¹.

A4.20 Mixed calibration and internal standard solutions.

A4.20.1 Into a 2 ml crimp-capped vial add 0.02 µg of internal standard, i.e. 10.0 ± 0.1 µl of internal standard solution (A4.18), 0.02 µg of recovery standard, i.e. 10.0 ± 0.1 µl of recovery standard solution (A4.19) and 500 ± 10 µl of the 1 mg l⁻¹ standard PCB stock solution (A4.17). Add 480 ± 10 µl of nonane (A4.5). Label the standard appropriately as the 0.5 mg l⁻¹ PCB calibration standard.

A4.20.2 Into a 2 ml crimp-capped vial add 0.02 µg of internal standard, i.e. 10.0 ± 0.1 µl of internal standard solution (A4.18), 0.02 µg of recovery standard, i.e. 10.0 ± 0.1 µl of recovery standard solution (A4.19) and 50 ± 5 µl of the 1 mg l⁻¹ standard PCB stock solution (A4.17). Add 930 ± 20 µl of nonane (A4.5). Label the standard appropriately as the 0.05 mg l⁻¹ PCB calibration standard.

A4.20.3 Into a 2 ml crimp-capped vial add 0.02 µg of internal standard, i.e. 10.0 ± 0.1 µl of internal standard solution (A4.18), 0.02 µg of recovery standard, i.e. 10.0 ± 0.1 µl of recovery standard solution (A4.19) and 5.00 ± 0.05 µl of the 1 mg l⁻¹ standard PCB stock solution (A4.17). Add 975 ± 20 µl of nonane (A4.5). Label the standard appropriately as the 0.005 mg l⁻¹ PCB calibration standard.

A4.20.4 If the above procedures have been followed the calibration solutions possess the concentrations shown in Table A1 of the PCBs being determined and the isotopically labelled-PCBs.

Table A1 Concentrations of PCBs in calibration solutions

PCBs of interest	Concentration (mg l ⁻¹)		
	0.5	0.05	0.005
Internal standards, ¹³ C ₁₂ -PCB 52 and ¹³ C ₁₂ -PCB 153	0.02	0.02	0.02
Recovery standard, ¹³ C ₁₂ -PCB 118	0.02	0.02	0.02

A4.21 Florisil 60-100 mesh.

A4.22 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.

A4.23 De-activated alumina. Add 7.0 ± 0.2 % m/m of water (A4.1) to alumina (A4.22) 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 7 days. After this period, unused amounts may be reprocessed as described in section A4.22 and above.

A5 Apparatus

A5.1 Sample containers. Plastic containers should not be used as PCB losses from some matrices may occur through adsorption onto the walls of the sample container. Wide-necked amber glass jars are suitable for soil samples.

A5.2 Oven. Capable of operating at 130 ± 20 °C.

A5.3 Muffle furnace. Capable of operating at up to 500 ± 50 °C.

A5.4 Desiccator.

A5.5 Filter papers. For example, Whatman 542, 125 mm diameter, or equivalent.

A5.6 Chromatography column for clean-up. Glass, 130 mm by 5.6 mm internal diameter, fitted with a sintered glass frit and a glass or polytetrafluoroethylene tap is suitable. Alternatively, for the “combination column” clean-up procedure, a 500 mm by 17 mm internal diameter glass column is suitable.

A5.7 Micro-syringes. Used for sample spiking and preparation of standard solutions.

A5.8 Ultrasonic bath.

A5.9 Rotary evaporating system. An alternative evaporation system may be used.

A5.10 Gas chromatography-mass spectrometry equipment. A gas chromatographic-mass spectrometric system capable of operating at a resolution of at least 5000 (i.e. 10 % valley definition) with a heated injector system.

A5.11 Gas chromatography column. A fused silica capillary column, for example 30 m by 0.25 mm internal diameter, 0.25 μm film thickness, such as a DB-5ms column or equivalent. Other capillary columns and conditions can be used. A longer or alternative phase column may be used to improve the separation efficiency if required. If specific congener information is required, a second determination using a column of different polarity may need to be carried out.

Typical conditions for the DB-5ms type column are as follows:

Carrier gas	:helium, 1 ml min ⁻¹ .
Injection volume	:1 μl .
Column temperature	:programmed, 80 °C for 1 minute, then to 200 °C at 15 °C per minute, then at 5 °C per minute to 300 °C, held for 5 minutes.
Injector temperature	:280 °C.
Source temperature	:260 °C.

A5.12 Pasteur pipette for clean-up chromatography. Glass, 150 mm by 5 mm internal diameter.

A6 Sample collection and storage

Samples should be collected in amber glass jars with polytetrafluoroethylene lids (A5.1). Given their persistence in the environment, PCBs are unlikely to degrade during sampling and transportation. Whilst transport and storage under cool conditions constitute recommended best practice, it is not, in this case, considered essential.

This method has been performance tested using air-dried samples and may not be suitable for samples containing significant amounts of water. The use of an air-dried sample rather than an “as received” sample enables a more homogeneous sub-sample to be taken for analysis. The procedures used to prepare crushed, ground, sieved and/or air-dried samples may, however, adversely affect some PCBs present in the original sample prior to analysis. This is particularly the case for monochlorobiphenyls, and possibly others, which may be lost in the drying process used to prepare the air-dried sample, even at ambient temperatures. Analysts should, therefore, ascertain whether the procedures used to prepare crushed, ground, sieved and/or air-dried samples affect the resulting determination of PCB concentrations. See “The preparation and pre-treatment of contaminated soil samples prior to chemical analysis”, in preparation within this series. Whilst clean-up procedures have been described, it may not be necessary to carry out these procedures on all types of samples. In addition, if the sample is heavily contaminated, a smaller quantity may need to be analysed. Great care should be taken to ensure that the sub-sample is homogeneous, and representative of the bulk material sampled.

A7 Analytical procedure

Step	Procedure	Note
A7.1	Sample extraction	
A7.1.1	Add 10.0 ± 0.1 g of the crushed, ground, sieved and/or air-dried soil sample to a 100 ml beaker, see section A6. Add $0.02 \mu\text{g}$ of each of the internal standards, i.e. $10.0 \pm 0.1 \mu\text{l}$ of the internal standard solution (A4.18) and 30 ± 2 ml of 50:50 v/v acetone:dichloromethane mixture (A4.7) to the beaker. Ultra-sonicate for 3 ± 1 minutes, see note a, then allow the soil particles to settle. The supernatant liquid is transferred to a 250 ml round-bottomed flask. The solvent extraction and transfer process is repeated with two further aliquots, each of 30 ml of solvent mixture (A4.7). All extracts are combined in the round-bottomed flask.	(a) Ensure that solvent is not lost during the sonication process.
A7.1.2	At the same time a blank sample, ideally, comprising a similar matrix to the soil under investigation, but containing negligible amounts of the PCBs being determined is similarly treated and analysed, see note b.	(b) The same type and amounts of internal standards should be added to the blank sample as used in the analysis of real samples.
A7.1.3	The extracts are dried by filtering into a round-bottomed flask through a filter paper (A5.5) containing approximately 10 g of sodium sulphate (A4.8). Wash the sodium sulphate with 30 ml of solvent mixture (A4.7) and allow the filtrate to collect in the round-bottomed flask.	
A7.1.4	The volume of dried extract is reduced to about 3-5 ml using a rotary evaporation system (A5.9), see note c. Remove the container and reduce the volume of solvent to approximately 1-2 ml using a stream of air (A4.16).	(c) Depending on the pressure, a temperature of about 60 C may be used. The procedures should be validated.
A7.1.5	If a clean-up procedure is not required, see note d, then quantitatively transfer the small volume of extract to a glass vial. Rinse the container with 2-3 ml of hexane and add the solvent washings to the vial. To the vial, add $0.02 \mu\text{g}$ of recovery standard, i.e. $10.0 \pm 0.1 \mu\text{l}$ of recovery standard solution (A4.19) see note e. Using a blow-down apparatus, reduce the volume of the solvent at ambient temperature to approximately 1 ml. Go to section A7.3.	(d) For less-contaminated soils, clean-up may not be required prior to the gas chromatographic analysis. The need for clean-up is often associated with the presence of large amounts of organic matter in the solvent extract following extraction. The colour and turbidity of the extract may suggest whether the extract needs to undergo clean-up.

(e) As given, the recovery of the internal standard, $^{13}\text{C}_{12}$ -PCB 52 and $^{13}\text{C}_{12}$ -PCB 153, added before extraction is determined relative to the amount of recovery standard, $^{13}\text{C}_{12}$ -PCB 118 added just prior to the gas chromatographic-mass spectrometric determination.

A7.2 Clean-up

Details of three procedures are described and it is left to users to choose which procedure is most appropriate for the circumstances. See note f. Following the gas chromatographic mass spectrometric determination, the presence of all PCBs in the "hexane fraction" from the Florisil clean-up will indicate the need for additional clean-up in a repeated determination.

Florisil column clean-up

A7.2.1 Prepare a Florisil column by packing a Pasteur pipette (A5.12) with a small amount of glass wool (A4.11). Pack the column, first with Florisil (A4.21) to a depth of 65 ± 5 mm and then with sodium sulphate (A4.8) to a depth of 50 ± 5 mm. The column is eluted with about 5 ml of dichloromethane (A4.4) and then allowed to drain. Heat the column at 130 ± 10 °C for in excess of 16 hours, see note g.

A7.2.2 Remove the column from the oven and allow it to cool. Place a suitable container below the base of the column. The extract in the container from section A7.1.4 is added to the top of the column. The container from section A7.1.4 is rinsed with 5 ml of hexane (A4.2) and the solvent washings added to the column. This procedure is then repeated. A further quantity (10 ml) of hexane is then added to the column and the column eluted until the solvent meniscus reaches the top of the column, see note h. The eluate is collected and labelled "hexane fraction", see note i. Evaporate the eluate to about 3-5 ml using a rotary evaporating system (A5.9). Remove the container and reduce the volume of solvent to approximately 1-2 ml using a stream of air (A4.16). Add 50 ± 5 ml of dichloromethane to the column and

(f) Other procedures may be used provided the performance is validated.

(g) The column should then be stored at this temperature until ready for use.

(h) Do not allow the meniscus of the solvent to run below the level of the column packing material.

(i) This extract usually contains all PCBs present except the non-ortho PCBs.

(j) This fraction usually contains the four non-ortho PCB congeners.

elute the column. Collect the eluate in a separate container and label "DCM fraction", see note j. Evaporate the eluate to about 3-5 ml using a rotary evaporating system (A5.9). Remove the container and reduce the volume of solvent to approximately 1-2 ml using a stream of air (A4.16).

Quantitatively transfer each extract to separate glass vials, rinsing each container with 2-3 ml of hexane and adding the solvent to the respective vials. To each vial, add 0.02 µg of recovery standard, i.e. 10.0 ± 0.1 µl of the recovery standard solution (A4.19) see note e. Using a blow-down apparatus, reduce the volume of the solvent in each vial at ambient temperature to approximately 1 ml. Go to section A7.3.

Alumina column clean up

A7.2.3 Close the tap at the base of the chromatography column (A5.6) and add 15 ml of hexane (A4.2) followed by 5.0 ± 0.1 g de-activated alumina (A4.23). Tap the column gently and allow the alumina to settle. Add sufficient sodium sulphate (A4.8) to form a layer 5 mm in depth. Elute the column until the hexane meniscus reaches the top of the sodium sulphate, see note h. Transfer the sample extract in the container from section A7.1.4 to the top of the column.

A7.2.4 Place a suitable container below the base of the column and elute the column see note h. Collect the eluate. Wash the container from section A7.1.4 with 1 ml of hexane (A4.2) and add the washings to the column. Elute the column, see note h, and collect the eluate in the container. When the hexane meniscus reaches the top of the sodium sulphate, add 40 ± 1 ml of hexane to the column and continue eluting and collecting the eluate. Evaporate the combined eluates to about 3-5 ml using a rotary evaporating system (A5.9). Remove the container and reduce the volume of solvent to approximately 1-2 ml using a stream of air (A4.16). Quantitatively transfer the small volume of extract to a glass vial, rinsing the container with hexane and adding the solvent to the vial. To the vial, add 0.02 µg of recovery standard, i.e. 10.0 ± 0.1 µl of the recovery standard solution (A4.19) see note e. Using a blow-down apparatus, reduce the volume of the solvent at ambient temperature to approximately 1 ml. Go to section A7.3.

Combination column clean-up

A7.2.5 A glass chromatography column (A5.6) is plugged with a small amount of glass wool (A4.11). The column is sequentially packed with separate layers of 2 g of sodium sulphate (A4.8), 2 g of silica gel (A4.10), 4 g of sulphuric acid-impregnated silica gel (A4.14), 2 g of silica gel (A4.10), 4 g of potassium silicate (A4.15), 2 g of silica gel (A4.10) and 2 g of sodium sulphate (A4.8). The column is eluted with about 70 ml of hexane (A4.2), see note h, and the hexane discarded.

A7.2.6 Place a suitable container (for example, a beaker) below the base of the column. Transfer the sample extract at section A7.1.4 to the top of the column. Wash the container from section A7.1.4 with 2-3 ml of hexane and add the washings to the column. Elute the column and collect the eluate in the beaker. Add 5 ml of hexane to the container from section A7.1.4, and when the solvent meniscus reaches the top of the column, add the washings to the column. Continue eluting the column and collecting the eluate in the beaker, see note h. Repeat this procedure with another 5 ml of hexane. Add 70 ± 5 ml of hexane to the column and collect the eluate, see note k.

A7.2.7 The combined eluates are evaporated to about 3-5 ml using the rotary evaporation system (A5.9). Remove the container and reduce the volume of solvent to approximately 1-2 ml using a stream of air (A4.16). Quantitatively transfer the small volume of extract to a glass vial rinsing the container with 2-3 ml of hexane and adding the solvent washings to the vial. To the vial, add 0.02 μg of recovery standard, i.e. $10.0 \pm 0.1 \mu\text{l}$ of the recovery standard solution (A4.19) see note e. Using a blow-down apparatus, reduce the volume of the solvent at ambient temperature to approximately 1 ml.

A7.3 Gas chromatography-mass spectrometry

A7.3.1 Install the gas chromatographic column and tune the mass spectrometer to 5000 resolution.

(k) If either the sulphuric acid-impregnated silica or potassium silicate layer of the column appears strongly coloured, a second column (prepared in the same way as the first column) should be used in sequence. If so, add the eluate from the first column to the top of the second column. Elute the second column. Wash the eluate-container with 70 ± 5 ml of hexane and add the washings to the top of the second column. Elute the second column and collect the eluate.

Check that the mass resolution is constant across the mass range of interest.

A7.3.2 Set up the gas chromatographic-mass spectrometric detection system according to manufacturer's instructions, to monitor a minimum of two mass fragmentation ions per level of chlorination. Selected masses are listed in Table A2 for all ten chlorination levels, see note l.

(l) Some mass spectrometers are typically limited to a mass range of a factor of two from the lowest mass ion monitored. Multiple groups may, therefore, need to be set up, if a wide range of PCBs and chlorination levels are to be determined.

A7.3.3 Undertake a multi-point calibration by injecting 1 µl of each of the PCB calibration standard solutions (A4.20.1, A4.20.2 and A4.20.3) and blank (nonane) solution into the gas chromatograph, see note m and section A7.4.5.

(m) It is good practice to analyse the standard solutions in order of increasing concentration.

A7.4 Calibration

A7.4.1 Quantification of the selected PCBs is carried out either via the isotope dilution technique for PCBs where isotopically labelled internal standards are used or by the internal standard technique for other PCBs. In the case where all of the PCBs within each chlorination level are to be determined and reported, the response of one PCB congener for each chlorination level may be used, see note n.

(n) This assumption is reasonable, since the change in response factors for individual congeners within a single chlorination level varies very little.

A7.4.2 Extraction clean-up recovery is determined from the addition of the recovery standard, $^{13}\text{C}_{12}$ -PCB 118, to the extract after the final concentration stage but prior to the gas chromatographic-mass spectrometric analysis.

A7.4.3 Prior to injecting sample extracts, calibration of the responses obtained for the series of standard solutions is required.

A7.4.4 Calculate relative response factors for the calibration standard solutions as described in section A8. Calculate the mean relative response factor for each PCB and the relative standard deviation (RSD). If the RSDs for all the PCBs are acceptable, for example, less than 20 %, then the mean relative response factor may be used for the sample analysis.

Pre sample analysis quality control measures

A7.4.5 Check the chromatography of the nonane “injection blank”, see section A7.3.3 and note o.

(o) The chromatogram of this solution should show no or minimal peaks (above background noise levels) that might interfere with the determination of the PCBs being analysed. In addition, a minimum acceptable gas chromatographic-mass spectrometric response for the PCBs should be recognised. For example, a signal to noise ratio of approximately 200:1 for the 0.005 mg l⁻¹ standard solution should be obtained. If these two criteria are satisfied, the calibration linearity should be acceptable and sample analysis may proceed. The limit of detection, reported in section A1.6, should then be capable of being achieved.

A7.4.6 Inject sample extracts (from A7.2.2, A7.2.4 or A7.2.7) and the blank extract, see section A7.1.2.

A8 Acceptance criteria and sample quality control measures

The confirmation of a PCB of interest is based upon a number of criteria. These include:

- (i) The peaks in the individual ion chromatograms for the ions monitored for each PCB group should maximise within 1 second of each other.
- (ii) The retention times of corresponding peaks should be within 3 seconds, relative to an isotopically labelled PCB.
- (iii) The isotope ratio should be within 20% of the theoretical value given in Table A2. If this is not the case, it may be that interference can be suspected.

In most commercial data systems, if a PCB elutes close to the correct time but with an incorrect isotope ratio, analysts may be automatically alerted by the system to enable them to inspect the raw data, and hence make an assessment of any remedial action that may be necessary.

The monitoring of the “lock mass check” channel can be used to monitor and possibly identify significant interferences with the source sensitivity. This may suggest that appropriate clean-up of the extract is required.

The recovery of the internal standards should be determined for each sample. If the recovery falls outside an acceptable range, a second sub-sample should be extracted and analysed.

A8.1 Relative response factors

The relative response factor (RRF) is defined by the following equation:

$$\text{RRF} = \frac{\text{Signal (analyte)} \times \text{Amount (internal standard)}}{\text{Signal (internal standard)} \times \text{Amount (analyte)}}$$

where the “signal” is the sum of the areas of the two isotope peaks (mass 1 and mass 2) for the specific isomer.

For recovery calculations

$$\text{RRF} = \frac{\text{Signal (internal standard)} \times \text{Amount (recovery standard)}}{\text{Signal (recovery standard)} \times \text{Amount (internal standard)}}$$

The average relative response factor is then simply the mean of the RRFs obtained for each of the calibration standard solutions.

A8.2 PCB quantification

Prior to extraction, a known amount of internal standard is added to a known amount of sample. Using these values and the calibration-based RRFs the amount of each PCB may be determined in the test sample.

The equation used to calculate the amount of PCB present in the sample is:

$$\text{Amount of PCB} = \frac{\text{Signal (PCB)} \times \text{Amount (internal standard added)}}{\text{Signal (internal standard)} \times \text{Amount (sample extracted)} \times \text{RRF}}$$

To determine the recovery of the internal standard, the following equation can be used:

$$\text{Recovery (\%)} = \frac{\text{Signal (internal standard)} \times \text{Amount (recovery standard added)} \times 100}{\text{Signal (recovery standard)} \times \text{Amount (internal standard added)} \times \text{RRF}}$$

A9 References

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2. “Analysis of polychlorinated biphenyls by glass capillary chromatography - Composition of technical ‘Aroclor’- and ‘Clophen’ mixtures”, *Fres Zeit fur Anal Chem.* 1980, **302**, pp20-31.
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4. “Toxic equivalency factors for PCBs, PCDDs, PCDFs for humans and wildlife”. K D Nolt *et al*, *Environmental Health Perspectives*, 1998, **106**, pp775-792.

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6. US EPA Method 1668, - “Revision A: Chlorinated biphenyl congeners in water, soil, sediment, and tissue by HRGC/HRMS”, 1999.
7. “A method for the determination of PCB congeners 77, 126 and 169 in biotic and abiotic matrices”, *Chemosphere*, 1992, **24**, pp1147-1154.

Table A2 Exact masses of suggested ions to be monitored

	Mass (Daltons)		Theoretical ratio of abundance of mass ions i.e peak area (or height) of mass ion 1 / peak area (or height) of mass ion 2)
	Mass ion 1	Mass ion 2	
monochlorobiphenyls	188.0393	190.0363	3.05
dichlorobiphenyls	222.0000	223.9970	1.54
trichlorobiphenyls	255.9613	257.9584	1.03
tetrachlorobiphenyls	289.9224	291.9194	0.78
¹³ C ₁₂ -PCB 52	301.9626	303.9597	0.78
pentachlorobiphenyls	325.8804	327.8774	1.55
¹³ C ₁₂ -PCB 118	337.9207	339.9177	1.55
hexachlorobiphenyls	359.8415	361.8395	1.24
¹³ C ₁₂ -PCB 153	371.8817	373.8788	1.24
heptachlorobiphenyls	393.8025	395.7995	1.04
octachlorobiphenyls	427.7640	429.7610	0.89
nonachlorobiphenyls	461.7250	463.7220	0.78
decachlorobiphenyl	497.6820	499.6800	1.17
Column Bleed	263.9810		n/a
FC43	263.9871		n/a

Note 1 :If the column bleed is of insufficient size, then m/z 263.9871 from FC43 may be monitored. PFK is not a good lock mass material owing to interferences, for example trichlorobiphenyls at m/z 256.

Note 2 :Small variations in the calculated and theoretic ratios may be noted.

Table A3 Summary of performance data

Chlorination level	PCB	Mean ($\mu\text{g kg}^{-1}$)	RSD (%)	CRM* value	Bias (%)
trichloro	PCB 28	46	10 (4)	44	5
tetrachloro	PCB 52	36	9 (5)	38	-5
pentachloro	PCB 101	41	7 (8)	44	-7
pentachloro	PCB 118	26	5 (12)	28	-7
hexachloro	PCB 138	32	3 (8)	27	18
hexachloro	PCB 153	48	3 (11)	50	-4
heptachloro	PCB 180	20	4 (10)	22	-9

Data (based on 6 replicate analyses) provided by SAL Ltd, Manchester.

Figures in brackets represent %RSD values (based on 12 duplicate analyses) of quality control samples of an in-house material.

* Certified reference material values from BCR CRM 536.

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B The determination of polychlorinated biphenyls in complex matrices by solvent extraction using gas chromatography with mass spectrometric detection

B0 Introduction

See section 1. The use of this method (and Method C) enables PCBs in a sample to be detected according to the level of chlorination. Individual PCBs are detected via their retention times and mass to charge ratios. Chlorination levels 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 all PCBs determined is then obtained by summation of the individual values calculated for each chlorination level. This method is basically Method F in “The determination of organochlorine pesticides and polychlorinated biphenyls in waters and complex matrices (2000)”, in this series⁽¹⁾.

B1 Performance characteristics of the method

B1.1 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^(2, 3). See Table B1. The peak retention time should also be within the experimental range established.

B1.2 Type of sample

Sludges, sediments and fish tissues. Soils may also be determined.

B1.3 Basis of method

PCBs are extracted from the dried sample with hexane and the extract cleaned-up, if necessary, concentrated and analysed by gas chromatography with mass spectrometric detection. The amount of PCBs determined for each chlorination level are summed to give a figure for all PCBs determined.

B1.4 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.

B1.5 Linear range

Linear over range of application.

B1.6 Standard deviation

See Table B2.

B1.7 Limit of detection

Typically, values for all PCBs determined are 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 B2.

B1.8 Sensitivity

Dependent on the instrument used.

B1.9 Bias

Extraction efficiencies are normally less than 100 %. See Table B2.

B1.10 Time required for analysis

Drying times excluded, and assuming parallel extraction, typically, 6 samples may be analysed per day.

B2 Principle

Sludges and sediments are air-dried (see section 1) and ultra-sonically extracted with hexane. Fish tissues, including freeze dried tissues, are mixed and ground with sodium sulphate. The dried sample is Soxhlet extracted with hexane, the extract dried, if necessary, with sodium sulphate, reduced to a low volume and cleaned-up. The determination is carried out using gas chromatography with mass spectrometric detection operating in electron impact mode with selected ion monitoring. Quantification is by internal standard added to the extract after evaporation. A $^{13}\text{C}_{12}$ -PCB recovery standard (added before extraction) is used to monitor the performance of the analysis for each sample.

B3 Interferences

Any co-extracted material which has a gas chromatographic retention time within the ranges established for the PCBs 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.

B4 Hazards

See section 2. In addition, care should be taken with sludges which may be biologically hazardous.

B5 Reagents

See section 3.

B5.1 Water.

B5.2 Hexane.

B5.3 2,2,4-trimethylpentane (iso-octane).

B5.4 Acetone.

B5.5 Sodium sulphate. To remove traces of organic material, the anhydrous and granular sodium sulphate may need to be washed with hexane and allowed to dry, prior to heating 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 dessicator. Store in a sealed glass container and use within 3 months.

B5.6 Florisil. 60 - 100 mesh.

B5.7 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 mg l^{-1} . Stock solutions of congeners representing each level of chlorination, for

example, monochlorobiphenyls PCB 1, PCB 3; dichlorobiphenyls PCB 7; trichlorobiphenyls PCB 30; tetrachlorobiphenyls PCB 50; pentachlorobiphenyls PCB 97; hexachlorobiphenyls PCB 143; heptachlorobiphenyls PCB 183; octachlorobiphenyls PCB 202; nonachlorobiphenyls PCB 207 and decachlorobiphenyl PCB 209 are available. See Table 3.

- B5.8 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 mg l^{-1} . Alternatively, certified solutions can be obtained commercially or substituted $^{13}\text{C}_{12}$ -PCBs considered.
- B5.9 Stock spiking solution of recovery standard.** A suitably certified solution of an appropriate recovery standard (to be added before extraction). For example, $^{13}\text{C}_{12}$ -PCB 97 representing pentachlorobiphenyls or $^{13}\text{C}_{12}$ -PCB 138 for hexachlorobiphenyls in 2,2,4-trimethylpentane, at a concentration of 10 mg l^{-1} .
- B5.10 Stock solution of Aroclor.** Prepare a stock solution of certified Aroclor 1242 or Aroclor 1260 in 2,2,4-trimethylpentane at, for example a concentration of 50 mg l^{-1} .
- B5.11 Mixed working standard solutions of PCBs with standards.** By dilution of the mixed standard stock solution (B5.7) and internal standard (B5.8) and recovery standard (B5.9) solutions prepare a series of at least five mixed PCB working standard solutions with internal and recovery standards in 2,2,4-trimethylpentane each containing, for example 0.25 mg l^{-1} of recovery standard. For example, a useful working range for each congener is 0.05 to 2 mg l^{-1} .

B6 Apparatus

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

- B6.1 Sample containers.** Glass containers. PCB 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, sediment and soil samples.
- B6.2 Vials.** Glass with polytetrafluoroethylene-lined cap, capacity 40 ml.
- B6.3 Soxhlet apparatus.** 250 ml flasks with disposable extraction thimbles, 33 x 100 mm.
- B6.4 Evaporating dishes.** 400 ml capacity.
- B6.5 Volumetric flasks, measuring cylinders, pipettes, syringes and glass vials.** Various sizes.
- B6.6 Kuderna-Danish evaporator.** An alternative evaporating system may also be used.
- B6.7 Chromatography column for clean-up.** 250 mm x 6 mm internal diameter.
- B6.8 Nitrogen.** Oxygen-free, filtered and dry.

B6.9 Gas chromatography-mass spectrometry equipment. A gas chromatograph-mass spectrometric system fitted with a capillary column and glass-lined injector, capable of operating in electron impact mode with selected ion monitoring 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, or equivalent.
Carrier gas	:helium, 1 ml min ⁻¹ .
Injection volume	:1 µl.
Injection mode	:on column.
Column temperature	:programmed, 75 °C for 2 minutes, then at 30 °C per minute to 120 °C, then at 10 °C per minute to 240 °C, held for 20 minutes.
Injector temperature	:300 °C.

A chromatogram showing PCBs (representing each level of chlorination) and the internal and recovery standards obtained under these conditions is given in Figure B1. Other columns and conditions can be used provided that an equivalent or better performance is obtained compared to that reported in this method.

B7 Sample preservation and storage

Samples should be taken in glass containers (B6.1) and extracted as soon as possible after sampling. If sludge or fish samples cannot be extracted on the day of collection, they should be stored in a refrigerator between 2-8 °C. Fish samples may be frozen. See also section A6.

B8 Analytical procedure

Step	Procedure	Notes
B8.1	Extraction of sludges and sediments	
	<i>Sludges</i>	
B8.1.1	Transfer sufficient sludge to an evaporating basin (B6.4) to give 4-5 g of dry solids after drying at 20-30 °C, see note a.	(a) A determination of the total solids content should be carried out on a separate portion of the sample to enable expression of results on a dry basis, if required. See also section A6.
B8.1.2	Transfer a known weight of the dried sludge, normally 3-5 g, to a 40 ml vial (B6.2). Add 5 µg of recovery standard to the sample, i.e. 0.50 ± 0.05 ml of the ¹³ C ₁₂ -PCB recovery standard	(b) The ¹³ C ₁₂ -PCB standard used in the performance testing was ¹³ C ₁₂ -PCB 97.

solution (B5.9) see note b. Proceed to section B8.1.5.

Sediments

B8.1.3 Transfer approximately 100 g of sediment to an evaporating basin (B6.4). Air-dry at 20-30 °C, see notes a and c. After drying, mix well, transfer the sediment to a 2 mm sieve and collect the material passing through the sieve. Mix thoroughly, see note d.

B8.1.4 Transfer a known weight of the dried, sieved sediment, normally, 3-5 g, to a 40 ml vial (B6.2). Add 5 µg of recovery standard to the sample, i.e. 0.50 ± 0.05 ml of $^{13}\text{C}_{12}$ -PCB recovery standard solution (B5.9) see note b.

Extraction

B8.1.5 The sample of dried sludge or sediment is now extracted. To the vial, add 10.0 ± 0.1 ml of hexane (B5.2) and cap. Transfer to an ultrasonic bath and ultra-sonicate for 10.0 ± 0.5 minutes. Leave the contents of the vial to settle, see note e.

B8.1.6 Transfer 5.00 ± 0.05 ml of the clear hexane extract into a pre-weighed flask and evaporate to dryness on a steam bath at 100 °C in a fume cupboard.

B8.1.7 Remove the flask, dry it on the outside and allow it to cool to room temperature. Re-weigh the flask and contents and calculate the total extractable solids, see note f.

B8.1.8 Transfer a known volume of the remaining clear extract, containing not more than 200 mg of total extractable solids into a clean flask, see note g. Place the flask on the steam bath and allow the solvent to evaporate to about 1-2 ml.

B8.1.9 Quantitatively transfer the extract

(c) The use of a large sample allows sub-sampling to be more representative of the bulk material sampled.

(d) It may be necessary to record details of the material not passing through the sieve, and whether this material undergoes analysis for PCB content.

(e) An alternative extraction procedure may be used provided it can be shown to give an equivalent or better performance.

(f) Use this value to determine the volume of extract to take for analysis.

(g) 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 of the sample. In these cases, the combined extracts from several columns should be combined and evaporated to 1-2 ml.

to a disposable glass tube. Rinse the flask with 2-3 ml of hexane and add the washings to the tube. Repeat this process if necessary. Gently evaporate the solvent to approximately 1 ml at 40 °C using a stream of nitrogen (B6.8). Proceed to section B8.3.

B8.2 Extraction of fish tissues

B8.2.1 Weigh 10.00 ± 0.05 g of macerated fish tissue into a mortar dish. Add approximately 90 g of sodium sulphate (B5.5) mix well and grind until the tissue-sulphate mixture “flows freely”. Add more sodium sulphate if necessary.

B8.2.2 Transfer the mixture to a disposable extraction thimble. Add 5 µg of recovery standard to the sample, i.e. 0.50 ± 0.05 ml of the $^{13}\text{C}_{12}$ -PCB recovery standard solution (B5.9). Place a small amount of glass wool or similar material into the top of the thimble sufficient to cover the surface of the mixture.

B8.2.3 Insert the packed thimble into the Soxhlet assembly fitted with a 250 ml flask containing approximately 120 ml of hexane and several anti-bumping granules. Extract the sample for 3 hours.

B8.2.4 Remove the flask and allow the solvent to cool to room temperature. Quantitatively transfer the solution to a 100 ml volumetric flask. Rinse the Soxhlet flask with 3-5 ml of hexane and add the washings to the volumetric flask. Repeat this process if necessary. Make to the mark with hexane.

B8.2.5 Add 50.0 ± 0.1 ml of the hexane extract into a pre-weighed flask and evaporate to dryness on a steam bath at 100 °C in a fume cupboard.

B8.2.6 Remove the flask, dry it on the outside and allow it to cool to room temperature. Re-weigh the flask and its contents and calculate the total extractable solids, see note f.

B8.2.7 Transfer a known volume of the remaining extract containing not more than 200 mg of total extractable solids into a clean flask, see note h. Place the flask on the steam bath and allow the solution to evaporate to 5-10 ml.

(h) See note g, but the combined extracts are evaporated to 5-10 ml.

B8.2.8 Quantitatively transfer the extract to a disposable glass tube. Rinse the flask with 2-3 ml of hexane and add the washings to the tube. Repeat this process if necessary. Gently evaporate the solution to about 1 ml at 40 °C using a stream of nitrogen (B6.8).

B8.3 Clean-up

B8.3.1 Insert a small plug of glass wool into the bottom of a column (B6.7) see note i. Add about 10 ml of hexane followed by 2.00 ± 0.05 g of Florisil (B5.6). Add sufficient sodium sulphate (B5.5) to give a layer, 10 mm in depth.

(i) An alternative clean-up procedure may be used, see for example section A7.2.

B8.3.2 Elute the column with the hexane until the meniscus of the solvent reaches the sodium sulphate. Discard the eluate. Using a Pasteur pipette, quantitatively transfer the extract in the tube from section B8.1.9 or B8.2.8 to the top of the column. Rinse the tube with 2-3 ml of hexane and add the washings to the column.

B8.3.3 Place a suitable collection vessel at the base of the column. Elute the column until the meniscus of the solvent reaches the sodium sulphate and collect the eluate.

B8.3.4 Rinse the tube with approximately 1 ml of hexane and transfer the washings to the column. Elute the column. When the meniscus reaches the sodium sulphate, add 20 ± 1 ml of hexane to the column and continue eluting the column.

B8.3.5 Collect all the eluate and allow the column to drain.

B8.3.6 Evaporate the eluate on a steam

(j) Since the internal standard is added

bath to about 1-2 ml and quantitatively transfer the solution to a glass vial. Rinse the vessel with 2-3 ml of hexane and add the washings to the vial. Repeat this process if necessary. Gently evaporate the extract at 40 °C using a stream of nitrogen (B6.8) to 1.00 ± 0.05 ml. Add $2.5 \mu\text{g}$ of internal standard, i.e. $25 \pm 2 \mu\text{l}$ of the internal standard solution (B5.8) see note j. The extract is now ready for gas chromatographic analysis.

B8.4 Gas chromatography-mass spectrometry

B8.4.1 Set up the instrument in accordance with the manufacturer's instructions using the conditions given in section B6.9.

B8.4.2 Set up the SIM programme by determining the time intervals over which the groups of PCBs at each chlorination level are eluted from the gas chromatographic column. For example, inject an aliquot of the Aroclor standard solution (B5.10) with the instrument operating in full scan mode (50-550 amu) see note k.

B8.4.3 Select two ions for each of the ten chlorination levels, see note l, and reprocess the data (from Figure B2) to produce two SIM chromatograms for each chlorination level. Note the retention times of the first and last peaks for each level of chlorination, see note m.

B8.4.4 Inject aliquots of standards and extracts from samples, blanks and recovery spikes into the gas

after extraction, an estimate of recovery may need to be determined to account for extraction and clean-up losses.

(k) A chromatogram of Aroclor 1242 obtained under these conditions is shown in Figure B2.

(l) Ten pairs of suitable ions are given in Table B1.

(m) The time intervals found for elution of PCBs under the conditions given in B6.9 are shown in Table B3 which lists the SIM programme used. Since the time windows overlap for different levels of chlorination, it is necessary to monitor two ions for two chlorination levels, i.e. using four ions at any one time over the chromatographic run. Figures B3 - B6 show examples of the SIM chromatograms for trichlorobiphenyls ($\text{Cl}_3\text{-PCB}$) and tetrachlorobiphenyls ($\text{Cl}_4\text{-PCB}$) obtained by reprocessing the data in Figure B2.

chromatograph and obtain the chromatographic data for the duration of the SIM programme.

B8.4.5 Construct calibration graphs for each of the PCB standards representing the ten chlorination levels using the primary ion listed in Table B3, see note n, using peak response ratios relative to the internal standard.

B8.4.6 Identify those PCB peaks in the sample extract chromatograms. This is achieved 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 B1, see note o.

B8.4.7 Where the ratios are within 20%, including those for the recovery standard, determine the response for the primary ion, see note n. For each chlorination level, add together the response values for all the peaks identified as PCBs within a given level. This gives a single combined value for each chlorination level.

B8.4.8 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. Calculate the concentration of all PCBs determined in the original sample.

B8.5 Blanks and recoveries

B8.5.1 Adequate blank values should be obtained, ideally, using a similar matrix to that being determined but containing negligible amounts of PCBs. Adequate recovery values should be obtained using spiked additions to samples or to matrices of a similar nature to those being analysed,

(n) In the case of the tetrachlorobiphenyls, the more abundant secondary ion should be used.

(o) 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. In order for PCBs to be identified the 20% criterion should be satisfied.

(p) To determine the recovery, spike a sample, similar in nature to that being analysed but expected to be low in PCBs, at an appropriate level with PCBs.

see note p. At least one reagent blank should be analysed with each batch of samples. Process these solutions under identical conditions to those used for samples. Recovery values should be determined for each sample. If the recovery falls outside an acceptable range, a second sub-sample should be extracted and analysed.

B8.6 AQC

B8.6.1 Carry out the entire procedure using either a matrix certified reference material similar in nature and concentration to the sample being analysed, or a substance similar in nature to the sample being analysed but spiked at appropriate concentrations of PCBs.

B9 Calculation

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

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

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

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

where

C_{Total} = concentration of all PCBs determined in the original sample (μgkg^{-1});

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

V = mass of sample extracted (in kg);

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).

Calculations are more easily performed using a laboratory data system. Results may then need to be expressed on a dry weight or wet weight basis, as appropriate.

B10 References

- 1 Standing Committee of Analysts, The determination of organochlorine pesticides and polychlorinated biphenyls in waters and complex matrices (2000), *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.
- 2 Determination of byproduct polychlorinated biphenyls in commercial products and wastes by high-resolution gas chromatography/electron impact mass spectrometry, *Environ Sci Technol*, M D Ericson, J S Stanley, J K Turman, J E Going, D P Redford and D T Heggem, 1988, **22**, pp71-76.
- 3 Analytical Chemistry of Polychlorinated Biphenyls, M D Ericson, Butterworth, Boston, 1986, pp219.

Table B1 Abundance ratios for ions monitored within the SIM programme

Chlorination level	Ions monitored (m/z)	Abundance ratio
monochlorobiphenyls	188.1 190.0	3.03 ± 0.6
dichlorobiphenyls	222.0 224.0	1.52 ± 0.32
trichlorobiphenyls	256.0 258.0	1.01 ± 0.2
tetrachlorobiphenyls	289.8 291.9	1.32 ± 0.26
pentachlorobiphenyls	325.8 327.9	1.52 ± 0.30
hexachlorobiphenyls	359.7 361.7	1.22 ± 0.24
heptachlorobiphenyls	393.8 395.8	1.32 ± 0.20
octachlorobiphenyls	429.8 431.9	1.52 ± 0.30
nonachlorobiphenyls	463.8 465.8	1.32 ± 0.26
decachlorobiphenyl	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 tetrachlorobiphenyls where the secondary ion is more abundant. These abundance ratios are taken from published values. They can also be calculated from the mass spectra of any individual PCB and small differences are noted depending on the actual fragment masses used; compare for example Table A2. Figure B7 shows the mass spectrum of a tetrachlorobiphenyl with ions m/z 289.9 and 291.9 (labelled as 290 and 292) indicated.

Table B2 Performance data

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

Table B2 Performance data continued

PCB	Chlorination level	Fish tissue			Mollusc tissue		
		Recovery (%)	LOD	RSD (%)	Recovery (%)	LOD	RSD (%)
PCB 1	monochlorobiphenyls	63	3.5(9)	5.7	44	3.7(9)	22.2
PCB 3	monochlorobiphenyls	66	3.8(8)	5.0	53	3.1(6)	16.8
PCB 7	dichlorobiphenyls	59	3.3(8)	3.9	49	2.2(8)	12.6
PCB 30	trichlorobiphenyls	64	4.4(9)	5.8	54	1.8(7)	9.7
PCB 50	tetrachlorobiphenyls	70	3.6(6)	5.3	59	1.3(5)	10.7
PCB 97	pentachlorobiphenyls	79	3.9(5)	9.8	66	1.3(4)	9.8
PCB 143	hexachlorobiphenyls	86	3.9(4)	26.9	70	3.8(4)	29.3
PCB 183	heptachlorobiphenyls	94	4.0(4)	20.9	74	2.8(4)	24.5
PCB 202	octachlorobiphenyls	101	4.2(4)	18.3	79	3.2(4)	21.2
PCB 207	nonachlorobiphenyls	97	4.1(4)	22.7	75	3.4(5)	23.1
PCB 209	decachlorobiphenyl	100	4.3(6)	23.3	78	8.5(8)	24.3

Units expressed in units of μgkg^{-1} unless otherwise stated.

Figures in brackets represent degrees of freedom.

LOD is calculated for individual PCBs, see B1.7 for all PCBs determined.

Table B3 Example of SIM programme of retention time intervals including ions monitored for the different levels of chlorination

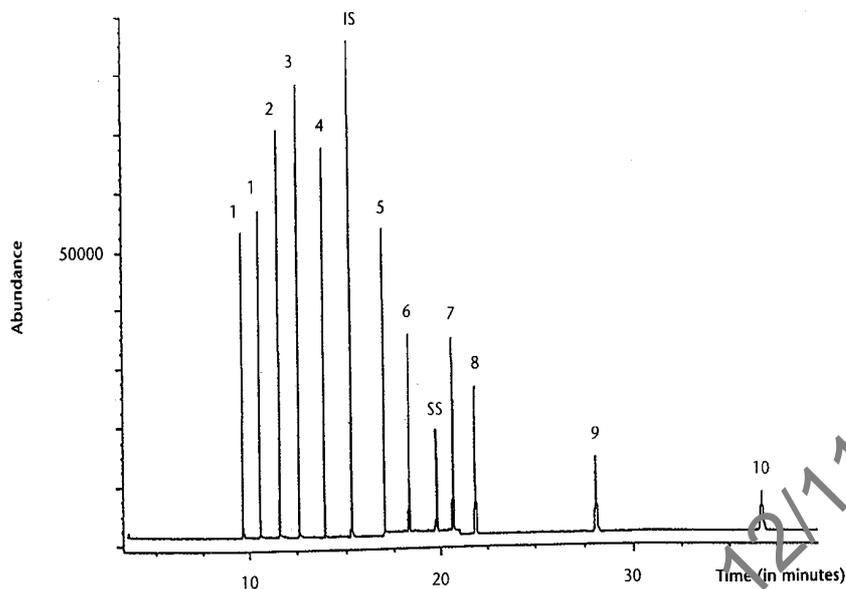
Chlorination level	Retention time intervals (minutes)		Ions monitored (m/z)		
	Start	End			
Cl ₁ : Cl ₂	3.5	11.0	188.1	190.0 : 222.0	224.0
Cl ₂ : Cl ₃	11.0	11.75	222.0	224.0 : 256.0	258.0
Cl ₃ : Cl ₄	11.75	13.6	256.0	258.0 : 289.8	291.9
Cl ₄ : IS	13.6	14.6	289.8	291.9 : 311.9	313.9
Cl ₄ : Cl ₅	14.6	15.7	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 (in this case, 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 above. Appropriate retention time intervals should, therefore, be selected for the system in use by inspection of the mass chromatograms of a PCB mixture.

Figure B1 Selected ion mass chromatogram of PCB standard mixture



Peaks are marked with the level of chlorination
IS is the internal standard (4,4'-dibromobiphenyl)
SS is the recovery standard ($^{13}\text{C}_{12}$ -PCB 138, i.e. a hexachlorobiphenyl)

Figure B2 GC-MS chromatogram of Aroclor 1242 in full scan mode

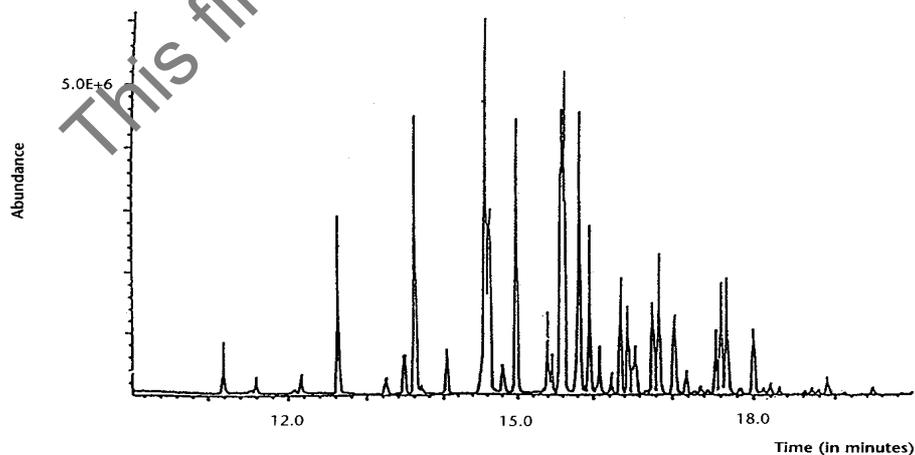


Figure B3 Mass chromatogram of ion 256 from figure C2 (GC-MS (SIM) of trichlorobiphenyls present in Aroclor 1242)

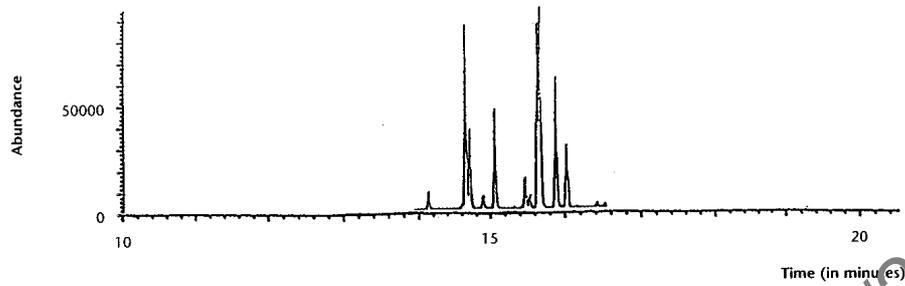
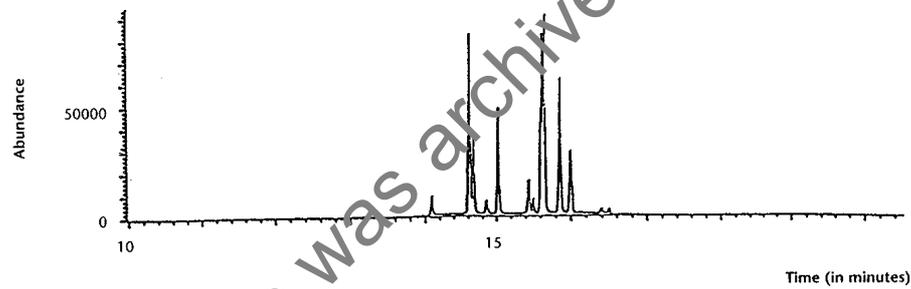


Figure B4 Mass chromatogram of ion 258 from figure C2 (GC-MS (SIM) of trichlorobiphenyls present in Aroclor 1242)

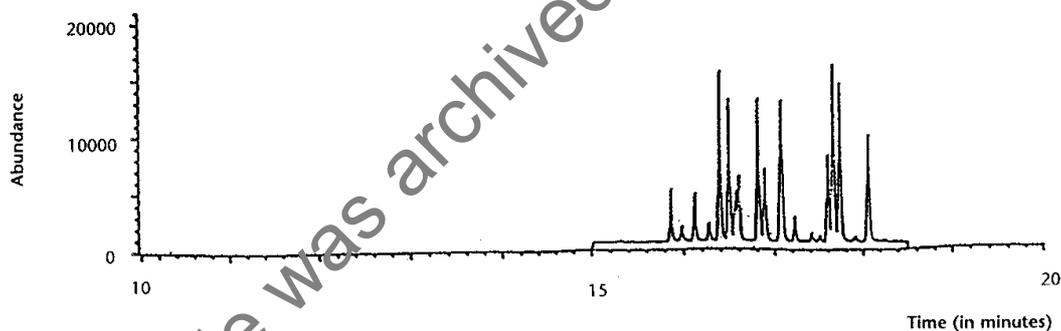


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Figure B5 Mass chromatogram of ion 291.9 from figure C2 (GC-MS (SIM) of tetrachlorobiphenyls present in Aroclor 1242)

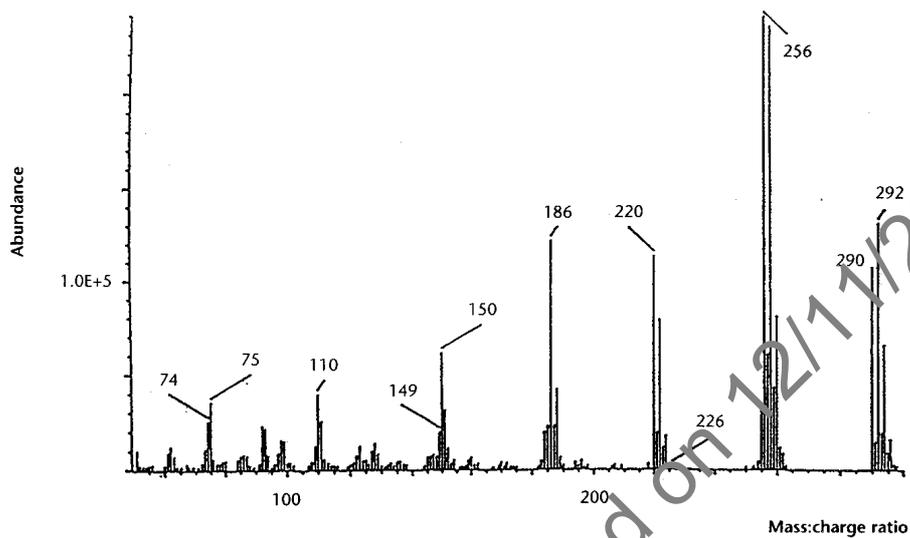


Figure B6 Mass chromatogram of ion 289.8 from figure C2 (GC-MS (SIM) of tetrachlorobiphenyls present in Aroclor 1242)



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Figure B7 Example of a mass spectrum from which the m/z abundance ratio can be calculated (in this case, the 291.9/289.8 ratio is used to indicate the presence of a tetrachlorobiphenyl)



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C A note on the determination of polychlorinated biphenyls in waters by solvent extraction using gas chromatography with mass spectrometric detection

C1 Introduction

This method is essentially as that described for Method B, which should be consulted and then suitably adapted for the analysis of potable, river and saline waters. PCBs are extracted into hexane. The extract is dried with anhydrous sodium sulphate, evaporated to low volume and re-constituted in 2,2,4-trimethylpentane. The resulting extract is analysed using gas chromatography with mass spectrometric detection operating in electron impact mode with selected ion monitoring. Quantification is by internal standard added to the extract solution after extraction and evaporation. A $^{13}\text{C}_{12}$ -surrogate standard added before extraction is used to monitor the performance of the analysis for each sample.

Samples should be taken in glass bottles (1.2 litre in capacity and marked at 1 litre) with glass stoppers or polytetrafluoroethylene-lined screw caps. The samples 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 2-8 °C. See also section 1.

C2 Analytical procedure

Step	Procedure	Note
C2.1	Extraction	
C2.1.1	To 1000 ± 10 ml of sample in the sample bottle, add 0.05 µg of surrogate standard in acetone (B5.4), i.e. a suitable volume of the diluted surrogate standard solution (B5.9). Add 40 ± 5 ml of hexane (B5.2). Stopper and shake vigorously for 120 ± 10 seconds, see notes a and b. 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 0.05 µg of surrogate standard in acetone (B5.4), i.e. a suitable volume of the diluted surrogate standard solution (B5.9) to 1000 ± 10 ml of sample in the sample bottle and mix thoroughly, see note b. 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. (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.
C2.1.2	Allow the phases to separate. When good separation has been achieved, if appropriate, run off and discard the lower aqueous phase, see note c. If not appropriate, repeat the extraction and combine the extracts.	(c) The efficiency of the extraction may be improved by repeating the extraction, especially in cases where samples contain particulate matter.

C2.2 Drying and concentration

C2.2.1 Transfer the hexane into a flask containing approximately 10 g of sodium sulphate (B5.5). Rinse the separating funnel with 10 ± 1 ml of hexane and add the washings to the flask. Swirl the flask and leave to stand. Occasionally, repeat this process.

C2.2.2 Transfer the dried extract to a Kuderna-Danish evaporator, see note d. Wash the sodium sulphate with 10 ± 1 ml of hexane. Decant the washings into the Kuderna, add an anti-bumping granule and evaporate the extract to about 1-5 ml.

C2.2.3 Reduce the volume of the extract using a stream of nitrogen (B6.8) to 1.00 ± 0.01 ml, see note e. Add $0.025 \mu\text{g}$ of internal standard, see note f, i.e. a suitable volume of the diluted internal standard solution (B5.8) if required. The solution is now ready for gas chromatographic determination. Refer to Method B, section B8.4.

(d) Alternative evaporating systems may be used.

(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) Since the internal standard is added after extraction, an estimate of recovery may need to be determined to account for extraction and clean-up losses.

C2.3 Blanks and recoveries

C2.3.1 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 (B5.1) see note g. Process these solutions under conditions identical with those used for samples under analysis.

C2.3.2 If the peak responses of extracted calibration solutions are used for comparison with those of the samples, an automatic correction is obtained. If not, recovery data should be established and recovery correction considered, see note h.

(g) To determine the recovery, spike the sample at an appropriate level with mixed PCBs (B5.7) ensuring that the volume of (water miscible) solvent used is between $20 \mu\text{l}$ and $100 \mu\text{l}$ per litre of aqueous sample.

(h) Use the surrogate standard to indicate whether the analysis has proceeded satisfactory for each individual sample.

C2.4 AQC

C2.4.1 Carry out the entire procedure using interference-free water spiked at an appropriate concentration with PCBs. Analyse a corresponding interference-free blank water.

C3 Calculation

Using equations similar to those in B9, calculate the concentration of all PCBs determined in the original sample.

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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 confirm the analysis remains in control. At least one control sample 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 (set at three times the standard deviation of the mean) or two consecutive values exceed the warning limit (set at twice the standard deviation of the mean). As more data are acquired, the standard deviation 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 have 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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Standing Committee of Analysts
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Landmere Lane, Edwalton
Nottingham
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Environment Agency Standing Committee of Analysts Members assisting with these methods

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