

1.5.5.8 Tools for coordinated monitoring of dioxins, planar CBs and PFOS

Request

Tools for coordinated monitoring of dioxins, planar CBs and PFOS (OSPAR 4-2008)

To prepare the following tools to support the coordinated monitoring of dioxins, planar CBs and PFOS under the OSPAR CEMP:

a. technical annexes to the JAMP Guidelines for monitoring Contaminants in Sediments (OSPAR agreement 2002-16) and JAMP Guidelines for monitoring Contaminants in Biota (OSPAR agreement 1992-2) according to the structure of the existing technical annexes covering the following:

- (i) monitoring of dioxins in biota in sediments, taking into account advice from SIME 2007 that monitoring of dioxins in sediments should only be carried out in specific areas (such as sedimentation areas or estuaries) because of time lag (10 – 12 years) in deposition of quantities required for sampling;*
- (ii) monitoring of [perfluorooctane sulfonate, ed.] PFOS in sediments, biota and water;*

b. to review the existing technical annexes on monitoring of chlorinated biphenyls [(CBs) ed.] in biota and sediment and propose revisions so that they are adequate for monitoring of planar CBs in these compartments, taking into account advice from SIME that monitoring in sediments should be undertaken only if levels of marker [polychlorinated biphenyls, ed.] PCBs are e.g. 100 times higher than the [Background Assessment Concentrations, ed.] BACs and that for biota monitoring of concentrations in seabird eggs could provide an alternative matrix;

c. to develop background concentrations for dioxins.

Source of information

ICES. 2009a. Report of the Marine Chemistry Working Group (MCWG 2009)

ICES. 2009b. Report of the Working Group on Marine Sediments in Relation to Pollution (WGMS 2009)

Summary

Technical annexes are provided for the analysis of perfluorinated compounds, including PFOS, (PFCs) in sediments and seawater, completing the suite of matrices to be covered as a technical annex for the analysis of PFCs. The guidelines for PFCs in biota was prepared in 2008. A technical annex is provided for the analysis of dioxins and furans in marine biota. A further technical annex for the analysis of dioxins in sediments will be completed in 2010. Technical annexes are also provided for the analysis of planar CBs in biota and sediments.

Information on background concentrations of dioxins was reviewed and recommendations are made based upon a very limited dataset.

ICES Response

Background information

a. technical annexes to the JAMP Guidelines for monitoring Contaminants in Sediments

A technical annex for the analysis of dioxins and furans in biota is attached (Annex 1). A draft technical annex for the analysis of dioxins and furans in sediments has been prepared and will be finalised in 2010.

In 2008 ICES provided advice and a technical annex on the monitoring of PFCs, including PFOS, in biota (item a(ii) above) and on background concentrations for dioxins (item c above). This present document includes technical annexes for monitoring of PFCs, including PFOS, in sediments (Annex 2) and water (Annex 3). It intended to publish the three sets of PFC technical guidelines in the ICES TIMES series, so as to make them more widely available.

b Monitoring of planar CBs in biota and sediments

A revised technical annex for the determination of chlorobiphenyls, including planar CBs in biota is attached as Annexes 4 and 5. The first section (Annex 4) outlining processes for species selection, sampling of biota, and sample transportation and storage is applicable to the analysis of organic contaminants more generally, i.e., all those determined within the OSPAR JAMP. It is proposed that this forms a supplement to the general OSPAR JAMP guidelines, to

which detailed technical sub-annexes for each of the organic contaminant groups determined would be attached at a later date. If OSPAR accepts that proposal the annex will require further expansion in order to fully describe sampling protocols. A revised technical annex for the determination of chlorobiphenyls, including planar CBs in sediments is attached as Annex 6.

A summary table of OSPAR technical annexes for contaminants prepared or revised over the period 2006-2009 is provided in Table 1.

Table 1 OSPAR technical annexes for contaminants prepared or revised and updated by ICES 2006-2009.

Technical Annex	Year
Polybrominated diphenyl ethers (PBDEs) in biota	2007
Hexabromocyclododecane (HBCD) in biota	2007
Polycyclic aromatic hydrocarbons, including alkylated PAH, in biota	2008
Polycyclic aromatic hydrocarbons, including alkylated PAH, in sediment	2008
Organotins in biota	2008
Perfluorinated compounds (PFCs), including PFOS, in biota	2008
Perfluorinated compounds (PFCs), including PFOS, in sediment	2009
Perfluorinated compounds (PFCs), including PFOS, in seawater	2009
Dioxins, furans and planar CBs in biota	2009
CBs, including planar CBs, in biota	2009
CBs, including planar CBs, in sediment	2009

c Background concentrations for dioxins in biota

At present, the primary source of dioxins in the environment is human activity. However, in the past, as today, natural events such as forest fires have produced some of these compounds. The background concentration for dioxins in environmental samples is therefore low, but not zero. In 2008 ICES conducted a preliminary assessment of the data available for dioxins in marine sediments and biota and noted that it would be a considerable challenge to define these background concentrations with any degree of rigor.

Minimum levels of dioxin contamination in fish, bivalves and crustacea were compiled from data available currently (Annex 6). It should be noted that these data do not necessarily represent “remote” or “pristine” areas and, that remote areas may not, in any case, be pristine due to long range atmospheric transport of PCDD/Fs. Furthermore, it was not practical to collate data for the preferred fresh or lipid weight concentrations of individual congeners, as opposed to toxic equivalents¹ summed from these, due to the manner in which the original data were reported. There was little information available on the concentrations of PCDD/Fs in fish liver, which is the recommended matrix for analysis of dioxins in most finfish species included in the OSPAR guidelines. OSPAR could consider the medians of minimum values as indicative low concentrations; 0.15 pg WHO-TEQ PCDD/F g⁻¹ wet weight for marine fish muscle, and 0.06 pg WHO-TEQ PCDD/F g⁻¹ wet weight for bivalve molluscs. Alternatively, a pragmatic limit of quantification, e.g. estimated from data within the ICES database, could be considered as a starting point for calculating Background Assessment Concentrations (BACs). Such approaches are hampered by the paucity of data available, a situation not likely to be rectified in the near future.

¹ Concentrations of dioxins, furans and planar CBs are often expressed as toxic equivalents of the most toxic congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The concentrations of individual congeners are multiplied by factors which relate their toxicity to that of the reference congener according to established factor schemes. The most commonly used is that established by the World Health Organisation, WHO-TEQ, as used here.

Annex 1: Technical Annex on the analysis of dioxins/furans and planar CBs in biota

1. Introduction

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) are ubiquitous in the environment, primarily as unintentional by-products of combustion and industrial processes. They enter the aquatic environment via several routes, including atmospheric deposition. Being strongly hydrophobic compounds, sediments are the eventual sink in the aquatic environment, providing a source of potential exposure to aquatic organisms (Hurst *et al.*, 2004). Generally highly resistant to metabolism, PCDD/Fs bioaccumulate and biomagnify and have reached high concentrations in e.g. fish from the Baltic Sea, which resulted in recommendations to restrict the use of those fish for human consumption (Verta *et al.*, 2007). This guideline only addresses the 17 tetra- through octa-chlorinated 2,3,7,8-substituted dioxin and furan congeners, and the non- and mono- *ortho* substituted polychlorinated biphenyl congeners (CBs), which are able to exhibit similar effects to the 2,3,7,8-substituted dioxins and furans. The general chemical structures of PCDD/Fs and planar CB congeners are given in Figure 1. When reviewing the literature, it should be noted that planar, coplanar and dioxin-like CBs / PCBs are all equivalent terms.

In this guideline, the term planar CBs is used for the non-*ortho* and mono-*ortho* CB congeners listed in Table 1. The coplanar structure of non-*ortho* substituted CB congeners allows a configuration similar to that of PCDD/Fs. Mono-*ortho* substituted CBs may take up a steric position close to coplanarity and are consequently less toxic (in a dioxin-like manner) than non-*ortho* CBs. Nevertheless, they have been considered due to their relatively high concentrations compared to those of non-*ortho* CBs or dioxins/furans (Daelemans *et al.*, 1992). The exposure to planar CBs is mainly via the food-chain, as the compounds are highly lipophilic and bioaccumulate and biomagnify in lipid-rich tissue (e.g. Dyke *et al.*, 2003).

Chlorinated dioxins/furans and planar CBs have been shown to produce various toxic responses, including immunotoxicity, developmental and reproductive effects, neurotoxicity and carcinogenesis (OSPAR, 2005). The initial mechanism of toxicity is via the aryl hydrocarbon receptor (AhR), leading to changes in gene expression, cell growth and cell differentiation (Nebert *et al.*, 1993; Hurst *et al.*, 2004). Due to their persistence, high toxicity, bioaccumulation potential and ability for long-range transport, they are controlled under the Stockholm Convention for Persistent Organic Pollutants (POPs). Their spatial and temporal monitoring in the aquatic environment is important to evaluate the risk to wildlife and human health (Hurst *et al.*, 2004). Due to the low concentrations at which adverse effects can be observed, the analytical methodology for the analysis of PCDD/Fs and planar CBs differs from those for other organochlorine compounds, as described in this guideline.

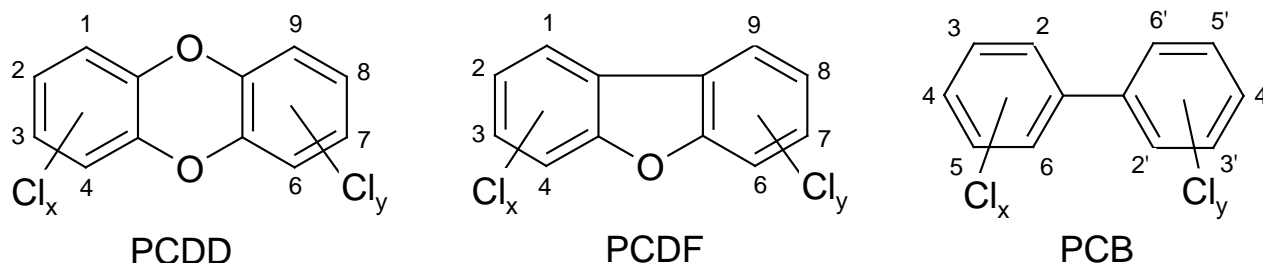


Figure 1 General formula of PCDDs, PCDFs and PCBs. The possible number of chlorine atoms results in 75 PCDD congeners, 135 PCDF congeners ($x=1-4$, $y=0-4$), and 209 PCB congeners ($x=1-5$, $y=0-5$).

2 Analytes and Toxicity Equivalent Factors (TEFs)

Environmental monitoring should include the 17 tetra- through octa-chlorinated 2,3,7,8-dibenzo-*p*-dioxins (CDDs) and dibenzofurans (CDFs) and the planar CBs listed in Table 1.

In the context of food and feed analysis and compliance checks with maximum residue limits, the concept of 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) Toxicity Equivalency Factors (TEFs) is commonly used, to account for mixtures of several PCDD/Fs and other compounds with dioxin-like activity usually present in these samples. Each congener has been assigned a TEF relative to that of the most toxic dioxin congener, 2,3,7,8-TCDD that was given a TEF of 1.0. The concentrations of the individual congeners are multiplied with their respective TEFs, and the sum of this gives the total concentration of dioxin-like compounds, expressed in TCDD Equivalents (TEQs). Thus, concentrations of mixtures can be expressed in terms of their dioxin-like activity in TEQs, relative to the most potent 2,3,7,8-TCDD. Two parallel TEF systems are currently in use: TEFs established by the World Health Organization (WHO-TEF/TEQ) and TEFs developed by NATO/CCMS (International TEFs or I-TEFs/TEQ). The use of I-TEFs,

however, is decreasing. The WHO-TEF-system is reviewed every five years, and Table 1 presents the most recent values, as of 2006 (Van den Berg *et al.* 2006).

According to OSPAR (2005), the scientific relevance of using TEQs to express results is greater for human exposure than for evaluation of pollution sources and emissions, for which information on congener patterns can be of more importance. Furthermore, the system assumes additive effects of the individual congeners, while both synergistic and antagonistic effects have also been reported (OSPAR, 2005). It is therefore recommended for environmental monitoring to report concentrations of individual PCDD/Fs and planar CB congeners in biota as absolute concentrations, i.e. pg/g wet weight (ww), with additional information on dry matter and lipid content (see chapter "Data reporting"). The TEF concept can be applied in a subsequent risk assessment, if appropriate.

As part of the TEQ approach, there are different ways of handling results below limits of quantitation:

- The concept of *upper bound* requires using the limit of quantification for the contribution of each non-quantified congener to the TEQ.
- The concept of *lower bound* requires using zero for the contribution of each non-quantified congener to the TEQ.
- The concept of *medium bound* requires using half of the limit of quantification calculating the contribution of each non-quantified congener to the TEQ.

As mentioned above, results of environmental monitoring should preferably be reported for individual congeners, in absolute concentrations. However, OSPAR (2005) mentioned the ongoing food analysis programmes which might be complementary to environmental monitoring. In this context, information on the handling of concentrations below quantitation limits will be important. Thus, results expressed as TEQ values should be reported as both upper bound and lower bound values (at least, indication of which calculation mode was used should be given). EU directive 2002/69/EC (2002) specifies that, for samples containing 1 pg WHO TEQ/g fat, the difference between upper bound and lower bound level should not exceed 20%. For lower contamination levels, this difference may be in the range of 25 to 40%.

Table 1

Chlorinated dibenzo-*p*-dioxins (CDDs), chlorinated dibenzofurans (CDFs) and planar CBs with their Toxicity Equivalent Factors (TEFs) according to the systems developed by the World Health Organization (WHO₂₀₀₅-TEF, Van den Berg *et al.* 2006) and NATO/CCMS (I-TEF) for human risk assessment. EC Regulation 1881/2006 setting maximum levels for certain contaminants in foodstuffs calculates WHO-TEQs using 1998 WHO TEFs. Maximum limits are set for PCDD/PCDFs and PCDD/PCDFs/Dioxin-like PCBs in various foodstuffs including fish and fishery products

Homologous group	Congener	I-TEF	WHO ₂₀₀₅ -TEF	IUPAC no.
PCDDs				
TCDD	2,3,7,8	1	1	
PeCDD	1,2,3,7,8	0.5	1	
HxCDD	1,2,3,4,7,8	0.1	0.1	
	1,2,3,6,7,8	0.1	0.1	
	1,2,3,7,8,9	0.1	0.1	
HpCDD	1,2,3,4,6,7,8	0.01	0.01	
OCDD	1,2,3,4,6,7,8,9	0.001	0.0003	
PCDFs				
TCDF	2,3,7,8	0.1	0.1	
PeCDF	1,2,3,7,8	0.05	0.03	
HxCDF	2,3,4,7,8	0.5	0.3	
	1,2,3,4,7,8	0.1	0.1	
	1,2,3,6,7,8	0.1	0.1	
	1,2,3,7,8,9	0.1	0.1	
HpCDF	2,3,4,6,7,8	0.1	0.1	
	1,2,3,4,6,7,8	0.01	0.01	
	1,2,3,4,7,8,9	0.01	0.01	
OCDF	1,2,3,4,6,7,8,9	0.001	0.0003	
Non-ortho PCBs				
TeCB	3,3',4,4'		0.0001	77
TeCB	3,4,4',5		0.0003	81
PeCB	3,3',4,4',5		0.1	126
HxCB	3,3',4,4',5,5'		0.03	169
Mono-ortho PCBs				
PeCB	2,3,3',4,4'		0.00003	105
	2,3,4,4',5		0.00003	114
	2,3',4,4',5		0.00003	118
	2',3,4,4',5		0.00003	123
HxCB	2,3,3',4,4',5		0.00003	156
	2,3,3',4,4',5		0.00003	157
	2,3',4,4',5,5'		0.00003	167
HpCB	2,3,3',4,4',5,5'		0.00003	189

3. Biota samples

OSPAR (2005) presented a monitoring strategy for PCDD/Fs, which identified biota as one of the important matrices for environmental monitoring (the other one being marine sediments). Aquatic organisms can accumulate hydrophobic compounds like dioxins/furans and reach concentrations considerably above those of the surrounding waters. The ratio between the concentration in biota and in the water is the bioconcentration factor (BCF), which is between 2000 and 9000 for PCDD/Fs (OSPAR, 2005). As the BCF varies with species and compound, it is important to design a sampling programme which minimises confounding factors, i.e. to choose the same species, sampling area and sampling period. The species selected for monitoring should fulfil certain requirements:

- 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.
- Representative of and abundant in the area (to ensure sufficient sample material for analysis).

- Relatively easy to handle.

Analogous to the monitoring of other organohalogen compounds, mussels and fish are suitable and commonly used for monitoring of PCDD/Fs and planar CBs (OSPAR, 2005). Highest dioxin concentrations are found in fish liver and muscle tissue of fatty fish such as herring and salmon. National food agencies often analyse PCDD/Fs and planar CBs in commercial fish and fish products, in order to monitor compliance with EU limit values. While different approaches will be necessary in environmental analyses, OSPAR (2005) recommends the monitoring of fish and shellfish as part of the monitoring strategy for dioxins.

In general, the same recommendations are valid as described for other organochlorine compounds, i.e. in the OSPAR JAMP guideline on organic compounds (OCs) in biota, which also contains details on sample dissection and homogenization. It should be pointed out, however, that the risk of sample contamination is considerably higher, given the extremely low concentrations of PCDD/Fs and planar CBs in most biota samples. The staff collecting and handling the samples should be well-trained and properly instructed in how to avoid any contamination.

For mussel samples, it is important to remove any sediment particles from their intestinal system, by depuration in a glass aquarium with filtered water from the sampling location for approximately 24 hours. Mussel samples must not be frozen prior to dissection, but should be transported at temperatures between 5 and 15°C, suitable for 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). More details on the practical aspects of sample handling and preparation are given in the OSPAR JAMP guideline on OCs in biota.

4. Analytical methods

According to COMMISSION DIRECTIVE 2002/69/EC laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of planar CBs in foodstuffs, a two-step approach can be chosen: This approach includes an initial screening aiming to detect the presence of PCDD/Fs and planar CBs in the sample (EU, 2002). Positively tested samples will then be subjected to verification methods (e.g. HRGC-HRMS), in order to identify and quantify individual congeners and compounds. This approach has not been applied in the monitoring of dioxins/furans under the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP), but will be briefly described in this guideline (see “Screening methods based on bioassays”). Primarily, it is applied for the screening of a high number of samples, as the method, based on cell lines, is relatively costly to maintain or to use for lower sample numbers. In environmental monitoring, it might for instance be useful for the identification of suitable sampling locations.

An example of a suitable method for the analysis of biota samples is summarised in Figure 2.

4.1 Preparatory steps

It is essential to avoid contamination during all analytical steps. Where possible, reagents should be of high purity or cleaned by extraction or solvent rinse. All solvents used must be checked for presence of residues of target or interfering compounds (e.g. polychlorinated diphenyl ethers). The purity of standards should be checked. Reusable glassware should be rinsed with solvent, disassembled, washed with a detergent solution and further rinsed with Milli Q grade water and solvent. Baking glassware is common practice as part of the cleaning process, but the formation of active sites on the glass surface that may adsorb the target compounds has been reported (USEPA, 1994).

The preparation of stock solutions and standards can follow the guidelines developed for OCs in biota. However, care has to be taken to monitor and to avoid contamination. Furthermore, the high toxicity of the compounds might require a particularly careful handling; see comments under “Safety”. Commercially available diluted stock solutions can be used to reduce safety issues. As valid for the entire analytical method, only trained personnel should perform these steps.

PCDD/Fs and planar CBs are normally determined by isotope dilution, using high resolution gas chromatography and high resolution mass spectrometry (HRGC-HRMS). ¹³C-labelled standards of all the congeners to be analysed are added prior to extraction of the samples. These internal standards are used to quantify the native PCDD/Fs and PCB congeners and to check the method performance in each sample (recovery surrogates). Table 2 provides a list of all ¹³C₁₂ labelled congeners available for use as internal standards while Table 3 provides the minimum number of internal standards to be used for the quantification of PCDD/Fs congeners.

Table 2¹³C₁₂ labelled congeners that can be used as the internal standards

PCDD/F congeners	PCB congeners
2,3,7,8- ¹³ C ₁₂ -TCDD	¹³ C ₁₂ -CB77
1,2,3,7,8- ¹³ C ₁₂ -PeCDD	¹³ C ₁₂ -CB81
1,2,3,4,7,8- ¹³ C ₁₂ -HxCDD	¹³ C ₁₂ -CB126
1,2,3,6,7,8- ¹³ C ₁₂ -HxCDD	¹³ C ₁₂ -CB169
1,2,3,7,8,9- ¹³ C ₁₂ -HxCDD	
1,2,3,4,6,7,8- ¹³ C ₁₂ -HpCDD	¹³ C ₁₂ -CB105
¹³ C ₁₂ -OCDD	¹³ C ₁₂ -CB114
	¹³ C ₁₂ -CB118
2,3,7,8- ¹³ C ₁₂ -TCDF	¹³ C ₁₂ -CB123
1,2,3,7,8- ¹³ C ₁₂ -PeCDF	¹³ C ₁₂ -CB156
2,3,4,7,8- ¹³ C ₁₂ -PeCDF	¹³ C ₁₂ -CB157
1,2,3,4,7,8- ¹³ C ₁₂ -HxCDF	¹³ C ₁₂ -CB167
1,2,3,6,7,8- ¹³ C ₁₂ -HxCDF	¹³ C ₁₂ -CB189
2,3,4,6,7,8- ¹³ C ₁₂ -HxCDF	
1,2,3,7,8,9- ¹³ C ₁₂ -HxCDF	
1,2,3,4,6,7,8- ¹³ C ₁₂ -HpCDF	
1,2,3,4,7,8,9- ¹³ C ₁₂ -HpCDF	
¹³ C ₁₂ -OCDF	

Table 3

Minimum number of internal standards to be used for calibration of PCDD and PCDF homologue groups

Substance	PCDD-Homologues		PCDF-Homologues	
	Native	¹³ C ₁₂ -labelled	Native	¹³ C ₁₂ -labelled
Tetrachloro homologues	2,3,7,8	2,3,7,8	2,3,7,8	2,3,7,8
Pentachloro homologues	1,2,3,7,8	1,2,3,7,8	1,2,3,7,8	2,3,4,7,8 1,2,3,7,8
Hexachloro homologues	1,2,3,4,7,8 1,2,3,6,7,8 1,2,3,7,8,9	1,2,3,7,8,9	1,2,3,4,7,8 1,2,3,6,7,8 1,2,3,7,8,9 2,3,4,6,7,8	2,3,4,6,7,8
Heptachloro homologues	1,2,3,4,6,7,8	1,2,3,4,6,7,8	1,2,3,4,6,7,8 1,2,3,4,7,8,9	1,2,3,4,6,7,8

4.2 Lipid determination

The total lipid content should be determined in all biota samples, using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963) or an equivalent method such as Smedes (1999). For normalisation purposes, the total lipid content is preferred to the extractable lipid content (De Boer, 1988).

4.3 Extraction

Soxhlet extraction is commonly used for biota samples. Immediately prior to use, the Soxhlet apparatus should be pre-extracted with e.g. dichloromethane:hexane (1:1) for approximately 3 hours (USEPA, 1994). An adequate amount of tissue (e.g. 10 g of wet tissue) is spiked with the labelled compounds and mixed with sodium sulphate. The sample is allowed to dry for 12-24 hours and should be remixed prior to transfer to a glass Soxhlet thimble. Soxhlet extraction proceeds for 18-24 hours using e.g. fresh dichloromethane:hexane (1:1) (USEPA, 1994).

More recently, pressurised liquid extraction (PLE) has become a common and faster alternative to Soxhlet extraction (Focant *et al.* 2004). PLE uses organic solvents at temperatures above their boiling point maintained in the liquid phase under high pressure. The extraction cell which contains the sample is heated (ex: 100°C) and filled up with an appropriate solvent (example: toluene, DCM) up to a pressure of 140 bars. The minimum extraction time should be 10 minutes in static mode, and several extraction cycles are recommended (n = 2-3). To further reduce analysis time, PLE can be combined with in-line clean-up procedures using preferably sulphuric acid impregnated silica as fat retainer (Björklund *et al.* 2006). Proper fat-fat retainer ratios are important to avoid fat remaining in the sample after extraction.

Mixed (polar/non-polar) solvent combinations cannot be used with this technique. It is understood that the combination of pressure and temperature is sufficient to remove all dioxins, furans and dl-PCBs from the matrix.

4.4 Clean-up

The extracts are concentrated using suitable evaporation devices, e.g. rotary evaporation, Turbovap, Syncore, Kuderna-Danish. The risk of cross-contamination is fairly high for rotary evaporation, so the evaporator should be pre-cleaned, e.g. by 100 ml of clean solvent. If the extracts are to be cleaned up by adsorption chromatography on e.g. silica gel, a solvent change to hexane is recommended. The purification procedures have two objectives: i) removal or destruction of lipids and ii) removal of interfering compounds. Due to the very low levels of PCDD/Fs in biota samples, the elimination of interferences is essential. Prior to column chromatography clean-up, the precision and recovery of this step should be assessed.

For the first part, addition of concentrated sulphuric acid is commonly applied, either in terms of a column chromatography clean up or by direct addition of silica impregnated with sulphuric acid to the extracts. The column chromatography clean up suggested by USEPA (1994) for lipid removal in biota extracts includes 2 g of silica gel, 2 g of potassium silicate, 2 g of anhydrous Na₂SO₄, 10 g of silica gel (impregnated with sulphuric acid) and another 2 g of anhydrous sodium sulphate, to be packed bottom-to-top into a column of 25 mm ID. The column is pre-eluted with 100 ml of hexane and after loading of the sample, eluted with 200 ml of hexane. Ready to use, multilayer clean-up columns are also available commercially. Alternatively, approximately 30 -100g of sulphuric acid impregnated silica gel can be added to the extract (200 ml), while stirring for 2-3 hours. The treatment with sulphuric acid impregnated silica requires strict safety procedures as the small particles can cause serious health damage after inhalation. Gel permeation chromatography (GPC) has also been applied for lipid removal, but often a series of GPC columns is needed to ensure a 100% fat removal. Alternatively, an additional cleanup step using concentrated sulphuric acid might be applied after GPC to remove residual lipids from samples having a higher lipid content.

For removal of interferences, HPLC, GPC, and column chromatography using alumina, silica gel, Florisil and activated carbon are possible alternatives. USEPA (1994) suggests adsorption chromatography on alumina or Florisil and carbon as minimum additional clean-up steps after lipid removal. Depending on whether acid or basic alumina is chosen, the eluents should be dichloromethane:hexane (1:4) or (1:1), respectively. The material for the carbon column can be e.g. Carbo-pack™-C. Interferences are removed in a washing step with e.g. hexane, dichloromethane:cyclohexane and dichloromethane:toluene. Then, the column is inverted and the analytes are eluted with toluene. HPLC can also be used for purification and fractionation of the extracts. 2-(1-pyrenyl)ethyl dimethylsilylated (PYE) silica columns and porous graphitised carbon are suitable columns for this purpose (Echols *et al.*, 1998). When coupled in series, nitrophenylpropylsilica column (Nucleosil, 5 µm particles, 250 x 4.6 mm) and PYE (Cosmosil, 5 µm particles, 150 x 4.6 mm) enables the separation of PCDD/Fs from planar CBs (Bandh *et al.* 1996). Fully automated clean-up systems are also available commercially (e.g. PowerPrep™ system). The European research project DIFFERENCE recommended at least three clean up or fractionation steps to ensure sufficiently clean extracts (van Leeuwen *et al.*, 2007).

4.5 Concentration and syringe standards

After clean-up, a keeper is added (e.g. iso-octane or nonane) and the extracts are concentrated to near dryness, i.e. 10-20 µl. A syringe standard mix should also be added to evaluate the recovery of labelled internal standards. For example ¹³C₁₂-1,2,3,4-TCDD can be used for recovery determinations of TCDD/Fs and PeCDD/Fs internal standards while ¹³C₁₂-1,2,3,7,8,9-HxCDD can be used for recovery determinations of HxCDD/Fs, HpCDD/Fs and OCDD/F internal standards.

4.6 HRGC-HRMS

The dioxin/furan content in environmental samples is commonly monitored using high resolution gas chromatography (HRGC) and high resolution mass spectrometry (HRMS), but low resolution mass spectrometry (LRMS) may be a suitable and cost effective alternative if the required minimum performance criteria are met (see "HRGC-LRMS").

4.7 GC analysis

The GC analysis should be optimised with regard to separation and sensitivity. Fishman *et al.* (2007) provided a comprehensive review of GC columns available for dioxins analysis. Generally 50-60 m, 5% diphenyl 95% dimethylpolysiloxane columns are a common choice. However, these columns could exhibit multiple coelutions for both CBs and PCDD/Fs (Reiner *et al.*, 2006), depending on the matrix to be analyzed. The use of RTX-Dioxin 2 column has been reported in the literature as a suitable alternative to DB-5 columns. Combining this phase with reduced inner diameter and phase thickness (for example a 40m x 0.18mm x 0.18µm) enables the analysis of the 17 PCDD/F congeners in 40 minutes, with data fulfilling QA/QC requirements and providing better selectivity, especially for 2,3,7,8-TCDD and 2,3,7,8-TCDF (Reiner *et al.*, 2006; Cochran *et al.* 2007).

Potential interferences for planar CBs on common GC columns are summarized in Table 4 (Reiner *et al.*, 2006). Complete separation can be achieved by multi-analysis on columns of different polarity. The GC separation of congener CB123 from interferences is critical. Due to the minor contribution of this congener to the overall TEQ, possible interferences only lead to a marginal increase in the uncertainty of the total TEQ. Recent developments indicate possibilities of full separation of relevant CB congeners on one column, e.g. on an SGE HT8-PCB capillary column. A full separation of all CB congeners is also possible by using comprehensive multi-dimensional GC (GC x GC).

Table 4 Possible interferences for selected planar CBs using a 5% phenyl column (Reiner *et al.* 2006)

PCB congener	Potential interference
CB-81	CB-87
CB-77	CB-110
CB-123	CB-149
CB-126	CB-178 and CB-129
CB-156	CB-171
CB-157	CB-201

Various injection techniques are possible, e.g. on-column injection, splitless injection, pressure-pulsed splitless injection and programmed temperature vaporizing (PTV) injection. The most suitable injection volume depends on the dioxin concentrations in the sample and the sensitivity of the instrumental analysis. In HRGC-HRMS analysis, 1 - 2 μ l is a common injection volume.

4.8 Compound identification

The HRMS system should be operated at a minimum of 10,000 resolving power throughout all the runs, and resolution should be checked regularly during the sequence of runs. The individual planar CBs, PCDD/Fs or labelled compounds are identified by comparing the GC retention time and ion abundance ratio of two exact masses monitored (Tables 5 and 6) with the corresponding retention time of an authentic labelled internal standard and the theoretical or acquired ion abundance ratio of the two exact masses. The congeners for which there are no labelled analogues are identified when relative retention time and ion abundance ratios agree within predefined limits. The following criteria should be met for identification of an individual planar CBs, PCDD/Fs or labelled compounds in a standard, blank or sample:

- The signal for the two exact masses specified in Tables 5 and 6 should be present and within ± 2 s.
- The signal-to-noise ratio (S/N) for the GC peak at each exact mass has to be at least 3 for each congener detected in a sample extract, and at least 10 for all congeners in the calibration standard.
- The ratio of the integrated areas of the two exact masses specified in Tables 5 and 6 has to be within 15% of the theoretical shown in Table 7.
- The relative retention time of a native planar CB or PCDD/F congener has to be within a time window of ± 3 s based on the retention time of the corresponding $^{13}\text{C}_{12}$ -labelled standard. The relative retention time of congeners for which there are no labelled analogues has to be within ± 0.002 s.

If interferences preclude identification, extract a new, further cleaned up aliquot and analyse again. If interferences cannot be removed flag the data to indicate results are maximum concentrations.

4.9 Compound quantification

Quantitative analysis is performed using selected ion monitoring (SIM) area, in one of the two following ways:

- For the planar CBs and PCDD/Fs for which labelled analogues have been added to the sample (Table 2), the GC-MS system is calibrated, and the concentration of each compound is determined using the isotope dilution technique.
- For the planar CBs and PCDD/Fs for which labelled analogues are not added to the sample (see Table 3 for PCDD/Fs), the GC-MS system is calibrated for each compound using a labelled isomer with the most similar structure and the concentration of each compound is determined using the internal standard technique.

Calibration curves should be based on a minimum of 5 calibration points. Mass drift correction is mandatory, usually based on a lock-mass m/z of perfluorokerosene (PFK) or perfluorotributylamine (PFTBA, FC43).

Table 5 Masses for the detection and quantification of PCDD/Fs

Substance	Dibenzofurans		Dibenzo-p-dioxins	
	Native	¹³ C ₁₂ -labelled	Native	¹³ C ₁₂ -labelled
Tetra-CDD/F	303.9016	315.9419	319.8965	331.9368
	305.8987	317.9389	321.8937	333.9339
Penta-CDD/F	339.8598	351.9000	355.8547	367.8949
	341.8569	353.8970	357.8518	369.8919
Hexa-CDD/F	373.8208	385.8610	389.8157	401.8559
	375.8179	387.8580	391.8128	403.8529
Hepta-CDD/F	407.7818	419.8220	423.7767	435.8169
	409.7789	421.8190	425.7738	437.8140
Octa-CDD/F	441.7428	453.7830	457.7377	469.7779
	443.7399	455.7801	459.7348	471.7750

Table 6 Masses for the detection and quantification of CB congeners

Homologue groups	Native CBs	¹³ C ₁₂ -labeled CBs
Tetrachlorobiphenyls	289.9223	301.9626
	291.9194	303.9597
Pentachlorobiphenyls	325.8804	337.9207
	327.8775	339.9177
Hexachlorobiphenyls	359.8415	371.8817
	361.8385	373.8788
Heptachlorobiphenyls	393.8025	405.8427
	395.7995	407.8398

The isotope ratio between the two ions of the molecular isotope cluster, which are recorded has to match the theoretical value within $\pm 15\%$ (see Table 7).

Table 7 Tolerance limits of isotope ratios for PCDD/Fs and planar CBs

Chlorine atoms	Isotope Ratio Lower Limit	Isotope Ratio Theoretical Value	Isotope Ratio Upper Limit
4	0.65	0.77 (M/M+2)	0.89
5	0.55	0.64 (M+4/M+2)	0.75
6	0.69	0.81 (M+4/M+2)	0.94
7	0.83	0.96 (M+4/M+2)	1.10
8	0.76	0.89 (M+2/M+4)	1.02

4.10 HRGC-LRMS

Low resolution mass spectrometry (LRMS) has also been applied to the analysis of PCDD/Fs and/or planar CBs. Limits of detections are higher than those obtained with HRMS detectors, but this can be compensated for by e.g. larger injection volumes. A very efficient extract clean up is of utmost importance so as to exclude any interferences. A technique commonly applied is GC-LRMS using ion trap mass analysers working in tandem mode (Focant *et al.*, 2005; Malavia *et al.*, 2008, Eppe *et al.*, 2004). Table 8 provides information on precursor and product ions obtained by GC-ion trap MS. GC-LRMS (quadrupole) can be an option for planar CBs in particular.

Both HRMS and LRMS techniques have to demonstrate that they meet the requirements regarding separation and sensitivity described in the monitoring programme, see also comments under "Quality Assurance and Quality Control". When using LRMS the maintenance of the instrument is crucial and could be time consuming (e.g. frequent cleaning of the ion source). The sensitivity for 2,3,7,8-TCDD may be critical. For matrices with relatively high dioxin and planar CB levels LRMS may be very useful, and is much cheaper to purchase and operate than HRMS.

Table 8

Precursor ions and product ions for the determination of PCDD/Fs and planar CBs using HRGC-ion trap tandem MS

Target Compounds	Native			¹³ C ₁₂ -labelled		
	Precursor (m/z)	Ion	Product Ions (m/z)	Precursor (m/z)	Ion	Product Ions (m/z)
TCDD	322 (M+2)		257 + 259	334 (M+2)		268 + 270
PeCDD	356 (M+2)		291 + 293	368 (M+2)		302 + 304
HxCDD	390 (M+2)		325 + 327	402 (M+2)		336 + 338
HpCDD	424 (M+2)		359 + 361	436 (M+2)		370 + 372
OCDD	460 (M+4)		395 + 397	472 (M+4)		406 + 408
TCDF	306 (M+2)		241 + 243	318 (M+2)		252 + 254
PeCDF	340 (M+2)		275 + 277	352 (M+2)		286 + 288
HxCDF	374 (M+2)		309 + 311	386 (M+2)		320 + 322
HpCDF	408 (M+2)		343 + 345	420 (M+2)		354 + 356
OCDF	444 (M+4)		379 + 381			
CB81, CB77	292 (M+2)		220 + 222	304 (M+2)		232 + 234
CB123, CB118, CB114, CB105, CB126	326 (M+2)		254 + 256	338 (M+2)		266 + 268
CB167, CB156, CB157, CB169	360 (M+2)		288 + 290	372 (M+2)		300 + 302
CB189	394 (M+2)		322 + 324	406 (M+2)		334 + 336

5 Quality Assurance and Quality Control

The laboratory is required to operate a formal quality assurance programme. Indicative values for accuracy and precision are given under "Verification method" in Table 9. An example of a comprehensive QA/QC approach is described in method 1613 by USEPA (1994).

The analytical method requires high sensitivity and low detection limits, usually in the pg-TEQ-range, for both dioxins/furans and planar CB congeners (OSPAR, 2005), and should meet the requirements for limits of quantitation specified in the monitoring programme. For individual PCDD/Fs, limits of quantification (LoQ) of 0.3 pg/g wet weight should be achievable, with the exception of OCDD (1 pg/g wet weight). For non-*ortho* PCBs, LoQ should be as low as 5 pg/g wet weight, while for mono-*ortho* PCBs, requirements on LoQ are less strict as their concentrations in biota samples are usually higher, in particular concentrations of congeners CB105, CB118 and CB156. The selectivity of the method should be sufficient to avoid interfering compounds, i.e. the individual congeners should be separated from each other and any interferences present.

All sample series should include procedural blanks and measurements of certified/laboratory reference materials. Certified reference materials should be analysed regularly, although only few are available for the determination of PCDD/Fs and planar CBs in biota, for example from NIST (cod liver oil), Cambridge Isotope Laboratories (fish) and the National Research Council Canada (fish) (de Boer and McGovern, 2001). The laboratory should further prove its competence by regular participation in relevant laboratory proficiency tests. It is essential that the matrix and concentration range of the proficiency testing samples are comparable with the samples routinely analysed within the monitoring programme (de Boer, 2001, Wells and de Boer, 2006).

The recovery of the individual internal standards added prior to extraction should be between 60-120%. According to Commission Directive 2002/69/EC, lower or higher recovery rates can be accepted for the hepta- and octa-PCDD/Fs, as long as their TEQ contributions are below 10% of the total TEQ value (EU, 2002). Blanks should be as low as possible, at least below 20% of the lowest concentration of interest.

6 Screening methods based on bioassays

As mentioned above, bioassays are not currently applied in the monitoring under OSPAR CEMP, but have been suggested as screening tools for monitoring PCDD/F and planar CBs in foodstuffs (Commission Directive 2002/69/EC), with the requirement to meet the criteria given in Table 9. Screening tools might be useful in, for instance, choice of sampling sites, and will therefore be briefly discussed in this guideline. Hurst *et al.* (2004) also emphasised that monitoring programmes were moving towards effect-based monitoring, with biological relevance becoming more important. The authors list the following requirements for bioassays to be included in monitoring programmes: The tool must be capable of rapid, inexpensive and high-throughput screening producing interpretable and meaningful results (Hurst *et al.*, 2004).

Table 9 Quality criteria for screening and verification methods (EU, 2002)

	Screening method	Verification method
False negatives	< 1%	
Accuracy		± 20%
Precision (expressed as the coefficient of variation between repeated measurements)	< 30%	< 15%

The dioxin responsive chemically activated luciferase expression (DR-CALUX) assay is mechanism specific and utilises the interaction of compounds with the AhR. However, it is not compound specific and produces a response with all compounds capable of interactions with the AhR. While Commission Directive 2002/69/EC demands that the TEQ-values determined by bioassays should be the sum of PCDD/Fs and planar CBs in the sample, Hurst *et al.* (2004) and van Leeuwen *et al.* (2007) demonstrated some disagreement between the results of the bioassay and the conventional targeted HRGC-HRMS analysis. The differences may be caused by unknown compounds producing a dioxin-like response in the CALUX assay (e.g. brominated or mixed halogenated dioxin analogues, polychlorinated naphthalenes, PAHs etc.) – or compounds antagonising the AhR (e.g. di-*ortho*-substituted CB congeners). These deviations from results of chemical analysis were also considered as an advantage by Hurst *et al.* (2004), as the assay allows a more accurate assessment of the true potency of dioxin-like compounds present in the samples. In order to obtain specific responses to PCDD/Fs and planar CBs in the sample, the extracts require specific clean up methods to exclude interferences from other dioxin-like compounds. As mentioned above, environmental monitoring aims at presenting concentrations of individual compounds in the respective samples, rather than toxicity assessments.

7. Safety

The chemical compounds dealt with in this guideline are hazardous and must only be handled by trained personnel familiar with handling of PCDD/F and planar CBs, and associated risks as well as precautionary measures. USEPA (1994) recommends that laboratories purchase diluted standard solutions instead of preparing primary solutions.

8. Data reporting

Results are reported on a congener basis in pg g^{-1} ww. The lipid content and water content of the samples should be reported as well. For normalizing purposes the total lipid content should be determined, rather than the extractable lipid content (de Boer, 1988). 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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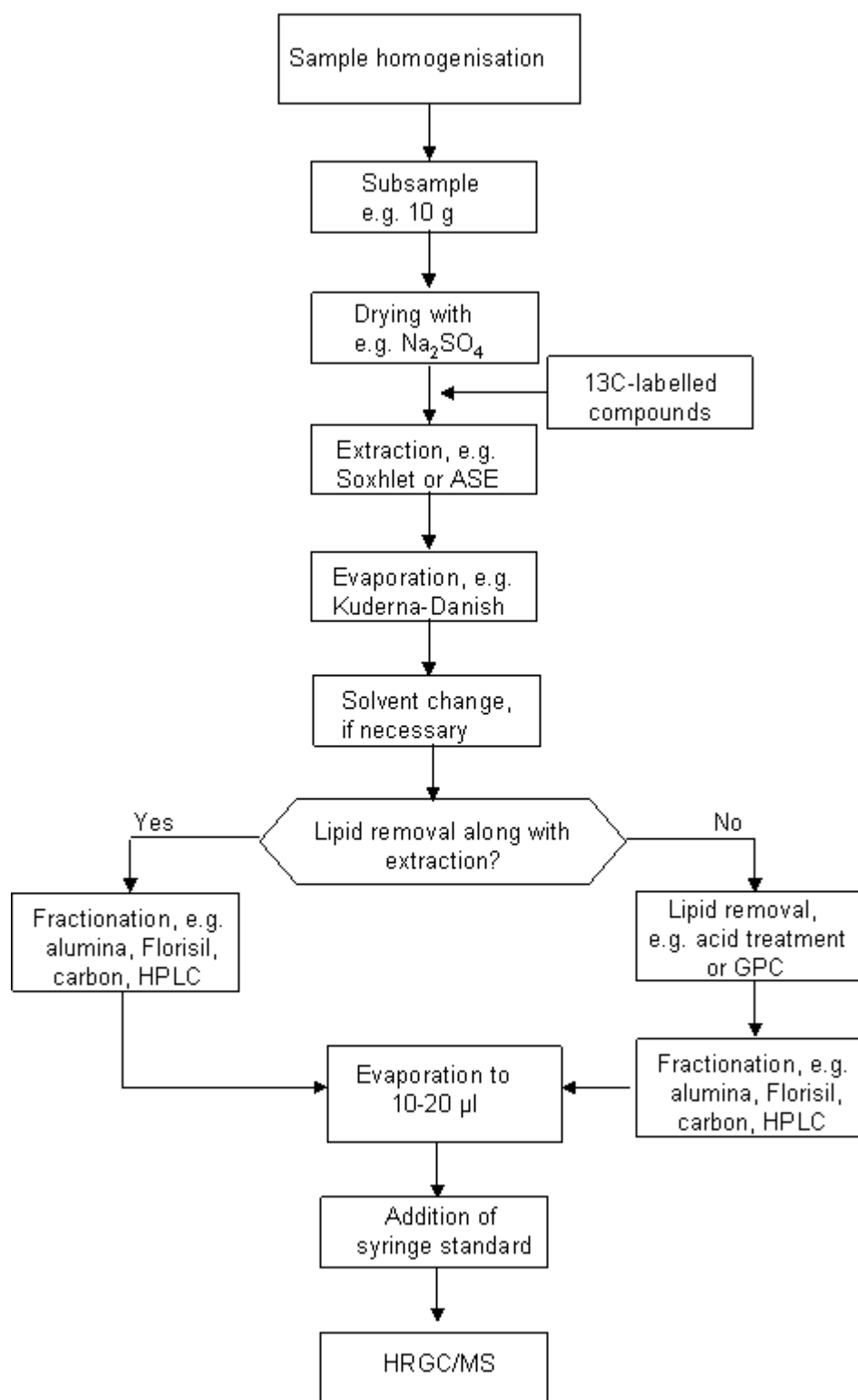


Figure 2 Analytical method recommended for analysis of biota samples within environmental monitoring.

Annex 2: Technical annex on the analysis of perfluorinated compounds (PFCs) in sediment

1. Introduction

Perfluorinated and polyfluorinated compounds (PFCs) are man-made chemicals and are ubiquitous in the environment (Giesy and Kannan, 2001). PFCs are widely used as processing additives during fluoropolymer production and as surfactants in consumer applications, including surface coatings for carpets, furniture and paper products. They are also components in breathable, waterproof fabrics, fire-fighting foams and insulators for electric wires (Kissa, 2001). From the production and use of these products, PFCs can be released into the environment.

In this document, the name PFCs refers to compounds with a hydrophilic functional group and a hydrophobic fully fluorinated chain which can vary in chain length. The polyfluorinated acids have moderate water solubilities, low pK_a values and are therefore dissociated at environmentally relevant pH values (Kissa, 2001). Perfluorinated sulfonamides and fluorotelomer alcohols (FTOHs) are neutral compounds with a moderate vapour pressure currently discussed as precursors to perfluorooctane sulfonate (PFOS) and perfluorinated carboxylic acids (PFCAs) (Ellis *et al.*, 2004).

The objective of this technical annex is to provide advice on the analysis of PFCs in sediment. The detection of PFCs at ppb to ppt levels is complex because of a risk of contamination during sample handling, storage, preparation and instrumental analysis. Only a few methods to determine PFCs in sediment are applied in various laboratories, but they generally apply extraction with polar solvents, clean-up steps and liquid-chromatography (LC) with mass spectrometric detection (MS). The International Standards Organisation (ISO) has already promoted a standard for the determination of PFOS and perfluorooctanoic acid (PFOA) in water (ISO 25101, 2009), but at present no standard is available for the analysis of these compounds in sediment samples.

2. Analytes

Table 1 gives an overview of relevant PFCs for analysis in sediments. They are chosen from the following groups: Perfluorinated sulfonates (PFSAs), perfluorinated sulfinates (PFSiAs), PFCAs and perfluorinated sulfonamides. For monitoring purposes, the high-volume chemicals PFOS and PFOA are considered the most important PFCs and are highly recommended to be included in sediment monitoring. Although most studies have focused mainly on PFOS, it is suggested that long-chained PFCs ($\geq C_8$) should be included in analysis due to their adsorption potential to sediment.

Table 1 Full names, acronyms, formulas and Chemical Abstract System (CAS) numbers of native and mass-labelled PFCs relevant for sediment analysis.

Analyte	Acronym	Formula	CAS ^a -Number
Perfluorobutanoic acid	<i>PFBA</i>	C ₃ F ₇ COOH	375-22-4
Perfluoropentanoic acid	<i>PFPA</i>	C ₄ F ₉ COOH	2706-90-3
Perfluorohexanoic acid	<i>PFHxA</i>	C ₅ F ₁₁ COOH	307-24-4
Perfluoroheptanoic acid	<i>PFHpA</i>	C ₆ F ₁₃ COOH	375-85-9
Perfluorooctanoic acid	<i>PFOA</i>	C ₇ F ₁₅ COOH	335-67-1
Perfluorononanoic acid	<i>PFNA</i>	C ₈ F ₁₇ COOH	375-95-1
Perfluorodecanoic acid	<i>PFDA</i>	C ₉ F ₁₉ COOH	335-76-2
Perfluoroundecanoic acid	<i>PFUnDA</i>	C ₁₀ F ₂₁ COOH	4234-23-5
Perfluorododecanoic acid	<i>PFDoDA</i>	C ₁₁ F ₂₃ COOH	307-55-1
Perfluorotridecanoic acid	<i>PFTriDA</i>	C ₁₅ F ₂₅ COOH	72629-94-8
Perfluorotetradecanoic acid	<i>PFTeDA</i>	C ₁₃ F ₂₇ COOH	376-06-7
Perfluorohexadecanoic acid	<i>PFHxDA</i>	C ₁₅ F ₃₁ COOH	67905-19-5
PERFLUOROBUTANE SULFONATE	<i>PFBS</i>	C ₄ F ₉ SO ₂ O ⁻	29420-49-3 (potassium salt)
Perfluorohexane sulfonate	<i>PFHxS</i>	C ₆ F ₁₃ SO ₂ O ⁻	3871-99-6 (potassium salt)
Perfluoroheptane sulfonate	<i>PFHpS</i>	C ₇ F ₁₅ SO ₂ O ⁻	n.a.
Perfluorooctane sulfonate	<i>PFOS</i>	C ₈ F ₁₇ SO ₂ O ⁻	1763-23-1 (sodium salt)
Perfluoro-1-decanesulfonate	PFDS	C ₁₀ F ₂₁ SO ₂ O ⁻	13419-61-9 (sodium salt)
1H,1H,2H,2H-perfluorooctane sulfonate	THPFOS (6:2 FTS)	C ₆ F ₁₃ C ₂ H ₄ SO ₃ ⁻	27619-97-2
Perfluorooctane sulfinic acid	<i>PFOSi</i>	C ₈ F ₁₇ SO ₂ ⁻	n.a.
Perfluorooctane sulfonamide	PFOSA	C ₈ F ₁₇ SO ₂ NH ₂	754-91-6
Internal Standards			
Perfluoro-n-(1,2,3,4- ¹³ C ₄)butanoic acid	[¹³ C ₄]-PFBA	[2,3,4- ¹³ C ₃]F ₇ ¹³ COOH	n.a.
Perfluoro-n-(1,2- ¹³ C ₂)hexanoic acid	[¹³ C ₂]-PFHxA	C ₄ F ₉ [2- ¹³ C]F ₂ ¹³ COOH	n.a.
Perfluoro-n-[1,2,3,4- ¹³ C ₄]octanoic acid	[¹³ C ₄]-PFOA	C ₄ F ₉ [2,3,4- ¹³ C ₃]F ₈ ¹³ COOH	n.a.
Perfluoro-n-[1,2,3,4,5- ¹³ C ₅]nonanoic acid	[¹³ C ₅]-PFNA	C ₄ F ₉ [2,3,4,5- ¹³ C ₄]F ₈ ¹³ COOH	n.a.
Perfluoro-n-[1,2- ¹³ C ₂]decanoic acid	[¹³ C ₂]-PFDA	C ₈ F ₁₇ ¹³ CF ₂ ¹³ COOH	n.a.
Perfluoro-n-[1,2- ¹³ C ₂]undecanoic acid	[¹³ C ₂]-PFUnDA	C ₉ F ₁₉ ¹³ CF ₂ ¹³ COOH	n.a.
Perfluoro-n-[1,2- ¹³ C ₂]dodecanoic acid	[¹³ C ₂]-PFDoDA	C ₁₀ F ₂₁ ¹³ CF ₂ ¹³ COOH	n.a.
Perfluoro-1-hexane[¹⁸ O ₂]sulfonate	[¹⁸ O ₂]-PFHxS	C ₆ F ₁₃ S[¹⁸ O ₂]O ⁻	n.a.
Perfluoro-1-[1,2,3,4- ¹³ C ₄]octanesulfonate	[¹³ C ₄]-PFOS	C ₄ F ₉ [1,2,3,4- ¹³ C ₄]F ₈ SO ₂ O ⁻	n.a.
Perfluoro-1-[1,2,3,4- ¹³ C ₄]octanesulfinic acid	[¹³ C ₄]-PFOSi	<i>C₄F₉[1,2,3,4-¹³C₄]F₈SO₂⁻</i>	n.a.

^a, Chemical Abstract System; n.a., not available

3 Sampling, transportation and storage

The sampling should be carried out by trained personnel being aware of the risk of contamination of samples if incorrectly handled. Materials and clothes that contain or can adsorb fluorinated compounds must be avoided. In particular the containers or bags that come in direct contact with the sample should not contain fluorinated polymers like Teflon™. Instead, containers and equipment made of polypropylene, glass or stainless steel should be used. After collection, samples should be stored in closed containers at a temperature lower than -20°C until sample preparation. The handling time at room temperature should be short to minimize possible degradation of precursors to PFCAs and PFSA.

4. Sample preparation

Sample preparation requires clean conditions on a clean bench. The laboratory should be free from any material that can contain fluorinated compounds (e.g. Teflon™). Every material that can come in contact with the sample must be free of fluorinated compounds. Materials used in the PFC analysis should be cleaned with solvents such as methanol and acetone and covered with solvent rinsed aluminium foil to keep out any dust. The septa of vials should be Teflon™-free such as Barrier™ septa made of silicone polymer and aluminium. Solvents including water should be of highest purity and must be tested for residues of PFCs prior to use.

Within each sample batch, a method blank should be analysed. If measurable blanks occur, the analytical instrumentation and every sample preparation step have to be checked for contamination and appropriate measures have to be taken before analysis continues.

4.1 Pre-treatment

It is advised to determine the water content or to dry samples before extraction. Freeze-drying is becoming available in an increasing number of laboratories. However, its application should be carefully considered, since losses of volatile PFCs or contamination may occur. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0°C. Alternatively, air-drying to constant weight at room temperature on a clean bench or drying in an oven may be considered. However, degradation of precursor compounds can change the composition profile of PFCs in the sediment sample. In addition, PFCs can be lost by volatilization depending on temperature and drying time.

Before taking a subsample for analysis, the samples should be sufficiently homogenised. Depending on matrix and expected concentrations, an appropriate sample amount is weighed in polypropylene tubes for extraction. Then, the extract is spiked with a mass-labelled internal standard (IS) mixture at concentrations close to the environmental level. Before extraction, the sample should be incubated with the IS for about 12 h at 4° C so that the IS can interact with the matrix.

4.2 Extraction

Three methods have been described in the scientific literature for the extraction of PFCs from sediments (Powley *et al.*, 2005, Higgins *et al.* 2005; Washington *et al.* 2008). Powley *et al.* (2005) used a wrist-action shaker operated at maximum deflection to extract PFCs from sediments with methanol, followed by a graphitized carbon adsorbent clean-up. Higgins *et al.* (2005) used an acetic acid wash, followed by repeated extraction with methanol/1% acetic acid in water (90:10, v/v) in a heated (60 °C) sonication bath and subsequent clean-up using C₁₈ cartridges. The method described by Washington *et al.* (2008) includes sonication with acetonitrile/water (60:40, v/v) and ion pairing clean-up. Alternatively, extraction can be performed by shaking placed on a wrist-action shaker set at maximum deflection. However, all extraction methods should include a minimum of three extractions, each with a solvent volume that corresponds to ten times the sample volume and 30 min extraction time. After the extraction, the three extracts are combined prior to clean-up.

4.3 Clean-up

Because of matrix effects on ionisation (enhancement/suppression of signal) in electrospray tandem mass spectrometry (ESI-MS-MS), a clean-up of the extracts is necessary. Different methods can be used, either separately or in combination, depending on extraction solvent and concentration level.

An appropriate clean-up method is described by Powley *et al.* (2005). Briefly, 25 mg of graphitized carbon adsorbent (e.g. ENVI-Carb™, 100 m² g⁻¹, 120/400 mesh) and 50 µL acetic acid are added into a small tube. The extract is concentrated to 1 mL and transferred into this tube. The extract is mixed, centrifuged and finally, 0.5 mL of the supernatant is transferred to another flask. Additional clean-up might be required, depending on sample type and concentration levels (Higgins *et al.* 2005; Washington *et al.* 2008).

Sample extracts should be concentrated according to the required sensitivity. Concentration techniques at low temperature (< 40°C) and controlled pressure conditions are preferred in order to avoid losses of the more volatile PFCs. Evaporation to dryness should be avoided in all cases.

An injection standard (InjS) can be added to the final extract for correction of the injection volumes and calculation of the recoveries of the mass-labelled IS. The InjS should not occur in environmental samples, hence, the use of a mass-labelled InjS is recommended.

The solvent composition of the final extract should correspond to the mobile phase of the LC method in order to obtain a satisfactory peak shape of the compounds, in particular of early eluting short-chain PFCs. Unless the samples are analysed immediately, the vials should be kept at < 4°C and analysed within one week.

5. Instrumental analysis

LC coupled with a tandem mass spectrometer and interfaced with an electrospray ionisation source in a negative-ion mode (LC(-)ESI-MS/MS) (Hansen *et al.*, 2001) or LC coupled with an (-)ESI time-of-flight mass spectrometer (LC-ESI-QTOF-MS) (Berger and Haukas, 2005) can be used for PFC analysis. Tandem MS and QTOF-MS have the advantage of low instrumental noise with a high selectivity.

5.1 Liquid chromatography

For the liquid-chromatography C₈ or C₁₈ reserved phase columns can be used. A guard column may improve the peak performance and extend the lifetime of the chromatographic column. Mixtures of water and methanol or acetonitrile can be used as mobile phase, both with 2-10 mM ammonium acetate as an ionisation aid. Gradients from 10% to 100% methanol or acetonitrile are required for the separation of the compounds listed in Table 1.

Modifications of the instrument might be necessary to minimise contact with fluorine-containing materials (Yamashita *et al.*, 2004). For example, Teflon™-containing tubing, filters for the mobile phase solvents and degassers can be sources of contaminations. A scavenger cartridge can be installed between the pump and injector to trap contaminants originating from the degasser, connecting tubes and mobile phase.

To ensure stability of retention times, the use of a temperature controlled column oven is strongly recommended.

5.2 Detection methods

The most widely used technique for detection of PFCs is by tandem MS operated in the MRM mode. Typical precursor and product ions are given in Table 2. MS-parameters for the individual compounds, such as collision energy, declustering potential and cone voltage, have to be optimised for each instrument. The sensitivity of tandem MS is usually about one order of magnitude higher than that of QTOF-MS (Berger *et al.*, 2004).

Table 2 Precursor and product ions for PFCs analysed using LC-(-)ESI-MS/MS.

Analyte	Precursor ion (m/z)	Product ion (m/z)
<i>PFBA</i>	112.9	168.7
<i>PFPA</i>	262.8	218.9
<i>PFHxA</i>	312.9	268.8
<i>PFHpA</i>	362.9	318.9
<i>PFOA</i>	413.0	368.9
<i>PFNA</i>	462.9	418.9
<i>PFDA</i>	512.9	469.0
<i>PFUnDA</i>	562.9	519.0
<i>PFDoDA</i>	613.0	568.9
<i>PFTriDA</i>	663.1	618.9
<i>PFTeDA</i>	713.0	669.0
<i>PFHxDA</i>	812.8	769.1
<i>PFBS</i>	298.9	79.8
<i>PFHxS</i>	398.9	79.8
<i>PFHpS</i>	449.0	79.3
<i>PFOS</i>	499.0	79.7
PFDS	598.9	79.5
THPFOS (6:2 FTS)	426.9	406.7
<i>PFOSi</i>	482.8	418.9
PFOSA	497.9	77.9
[¹³ C ₄]-PFBA	216.8	171.8
[¹³ C ₂]-PFHxA	314.9	269.9
[¹³ C ₄]-PFOA	417.0	371.8
[¹³ C ₃]-PFNA	467.9	423.0
[¹³ C ₂]-PFDA	514.9	469.8
[¹³ C ₂]-PFUnDA	565.0	519.8
[¹³ C ₂]-PFDoDA	614.9	569.9
[¹⁸ O ₂]-PFHxS	403.0	83.9
[¹³ C ₄]-PFOS	502.9	79.5
[¹³ C ₄]-PFOSi	486.9	422.9

6. Calibration and quantification

6.1 Standards

The use of commercially available standards with a purity of > 99% is recommended. The purity of standards should be verified, as impurities from the same homologue group and isomers can occur.

Suggestions for mass-labelled IS are given in Table 1. It is strongly recommended to use IS for PFC-analysis, so as to compensate for signal enhancement/suppression or losses during sample preparation. The IS and InjS have to be added before the extraction and the measurement, respectively. If possible, the corresponding mass-labelled IS should be used for each target analyte. In case a mass-labelled standard is not available, an IS with physicochemical characteristics and recovery rates similar to that of the target compound can be used but matrix suppression/ enhancement effects must first be checked in LC-ESI-MS/MS.

6.2 Calibration

The calibration curves must include the IS and InjS in the same range as the spike level for the samples. Linearity has to be checked for the calibration range and the correlation coefficient (R) should be better than 0.99. The lower end of linear range is determined by the quantification limits and blank levels. The blank response should be lower than 20 % of the lowest calibration standard. A multilevel calibration should have at least five calibration levels.

In case of matrix effects, standard addition may be an alternative calibration option.

6.3 Quantification

Every detection and quantification must comply with defined criteria for quality assurance (U.S. DHHS/FDA/CDER/CVM, 2001). If possible, two mass transitions should be recorded for each target analyte, one for quantification (quantifier) and one for identification (qualifier). The abundance ratio of these two masses in the sample is compared with that of the calibration standards obtained under identical chromatographic conditions. A substance is considered identified:

- if the relative retention time of the target compound in the sample is within ± 0.3 min of that in the calibration standard and
- if the abundance ratio of the two masses in the sample deviates less than 30 % from the average abundance ratio calculated from the calibration standards.

For quantification, the signal to noise ratio for the HPLC peak has to be at least 10 for all target compounds. The peak height of the target compound should exceed the measured blank as a minimum by a factor of 5.

Some PFSA and sulfonamides show more than one peak in the chromatogram, which is due to the presence of branched isomers. The ratio of linear and branched isomers can differ between the calibration standard and environmental samples. Branched isomers cannot be quantified precisely because of the lack of proper calibration standards. If the peak area of the branched isomer exceeds 10 % of that of the linear isomer, it is recommended to estimate its concentration based on the response factor of the linear standard.

The PFCA and PFSA are almost completely dissociated in environmental matrices. If salts are used for the preparation of calibration standards, quantification results should be calculated for the corresponding acids.

7. Quality Assurance and Quality Control

Prior to the analysis of environmental samples, the method should be subject to a full in-house validation according to the requirements of the monitoring programme. This should include the determination of limits of detection, limits of quantification, trueness, precision, linearity of calibration, measurement uncertainty and robustness.

Every sample batch should include a procedural blank that is prepared in the same way as the samples. The number of samples per batch may differ between laboratories and depend on how many samples can be processed under comparable conditions.

If mass labelled internal standards are used, absolute recoveries between 50% and 150% are acceptable. In all other cases, recoveries should be between 70% and 120%.

Within each sample batch, at least one sample should be extracted in duplicate and a laboratory control samples should be included. The results should be recorded and monitored in control charts.

At present, no certified reference material is available for PFCs in sediment. Possible bias in the analytical method should be checked by the analysis of spiked laboratory control samples.

Laboratories should demonstrate their competence by participation in laboratory proficiency testing schemes relevant for the monitoring programme. Such exercises are still rarely offered by proficiency testing providers, but a recent interlaboratory study aiming at method validation demonstrated acceptable performance of laboratories in analysing PFC in biota and water (van Leeuwen *et al.*, 2009).

8. Data Reporting

For routine analysis, the data report should be in accordance with the relevant monitoring programme; it should e.g. include information about sampling, sample processing, storage and analysis. Results should be reported along with the associated measurement uncertainty.

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Annex 3: Technical annex on the analysis of PFCs in seawater

1. Introduction

Perfluorinated and polyfluorinated compounds (PFCs) are man-made chemicals and are ubiquitous in the environment (Giesy and Kannan, 2001). PFCs are widely used as processing additives during fluoropolymer production and as surfactants in consumer applications, including surface coatings for carpets, furniture and paper products. They are also components in breathable, waterproof fabrics, fire-fighting foams and insulators for electric wires (Kissa, 2001). From the production and use of these products, PFCs can be released into the environment.

In this document, the name PFCs refers to compounds with a hydrophilic functional group and a hydrophobic fully fluorinated chain which can vary in chain length. The polyfluorinated acids have moderate water solubilities, low pK_a values and are therefore dissociated at environmentally relevant pH values (Kissa, 2001). Perfluorinated sulfonamides and fluorotelomer alcohols (FTOHs) are neutral compounds with a moderate vapour pressure currently discussed as precursors to perfluorooctane sulfonate (PFOS) and perfluorinated carboxylic acids (PFCAs) (Ellis *et al.*, 2004).

A method for the determination of PFOS and perfluorooctanoic acid (PFOA) in unfiltered water is already described in the International Standard ISO 25101 (ISO 25101, 2009). The objective of this technical annex is to provide advice on the analysis of PFCs in water based on the ISO 25101. This method should be extended to more PFCs and to water samples with a higher amount of suspended particle matter (SPM).

2. Analytes

Table 1 gives an overview of relevant PFCs for analysis in water. They are chosen from the following groups: Perfluorinated sulfonates (PFSAs), perfluorinated sulfinates (PFSiAs), PFCAs and perfluorinated sulfonamides. For monitoring purposes, the high-volume chemicals PFOS and PFOA are considered the most important PFCs and are highly recommended to be included in water monitoring. Although most studies have focused mainly on PFOA and PFOS, it is suggested that short-chained PFCs ($C \leq 8$), in particular perfluorobutane sulfonate (PFBS) and perfluorobutanoic acid (PFBA), should be included in monitoring programmes due to their good water solubility and their use as substitutes for PFOS in various applications.

Table 1 Full names, acronyms, formulas and Chemical Abstract System (CAS) numbers of native and mass-labelled PFCs relevant for water analysis.

Analyte	Acronym	Formula	CAS ^a -Number
Perfluorobutanoic acid	PFBA	C ₃ F ₇ COOH	375-22-4
Perfluoropentanoic acid	PFPA	C ₄ F ₉ COOH	2706-90-3
Perfluorohexanoic acid	PFHxA	C ₅ F ₁₁ COOH	307-24-4
Perfluoroheptanoic acid	PFHpA	C ₆ F ₁₃ COOH	375-85-9
Perfluorooctanoic acid	PFOA	C ₇ F ₁₅ COOH	335-67-1
Perfluorononanoic acid	PFNA	C ₈ F ₁₇ COOH	375-95-1
Perfluorodecanoic acid	PFDA	C ₉ F ₁₉ COOH	335-76-2
Perfluoroundecanoic acid	PFUnDA	C ₁₀ F ₂₁ COOH	4234-23-5
Perfluorododecanoic acid	PFDoDA	C ₁₁ F ₂₃ COOH	307-55-1
Perfluorotridecanoic acid	PFTriDA	C ₁₅ F ₂₅ COOH	72629-94-8
Perfluorotetradecanoic acid	PFTeDA	C ₁₃ F ₂₇ COOH	376-06-7
Perfluorohexadecanoic acid	PFHxDA	C ₁₅ F ₃₁ COOH	67905-19-5
PERFLUOROBUTANE SULFONATE	PFBS	C ₄ F ₉ SO ₂ O ⁻	29420-49-3 (potassium salt)
Perfluorohexane sulfonate	PFHxS	C ₆ F ₁₃ SO ₂ O ⁻	3871-99-6 (potassium salt)
Perfluoroheptane sulfonate	PFHpS	C ₇ F ₁₅ SO ₂ O ⁻	n.a.
Perfluorooctane sulfonate	PFOS	C ₈ F ₁₇ SO ₂ O ⁻	1763-23-1 (sodium salt)
Perfluoro-1-decanesulfonate	PFDS	C ₁₀ F ₂₁ SO ₂ O ⁻	13419-61-9 (sodium salt)
1H,1H,2H,2H-perfluorooctane sulfonate	THPFOS (6:2 FTS)	C ₆ F ₁₃ C ₂ H ₄ SO ₃ ⁻	27619-97-2
Perfluorooctane sulfinic acid	PFOSi	C ₈ F ₁₇ SO ₂ ⁻	n.a.
Perfluorooctane sulfonamide	PFOSA	C ₈ F ₁₇ SO ₂ NH ₂	754-91-6
Internal Standards			
Perfluoro-n-(1,2,3,4- ¹³ C ₄)butanoic acid	[¹³ C ₄]-PFBA	[2,3,4- ¹³ C ₃]F ₇ ¹³ COOH	n.a.
Perfluoro-n-(1,2- ¹³ C ₂)hexanoic acid	[¹³ C ₂]-PFHxA	C ₄ F ₉ [2- ¹³ C]F ₂ ¹³ COOH	n.a.
Perfluoro-n-[1,2,3,4- ¹³ C ₄]octanoic acid	[¹³ C ₄]-PFOA	C ₄ F ₉ [2,3,4- ¹³ C ₃]F ₆ ¹³ COOH	n.a.
Perfluoro-n-[1,2,3,4,5- ¹³ C ₅]nonanoic acid	[¹³ C ₅]-PFNA	C ₄ F ₉ [2,3,4,5- ¹³ C ₄]F ₈ ¹³ COOH	n.a.
Perfluoro-n-[1,2- ¹³ C ₂]decanoic acid	[¹³ C ₂]-PFDA	C ₈ F ₁₇ ¹³ CF ₂ ¹³ COOH	n.a.
Perfluoro-n-[1,2- ¹³ C ₂]undecanoic acid	[¹³ C ₂]-PFUnDA	C ₉ F ₁₉ ¹³ CF ₂ ¹³ COOH	n.a.
Perfluoro-n-[1,2- ¹³ C ₂]dodecanoic acid	[¹³ C ₂]-PFDoDA	C ₁₀ F ₂₁ ¹³ CF ₂ ¹³ COOH	n.a.
Perfluoro-1-hexane[¹⁸ O ₂]sulfonate	[¹⁸ O ₂]-PFHxS	C ₆ F ₁₃ S[¹⁸ O ₂]O ⁻	n.a.
Perfluoro-1-[1,2,3,4- ¹³ C ₄]octanesulfonate	[¹³ C ₄]-PFOS	C ₄ F ₉ [1,2,3,4- ¹³ C ₄]F ₈ SO ₂ O ⁻	n.a.
Perfluoro-1-[1,2,3,4- ¹³ C ₄]octanesulfinic acid	[¹³ C ₄]-PFOSi	C₄F₉[1,2,3,4-¹³C₄]F₈SO₂⁻	n.a.

^a, Chemical Abstract System; n.a., not available

3 Sampling, transportation and storage

The sampling should be carried out by trained personnel being aware of the risk of contamination of samples if incorrectly handled. It is recommended that sampling containers made of polypropylene, glass or stainless steel are used (for further details see ISO 25101).

4. Sample preparation

4.1 Pre-treatment

It is recommended that the samples are filtered prior to extraction to remove SPM using glass fibre filters (GFFs), and after filtration, to rinse the equipment with a polar solvent.

After filtration the sample should be spiked with a mass-labelled internal standard (IS) mixture at concentrations close to the environmental level. Before extraction, the sample should be incubated with the IS for about 1 h at 4° C so that the IS can interact with the matrix.

4.2 Extraction

The extraction is carried out using solid phase extraction (SPE) according to the ISO 25101 method.

4.3 Clean-up

The sample matrix may affect ionisation yield (enhancement/suppression of the signal) in electrospray tandem mass spectrometry (ESI-MS-MS), and so clean-up of the extracts is usually necessary. An appropriate clean-up method has been described by Powley *et al.* (2005). Briefly, 25 mg of graphitized carbon adsorbent (e.g., ENVI-Carb™, 100 m² g⁻¹, 120/400 mesh) and 50 µL acetic acid are added into a small tube. The extract is concentrated to 1 mL and transferred into this tube. The suspension is mixed vigorously, centrifuged and finally, 0.5 mL of the supernatant is transferred to another flask.

An injection standard (InjS) can be added to the final extract for correction of the injection volumes and calculation of the recoveries of the mass-labelled IS. The InjS should not occur in environmental samples, hence, the use of a mass-labelled InjS is suggested.

The solvent composition of the final extract should correspond to the mobile phase of the liquid-chromatography (LC) method in order to obtain a satisfactory peak shape of the compounds, in particular of early eluting short-chain PFCs. Unless the samples are analysed immediately, the vials should be kept at < 4°C and analysed within one week.

5. Instrumental analysis

LC coupled with a tandem mass spectrometer and interfaced with an electrospray ionisation source in a negative-ion mode (LC-(-)ESI-MS/MS) (Hansen *et al.*, 2001) or LC coupled with an (-)ESI time-of-flight mass spectrometer (LC-ESI-QTOF-MS) (Berger and Haukas, 2005) can be used for PFC analysis. Tandem MS and QTOF-MS have the advantage of low instrumental noise with a high selectivity (for further details see ISO 25101).

5.1 Liquid chromatography

Modifications of the instrument might be necessary to minimise contact with fluorine-containing materials (Yamashita *et al.*, 2004). A scavenger cartridge can be installed between the pump and injector to trap contaminants originating from the degasser, connecting tubes and the mobile phase.

5.2 Detection methods

The most widely used technique for detection of PFCs is tandem MS operated in the MRM mode. Typical precursor and product ions are given in Table 2. The sensitivity of tandem MS is usually about one order of magnitude higher than that of QTOF-MS (Berger *et al.*, 2004).

Table 2

Precursor and product ions for PFCs analysed using LC(-)ESI-MS/MS.

Analyte	Precursor ion (m/z)	Product ion (m/z)
<i>PFBA</i>	112.9	168.7
<i>PFPA</i>	262.8	218.9
<i>PFHxA</i>	312.9	268.8
<i>PFHpA</i>	362.9	318.9
<i>PFOA</i>	413.0	368.9
<i>PFNA</i>	462.9	418.9
<i>PFDA</i>	512.9	469.0
<i>PFUnDA</i>	562.9	519.0
<i>PFDoDA</i>	613.0	568.9
<i>PFTrIDA</i>	663.1	618.9
<i>PFTeDA</i>	713.0	669.0
<i>PFHxDA</i>	812.8	769.1
<i>PFBS</i>	298.9	79.8
<i>PFHxS</i>	398.9	79.8
<i>PFHpS</i>	449.0	79.3
<i>PFOS</i>	499.0	79.7
PFDS	598.9	79.5
THPFOS (6:2 FTS)	426.9	406.7
<i>PFOSi</i>	482.8	418.9
PFOSA	497.9	77.9
[¹³ C ₄]- <i>PFBA</i>	216.8	171.8
[¹³ C ₂]- <i>PFHxA</i>	314.9	269.9
[¹³ C ₄]- <i>PFOA</i>	417.0	371.8
[¹³ C ₃]- <i>PFNA</i>	467.9	423.0
[¹³ C ₂]- <i>PFDA</i>	514.9	469.8
[¹³ C ₂]- <i>PFUnDA</i>	565.0	519.8
[¹³ C ₂]- <i>PFDoDA</i>	614.9	569.9
[¹⁸ O ₂]- <i>PFHxS</i>	403.0	83.9
[¹³ C ₄]- <i>PFOS</i>	502.9	79.5
[¹³ C ₄]- <i>PFOSi</i>	486.9	422.9

6. Calibration and quantification

6.1 Standards

The use of commercially available standards with a purity of > 99% is recommended. Suggestions for mass-labelled IS are given in Table 1. It is strongly recommended to use IS for PFC-analysis, to compensate for signal enhancement/suppression or losses during sample preparation. If possible, the corresponding mass-labelled IS should be used for each target analyte. In case a mass-labelled standard is not available, an IS with physicochemical characteristics and recovery rates similar to that of the target compound can be used but matrix suppression/enhancement effects must first be checked in LC-ESI-MS/MS (for further details see ISO 25101).

6.2 Calibration and quantification

The calibration and quantification should be carried out using the method of internal standard according to the ISO 25101 method. In case of matrix effects, standard addition may be an alternative calibration option.

Some PFCs show more than one peak in the chromatogram, which is due to the presence of branched isomers. The ratio of linear to branched isomers can differ between the calibration standard and environmental samples. Branched isomers cannot be quantified precisely because of the lack of proper calibration standards. If the peak area of the branched isomer exceeds 10 % of that of the linear isomer, it is recommended to estimate its concentration based on the response factor of the linear standard.

The PFCAs and PFSAs are almost completely dissociated in environmental matrices. If salts are used for the preparation of calibration standards, quantification results should be calculated for the corresponding acids.

7. Quality Assurance and Quality Control

Prior to the analysis of environmental samples, the method should be subject to a full in-house validation according to the requirements of the monitoring programme. This should include the determination of limits of detection, limits of

quantification, trueness, precision, linearity of calibration, measurement uncertainty and robustness (for further details see ISO 25101).

Within each sample batch, at least one sample should be extracted in duplicate and a laboratory control samples should be included. The results should be recorded and monitored in control charts.

Laboratories should demonstrate their competence by participation in laboratory proficiency testing schemes relevant for the monitoring programme. Such exercises are still rarely offered by proficiency testing providers, but a recent interlaboratory study aiming at method validation demonstrated acceptable performance of laboratories in analysing PFC in biota and water (van Leeuwen *et al.*, 2009).

8. Data Reporting

For routine analysis, the data report should be in accordance with the relevant monitoring program; it should e.g. include information about sampling, sample processing, storage and analysis. Results should be reported along with the associated measurement uncertainty.

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Annex 4: Revised technical annex on the analysis of organic contaminants in biota

This annex is intended as a supplement to the general OSPAR JAMP guidelines. The Annex applies to analysis of all types of organic contaminants but is an overview and is not intended as a complete description or a substitute for detailed instructions. Advice and recommendations given in documents prepared through the QUASIMEME project (Quality Assurance of Information for Marine Environmental Monitoring in Europe) are frequently cited.

1. Species

1.1 Fish and shellfish

1.1.1 Criteria for the selection of species for temporal trend monitoring

Species for temporal trend monitoring can only be selected in the light of information on fish stock composition and history. It is essential that long time series with one species are obtained. Care should be taken that the sample is representative of the population and can be repeated annually. Fish and shellfish species currently used for trend monitoring are listed in Tables 1 and 2 of the main guidelines.

1.1.2 Criteria for the selection of species for spatial distribution monitoring

In order to standardise results the first choice species *Limanda limanda*, *Gadus morhua* and *Mytilus edulis* or *Mytilus galloprovincialis* should be used whenever possible. The second choice species *Merlangius merlangus*, *Merluccius merluccius*, *Platichthys flesus* and *Crassostrea gigas* should only be used when none of the first choice species are available.

First choice species

Limanda limanda (dab)

Dab is a ground dwelling species confined to the shelf seas. It has replaced the previously recommended plaice and flounder for the following reasons:

- its migration is less pronounced, thus it is more likely to represent the area in which it is caught;
- it has been used successfully in disease studies, thus complementary information from such studies would be available (in fish disease studies a length range for individual fish of 20-25 cm is used).

The southern distribution limit of dab is the north coast of Spain.

Gadus morhua (cod)

Cod normally live near the seabed but may also be pelagic. Cod occur in coastal areas and to 600 m depth. Cod may also be found in the open ocean and so may also be used for monitoring oceanic regions of the Maritime Area. The southern distribution limit of cod is at 45°N. A sampling size range of 30-45 cm is specified because cod of that size and age tend to feed on a fairly uniform diet.

Mytilus sp. (mussel)

Mytilus edulis occurs in shallow waters along almost all coasts of the Contracting Parties. It is therefore suitable for monitoring in nearshore waters. No distinction is made between *Mytilus edulis* and *Mytilus galloprovincialis* because the latter, which may occur along Spanish and Portuguese coasts, cannot easily be discerned from *Mytilus edulis*. A sampling size range of 3-6 cm is specified to ensure availability throughout the whole maritime area.

Second choice species

Platichthys flesus (flounder)

The distribution of flounder extends further south than that of dab and might therefore represent the flatfish of choice for certain Portuguese coastal areas and Spain's northwestern coastal areas. Flounder is not suitable for monitoring in open sea areas due to its migration pattern. A sampling size range of 15-35 cm ensures individuals of the 2-3 year age class.

Merlangius merlangus (whiting)

Whiting can be caught in coastal waters and to 200 m depth. Its distribution is from Portugal to Iceland and Norway, thus covering all the maritime area subject to monitoring by Contracting Parties. It is a suitable substitute for Cod. The sampling size range, 20-35 cm, may need adjustment in the light of future experience.

Merluccius merluccius (*hake*)

Hake live at 100-300 m along the shelf margins. The sampling size range is 20-35 cm. The sampling size interval suggested is arbitrary and may need adjustment in the light of future experience.

Crassostrea gigas (*Pacific oyster*)

The Pacific oyster should be sampled in areas where *Mytilus sp.* is not available. The sampling size should be within the length range 9-14 cm to ensure individuals of the 2 year age class.

1.2 Seabirds

Relevant references concerning the use of seabirds in contaminant monitoring programmes include Gilbertson, 1987; Becker, 1989 and 1991; Becker *et al.*, 1991 and 1992; Walker, 1992 and Bignert *et al.*, 1995.

Sterna hirundo (*common tern*)

The common tern is widely distributed over the European and North American Atlantic coasts as well as the Baltic Sea, but does not occur in Iceland. It feeds in marine, brackish and fresh waters.

Haematopus ostralegus (*oystercatcher*)

The oystercatcher is widely distributed along the coasts of the North-west Atlantic, including Iceland, and also occurs in the Baltic Sea. The species is not strictly marine as it also feeds inland. It feeds on benthos. In contrast to other seabirds, nest sites are accessible and the eggs within reach.

Uria aalge (*guillemot*)

The guillemot feeds in the open sea and nests on the coasts of northern Europe, in the Baltic Sea and on the North American coast.

2. Sampling

Two alternative sampling strategies are described: sampling to minimise natural variability and length-stratified sampling. References of relevance to sampling and statistics include Gilbert, 1987; Bignert *et al.*, 1993 and 1994; Tema Nord, 1995 and Nicholson *et al.*, 1996 and 1997.

2.1 Sampling to minimise natural variability

Gain in precision of the contaminant data can be obtained by minimising variance from the biological covariables. For fish, this can be achieved by sampling and analysing individually at least 12 young fish of the same sex, *e.g.* 2-3 year old female fish. For shellfish, a sample should be collected with the number of individuals large enough to be divided into at least 3 equal pools with each pool consisting of at least 20 animals and enough soft tissue for all analyses. The length of the individuals collected should be constant from year to year at each station, or should at least fall within a very narrow range, *e.g.* within 5 mm. To reflect recent levels of contamination, young individuals should be chosen. In selecting the sample, care should be taken to ensure that it is representative of the population and that it can be obtained annually.

2.2 Length-stratified sampling

Where successfully ongoing, length-stratified time series should be continued.

2.2.1 Fish

Gain in precision of the contaminant data can also be obtained from stratification using biological variables. Although several biological parameters are appropriate, length appears to be the parameter which can most easily be applied onshore and at sea and which has also been shown to be significant in many analyses. Much discussion has been devoted as to whether simple linear or log-linear (multiplicative) models give the better fit. General experience with other fish and other types of data indicate a preference for the log-normal model at least for the present. As the length dependence of the contaminant concentration is not well understood, sampling should keep the length/contaminant relationship under constant surveillance, *i.e.* the entire length range should be covered evenly. Care should be taken that the samples are not unduly clustered within a particular length interval. More length intervals could be used and the test of the hypothesised contaminant/length relationship becomes stronger if the lengths are evenly distributed. It is essential to keep the length stratification identical from one year to the next. The length range should be defined on the basis of practical considerations. For fish, the upper limit should be chosen in such a way that at least 5 fish in the largest length interval can easily be found. The length stratification should be determined in such a way that it can be maintained over many years. The length interval should be at least 2 cm in size. The length range should be split into 5 length intervals,

which are of equal size after log transformation. For example, if the length range is 18-36 cm, then the interval boundaries could be (rounded to 0,1 cm) as follows:

18 - 20,720,8-23,8 23,9-27,3 27,4-31,3 31,4-36 cm.

2.2.2 Shellfish

For shellfish, the upper limit should be chosen in such a way that at least 20 mussels in the largest length interval can easily be found. The length stratification should be determined in such a way that it can be maintained over many years. The length interval should be at least 5 mm in size. The length range should be split into at least 3 length intervals (small, medium and large) which are of equal size after log transformation. For example, if the length range is 40-70 mm, then the interval boundaries could be (rounded to 1 mm) as follows:

5 intervals: 40 - 45 46 - 50 51 - 56 57 - 63 64 - 70
3 intervals: 40 - 48 49 - 58 59 - 70

2.3 Seabird eggs

2.3.1 Permission

Permission to collect the eggs must be received from the appropriate national authorities.

2.3.2 Sampling period and frequency

Eggs should be sampled annually at each site in May or June. Only clutches from the first laying cycle within a single year should be selected.

2.3.3 Number of eggs and sampling procedure

Eggs should only be taken from full clutches (*i.e.* common tern 3 eggs, oystercatcher 3-4 eggs). Eggs should not be taken from abandoned clutches. Only one egg should be taken from each clutch. Ten eggs should be selected in total (*i.e.* one egg from 10 separate clutches) and it is important to choose the egg from each clutch randomly. As the eggs must be fresh (*i.e.* between 1-5 days incubation) information about the incubation stage of each egg is required. Two methods are recommended for determining incubation stage:

locate 12-15 clutches containing one egg only and mark these by placing a peg about 1 m from the nest. Check the clutches every other day until they are complete. Take one egg randomly from the completed clutch;
fill a 1 litre plastic beaker with water and place the egg in the water:
fresh eggs (*i.e.* of 1-2 days incubation) will lie on the bottom with the long axis parallel to the bottom;
eggs of 3-6 days incubation will rest with the small end on the bottom of the beaker and the long axis forming an angle of 30-45°;
eggs which float or stand vertically with the small end on the bottom are of more than 7 days incubation and should not be selected.

Each nest from which an egg has been taken should be marked, using a peg or some other type of marker, to ensure that a second egg is not taken. While still in the field the egg selected should be put into a numbered plastic egg box. The number of the box should be written on the shell of the egg in soft pencil. The clutch size from which the egg was taken, the nest number and the sampling date should be recorded.

2.3.4 Materials

For each species, area and year the following are required:

- nest pegs;
- a non-toxic, waterproof marker;
- a 1 litre plastic beaker;
- numbered egg boxes (*e.g.* for oystercatcher: 100 ml, polypropylene polyethylene, ø 55*73 mm, and for common tern: 50 ml, polystyrol/ polyethylene, ø 41*49 mm).

3. Transportation

3.1 Fish and shellfish

Samples should be kept cool and frozen at $< -20^{\circ}\text{C}$ as soon as possible after collection. Length and weight should be determined before freezing. Live mussels should be transported in closed containers at temperatures between $5 - 15^{\circ}\text{C}$, preferably $< 10^{\circ}\text{C}$. Frozen samples should be transported in closed containers at temperatures $< -20^{\circ}\text{C}$. More rigorous conditions will be necessary for samples taken for biological effects monitoring, *e.g.* storage in liquid nitrogen.

3.2 Seabird eggs

Eggs should be kept cool and frozen at -18°C as soon as possible after collection.

4. Pre-treatment and storage

4.1 Contamination

Sample contamination may occur during sampling, sample handling, pre-treatment and analysis (Oehlenschläger, 1994), due to the environment, the containers or packing material used, the instruments used during sample preparation or from the chemical reagents used during the analytical procedures. The risk of contamination is particularly high for dioxin-like PCBs which occur at ultra-trace levels in the environment. Controlled conditions are therefore required for all procedures, including the dissection of fish organs on board ship. One way of minimising the risk is to conduct dissection in a clean area, such as within a laminar-flow hood away from the deck areas of the vessel.

4.2 Fish

4.2.1 Dissection and storage

Ungutted fish should be wrapped separately in suitable material (*e.g.* pre-cleaned aluminium foil) and frozen. Plastic materials, except polyethylene or polytetrafluorethylene, must not be used (*cf.* Smedes and de Boer, 1994). The frozen samples should be stored in suitable containers to avoid damage. Sub-samples (*e.g.* of liver) should be stored in pre-cleaned containers made of glass (*e.g.* borosilicate glass), stainless steel or aluminium, or should be wrapped in pre-cleaned aluminium foil and frozen quickly in liquid nitrogen or a blast freezer. The individual samples should be clearly labelled and stored together in a suitable container placed in a deep freeze at $< -20^{\circ}\text{C}$ until analysis. Sub-samples for enzyme tests should be stored in vials suitable for storage in liquid nitrogen, labelled clearly and stored in liquid nitrogen until analysis.

When samples are processed at sea the dissection must be done by trained personnel preferably on a clean bench wearing clean gloves and using clean stainless steel knives. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, the tools should be cleaned. Washing in acetone or alcohol and high purity water is the procedure recommended.

4.2.2 Sub-sampling

To sample fish muscle, care should be taken to avoid including any epidermis or subcutaneous fatty tissue in the sample. Samples should be taken underneath the red muscle layer. In order to ensure uniformity, the right side dorso-lateral muscle should be sampled. If possible, the entire right dorsal lateral filet should be homogenised and sub-samples taken for replicate dry weight and contaminant determinations. If however the amount of material to be homogenised would be too large, a specific portion of the dorsal musculature should be chosen. It is recommended that the portion of the muscle lying directly under the first dorsal fin be used in this case. As both fat and water content vary significantly in the muscle tissue from the anterior to the caudal muscle of the fish, in order to ensure comparability it is important to obtain the same portion of the muscle tissue for each sample (see Oehlenschläger, 1994).

When dissecting the liver, care should be taken to avoid contamination from other organs. The whole liver should be homogenised or freeze-dried. If however the amount of material homogenised was too large, a specific portion of the liver should be chosen in order to ensure comparability.

Where pooling of tissues is necessary, an equivalent quantity of tissue must be taken from each fish, *e.g.* a whole fillet from every fish. If the total quantity of tissue so yielded would be too large to be handled conveniently, the tissue may be sub-sampled, but a fixed proportion of each tissue must then be taken, *e.g.* 10% of each whole fillet or 10% of each whole liver or for muscle tissue 10% of the fish.

Personnel must be capable of identifying and removing the desired organs according to the requirements of the investigations.

4.3 Shellfish

4.3.1 Depuration

Mussels should be placed on a polyethylene tray elevated above the bottom of a glass aquarium. The aquarium should be filled with filtered sub-surface sea water collected from the same site as the samples and which has not been subject to contamination from point sources if possible. The aquarium should be aerated and the mussels left for 20-24 hours at water temperatures and salinity close to those from which the samples were removed.

4.3.2 Opening of the shells

Mussels should be shucked live and opened with minimum tissue damage by detaching the adductor muscles from the interior of one valve. The mussels should be inverted and allowed to drain on a clean towel or funnel for at least 5 minutes in order to minimise influence on dry weight determinations.

4.3.3 Dissection and storage

The soft tissues should be removed and deep frozen ($< -20^{\circ}\text{C}$) as soon as possible in containers appropriate to the intended analysis. The dissection of the soft tissue must be done under clean conditions on a clean bench by trained personnel, wearing clean gloves and using clean stainless steel knives. After each sample has been prepared, the tools should be cleaned. Washing in acetone or alcohol and high purity water is recommended. When the analysis is eventually undertaken, all fluids that may initially separate on thawing should be included with the materials homogenised. Homogenisation should be performed immediately prior to any sub-dividing of the sample.

4.4 Seabird eggs

Before thawing, each egg should be placed in a previously weighed goblet. The weight of the egg (to the nearest 0.1 g inclusive of shell), the length of the egg between poles and the breadth of the egg at the equator (to the nearest 0.1 mm using callipers) should be recorded. The egg should then be opened (if this has not already happened during thawing) and the content carefully separated from the shell. If the egg contains an embryo, the eye diameter or the "crown-tail" length of the embryo should be measured (to the nearest 0.1 mm using callipers). The content of the egg (*i.e.* the albumen and yolk) should be weighed (to the nearest 0.1 g) and homogenised in the same goblet for each egg (*e.g.* by an Ultra Turrax). Any sub-sampling should be done immediately after homogenisation. The samples can then be analysed or deep frozen for later analysis. The shell (including the shell-skin) should be washed with water and dried in laboratory air for at least a week before weighing (to the nearest 0.01 g). The shell thickness should be measured at three points along the egg equator.

Annex 5 : Technical annex of the determination of chlorobiphenyls in biota – analytical method

1. Introduction

This technical annex provides advice on chlorobiphenyl (CB) analysis for all biota samples. The guideline is an update of an earlier version (OSPAR, 1999) taking into account evolutions in the field of analytical chemistry and also covering the determination of the planar CBs, i.e. the mono-*ortho* (CB105, CB114, CB118, CB123, CB156, CB157, CB167 and CB189) and non-*ortho* substituted CBs (CB81, CB77, CB126 and CB169). When reviewing the literature, it should be noted that planar, coplanar and dioxin-like CBs / PCBs are all equivalent terms.

The analysis of CBs in biota generally involves extraction with organic solvents, clean-up (removal of lipids and fractionation), and gas chromatographic separation with electron capture or mass-spectrometric detection. All stages of the procedure are susceptible to insufficient recovery and/or contamination. Where possible, quality control procedures are required in order to check the method's performance. These guidelines 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. Due to the low concentrations of, particularly, non-*ortho* substituted CBs in biota compared to those of other CBs, their determination requires an additional separation and concentration step. Therefore, in the relevant sections a distinction will be made between the non-*ortho* CBs and the others.

These guidelines can also be used for several other groups of organochlorine compounds, e.g. DDTs and their metabolites, chlorobenzenes and hexachlorocyclohexanes. Recoveries in the clean-up procedures must be checked carefully. In particular, treatment with H₂SO₄ results in a loss of some compounds (e.g. dieldrin and endosulfan (de Boer and Wells, 1996)).

These guidelines are not intended as a complete laboratory manual. If necessary, further guidance should be sought from specialised laboratories. Whichever analytical procedure is adopted, the laboratory must demonstrate the validity of the procedure. Analyses must be carried out by trained staff.

2. Analysis

2.1. Precautionary measures

Solvents, chemicals and adsorption materials must be free of CBs or other interfering compounds. If not they should be purified using appropriate methods. Solvents should be checked by concentrating the volume normally used in the procedure to 10% of the final volume if practical and then analysing for the presence of CBs and other interfering compounds. If necessary, the solvents can be purified by redistillation but this practice is not favoured by most analytical laboratories as they generally opt to purchase high quality solvents directly. 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. Alternatively, glass thimbles with a G1 glass filter at the bottom can be used. Generally, paper filters should be avoided in filtration and substituted for by appropriate glass filters. As all pre-cleaned materials are prone to contamination (e.g. by the adsorption of CBs and other compounds from laboratory air), materials ready for use should not be stored for long periods. All containers, tools, glassware *etc.* 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, heated at >250°C and rinsed immediately before use with organic solvents or mixtures such as hexane/acetone. In addition all glassware should preferably be covered with aluminium foil and stored in cupboards to keep out any dust. Old and scratched glassware is more likely to cause blank problems because of the larger surface and therefore greater chance of adsorption. Furthermore, scratched glassware can be more difficult to clean effectively, as shown during analysis of brominated flame retardants (QUASIMEME, 2007).

2.2 Lipid determination

The determination of the lipid content of tissues can be of use in characterising the samples and reporting concentrations in biota on a wet weight or lipid weight basis. The total lipid content of fish or shellfish should be determined using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963) or an equivalent method such as Smedes (1999). Extractable lipid methods may be used, particularly if the sample size is small and lipid content is high. It has been shown that if the lipid content is high (> 5%) then the extractable lipid content will be comparable to that of the total lipid. If extraction techniques are applied which destroy or remove lipid materials (e.g., PLE with fat retainers), the lipid content should be determined on a separate subsample of the tissue homogenate. Other relevant information concerning lipid determination are provided by QUASIMEME, 1994 and Roose *et al.*, 1996.

2.3. Dry weight determination

Dry weight determinations should be carried out by drying homogenised sub-samples of the material to be analysed to constant weight at 105°C.

2.4. Homogenisation and drying

Prior to analysis, the samples should be sufficiently homogenised. Homogenisation is generally carried out on fresh tissue. Care should be taken that the sample integrity is maintained during the actual homogenisation. When the analysis is undertaken, all fluids that may initially separate on thawing should be included with the materials homogenised. Homogenisation should be performed prior to extraction and clean-up procedures. When homogenising samples after drying, classical techniques using a ball mill can be used. Cryogenic homogenisation of dried or fresh materials at liquid nitrogen temperatures using a PTFE device (*cf.* Iyengar and Kasperek, 1977) or a similar technique is also possible (*cf.* Iyengar, 1976; Klussmann *et al.*, 1985).

CBs can be extracted from either wet or dried samples, although storage, homogenisation and extraction are easier when the samples are dry. Drying the samples may, however, alter the CB concentrations e.g. by the loss of compounds through evaporation or by contamination. Potential losses and contamination should be checked as part of the method validation.

Chemical drying can be performed by grinding with e.g. Na₂SO₄ or MgSO₄ until the sample reaches a free-flowing consistency. It is essential that at least several hours elapse between grinding and extraction to allow for complete dehydration of the sample, as the presence of residual water will decrease the extraction efficiency.

Freeze-drying is also a popular technique, although its application should be carefully considered. Possible losses or contamination must be checked. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0°C. Contamination during freeze-drying can be reduced by putting a lid, with a hole of about 3 mm in diameter, on the sample container.

2.5. Extraction

Recovery standards should be added prior to extraction. When using Soxhlet extraction, a combination of polar and apolar solvents is recommended. Alternatively, saponification may be used. This technique is highly effective, but conditions should be controlled as saponification could result in the decomposition of some pesticides and, under certain conditions, of some CB congeners.

Although the use of binary non-polar/polar solvent mixtures and Soxhlet extraction is still the benchmark for CB extraction, there have been numerous attempts to find alternative procedures, which are less time-consuming, use less solvent and/or enable miniaturisation. 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).

From among the techniques mentioned, PLE or Accelerated Solvent Extraction (ASE) has – so far – been most successful. 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 acid-impregnated silica, florisil or alumina, to the cell. New promising techniques have been described, e.g. the use of a small carbon column in the extraction cell, which selectively adsorbs dioxin-like compounds (subsequently isolated by back-flushing with toluene), but these are not established for routine analysis (Sporring *et al.*, 2003). PLE and MAE have the shared advantage over SFE that they are matrix-independent, which 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 satisfactory, and sonication time often is 30 min or less (Roose and Brinkman, 2005).

All the methods described above are in principle suitable for extracting CBs from biota. However, Soxhlet extraction is still the reference for alternative approaches.

2.6 Clean-up

The extraction procedures above will result in the co-extraction of lipids, which will need to be removed from the extract. Furthermore, tissue extracts will always contain many compounds other than CBs, 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. Most CBs are stable under acid conditions; therefore treatment with sulphuric acid or acid impregnated silica columns may be used in the clean-up.

The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography, but gel permeation chromatography (GPC) is also employed. Any water residues in the extract should be removed prior to clean-up, e.g. by adding Na₂SO₄.

As CBs are apolar, clean-up using normal-phase chromatography is the most appropriate technique for their separation from other compounds. Using an apolar solvent (e.g. hexane or *iso*-octane) as an eluent, CBs normally elute very rapidly. All polar solvents used in the extraction should be removed before further clean-up. The last concentration step is usually performed by evaporation with a gentle stream of nitrogen. Evaporation to dryness should always be avoided but, for the analysis of planar CBs, very small final sample extract volumes might be necessary to achieve detectable concentrations.

Deactivated Al₂O₃ (5-10% water) is often used as a primary clean-up technique. Al₂O₃ can yield a sufficiently clean extract for a GC-ECD analysis of the sample. Al₂O₃ effectively removes lipid compounds from the extracts (although samples with a very high lipid content and low CB concentrations may require additional clean-up).

Deactivated silica (1-5% water) does not retain CBs (including non-*ortho* CBs) and only retains polycyclic aromatic hydrocarbons (PAHs) slightly when eluted with hexane or *iso*-octane. When organochlorine pesticides are also to be determined in the same extract, deactivation of the silica with a few percent of water is necessary.

For high activity silica (heated overnight at 180°C) the retention of CBs is negligible, while PAHs are more strongly retained. The CBs and a few other organochlorine compounds can be eluted with apolar solvents. More polar solvents (e.g. hexane/acetone) should be avoided as some interfering organochlorine pesticides would be eluted as well.

For the separation of CBs from lipids or oil components, reversed-phase HPLC can be used. In reversed-phase chromatography, CBs elute during a solvent gradient of 80 to 90% methanol together with numerous other compounds of the same polarity. Most of the above mentioned extraction methods and clean-up procedures yield an extract containing an apolar solvent. These cannot be injected directly for reversed-phase chromatography, and so compounds must be transferred between solvents several times e.g. before injection and after elution. When using polar solvents for extraction, reversed-phase columns could be used directly for clean-up. When eluting an acetonitrile extract from a C₁₈ solid phase extraction (SPE) column with acetonitrile, high molecular hydrocarbons are strongly retained while CBs elute in the first few column volumes.

The above mentioned normal-phase chromatographic procedures on silica and Al₂O₃ can be transferred to HPLC having the advantages of higher resolution and better reproducibility.

When using GPC, the elution of CBs should be carefully checked. 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, a second clean-up step is often required to separate the CBs from other organohalogenated compounds and/or to remove residual lipids.

One advantage of using PLE extraction is that it is possible to combine the clean up with the extraction, especially where mass spectrometry will be used as the detection method. If Soxhlet extraction is used for biota, then there is a much greater quantity of residual lipid to be removed than in the case of PLE with fat retainers. An additional clean-up stage may therefore be necessary. Methods have been developed for online clean-up and fractionation of dioxins, furans and CBs with PLE for food, feed and environmental samples (Sporring *et al.*, 2003), utilising a fat retainer for the on-line 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* CBs require a more specialised 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 intake results in the presence of even more co-extractive compounds and care has to be taken that the capacity of the adsorption columns is not exceeded and/or that lipids are adequately removed. Often, more rigorous procedures are applied to remove the excess material by e.g. shaking the sample with concentrated sulphuric acid. A more efficient alternative is to elute the sample over a silica column impregnated with sulphuric acid (40 % w/w).

Non-*ortho* CBs are nearly always separated from the other CBs using advanced separation techniques. One very efficient method is to inject the extracts (after concentrating them) into a HPLC system coupled to a PYE (2-(1-pyrenyl) ethyldimethylsilylated silica) column. Column dimensions are typically 4.6 x 150 mm, but combinations of several columns in-line are sometimes used. The use of PYE columns not only allow the separation of *ortho*, mono-*ortho* and non-*ortho* CBs from one another on the basis of structural polarity, but also from dibenzo-*p*-dioxins and dibenzofurans.

The eluting solvent is an apolar solvent such as *iso*-hexane. Coupled to a fraction collector, the use of a HPLC system allows the automatic clean-up of a considerable number of samples at a time. Alternatively, HPLC systems equipped with porous graphitised carbon can be used. Column sizes are in the order of 50 x 4.7 mm and care has to be taken that the column is not overloaded. Similarly to PYE columns, they will separate non-*ortho* CBs from the other CBs and from dioxins. Fully automated systems, such as Powerprep™, that combine several steps are routinely used.

2.7 Pre-concentration

Evaporation of solvents using a rotary-film evaporator was, until recently, the most common method. However, evaporation of solvents using this technique should be performed at low temperature (water bath temperature of $\leq 40^{\circ}\text{C}$) and under controlled pressure conditions, in order to prevent losses of the more volatile CBs. To reduce the sample to the final volume, solvents can be removed by blowing-down with a gentle stream of nitrogen. Only nitrogen of a controlled high quality should be used.

Turbovap sample concentrators can also be used to reduce solvent volume. This is a rapid technique, but needs to be carefully optimised and monitored to prevent both losses (both of volatiles and solvent aerosols) and cross-contamination. The use of rotary-film evaporators is more time consuming but more controllable. Here also, evaporation to dryness should be avoided at all costs. Syncore™ parallel evaporators (Buchi, Switzerland) can be used with careful optimisation of the evaporation parameters. The Buchi Syncore™ Analyst also uses glass tubes but the system is sealed, avoiding contamination from the laboratory air during evaporation. It does not use a nitrogen stream, thus reducing the loss of volatiles and if the flushback module is fitted the sides of the tubes are rinsed automatically thus reducing the loss of the heavier components. Again water-bath temperatures should be minimised to prevent losses. When reducing the sample to the required final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the gas chromatograph (GC) include hexane, heptane, toluene and *iso*-octane.

2.8 Calibration and preparation of calibrant solutions

Internal standards (recovery and quantification standards) should be added in a fixed volume or weight to all standards and samples. The ideal internal standard contains negligible (non-detectable) concentrations of CBs and does not coelute with other CBs. All CBs with a 2,4,6-substitution (e.g. CB112, CB155, CB198) are, in principle, suitable for this purpose. Alternatively, 1,2,3,4-tetrachloronaphthalene or homologues of dichloroalkylbenzylether can be used. For GC analysis with mass selective detection (GC-MS), ^{13}C labelled CBs should be used at each degree of chlorination. This is especially critical for the determination of the non-*ortho* CBs. If possible, the labelled calibrant solutions should correspond to the unlabelled determinants. For the non-*ortho* CBs, a labelled standard is available for each congener and use of all of them is recommended. When preparing a calibration solution for a new determinant for the first time, two independent stock solutions of different concentrations should always be prepared simultaneously to allow cross checking. A new calibration solution should also be cross-checked to the old standard solution. Crystalline CBs of known purity can be used for preparing calibration solutions but, for health and safety reasons, the purchase of solutions is recommended for planar CBs. In recent years, a lot of certified commercial custom made standards have become available and laboratories have been switching to these. If the quality of the standard materials is not guaranteed by the producer or supplier, it should be checked by GC preferably with mass spectrometric detection. Solid standards should be weighed to a precision of 10^{-5} grams. Calibration solutions should preferably be stored in ampoules in a cool and dark place. Commercially available screw-cap vials with a capillary opening (Certan™) combine of advantages of ampoules and vials, and, have proven to be reliable. When stored in containers the weight loss during storage should be recorded.

2.9 Instrumental determination

2.9.1 Injection techniques

The two modes commonly used are splitless and on-column injection as, in split 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 so as to avoid poor transfer to the column and losses by adsorption. Liners with a light packing of (silylated) glass wool may improve the performance for CBs, but may cause degradation of 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 negative effects previously associated with that technique, but should be thoroughly optimised before use. Increasing the injection volume will allow either the elimination of an extra evaporation step or the lowering of the analytical detection limits, or both.

2.9.2 Carrier gas

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

2.9.3 Columns

Only capillary columns should be used. The following parameters are recommended:

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 .

Columns which do not fulfil these requirements generally do not offer sufficient resolution to separate CB28, CB105 and CB156 from closely eluting CBs. A wide range of stationary phases can be used for CB separation. The chemical composition is different for columns from different producers and this influences the maximum temperature at which the column can be operated. Further advice may be found in the producers' catalogues, where compositions, applications and tables from which to compare products from different manufacturers are included.

In recent years, new chromatographic phases have become available that result in an improved separation of critical CB pairs. A good example is the HT-8 phase (1,7-dicarba-closo-dodecarborane phenylmethyl siloxane) (Larsen *et al.*, 1995) that shows a remarkable selectivity for CBs (Table 1). This column is currently recommended for CB analysis.

2.9.4 Detection

The electron capture detector (ECD) is still frequently used for CB analysis. Injection of chlorinated solvents or oxygen-containing solvents should be avoided when ECD is used due to the generation of large interfering signals. When using mass selective detectors (MSD), the electron-capture negative-ion chemical ionisation mode (ECNICI) is extremely sensitive for pentachlorinated to decachlorinated CBs, and is approximately ten fold more sensitive than ECD. However, the sensitivity of MS systems has improved considerably, allowing analysis also to be undertaken using electron impact ionisation (EI). Previously, the use of ECNICI was often necessary in order to detect the low concentrations of, in particular, the non-*ortho* CBs. Suggested target and qualifier ions for *ortho* CBs (including mono-*ortho* CBs) are shown in Table 1 and in Table 2 for non-*ortho* CBs..

Next to conventional GC-MS, the use of ion-trap with its tandem MS² option - i.e., yielding improved selectivity - is receiving increased attention. The use of GC-ITMS provides a less expensive alternative to high-resolution mass spectrometry (HRMS), which is commonly used to determine PCDD/Fs and, as such, is also ideally suited for the detection of all CB groups.

Table 1

Example of retention times for selected CB congeners using a 50 m HT8 column (0.25 mm i.d. and 0.25 µm film), along with possible target and qualifier ions. 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.

CB congener	MW	RT	Target Ion	Qualifier Ion	Number of chlorines
¹³C-CB28	270	28.371	268	270	3
CB31	258	28.071	256	258	3
CB28	258	28.388	256	258	3
¹³C-CB52	304	30.317	304	302	4
CB52	292	30.336	292	290	4
CB49	292	30.698	292	290	4
CB44	292	32.024	292	290	4
CB74	292	34.881	292	290	4
CB70	292	35.199	292	290	4
¹³C-CB101	340	36.612	338	340	5
CB101	326	36.630	326	328	5
CB99	326	37.062	326	328	5
CB97	326	38.267	326	328	5
CB110	326	39.277	326	328	5
CB123*	326	41.2	326	328	5
CB118*	326	41.563	326	328	5
CB105*	326	43.443	326	328	5
CB114*	326	42.2	326	328	5
¹³C-CB153	374	42.567	372	374	6
CB149	362	40.328	360	362	6
CB153	362	42.584	360	362	6
CB132	362	42.236	360	362	6
CB137	362	43.744	360	362	6
¹³C-CB138	374	44.437	372	374	6
CB138	362	44.487	360	362	6
CB158	362	44.663	360	362	6
CB128	362	46.307	360	362	6
¹³C-CB156	374	48.406	372	374	6
CB156*	362	48.366	360	362	6
CB167*	362	46.2	360	362	6
CB157*	362	48.698	360	362	6
¹³C-CB180	408	48.829	406	408	7
CB187	396	44.787	394	396	7
CB183	396	45.264	394	396	7
CB180	396	48.846	394	396	7
CB170	396	50.684	394	396	7
¹³C-CB189	406	53.182	406	408	7
CB189*	396	53.196	394	396	7
¹³C - CB194	442	57.504	442	440	8
CB198	430	50.347	430	428	8
CB194	430	57.514	430	428	8

*mono-ortho CBs

Table 2Possible target and qualifier ions for non-*ortho* CBs, including labelled internal standards

CB	Target ion (<i>m/z</i>)	Qualifier (<i>m/z</i>)	Qualifier (<i>m/z</i>)	Qualifier (<i>m/z</i>)
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

2.9.5 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, as column-specific co-elution of the target CBs with other CBs or other organochlorine compounds can occur on a single column. Using columns of differing selectivities can resolve these co-elution problems. The temperature programme used must be optimised for each column to achieve sufficient separation of the CB congeners to be determined. An isothermal period in the programme around 200-220°C of approximately 30 minutes is recommended. Care should be taken that CBs of interest do not coelute with other CB congeners (for example CB28 and CB31). 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. Shifts in retention times should be checked for different areas of the chromatogram by identifying characteristic, unmistakable peaks (*e.g.* originating from the internal standard or higher concentrated CBs such as CB153 and CB138). 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 resolved CBs. Since calibration curves for most CBs are usually non-linear when using GC-ECD, but should be linear when using GC-MS, a multilevel calibration of at least five concentrations is recommended. The calibration curve must be controlled and the best fit must be applied over the relevant concentration range. One should strive to work within the linear range of the detector. Analysis of the calibration solutions should be carried out in a mode encompassing the concentrations of the sample solutions (or alternatively by injecting matrix-containing sample solutions and matrix-free standard solutions distributed regularly over the series). When the chromatogram is processed with the help of automated integrators, the baseline may not always be set unambiguously and always needs to be inspected visually. When using GC-ECD, peak height is preferable to peak area for quantification purposes. From the two columns of different polarity the more reliable result (in terms of absence of co-elutions) should be reported.

Recent years have witnessed the emergence of so-called comprehensive two-dimensional gas chromatography (GC x GC) – a technique that can be used to considerably improve analyte/matrix as well as analyte/analyte separation. Briefly, a non-polar x (semi-)polar column combination is used, with a conventional 25 - 30 m long first-dimension, and a short, 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 the comprehensive approach are that the entire sample (and not one or a few heart-cuts, as in conventional multidimensional GC (Dallüge *et al.*, 2003) is subjected to a completely different separation, that the two-dimensional separation does not take any more time than the first-dimension run, and that the re-focusing in the modulator helps to increase analyte detectability. The most interesting additional benefit for CBs is, that structurally related as CB congeners show up as so-called ordered structures in the two-dimensional GC x GC plane. The very rapid second-dimension separation requires the use of detectors with sufficiently high data acquisition rates. Initially, only flame ionisation detectors could meet this requirement. However, today there is also a micro-ECD on the market that is widely used for GC x GC- μ ECD of halogenated compound classes. Even more importantly, analyte identification can be performed by using a time-of-flight mass spectrometer (Dallüge *et al.*, 2002) or - with a modest loss of performance, but at a much lower price - one of the very recently introduced rapid-scanning quadrupole mass spectrometers (Korytar *et al.*, 2005; Adahchour *et al.*, 2005). So far, the use of GC x GC has been limited to qualitative applications and still seems inappropriate for routine quantification of analytes.

3 Quality assurance

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. References of relevance to QA procedures include HELCOM, 1988; QUASIMEME 1992; Wells *et al.*, 1992; Oehlenschläger, 1994; Smedes *et al.*, 1994 and ICES, 1996.

3.1 System performance

The performance of the GC system should be monitored by regularly checking the resolution of two closely eluting CBs. A decrease in resolution points to deteriorating GC conditions. The signal-to-noise ratio yields information on the condition of the detector. A dirty ECD detector or MS ion source can be recognised by the presence of an elevated background signal together with a reduced signal-to-noise ratio. Chromatograms should be inspected visually by a trained operator.

3.2 Recovery

The recovery should be checked and reported. One method is to add an internal (recovery) standard to each sample immediately before extraction and a second (quantification) standard immediately prior to injection. If smaller losses occur in extraction or clean-up, or solutions are concentrated by uncontrolled evaporation of solvents (*e.g.* because vials are not perfectly capped), such losses can be compensated for by normalisation. If major losses are recognised and the reasons are unknown, the results should not be reported, as recoveries are likely to be irreproducible. A control for the recovery standard is recommended by adding the calibration solution to a real sample. Recoveries should be between 70 and 120%, if not, analysis of samples should be repeated.

3.3 Blanks

A procedural blank should be measured for each sample series and should be prepared simultaneously using the same chemicals and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will lead to errors in quantification. Even if an internal 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 standard cannot be adsorbed by a matrix. An alternative may be using a CB-free oil as a matrix blank.

3.4 Accuracy and precision

Analysis of a Laboratory Reference Material (LRM) should be included, at least one sample within each batch of samples. The LRM must be homogeneous, well characterised for the determinands in question and stability tests must have shown that it produces consistent results 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 determinant concentrations should occur in a comparable range to those of the samples. If the range of determinant concentrations in the sample is large (greater than a factor of 5) it is preferable to include two reference materials within each batch of analyses to cover the lower and upper concentrations. It is good practice to run duplicate analyses of a reference material to check within-batch analytical variability. A quality control chart should be recorded for a selected set of CBs. When introducing a new LRM or when it is suspected from the control chart that there is a systematic error possibly due to an alteration of the material, a relevant Certified Reference Material (CRM) of a similar matrix to the material analysed should be used to check the LRM. Additionally a duplicate of at least one sample should be run with every batch of samples. Each laboratory should participate in interlaboratory comparison studies and proficiency testing schemes on a regular basis, preferably at an international level.

3.5 Data collection and reporting

The calculation of results and the reporting of data can represent major sources of error. Control procedures should be established in order to ensure that data are correct and to obviate transcription errors. Data stored on databases should be checked and validated, and checks are also necessary when data are transferred between databases. If possible data should be reported in accordance with the latest ICES reporting formats.

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Annex 6: Revised technical annex on the analysis of PCBs in sediments

Determination of chlorobiphenyls in sediments - analytical method

1. Introduction

This annex provides advice on (chlorinated biphenyl) CB analysis for all sediment fractions and suspended particulate matter (*e.g.* < 2mm fraction and < 20µm fraction). The guideline is an update of the earlier version (Smedes and de Boer, 1994 and 1997) taking into account evolutions in the field of analytical chemistry and also covering the determination of planar CBs. Basically, these consist of mono-*ortho* (CB105, CB114, CB118, CB123, CB156, CB157, CB167 and CB189) and non-*ortho* substituted CBs (CB81, CB77, CB126 and CB169). When reviewing the literature, it should be noted that planar, coplanar and dioxin-like CBs / PCBs are all equivalent terms. OSPAR SIME has advised that monitoring for planar CBs in sediments should only take place when the concentrations of marker (non-planar) CBs are *e.g.* 100 times higher than the Background Assessment Concentrations for those compounds.

The analysis of CBs in sediments generally involves extraction with organic solvents, clean-up (removal of sulphur and column fractionation), and gas chromatographic separation with electron-capture or mass spectrometric detection. All stages of the procedure are susceptible to insufficient recovery of analytes and/or contamination. Quality control procedures are recommended in order to check the method's performance. These guidelines 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. Due to the low concentrations of, particularly, non-*ortho* substituted CBs in sediments compared to those of other CBs, their determination requires an additional separation and concentration step. Therefore, in the relevant sections a distinction will be made between the non-*ortho* substituted CBs and the others.

These guidelines can also be used for several other groups of organochlorine compounds, *e.g.* DDTs and their metabolites, chlorobenzenes and hexachlorocyclohexanes. Recoveries in the clean-up procedures must be checked carefully. In particular, treatment with H₂SO₄ results in a loss of some compounds (*e.g.* dieldrin and endosulfanes (de Boer and Wells, 1996). Also, the clean-up procedure with silver ions can result in low recoveries for some pesticides (*e.g.* hexachlorocyclohexanes).

These guidelines are not intended as a complete laboratory manual. If necessary, guidance should be sought from highly specialised research laboratories. Whichever analytical procedure is adopted, each laboratory must demonstrate the validity of each step in the procedure. In addition, the use of a second (and different) method, carried out concurrently with the routine procedure is recommended for validation. Analyses must be carried out by experienced staff.

2. Sampling and storage

Plastic materials (except polyethylene or polytetrafluorethene) must not be used for sampling due to the possible adsorption of contaminants onto the container material. Samples should be stored in solvent washed aluminium cans or glass jars. Aluminium cans are preferred, as glass jars are more susceptible to breakage. Samples should be transported in closed containers; a temperature of 25°C should not be exceeded. If samples are not analysed within 48 h after sampling, they must be stored in the short term at 4°C. Storage over several months or longer should be limited to those samples which have been frozen (< -20°C) and dried samples.

3. Precautionary measures

Solvents, chemicals and adsorption materials must be free of CBs or other interfering compounds. If not they should be purified using appropriate methods. Solvents should be checked by concentrating the volume normally used in the procedure to 10% of the final volume and then determining the presence of CBs and other interfering compounds by GC analysis. If necessary, the solvents can be purified by re-distillation but this practice is not favoured by most analytical laboratories as they generally opt to buy high quality solvents directly. Chemicals and adsorption materials should be purified by extraction and/or heating. Glass fibre materials (*e.g.* Soxhlet thimbles and filter papers used in pressurised liquid extraction (PLE)) should be cleaned by solvent extraction or pre-baked at 450°C overnight. Alternatively, glass thimbles with a G1 glass filter at the bottom can be used. Generally, paper filters should be avoided and substituted by appropriate glass filters. As all super cleaned materials are prone to contamination (*e.g.* by the adsorption of CBs and other compounds from laboratory air), materials ready for use should be held in sealed containers and should not be stored for long periods. All containers, tools, glassware *etc.* 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, heated at > 250°C and rinsed immediately before use with organic solvents or mixtures such as hexane/acetone. In addition, all glassware should preferably be covered with aluminium foil and stored in cupboards to keep out any dust. Old and scratched glassware is more likely to cause blank problems because of the larger surface and

therefore greater chance of adsorption. Furthermore, scratched glassware can be more difficult to clean. All glassware should be stored in clean cupboards, ensuring dust cannot enter (QUASIMEME, 2007)

4. Pre-treatment

Before taking a subsample for analysis, the samples should be sufficiently homogenised.

CBs can be extracted from wet or dried samples, although storage, homogenisation and extraction are much easier when the samples are dry. Drying the samples, however, may alter the concentrations *e.g.* by the loss of compounds through evaporation or by contamination (Smedes and de Boer, 1994 and 1997). Losses and contamination must be accounted for.

Chemical drying can be performed by grinding with Na_2SO_4 or MgSO_4 until the sample reaches a free-flowing consistency. It is essential that there are at least several hours between grinding and extraction to allow for complete dehydration of the sample; any residual water will decrease the extraction efficiency.

Freeze-drying is becoming a more popular technique, although its application should be carefully considered. Possible losses or contamination must be checked. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0°C . Contamination during freeze-drying is reduced by putting a lid, with a hole of about 3 mm in diameter, on the sample container.

5. Extraction

The target compounds must be extracted from the sediment with an organic solvent prior to analysis. Extraction methods do not differ for planar CBs but, because of the low concentrations, a substantially larger sample intake has to be considered. Generally, at least a 100 g sample of freeze-dried sediment is required.

5.1 Wet sediments

Wet sediments are extracted in a step-wise procedure by mixing them with organic solvents. Extraction is enhanced by shaking, Ultra Turrax mixing, ball mill tumbler or ultrasonic treatment. Water miscible solvents are used (especially in the first step) such as methanol, acetone, acetonitrile, *etc.* The extraction efficiency of the first step is low as there will be a considerable amount of water in the liquid phase at that stage. The extraction is continued with a mixture of polar and apolar solvents (*e.g.* acetone/hexane or methanol/dichloromethane). For adequate extraction of target compounds, wet sediments must be extracted with organic solvents at least three times. The contact time with the solvent should be sufficient to complete the desorption of the CBs from the sediment.

When using a Soxhlet, extraction of wet sediments should be done in two steps. A polar solvent, such as acetone, is first used to extract the water from the sediment and then the flask is replaced and the extraction continued with a polar/apolar mixture such as acetone/hexane.

In both cases water must be added to the combined extracts and the CBs must be extracted to an apolar solvent such as hexane.

5.2 Dry sediments

For dried sediments, Soxhlet extraction is the most frequently used technique. A mixture of a polar and an apolar solvent (*e.g.* acetone/hexane) is recommended for efficient extraction; a good choice is 25% acetone in hexane. A greater proportion of polar solvent increases the extraction efficiency, but the polar solvent must be removed prior to gas chromatographic analysis. Extraction can be carried out with a normal Soxhlet or a hot Soxhlet apparatus. A sufficient number of extraction cycles must be performed (ca. 8 h for the hot Soxhlet and ca. 12 to 24 h for normal Soxhlet extraction). The extraction efficiency must be checked for different types of sediments by a second extraction step. These extracts should be analysed separately.

Although the use of binary non-polar/polar solvent mixtures and Soxhlet is still the benchmark for CB extraction, there have been numerous attempts to find alternative procedures, which are less time-consuming, use less solvent and/or enable miniaturisation. 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).

From among the techniques mentioned, PLE or Accelerated Solvent Extraction (ASE) has – so far – been most successful. 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 florisisil

or alumina, to the cell, and the use of a small carbon column in the extraction cell, 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 that they are matrix-independent, which facilitates method development and changing-over from the classical Soxhlet extraction. Recent years have also seen an increased use of ultrasound-based techniques for analytes isolation from solid samples. With most applications, extraction efficiency is fully satisfactory, and sonication time often is 30 min or less (Roose and Brinkman, 2005).

All the methods described above are in principle suitable for extracting CBs from sediments. However, Soxhlet extraction is still the reference for alternative approaches.

6. Clean-up

6.1 Removal of sulphur and sulphur-containing compounds

An aqueous saturated Na_2SO_3 solution is added to a hexane extract. In order to allow the transfer of the HSO_3^- ions to the organic phase, tetrabutylammonium salts (TBA) and *iso*-propanol are then added to the mixture. Water is subsequently added to remove the *iso*-propanol. The aqueous phase must then be quantitatively extracted with hexane (Jensen *et al.*, 1977). If the extraction was performed by a polar solvent miscible with water, then a Na_2SO_3 solution can be added directly after extraction. If the extraction mixture also contains an apolar solvent, then depending on the ratio of the solvents, the addition of TBA and *iso*-propanol may or may not be necessary. Any excess Na_2SO_3 and reaction products can be removed by the addition of water and thus partitioning between apolar solvent and water.

Japenga *et al.* (1987) developed a column method for the removal of sulphur and sulphur-containing compounds. The column material is made by mixing an aqueous solution of Na_2SO_3 with Al_2O_3 . Some NaOH is also added to improve the reaction with sulphur. Subsequently the material is dried under nitrogen until a level of deactivation equivalent to 10 % water is reached. Storage must be under nitrogen because sulphite in this form may easily be oxidised to sulphate. Eluting the extract (hexane) through a column filled with this material results in removal of the sulphur in combination with further clean-up of the sediment extract. The sulphur removal properties are somewhat difficult to control.

Mercury, activated copper powder, wire or gauze (Smedes and de Boer, 1994 and 1997; Wade and Cantillo, 1996) remove the sulphur directly from an organic solvent. Although mercury is appropriate for removing sulphur, it should be avoided for environmental reasons. Copper can be applied during or after Soxhlet extraction. Ultrasonic treatment might improve the removal of sulphur. If sulphur appears to be present in the final extract the amount of copper or mercury used was insufficient and the clean-up procedure must be repeated.

Silver ions strongly bind sulphur and sulphur compounds. Loaded onto silica, AgNO_3 is a very efficient sulphur removing agent. It can be prepared by mixing dissolved AgNO_3 with silica and subsequently drying under nitrogen. Compounds containing aromatic rings are strongly retained, but for CBs this retention is reduced, probably due to shielding of the rings by the chlorine atoms. Retained compounds can easily be eluted by using cyclohexene, or another solvent with double bonds, as a modifier (Eganhouse, 1986; Japenga *et al.*, 1987).

Elemental sulphur is strongly retained on a polystyrene-divinylbenzene copolymer column as generally applied for gel permeation chromatography (GPC). In addition, GPC combines sulphur removal with a clean-up stage.

All these methods have advantages and disadvantages. For different samples, the use of multiple methods may sometimes prove necessary. Several of the methods leave some aromatic sulphur compounds in the extract. These compounds elute from the GC column at similar retention times to some of the lower-chlorinated CBs. The major part of these compounds can be removed by eluting an apolar extract over a column with silica loaded with concentrated H_2SO_4 . Other interfering compounds (*e.g.* phthalates and fatty acid esters) are also removed by using this procedure.

6.2 Further clean-up

The extraction procedures above will result in the co-extraction of many compounds other than CBs. The extract may be coloured due to pigments extracted from sediment, and may also contain sulphur and sulphur-containing compounds, oil, PAHs and many other natural and anthropogenic compounds which will need to be removed from the extract. Different clean-up 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. Most CBs are stable under acid conditions; therefore treatment with sulphuric acid or acid impregnated silica columns may be used in the clean-up.

The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography, but gel permeation chromatography (GPC) is also employed.

As CBs are apolar, clean-up using normal-phase chromatography is the most appropriate technique for the separation from other compounds. Using an apolar solvent (*e.g.* hexane or *iso*-octane) as an eluent, CBs normally elute very rapidly. All polar solvents used in the extraction or sulphur removal step should be removed before further clean-up. The last concentration step is usually performed by evaporation with a gentle stream of nitrogen. Evaporation to dryness should always be avoided.

Deactivated Al₂O₃ (with 5 - 10% water) is often used as a primary clean-up method. Provided that sulphur has been removed beforehand, Al₂O₃ clean-up sometimes yields a sufficiently clean extract for a GC-ECD analysis of the sample to be performed. Al₂O₃ removes lipid compounds from the extracts (although samples with a very high lipid content and low CB concentrations may require additional clean-up).

Deactivated silica (with 1 - 5% water) does not retain CBs (including planar CBs) and only slightly retains polycyclic aromatic hydrocarbons (PAHs) when eluted with hexane or *iso*-octane. When organochlorine pesticides are also to be determined in the same extract, deactivation of the silica with a few percent of water is essential.

For high activity silica (overnight at 180°C) the retention of CBs is negligible, while PAHs are more strongly retained. The CBs 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.

For the separation of CBs from lipids or oil components, reversed-phase HPLC can be used. In reversed-phase chromatography CBs elute during a solvent gradient of 80 to 90% methanol, together with numerous other compounds of similar polarity. Most of the above mentioned extraction methods and clean-up procedures yield an extract containing an apolar solvent. These cannot be injected directly for reversed-phase chromatography, and so compounds must be transferred between solvents several times *e.g.* before injection and after elution. When using polar solvents for extraction (*e.g.* for wet sediments) reversed-phase columns could be used directly for clean-up. When eluting an acetonitrile extract from a C₁₈ solid phase extraction (SPE) column with acetonitrile, high molecular hydrocarbons are strongly retained while CBs elute in the first few column volumes.

The above mentioned normal-phase chromatographic procedures on silica and Al₂O₃ can be transferred to HPLC having the advantages of higher resolution and better reproducibility.

When using GPC the elution of CBs 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, a second clean-up step is often required to separate the CBs 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 as the detection method. If Soxhlet extraction is used for biota, then there is a much greater quantity of residual lipid to be removed than in the case of PLE with fat retainers. An additional clean-up stage may therefore be necessary. Methods have been developed for online clean-up and fractionation of dioxins, furans and CBs with PLE for food, feed and environmental samples (Sporring *et al.*, 2003). The first method utilises a fat retainer for the on-line 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* CBs require a more specialised clean-up, similar to that which is generally associated with the analysis of dioxins and furans. Although initial clean-up may very well proceed along the lines described above, the larger sample intake results in even larger amounts of 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. Often, more rigorous procedures are applied to remove the excess material by *e.g.* shaking the sample with concentrated sulphuric acid. A more efficient and safer alternative is to elute the sample over a silica column impregnated with sulphuric acid (40 % w/w).

Non-*ortho* CBs are nearly always separated from the other CBs using advanced separation techniques. A very efficient method is to inject the extracts (after concentrating them) into a HPLC system coupled to PYE (2-(1-pyrenyl) ethyldimethylsilylated silica) column. Column dimensions are typically 4.6 x 150 mm column, but combinations of several columns in-line are sometimes used. PYE columns not only allow the separation of *ortho*, mono-*ortho* and non-*ortho* CBs on the basis of structural polarity from each other but also from dibenzo-*p*-dioxins and dibenzofurans. The eluting solvent is an apolar solvent such as *iso*-hexane. When coupled to a fraction collector, the use of a HPLC system allows the automatic clean-up of a considerable number of samples. Alternatively, HPLC systems equipped with porous graphite carbon. Column sizes are in the order of 50 x 4.7 mm and care has to be taken that the column is not overloaded. Similarly to PYE columns, they will separate non-*ortho* CBs from the others and from dioxins and furans. Fully automated systems, such as Powerprep, that combine several steps are routinely used (Focant and De Pauw, 2002).

7. Pre-concentration

Evaporation of solvents with a rotary-film evaporator was up until recent the common method. However, evaporation of solvents using this technique should be performed at low temperature (water bath temperature of $\leq 30^{\circ}\text{C}$) and under controlled pressure conditions, in order to prevent losses of the more volatile CBs. To reduce the sample to the final volume, solvents can be removed by blowing-down with gently streaming nitrogen. Only nitrogen of a controlled high quality should be used. As a solvent for the final solution to be injected into the GC, *iso*-octane is recommended.

Turbovap sample concentrators can also be used to reduce solvent volume. This is a rapid technique, but needs to be carefully optimised and monitored to prevent both losses (both of volatiles and solvent aerosols) and cross-contamination. The use of rotary-film evaporators is more time consuming but more controllable. Here also, evaporation to dryness should be avoided at all costs. Syncore parallel evaporators (Buchi, Switzerland) can be used with careful optimisation of the evaporation parameters. The Buchi Syncore Analyst also uses glass tubes but the system is sealed, avoiding contamination from the lab air during evaporation. It does not use a nitrogen stream, thus reducing the loss of volatiles and if the flashback module is fitted the sides of the tubes are rinsed automatically thus reducing the loss of the heavier components. Again water-bath temperatures should be minimised to prevent losses. When reducing the sample to the required final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the gas chromatograph (GC) include hexane, heptane, toluene and *iso*-octane.

7. Calibration and preparation of calibrant solutions

Internal standards (recovery and quantification standards) should be added in a fixed volume or weight to all standards and samples. The ideal internal standard contains no CBs or negligible (non-detectable) concentrations and does not co-elute with other CBs. All CBs with a 2,4,6-substitution (e.g. CB115, CB155, CB198) are, in principle, suitable. Alternatively, 1,2,3,4-tetrachloronaphthalene or homologues of dichloroalkylbenzylether can be used. For GC analysis with mass selective detection (GC-MS), ^{13}C labelled CBs should be used for each degree of chlorination. This especially critical for the non-*ortho* CBs. If possible, the labelled calibrant solutions should correspond to the unlabelled determinants. For the non-*ortho* CBs a labelled standard is available for each congener and use of all of them is recommended. When preparing a calibration solution for a new determinant for the first time, two independent stock solutions of different concentrations should always be prepared simultaneously to allow cross checking. A new calibration solution should also be cross-checked to the old standard solution. Crystalline CBs of known purity should always be used for preparing calibration solutions. If the quality of the standard materials is not guaranteed (e.g. as in the case for a Certified Reference Material) by the producer or supplier, it should be checked by GC preferably with mass spectrometric detection. Solid standards should be weighed to a precision of 10^{-5} grams. In recent years, a lot of certified commercial custom made standards have become available and laboratories have been switching to these. Calibration solutions should preferably be stored in ampoules in a cool and dark place. When stored in containers the weight loss during storage should be recorded.

8. Instrumental determination

8.1 Injection techniques

The two modes commonly used are splitless and on-column injection. In split 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 light packing of (silylated) glass wool may improve the performance for CBs, 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 negative effects previously associated with that, but should be thoroughly optimised before use. Increasing the injection volume will allow either or both the elimination of an extra evaporation step and lowering the detection limits.

8.2 Carrier gas

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

8.3 Columns

Only capillary columns should be used. The following parameters are recommended:

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 .

Columns which do not fulfil these requirements generally do not offer sufficient resolution to separate CB28, CB105 and CB156 from closely eluting CBs. A wide range of stationary phases can be used for CB separation. The chemical composition is different for many producers and depends on the maximum temperature at which the column can be operated. Further advice may be found in the producer's catalogues, where compositions, applications and tables to compare products from different manufacturers are included.

In recent years, new chromatographic phases have become available that result in an improved separation of critical CB pairs. A good example is the HT-8 phase (1,7-dicarba-closo-dodecarborane phenylmethyl siloxane) (Larsen *et al.*, 1995) that shows a remarkable selectivity for CBs. This column is currently recommended for CB analysis. Examples of the retention times for various CBs are given in Table 1.

8.4 Detection

The electron capture detector (ECD) is still frequently used for CB analysis. Injection of chlorinated solvents or oxygen-containing solvents should therefore be avoided due to the generation of large interfering signals. When using mass selective detectors (MSD) negative chemical ionisation mode (NCI) is extremely sensitive for pentachlorinated to decachlorinated CBs and is approximately ten fold better than ECD. However, MS systems have improved considerably allowing analysis by Electron impact ionisation (EI), whereas before, electron-capture negative ion chemical ionisation (ECNICI) was often necessary in order to detect the low concentrations of, in particular the planar CBs. Suggested target and qualifier ions for *ortho* CBs (including non-*ortho* CBs) are shown in Table 1 and in Table 2 for non-*ortho* CBs.

Table 1

Example of retention times for selected CB congeners using a 50 m HT8 column (0.25 mm i.d. and 0.25 µm film), along with possible target and qualifier ions. 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.

CB congener	MW	RT	Target Ion	Qualifier Ion	Number of chlorines
¹³C-CB28	270	28.371	268	270	3
CB31	258	28.071	256	258	3
CB28	258	28.388	256	258	3
¹³C-CB52	304	30.317	304	302	4
CB52	292	30.336	292	290	4
CB49	292	30.698	292	290	4
CB44	292	32.024	292	290	4
CB74	292	34.881	292	290	4
CB70	292	35.199	292	290	4
¹³C-CB101	340	36.612	338	340	5
CB101	326	36.630	326	328	5
CB99	326	37.062	326	328	5
CB97	326	38.267	326	328	5
CB110	326	39.277	326	328	5
CB123*	326	41.2	326	328	5
CB118*	326	41.563	326	328	5
CB105*	326	43.443	326	328	5
CB114*	326	42.2	326	328	5
¹³C-CB153	374	42.567	372	374	6
CB149	362	40.328	360	362	6
CB153	362	42.584	360	362	6
CB132	362	42.236	360	362	6
CB137	362	43.744	360	362	6
¹³C-CB138	374	44.437	372	374	6
CB138	362	44.487	360	362	6
CB158	362	44.663	360	362	6
CB128	362	46.307	360	362	6
¹³C-CB156	374	48.406	372	374	6
CB156*	362	48.366	360	362	6
CB167*	362	46.4**	360	362	6
CB157*	362	48.698	360	362	6
¹³C-CB180	408	48.829	406	408	7
CB187	396	44.787	394	396	7
CB183	396	45.264	394	396	7
CB180	396	48.846	394	396	7
CB170	396	50.684	394	396	7
¹³C-CB189	406	53.182	406	408	7
CB189*	396	53.196	394	396	7
¹³C - CB194	442	57.504	442	440	8
CB198	430	50.347	430	428	8
CB194	430	57.514	430	428	8

*mono-ortho CBs, ** to be checked

Next to conventional GC-MS, the use of ion-trap with its tandem MS² option - i.e., yielding improved selectivity - is receiving increased attention. The use of GC-ITMS provides a less expensive alternative to high-resolution mass spectrometry (HRMS), which is commonly used to determine PCDD/F and, as such, also ideally suited for all CB groups (Epe *et al.*, 2004).

Table 2Possible target and qualifier ions for non-*ortho* CBs, including labelled internal standards

CB	Target ion (<i>m/z</i>)	Qualifier (<i>m/z</i>)	Qualifier (<i>m/z</i>)	Qualifier (<i>m/z</i>)
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

8.5 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, as column-specific coelution of the target CBs with other CBs or organochlorine compounds occurs. The temperature programme must be optimised for each column to achieve sufficient separation of the CB congeners to be determined. An isothermal period in the programme around 200-220°C of approximately 30 minutes is recommended. Care should be taken that CBs of interest do not co-elute with other CB congeners (for example CB28 and CB31). 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. Shifts in retention times should be checked for different areas of the chromatogram by identifying characteristic, unmistakable peaks (*e.g.* originating from the internal standard or higher concentrated CBs such as CB153 and CB138). When 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 CBs. Since calibration curves of most CBs normally non-linear when using a GC-ECD, but should be linear for GC-MS, a multilevel calibration of at least five concentrations is recommended. The calibration curve must be controlled and the best fit must be applied for the relevant concentration range. Otherwise, one should strive to work in the linear range of the detector must. Analysis of the calibration solutions should be carried out in a mode encompassing the concentrations of the sample solutions (or alternatively by injecting matrix-containing sample solutions and matrix-free standard solutions distributed regularly over the series). When the chromatogram is processed with the help of automated integrators the baseline is not always set unambiguously and always needs to be inspected visually. When using GC-ECD, peak height is preferable to peak area for quantification purposes. From the two columns of different polarity the more reliable result should be reported.

Recent years have witnessed the emergence of so-called comprehensive two-dimensional gas chromatography (GC x GC) – a technique that can be used to considerably improve analyte/matrix as well as analyte/analyte separation. Briefly, a non-polar x (semi-)polar column combination is used, with a conventional 25-30 m long first-dimension, and a short, 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 the comprehensive approach are that the entire sample (and not one or a few heart-cuts, as in conventional multidimensional GC (Dallüge *et al.* 2003) is subjected to a completely different separation, that the two-dimensional separation does not take any more time than the first-dimension run, and that the re-focusing in the modulator helps to increase analyte detectability. The most interesting additional benefit for CBs is, that structurally related as CB congeners show up as so-called ordered structures in the two-dimensional GC x GC plane. The very rapid second-dimension separation requires the use of detectors with sufficiently high data acquisition rates. Initially, only flame ionisation detectors could meet this requirement. However, today there is also a micro-ECD on the market that is widely used for GC x GC- μ ECD of halogenated compound classes. Even more importantly, analyte identification can be performed by using a time-of-flight mass spectrometer [Dallüge *et al.*, 2002] or - with a modest loss of performance, but at a much lower price - one of the very recently introduced rapid-scanning quadrupole mass spectrometers [Korytar *et al.*, 2005; Adachour *et al.*, 2005). So far, the use of GC x GC has been limited to qualitative applications and still seems inappropriate for routine quantification of analytes.

9. Quality assurance

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. The limit of determination should depend on the purpose of the

investigation. A limit of at least 0.1 ng/g (dry weight, fraction < 2mm) should be reached, but detection limits of 0.01 ng/g are achievable nowadays. The method for calculating the limit of determination should reflect QUASIMEME advice (Topping *et al.*, 1992). The limit of determination that can be achieved depends on the blank, on the sample matrix, on concentrations of interfering compounds and on the mass of sediment taken for analysis. References of relevance to QA procedures include HELCOM, 1988; QUASIMEME 1992; Wells *et al.*, 1992; Oehlenschläger, 1994; Smedes *et al.*, 1994 and ICES, 1996.

9.1 System performance

The performance of the GC system should be monitored by regularly checking the resolution of two closely eluting CBs. A decrease in resolution points to deteriorating GC conditions. The signal-to-noise ratio yields information on the condition of the detector. A dirty ECD detector or MS ion source can be recognised by the presence of an elevated background signal together with a reduced signal-to-noise ratio. Chromatograms should be inspected visually by a trained operator.

9.2 Recovery

The recovery should be checked and reported. One method is to add an internal (recovery) standard to each sample immediately before extraction and a second (quantification) standard immediately prior to injection. If smaller losses occur in extraction or clean-up or solutions are concentrated by uncontrolled evaporation of solvents (*e.g.* because vials are not perfectly capped) losses can be compensated for by normalisation. If major losses are recognised and the reasons are unknown, the results should not be reported, as recoveries are likely to be irreproducible. A control for the recovery standard is recommended by adding the calibration solution to a real sample. Recoveries should be between 70 and 120%, if not, samples should be repeated.

10. References

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Annex 7. Status of work to define background concentrations for dioxins in biota

Based upon an analysis of data available currently, a series of minimum levels for dioxins, furans and planar CBs (summed as TEQs – toxic equivalents of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) in biota have been defined. These data were mostly derived from consumer protection studies. It is proposed that these be used by OSPAR as interim assessment criteria pending the development of Background Concentrations and/or Background Assessment Concentrations. These will be difficult to establish due to a paucity of background data from remote or pristine areas.

Table 1 Minimum values for sum of PCDD/Fs from selection of lowest contaminated locations of reviewed data sets (pg WHO-TEQ PCDD/F g⁻¹ wet weight)

	Fish muscle	Bivalves	Crustaceans
Mean	0,17	0,09	0,17
SD	0,13	0,09	0,08
RSD,%	75	98	50
Median	0.150	0.059	0.149
1.quartile (25%)	0.062	0.022	0.100
10-percentile	0.016	0.012	0.089
Minimum	0.010	0.001	0.060
Maximum of selected data (cut off)	0.5	0.323	0,3
n	49	17	10
<i>Mean PCDD/F limi of quamtification*</i>		<i>0.024 ± 0.022 pg g⁻¹ ww ; sample intake ca. 24 g</i>	

OSPAR's MON Working Group could consider the medians of minimum values as indicative low concentrations; 0.15 pg WHO-TEQ PCDD/F g⁻¹ wet weight for marine fish muscle, and 0.06 pg WHO-TEQ PCDD/F g⁻¹ wet weight for bivalve molluscs. These are crude estimates (e.g. species differences are not accounted for), based on extremely limited data, but these values could be used on a trial basis.