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DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCBs) IN SEDIMENT AND BIOTA

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This series presents detailed descriptions of methods and procedures relating to chemical and biological measurements in the marine environment. Most techniques described have been selected for documentation based on performance in ICES or other intercalibration or intercomparison exercises: they have been carefully evaluated and shown to yield good results when correctly applied. They have also been subject to review by relevant ICES working groups, but this is not to be construed as constituting official recommendation by the Council.

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Abstract

Polychlorinated biphenyls (PCBs) are environmental contaminants regulated by the Stockholm Convention of Persistent Organic Pollutants (POPs), and are included on the OSPAR List of Chemicals for Priority Action due to their persistence, potential to bioaccumulate, and toxicity. Analysis of the ICES-7 PCBs (CB28, 52, 101, 118, 138, 153, and 180), in sediment and biota, is a mandatory requirement of the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP). Three of the four non-*ortho* (CB77, 126 and 169) are classed as pre-CEMP determinands at the time of publication, and analysis in biota is recommended but on a voluntary basis.

This document provides advice on the analysis of PCBs in biota and sediment, including non-*ortho* PCBs. The determination of PCBs in sediment and biota generally involves extraction with organic solvents, clean-up, and gas chromatographic separation with electron capture detection or mass spectrometry. Due to the low concentrations of non-*ortho* substituted PCBs compared to those of other PCBs, their determination may require an additional separation step.

All stages of the procedure are susceptible to insufficient recovery and/or contamination. Therefore, quality control procedures are important in order to check method performance. These guidelines have been prepared by members of the ICES Marine Chemistry Working Group (MCWG) and the Working Group on Marine Sediment (WGMS) and are intended to encourage and assist analytical chemists to reconsider their methods and to improve their procedures and/or the associated quality control measures where necessary.

Keywords: polychlorinated biphenyls, sediment, biota, storage, extraction, clean-up, calibration, gas chromatography, electron capture detection, mass spectrometry.

1 Introduction

Commercial formulations of polychlorinated biphenyls (PCBs), such as Aroclor mixtures, were widely used in the past in transformers, capacitors, hydraulic fluids and as plasticizers in paints, plastics and sealants. It has been estimated that globally 1.3 million tonnes of PCB compounds have been produced (Breivik *et al.*, 2007). Historically the main sources of PCBs in the marine environment include energy production, combustion industries, production processes, and waste (landfill, incineration, waste treatment, and disposal). Due to concerns about the environmental impact of PCBs, production in Western Europe and North America ceased in the late 1970s and in Eastern Europe and Russia in the early 1990s. PCBs can still enter the marine environment following the destruction and disposal of industrial plants and equipment or from emissions from construction materials (Kohler *et al*., 2005) and old electrical equipment (for example from landfill sites). PCBs are included in the Stockholm Convention (UNEP, 2009) due to their persistence, bioaccumulation, and toxicity (PBT).

Theoretically 209 individual PCB congeners can be produced, depending on the number and position of chlorine that is substituted onto the biphenyl moiety. Individual congeners are generally named according to the short-hand system Ballschmiter and Zell (1980) developed for PCB congeners. For this naming system a number from 1 to 209, often prefixed with "CB", was applied to each congener after the congeners had been sorted on the basis of their structural names. This system was summarized more recently by Mills *et al.* (2007). The seven ICES (International Council for the Exploration of the Sea) PCBs (CB28, 52, 101, 118, 153, 138, and 180) were recommended for monitoring by the European Union Community Bureau of Reference; these PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The ICES-7 PCBs have been part of the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP) since 1998. Of the 209 PCB congeners, the most toxic are the so-called 'dioxin-like' PCBs (DL-PCBs). The DL-PCBs are the four non-*ortho* (CB77, 81, 126, and 169) and eight mono-*ortho* (CB105, 114, 118, 123, 156, 157, 167, and 189) PCBs that also have chlorines in both *para* and at least two *meta* positions. The non-*ortho* PCBs can obtain a planar configuration and the mono-*ortho* PCBs can obtain a near planar configuration. As a result, the twelve DL-PCBs are stereochemically similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and therefore have similar toxic and biological responses to those of dioxins (Safe *et. al*, 1985; Kannan *et. al*, 1989). However, they are normally found at much lower concentrations when compared to the *ortho*-PCBs. At the time of publication three of the four non-*ortho* (CB77, 126, and 169) PCBs are classed as pre-CEMP determinands, and analysis in biota is recommended but on a voluntary basis.

This paper provides advice on PCB analysis of biota and sediment samples. The guideline is an update of an earlier version (Smedes and de Boer, 1998), which takes into account evolutions in the field of analytical chemistry and also covers the determination of DL-PCBs. The analysis of PCBs in biota and sediment generally involves extraction with organic solvents, clean-up (removal of lipids and fractionation), and gas chromatographic separation with electron capture or mass-spectrometric detection. Due to the low concentrations, particularly of non-*ortho* substituted PCBs in biota compared to those of other PCBs, their determination usually requires an additional separation and concentration step. Therefore, in the

relevant sections a distinction will be made between the procedures for non-*ortho* PCBs and the others.

2 Sampling and sample handling

OSPAR (2004) presented a monitoring strategy for PCBs for which contracting parties should continue to measure PCBs under the CEMP on a mandatory basis in biota (fish and mussels) and sediments for temporal trends and spatial distribution, covering PCB congeners 28, 52, 101, 118, 138,153, and 180. Three of the four non-*ortho* PCBs (CB77, 126, and 169) are classed as pre-CEMP, and their analysis in biota is on a voluntary basis. Details on sample handling and preparation as described for the analysis of polybrominated diphenyl ethers (PBDEs) will also be valid for PCBs and DL-PCBs (Webster *et al.*, 2009).

2.1 Biota samples

Aquatic organisms can accumulate hydrophobic compounds like PCBs and reach concentrations considerably higher than those of the surrounding waters. Therefore mussels and fish are suitable and commonly used for monitoring of PCBs (OSPAR, 2004). PCBs are bioaccumulated and biomagnified by marine organisms, leading to high concentrations within the fatty tissues of piscivorous birds and marine mammals. For fish, the highest PCB concentrations are found in lipid-rich tissues like the liver and in muscle tissue of fatty fish such as herring and salmon.

The species selected for monitoring of biota should fulfil the same requirements as for dioxins monitoring (Vorkamp *et al*, 2012):

- Reflect concentration changes in the sampling area, i.e. ensure a link between exposure and concentration in the organisms.
- Accumulate compounds without showing adverse effects.
- Be representative of and abundant in the area (to ensure sufficient sample material for analysis).
- Be relatively easy to handle.

For mussel samples, it is important to remove any sediment particles from their intestinal system. This is accomplished by depuration in a glass aquarium with filtered water from the sampling location for approximately 24 hours. Mussel samples should preferably not be frozen prior to dissection but should be transported at temperatures between 5 and 15°C, a scale representative of the area of origin, in a clean container. After dissection, all samples should be stored in the dark at < -20°C prior to analysis. Under these conditions, long-term storage of tissue samples is possible (De Boer and Smedes, 1997).

2.2 Sediment samples

Marine sediments, in particular those with a high organic carbon content, may accumulate hydrophobic compounds like PCBs to considerably higher levels than those of the surrounding waters. The sampling strategy depends on the purpose of the monitoring programme and the natural conditions of the region to be monitored. Typically sampling approaches include fixed-station sampling, stratified random sampling, or stratified fixed sampling. Muddy sediments, i.e. those containing a high proportion of fine material, are preferable for organic contaminant monitoring, although sieving of sediments may be an alternative (OSPAR, 2002).

2.3 Determination of lipid and organic carbon content

The total lipid content of biota samples should be determined to allow normalisation to lipid and for expressing PCB concentrations on a lipid weight basis. Suitable methods for lipid determination have been described in the ICES guidelines for polybrominated diphenyl ethers (Webster *et al.*, 2009) and include the methods by Bligh and Dyer (1959), as modified by Hanson and Olley (1963), and Smedes (1999). Extractable lipid may be used, particularly if the sample size is small and lipid content is high. It has been demonstrated that if the lipid content is high (> 5 %), extractable lipid will be comparable with the total lipid (Roose and Smedes, 1996). If extraction techniques are applied that destroy or remove lipid (e.g. PLE with fat retainers), the lipid content should be determined on a separate subsample of the tissue homogenate.

For comparison to assessment criteria, PCB concentrations in sediment should be normalized to organic carbon. Therefore, total organic carbon (TOC) should be determined for normalization and for characterization of the sediment (Schumacher, 2002; Leach *et al.*, 2008). In sediment samples, dry weight should also be determined for normalization purposes, for instance through weight loss after drying at 105°C for 22 ± hours.

3 Analytical methods

3.1 Precautionary measures

The subject of this section has been covered in the ICES guidelines for polybrominated diphenyl ethers (Webster *et al.*, 2009), but key aspects are emphasized here due to their critical nature. Solvents, chemicals, and adsorption materials must be free of PCBs or other interfering compounds. Solvents should be checked for purity by first concentrating the volume normally used in the procedure to 10% of the final volume and then analyzing them using a gas chromatograph (GC) for the presence of PCBs and other interfering compounds. Solvents could be purified using appropriate methods (e.g. using a rectification column). Chemicals and adsorption materials should be purified by extraction and/or heating. Glass fibre materials (e.g. thimbles for Soxhlet extraction) should be pre-extracted. All containers and glassware which come into contact with the sample must be made of appropriate material and must have been thoroughly pre-cleaned. Glassware should be extensively washed with detergents and either rinsed with organic solvents or mixtures such as hexane/acetone or heated at >450°C. As all super cleaned materials are prone to contamination (e.g. by the adsorption of PCBs and other compounds from laboratory air), materials ready for use should not be stored for long periods; instead, such materials may be stored for brief periods in cupboards covered with aluminium foil in order to keep out any dust. Old and scratched glassware is more likely to cause blank problems it is also more difficult to clean.

3.2 Extraction

The target compounds must be extracted from the sediment or biota with an organic solvent prior to analysis. Generally, 40–100 g of freeze-dried sediments is required. Typical biota sample weights extracted are 5–10 g for fish, flesh, or mussel and 0.5–1 g for liver. For small sample sizes such as liver, pooling of samples may be necessary. In this case an equivalent quantity of tissue should be taken from each fish, for example, 10 % from each liver. Extraction methods do not differ for DL-PCBs but,

because of the low concentrations, a substantially larger sample size has to be considered.

Recovery and internal standards should always be added prior to extraction. Although Soxhlet extraction using a combination of polar and apolar solvents is still the benchmark for PCB extraction, there have been numerous attempts to find alternative procedures which are less time-consuming, use less solvent, and/or enable miniaturization. Amongst these novel approaches are pressurized liquid extraction (PLE) and related subcritical water extraction (SWE), microwave-assisted extraction (MAE), matrix solid-phase dispersion (MSPD), ultrasound extraction (US), and supercritical fluid extraction (SFE). At present PLE is the most widely-used (Roose and Brinkman, 2005). Soxhlet methods are easily translated into PLE as the same solvent compositions can be used. The method further allows interesting modifications that include in-cell clean-up of samples by adding fat retainers such as florisil or alumina to the cell and the use of a small carbon column in the extraction cell, the latter of which selectively adsorbs dioxin-like compounds (subsequently isolated by back-flushing with toluene) (Sporring *et al*., 2003). PLE and MAE have the shared advantage over SFE in that they are matrix-independent. This facilitates method development and changing-over from the classical Soxhlet extraction. Recent years have also seen an increased use of ultrasound-based techniques for the isolation of analytes from solid samples. With most applications, extraction efficiency is fully satisfactory, and sonication time is often 30 minutes or less (Roose and Brinkman, 2005).

All the methods described above are, in principle, suitable for extracting PCBs from both sediment and biota.

As an alternative, saponification has also been used (Booij and van den Berg, 1994). This technique is highly effective, but conditions must be controlled as saponification can result in the decomposition of certain pesticides (if analyzed) and, under certain conditions, of some PCB congeners. This method is therefore not recommended.

3.3 Clean-up

Extracts are concentrated using suitable evaporation devices, e.g. rotary evaporation, Turbovap®, Syncore®, or Kuderna-Danish. A more detailed description of concentration procedures is given in the ICES guidelines for polybrominated diphenyl ethers (Webster *et al*., 2009).

Tissue or sediment extracts will always contain many compounds other than PCBs, (including lipids and sulphur) and a suitable clean-up is necessary to remove those compounds which may interfere with the subsequent analysis. Different techniques may be used, either singly or in combination, and the choice will be influenced by the selectivity and sensitivity of the final measurement technique and also by the extraction method employed. All PCBs are stable under acid conditions; therefore treatment with sulphuric acid or acid impregnated silica columns may be used in the clean-up, primarily for the removal of lipid. Sulphur should be removed from sediment samples to reduce interferences and protect the detector. This may be achieved by the addition of copper (granules, wire, or gauze) during or after extraction.

The most commonly used clean-up methods involve the use of alumina (Al_2O_3) or silica adsorption chromatography. Deactivated Al_2O_3 (5–10% water) is often used as a primary clean-up (Van Leeuwen and de Boer, 2008). For sediment, provided that sulphur has been removed, Al_2O_3 sometimes gives a sufficiently clean extract for a

GC-ECD or GC-MS analysis of the sample. However, when analyzing by ion trap, GC-MS additional clean-up steps may be required (Pico, 2008). Al_2O_3 also removes lipids from the extracts of biota samples, although samples with a very high lipid content and low PCB concentration may require additional clean-up.

Silica columns are often used as a secondary clean-up to separate PCBs from other contaminant groups such as pesticides (Wells and Hess, 2000). Deactivated silica (1–5% water) does not retain PCBs (including non-*ortho* PCBs) and only slightly retains polycyclic aromatic hydrocarbons (PAHs) when eluted with hexane, *iso*-hexane, or *iso*-octane. The PCBs and a few other organochlorine compounds are eluted with apolar solvents. More polar solvents (e.g. hexane/acetone) should be avoided as some interfering organochlorine pesticides would be eluted.

Gel Permeation Chromatography (GPC) is another commonly used clean-up method and for sediment samples it also has the advantage that it removes sulphur (Wells and Hess, 2000; Muir and Sverko, 2006). When using GPC, the elution of PCBs should be carefully checked. When applying GPC, two serial columns are often used for improved lipid separation. Solvent mixtures such as dichloromethane/hexane or cyclohexane/ethyl acetate can be used as eluents for GPC. However, an additional fractionation step is often required to separate the PCBs from other organohalogenated compounds.

One advantage of using PLE extraction is that it is possible to combine the clean-up with the extraction, especially when mass spectrometry will be used due to the detection method being less affected by interfering compounds. PLE methods have been developed for online clean-up and fractionation of dioxins, furans, and PCBs in food, feed, and environmental samples (Sporring *et al*., 2003). A fat retainer can be used during PLE extractions for the online clean-up of fat. Silica impregnated with sulphuric acid, alumina, and florisil have all been used as fat retainers. A non-polar extraction solvent such as hexane should be used if fat retainers are used during PLE.

Non-*ortho* PCBs require a more specialized clean-up that is generally associated with the analysis of dioxins. Although initial clean-up may very well proceed along the lines described above, the larger sample size results in even more co-extractives and care has to be taken that the capacity of the adsorption columns is not exceeded and/or that sulphur is adequately removed in the case of sediment samples. Often, more rigorous procedures such as shaking the sample with concentrated sulphuric acid are applied to remove the excess lipid. A more efficient and safer alternative is to elute the sample over a silica column impregnated with sulphuric acid (*ca* 40% w/w).

Non-*ortho* PCBs are nearly always separated from the other PCBs using advanced separation techniques. A particularly efficient method is to inject the extracts (after concentrating them) into an HPLC system coupled to a PYE (2-(1-pyrenyl) ethyldimethylsilylated silica) column (Hess *et al*., 1995). Column dimensions are typically 4.6 × 150 mm, but combinations of several columns are sometimes used. PYE columns not only allow the separation of *ortho*, mono-*ortho*, and non-*ortho* PCBs on the basis of structural polarity from each other, but they also separate these PCBs from dibenzodioxins and furans. The eluting solvent must be apolar, e.g. *iso*-hexane. Coupled to a fraction collector, the use of an HPLC system allows the automatic clean-up of a large number of samples. Similarly to PYE columns, HPLC systems equipped with porous graphite carbon will separate non-*ortho* PCBs from other PCBs and from dioxins/furans (Kannan *et al*., 1987; de Boer *et al*. 1992; Hess *et al*., 1995). Column sizes are in the order of 50 × 4.7 mm and care has to be taken that the column

is not overloaded. Fully automated clean-up systems that combine several steps are also routinely used and available commercially (e.g. PowerPrep™ system).

4 Instrumental analysis

The PCB content of environmental samples is commonly monitored using gas chromatography with electron capture detection (GC-ECD) or gas chromatography with mass spectrometry (GC-MS). DL-PCBs should be analyzed by GC-MS, preferably by high-resolution MS, although low-resolution mass spectrometry may be a suitable alternative (Vorkamp *et al*., 2012).

4.1 Internal/recovery standards

Internal standards and recovery standards should be added in a defined amount to all calibration solutions and samples. Ideally, these standards should fall within the range of compounds to be determined and should not include compounds which may be present in the samples or co-elute with other PCBs.

For analysis by GC-ECD, all PCBs with a 2,4,6-substitution (e.g. CB112, CB155, CB198) are generally used as they are not found in the environment. In addition, 1,2,3,4-tetrachloronaphthalene (TCN) or homologues of dichloroalkylbenzylether can be used as injection standards. Additionally, contamination by graphite (e.g. from ferrules) should be avoided in the liner as it may adsorb DL-PCBs and TCN.

For GC-MS, labelled internal standards must be used. ^{13}C labelled PCBs should be used for each degree of chlorination. If possible, the labelled internal standard solutions should correspond to the unlabelled determinands. For the non-*ortho* PCBs, a labelled standard is available for each of the four congeners and use of all of them is recommended.

When preparing a calibration solution for a new determinand for the first time, two independent stock solutions of different concentrations should always be prepared simultaneously to allow cross-checking.

4.2 GC-Analysis

The two most commonly used GC injection techniques for PCBs analysis are splitless and on-column injection. In splitless injection, strong discrimination effects may occur. The liner should possess sufficient capacity with respect to the injected volume after evaporation but should not be oversized to avoid poor transfer to the column and losses by adsorption. Liners with a light packing of silylated glass wool may improve the PCB performance but may degrade some organochlorine compounds like DDT, which are often included in national monitoring programmes.

Recently, other techniques such as temperature-programmed or pressure-programmed injection have become more prominent. They offer additional advantages such as an increased injection volume without the previously associated negative effects, but these methods should be thoroughly optimized before use. Increasing the injection volume will allow either the elimination of an extra evaporation step and/or the lowering of the detection limits.

Hydrogen is the preferred carrier gas and is essential for columns with very small inner diameters. Helium is also acceptable and the standard carrier gas for GC-MS.

The recommended column parameters for PCB analysis are shown in Table 1. Columns which do not fulfil these requirements generally do not offer sufficient resolution to separate CB28, CB105, and CB156 from closely eluting PCBs.

Table 1. Recommended column parameters for the analysis of PCBs

MINIMUM LENGTH	50 M (FOR MICROCOLUMNS OF INTERNAL DIAMETER <0.1 MM, SHORTER COLUMNS CAN BE SUITABLE).
Maximum internal diameter	0.25 mm. (Note that for diameters <0.15 mm the elevated pressure of the carrier gas needs special instrumental equipment as most of the instruments are limited to 400 kPa.)
Film thickness	0.2–0.4 μm.

A wide range of stationary phases can be used for PCB separation, such as HP-5 or CP-Sil8 (Verenitch *et al.*, 2007). The chemical composition is different for many manufacturers and affects the maximum temperature at which the column can be operated. Further advice may be found in the suppliers' catalogues, where compositions, applications, and tables to compare products from different manufacturers are included. A good example is the HT-8 phase (1,7-dicarba-closo-dodecarborane phenylmethyl siloxane) (Larsen *et al.*, 1995), which shows a remarkable selectivity for PCBs and improved separation of critical PCB pairs (Table 2). This column is currently recommended for PCB analysis.

4.3 Detection

The electron capture detector (ECD) is still frequently used for PCB analysis. However, in recent years MS techniques are being increasingly applied. For most PCBs, low-resolution mass spectrometry (LRMS) provides sufficient sensitivity for biota and sediment samples. For DL-PCBs, HRMS is preferred due to the low concentrations found in the environment. However, LRMS may be a suitable alternative, particularly for the screening of samples. Analysis of DL-PCBs by GC-HRMS is described in more detail by Vorkamp *et al.* (2012).

When using MS, electron-capture negative ionisation (ECNI) is extremely sensitive for pentachlorinated to decachlorinated PCBs and is approximately tenfold more sensitive than ECD. However, MS systems have improved considerably allowing analysis by electron impact ionisation (EI), whereas before ECNI was often necessary in order to detect the low concentrations of, in particular, the non-*ortho* PCBs (Wells and Hess, 2000; Van Leeuwen and de Boer, 2008). Suggested target and qualifier ions for *ortho* PCBs (including mono-*ortho* PCBs) are shown in Table 2 and in Table 3 for non-*ortho* PCBs.

Next to conventional GC-MS, the use of ion-trap (GC-ITMS) with its MS^2 option—i.e., increased selectivity—is receiving increased attention. GC-ITMS is a less expensive alternative to high-resolution mass spectrometry (HRMS). HRMS is commonly used to determine PCDD/Fs and as such also ideally suited for all PCB groups (Eppe *et al.*, 2004).

Table 2. Example of retention times for selected PCB congeners using a 50 m HT8 column (0.25 mm i.d. and 0.25 μm film), along with possible target and qualifier ions (based on a quadrupole MS operated in electron impact mode). Temperature programme: 80˚C, hold for 1 minute, ramp 20˚C/minute, to 170˚C, hold 7.5 minutes, ramp 3˚C/minute to 300˚C, hold for 10 minutes.

PCB congener	MW	RT	Target Ion	Qualifier Ion	Number of chlorines
^{13}C-CB28	**270**	28.4	268	270	3
CB31	**258**	28.1	256	258	3
CB28	**258**	28.4	256	258	3
^{13}C-CB52	**304**	30.3	304	302	4
CB52	**292**	30.3	292	290	4
CB49	**292**	30.7	292	290	4
CB44	**292**	32.1	292	290	4
CB74	**292**	34.9	292	290	4
CB70	**292**	35.2	292	290	4
^{13}C-CB101	**340**	36.6	338	340	5
CB101	**326**	36.6	326	328	5
CB99	**326**	37.1	326	328	5
CB97	**326**	38.3	326	328	5
CB110	**326**	39.3	326	328	5
CB123*	**326**	41.2	326	328	5
CB118*	**326**	41.6	326	328	5
CB105*	**326**	43.4	326	328	5
CB114*	**326**	42.2	326	328	5
^{13}C-CB153	**374**	42.6	372	374	6
CB149	**362**	40.3	360	362	6
CB153	**362**	42.6	360	362	6
CB132	**362**	42.2	360	362	6
CB137	**362**	43.7	360	362	6
13C-CB138	**374**	44.4	372	374	6
CB138	**362**	44.5	360	362	6
CB158	**362**	44.7	360	362	6
CB128	**362**	46.3	360	362	6
^{13}C-CB156	**374**	48.4	372	374	6
CB156*	**362**	48.4	360	362	6
CB167*	**362**	46.2	360	362	6
CB157*	**362**	48.7	360	362	6
^{13}C-CB180	**408**	48.8	406	408	7
CB187	**396**	44.8	394	396	7
CB183	**396**	45.3	394	396	7
CB180	**396**	48.8	394	396	7
CB170	**396**	50.7	394	396	7

^{13}C-CB189	**406**	53.2	406	408	7
CB189*	**396**	53.2	394	396	7
^{13}C – CB194	**442**	57.5	442	440	8
CB198	**430**	50.3	430	428	8
CB194	**430**	57.5	430	428	8

***mono-*ortho* PCBs**

Table 3. Possible target and qualifier ions (based on a quadrupole MS operated in electron impact mode) for non-ortho PCBs, including labelled internal standards

CB	Target ion (*m/z*)	Qualifier (*m/z*)	Qualifier (*m/z*)	Qualifier (*m/z*)
^{13}CB81	304	302	NA	NA
CB81	292	290	220	222
^{13}CB77	304	302	NA	NA
CB77	292	290	220	222
^{13}CB126	338	340	NA	NA
CB126	326	328	254	256
^{13}CB169	372	374	NA	NA
CB169	360	362	218	220

4.4 Separation, identification and quantification

When using GC-ECD, and to a certain extent GC-MS, two columns with stationary phases of different polarity should be used since column-specific co-elution of the target PCBs with other PCBs or organochlorine compounds can occur. The temperature programme must be optimized for each column to achieve sufficient separation of the PCB congeners to be determined. An isothermal period of approximately 30 minutes in the programme at around 200–220°C, when the critical pairs of PCBs are eluting, is generally recommended. Alternatively, a gentle increase in temperature (e.g. 3°C/minute) can also be used, which will result in a shorter analysis time. Care should be taken that PCBs of interest do not co-elute with other PCB congeners (for example CB28 and CB31). CB138 and CB163 will co-elute on many GC columns and are often mistakenly reported as just CB138, when in fact it should be CB138 and CB163. However, it is possible to separate CB138 and CB163 using an HT-8 column. When using two columns of different polarity, the most reliable result should be reported.

When using GC-ECD, compounds are identified by their retention time in relation to the standard solutions under the same conditions. Therefore GC conditions should be constant. Retention times should be checked for shifts throughout the chromatogram with the help of characteristic, unmistakable peaks (e.g. originating from the recovery, internal standard, or more highly concentrated PCBs such as CB153 and CB138) to ensure correct peak identification. Using a GC-MS system, the molecular mass or characteristic mass fragments or the ratio of two ion masses can be used to confirm the identity of separated PCBs.

The calibration curves of most PCBs analyzed by GC-ECD are linear over a limited concentration range, meaning that using a multilevel calibration of at least five

concentration standards is recommended. When analyzing by GC-MS, the linear range is wider and, therefore, it may be possible to use fewer calibration levels.

As baseline separation is not always achievable when using GC-ECD, peak height is preferable to peak area for quantification purposes.

Recent years have witnessed the emergence of commercially available instruments for comprehensive two-dimensional gas chromatography (GC × GC) — a technique that can be used to considerably improve analyte/matrix as well as analyte / analyte separation (Korytar *et al*., 2006; Van Leeuwen and de Boer, 2008). Briefly, a non-polar and semi-polar column combination is used, with a conventional 25–30 m long first-dimension column and a shorter, 0.5–1 m long, second-dimension column. The columns are connected via an interface called a modulator. The latter device serves to trap and focus each subsequent small effluent fraction from the first-dimension column and then to launch it into the second column. The main advantages of this comprehensive approach are that the entire sample is subjected to a completely different separation, that the two-dimensional separation does not take more time than the first-dimension run, and that the refocusing in the modulator helps to increase analyte separation. The most interesting additional benefit for PCB analysis is that structurally related analytes, i.e. PCB congeners, show up as ordered structures in the two-dimensional GC × GC plane. The very rapid second-dimension separation requires the use of detectors with sufficiently high data acquisition rates. Initially, only flame ionization detectors could meet this requirement. However, today there is also a micro-ECD on the market that is widely used for GC × GC-μECD of halogenated compound classes. Even more importantly, analyte identification can be performed by using a time-of-flight mass spectrometer (Roose and Brinkman, 2005) or, with a modest loss of performance but at a much lower price, one of the more recently introduced rapid-scanning quadrupole mass spectrometers (Roose and Brinkman, 2005). So far, the use of GC × GC has been mostly limited to qualitative purposes and still seems to be rarely used for routine quantification.

When using an internal standard, it should be added to each calibration level at the same concentration. Calibration curves are drawn by plotting the ratio of the response of the analytes to the response of the internal standard against the ratio of the concentration of the analytes to the concentration of internal standard. A known amount of internal standard is added to all samples, prior to extraction to correct for any losses during extraction and clean-up. The ratio of the analyte response to internal standard response is then used to obtain the analyte concentrations from the calibration curve.

5 Quality assurance

Planners of monitoring programmes must decide on the accuracy, precision (within-lab reproducibility), measurement uncertainty, and limits of detection (LOD) and determination/quantification (LOQ) which will be acceptable. References of relevance to QA procedures include QUASIMEME (1992); Wells *et al*. (1992); Oehlenschläger (1994); Smedes and de Boer (1994) and ICES (1996).

5.1 System performance

It is standard QA practice to monitor GC performance. For instance, the performance of the GC system should be monitored by regularly checking the resolution of two closely eluting PCBs (e.g. CB28 and CB31). A decrease in resolution points to a

deteriorated column. A dirty EC detector or MS source can be recognized by the presence of an elevated background signal together with a reduced signal-to-noise ratio.

5.2 Recovery

The recovery of PCBs from samples should be monitored. One method is to add an internal or recovery standard to each sample immediately before extraction. If small losses occur during extraction or clean-up, or solutions are concentrated by the uncontrolled evaporation of solvents, e.g. because vials are not perfectly capped, losses can be compensated for by normalization. If major losses are recognized and the reasons are unknown, the results should not be reported, as recoveries are likely to be irreproducible. Recoveries should be between 70 and 120%; if not, sample extractions should be repeated.

Periodic checks of the method performance are recommended. This can be done by adding a calibration solution to a real sample prior to extraction or by the extraction and analysis of a Certified Reference Material (CRM) (see Section 5.4).

5.3 Procedural blanks

A procedural blank should be determined for each sample batch and should be prepared simultaneously using the same chemicals and solvents as for the samples. The purpose of the procedural blank is to reveal any sample contamination by interfering compounds which would lead to errors in quantification. There is some debate about whether blank corrections should be performed on samples or not. One argument is that even if an internal standard / recovery standard has been added to the blank at the beginning of the procedure, a quantification of peaks in the blank and subtraction from the values obtained for the determinands must not be performed, as the added internal/recovery standard cannot be adsorbed by a matrix. However, it is generally recommended that if concentrations of PCBs in the blanks are significant (for example, averaging greater than 10% of the average level of total PCBs in the samples), then blank correction should be carried out.

5.4 Precision and accuracy

A Laboratory Reference Material (LRM) should be included, at least one sample for each batch of identically prepared samples. The LRM must be homogeneous, well characterized for the determinands in question, and must have been shown to produce consistent results in stability tests over time. The LRM should be of the same type of matrix (e.g. liver, muscle tissue, fat or lean fish) as the samples, and the determinand concentrations should occur in a comparable range to those of the samples. If this is not possible, e.g. if the range of determinand concentrations in the sample is large (> factor of 5), two different reference materials could be included in each batch of analyses to cover the lower and upper concentrations. A quality control chart should be recorded for a selected set or preferable all PCBs.

It is good practice to run duplicate analyses of a reference material or one of the samples to check within-batch analytical variability.

When introducing a new LRM or when it is suspected from the control chart that there is a systematic error, a relevant Certified Reference Material (CRM) of a similar matrix to the material analyzed should be used to check the LRM.

Each laboratory should participate in interlaboratory comparison studies or proficiency testing schemes on a regular basis, preferably at an international level.

5.5 Data collection and reporting

For biota analysis, results are typically reported in μg/kg wet weight (ww). The lipid content of the samples should be reported as well. For sediment analysis, results are typically reported in μg/kg dry weight (dw). The water and organic carbon content of the samples should be reported as well, the latter being used for normalizing purposes.

Concentrations are reported to two significant figures. Minimum performance criteria such as LOQ and measurement uncertainty along with information on blanks and reference materials should be included in the report.

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7 Abbreviations and technical terminology

CEMP	OSPAR Co-ordinated Environmental Monitoring Programme
CRM	certified reference material
DDT	dichlorodiphenyltrichloroethane
DL-PCB	dioxin-like polychlorinated biphenyl(s)
ECD	electron capture detector
ECNI	electron-capture negative ionization
EI	electron ionization
GC	gas chromatograph
GC-ECD	gas chromatography electron-capture device
GC-HRMS	gas chromatography high-resolution mass spectrometry
GC-ITMS	gas chromatography ion-trap mass spectrometry
GC-MS	gas chromatography-mass spectrometry
GPC	gel permeation chromatography
HELCOM	Baltic Marine Environment Protection Commission (Helsinki Commission)
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
LRM	laboratory reference material
LRMS	low-resolution mass spectrometry
MAE	microwave-assisted extraction
MS	mass spectrometry
MSPD	matrix solid-phase dispersion
OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlantic
PAH	polycyclic aromatic hydrocarbon(s)
PBDE	polybrominated diphenyl ether(s)
PCB	polychlorinated biphenyl(s)
PCDD/Fs	Polychlorinated dibenzo-*p*-dioxins and dibenzo-furans
PLE	pressurized liquid extraction
PYE	2-(1-pyrenyl)ethyldimethylsilylated silica
QUASIMEME	Quality Assurance of Information for Marine Environmental Monitoring in Europe

SFE	supercritical fluid extraction
SWE	subcritical water extraction
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TCN	1,2,3,4-tetrachloronaphthalene
TOC	total organic carbon
US	ultrasound extraction

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