ICES TECHNIQUES IN MARINE ENVIRONMENTAL SCIENCES

NO. 50

MAY 2012

Determination of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in biota and sediment

Katrin Vorkamp • Patrick Roose • Philippe Bersuder
Lynda Webster • Peter Lepom • Catherine Munschy
Rossana Bossi • Jacek Tronczynski • Jacob de Boer

ICES International Council for the Exploration of the Sea
CIEM Conseil International pour l'Exploration de la Mer

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

Recommended format for purposes of citation:

Vorkamp, K., Roose, P., Bersuder, P., Webster, L., Lepom, P., Munschy, C., Bossi, R., Tronczynski, J., and de Boer, J. 2012. Determination of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in biota and sediment. ICES Techniques in Marine Environmental Sciences No. 50. 23 pp.

Series Editor: Paul D. Keizer

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

ISBN 978-87-7482-109-0

ISSN 0903-2606

Contents

Abstract

Polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) are environmental contaminants regulated by the Stockholm Convention of Persistent Organic Pollutants. Being hydrophobic and lipophilic, these compounds accumulate in the marine environment in sediments and lipid-rich tissue of marine organisms, making these matrices preferred media for environmental monitoring. This document focuses on the analysis of PCDD/Fs and dioxin-like PCBs (i.e. non-*ortho* and mono-*ortho* PCBs), which have a similar planar molecular structure to PCDD/Fs and, therefore, exhibit similar toxic effects. Because concentrations in the environment are low and common analytical methods result in co-extractions of a large variety of potentially interfering compounds, analytical procedures are complex. This document includes comments and advice on sampling and sample pretreatment steps, suitable extraction and clean-up procedures as well as preconcentration methods. It highlights the importance of extract clean-up and the risk of contamination. Furthermore, suitable methods for instrumental analysis are discussed for gas chromatographic separation, compound identification, and quantification and detection methods. Although high-resolution mass spectrometry often is the method of choice, low-resolution mass spectrometry can also provide sufficiently sensitive analyses, in particular for screening purposes. In this context, bioassays can also play a role, reflecting a cumulative toxicity rather than concentrations of individual congeners. The paper also discusses general aspects of good laboratory practice, quality assurance/quality control, and laboratory safety.

1 Introduction

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans [dioxins/furans (PCDD/Fs)] are ubiquitous in the environment, primarily as unintentional byproducts of combustion and industrial processes. Because they are 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 relatively 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; Pandelova *et al.*, 2008). This guideline only addresses the 17 tetra- through octa-chlorinated 2,3,7,8-substituted PCDD/F congeners and the non- and mono-*ortho* substituted polychlorinated biphenyls (PCBs). The general chemical structures of PCDD/Fs and PCBs are given in Figure 1.

In this document, the term "dioxin-like PCBs" (dl-PCBs) is used for the non- and mono-*ortho* PCB congeners listed in Table 1. The coplanar structure of non-*ortho* substituted PCB congeners allows a configuration similar to that of PCDD/Fs. Mono-*ortho* substituted PCBs may take a steric position close to coplanarity and are consequently less toxic than non-*ortho* PCBs. Nevertheless, they may exhibit some dioxin-like effects, because of their relatively high concentrations compared with those of non-*ortho* PCBs or PCDD/Fs (Daelemans *et al.*, 1992). As with PCDD/Fs, the human exposure to dl-PCBs is mainly via the food chain, because the compounds are highly lipophilic and bioaccumulate in lipid-rich tissue and biomagnify through the food chain (Dyke *et al.*, 2003).

PCDD/Fs and dl-PCBs have been demonstrated to produce various toxic responses, including immunotoxicity, developmental and reproductive effects, and carcinogenesis (OSPAR, 2005). Because of their persistence, high toxicity, bioaccumulation potential, and ability for long-range transport, they are controlled under the Stockholm Convention for Persistent Organic Pollutants. Their spatial and temporal monitoring in the aquatic environment is important to evaluate the risk to wildlife and human health (Hurst *et al.*, 2004).

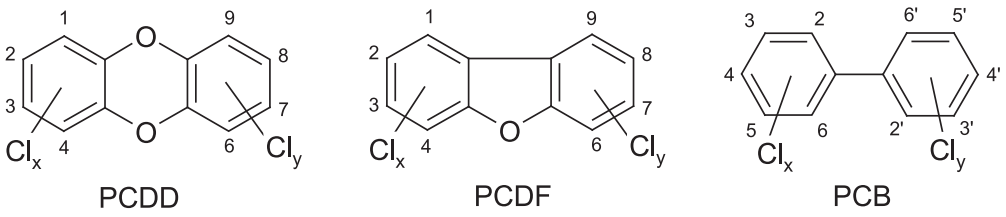

Figure 1. General formula of PCDDs, PCDFs, and PCBs. The possible number of chlorine atoms results in 75 PCDD congeners (x = 1–4, y = 0–4), 135 PCDF congeners (x = 1–4, y = 0–4), and 209 PCB congeners (x = 1–5, y = 0–5).

The objective of this document is to give general advice on the analysis of PCDD/Fs and dl-PCBs in marine biota and sediments. Because of the very low concentrations at which adverse effects can be observed, the analytical methodology for the analysis of PCDD/Fs and dl-PCBs differs from those for other organochlorine compounds.

2 Analytes

Table 1 provides a list of the 17 tetra- through octa-chlorinated 2,3,7,8-substituted PCDD/Fs and the dl-PCBs congeners that are of highest environmental relevance and typically included in environmental monitoring. In this context, results should be reported as concentrations of individual PCDD/Fs and dl-PCB congeners in biota and sediment, i.e. in units of mass/mass, with relevant additional information on e.g. dry matter content of the sample (see Section 9 "Data reporting").

In the context of food and feed analysis and compliance checks with maximum residue limits, the concept of TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) toxicity equivalency factors (TEFs) is commonly used, to account for the combined toxicity of mixtures of 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, which 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 through their dioxin-like activity in TEQs, relative to the most potent 2,3,7,8-TCDD. The most commonly used TEF system is that of the World Health Organization (WHO-TEF/TEQ). It is reviewed regularly, and Table 1 presents the most recent values (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. For sediment samples in particular, the use of TEQs is of limited relevance (Van den Berg *et al.*, 2006). If appropriate, the TEF concept can be applied in a risk assessment of environmental concentrations. In this context, it will be important to state how concentrations below limits of quantification (LoQ) have been handled. To maximize information, results expressed as TEQ values should be reported as both upper bound and lower bound values (i.e. non-quantifiable concentrations have been replaced by LoQ and zero, respectively).

Table 1. Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dl-PCBs with their toxicity equivalency factors (TEFs) according to the systems developed by the World Health Organization (WHO_{2005}-TEF; Van den Berg *et al.*, 2006) and NATO/CCMS (I-TEF).

Homologue group	Congener	WHO_{2005}-TEF	I-TEF	IUPAC no.
PCDDs				
Tetra-CDD	2,3,7,8	1.0	1.0	
Penta-CDD	1,2,3,7,8	1.0	0.5	
Hexa-CDD	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	
Hepta-CDD	1,2,3,4,6,7,8	0.01	0.01	
Octa-CDD	1,2,3,4,6,7,8,9	0.0003	0.001	
PCDFs				
Tetra-CDF	2,3,7,8	0.1	0.1	
Penta-CDF	1,2,3,7,8	0.03	0.05	
	2,3,4,7,8	0.3	0.5	
Hexa-CDF	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	
	2,3,4,6,7,8	0.1	0.1	
Hepta-CDF	1,2,3,4,6,7,8	0.01	0.01	
	1,2,3,4,7,8,9	0.01	0.01	
Octa-CDF	1,2,3,4,6,7,8,9	0.0003	0.001	
Non-*ortho* PCBs				
Tetra-CB	3,3',4,4'	0.0001		77
	3,4,4',5	0.0003		81
Penta-CB	3,3',4,4',5	0.1		126
Hexa-CB	3,3',4,4',5,5'	0.03		169
Mono-*ortho* PCBs				
Penta-CB	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
Hexa-CB	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
Hepta-CB	2,3,3',4,4',5,5'	0.00003		189

3 Sampling and sample handling

OSPAR (2005) presented a monitoring strategy for PCDD/Fs, which identified biota and sediment as important matrices for environmental monitoring, as a consequence of their accumulation. Generally, the same recommendations are valid as described for other organochlorine compounds (OSPAR, 2010a, 2010b). Details on sample handling and preparation as described for the chemical analysis of polybrominated diphenyl ethers (PBDEs) will also be valid for PCDD/Fs and dl-PCBs (Webster *et al.*, 2009). However, particular attention should be paid to the risk of sample contamination, given the lower concentrations of PCDD/Fs and dl-PCBs in most environmental samples.

3.1 Biota samples

Aquatic organisms can accumulate hydrophobic compounds like PCDD/Fs and dl-PCBs 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). Because the BCF varies with species and compound, it is important to design a biota-sampling programme that minimizes confounding factors, i.e. to choose the same species of the same age and the same time of year for sampling.

The species selected for monitoring of biota should fulfil the following requirements.

- Reflect concentration changes in the sampling area, i.e. ensure a link between exposure and concentration in the organisms;
- Accumulate compounds without being severely affected;
- Be representative of and abundant in the area (to ensure sufficient sample material for analysis);
- Be 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 dl-PCBs (OSPAR, 2005). The highest PCDD/F fresh-weight concentrations are found in muscle tissue of fatty fish, such as herring and salmon or in fatty liver of some fish, such as cod or haddock. National food agencies often analyse PCDD/Fs and dl-PCBs in commercial fish and fish products, to monitor compliance with EU limit values. Although different approaches will be necessary in environmental analyses, OSPAR (2005) also recommends the monitoring of fish and shellfish as part of the environmental monitoring strategy for dioxins.

Mussel samples must not be frozen prior to dissection, but should be transported at temperatures between 5 and 15°C, similar to those of the area of origin, in a clean container. It is important to remove any sediment particles from their intestinal system, by depuration in a glass aquarium with filtered water, preferably from the sampling location, for approximately 24 h. 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).

3.2 Sediment samples

The sampling strategy for sediments depends on the purpose of the monitoring programme and the natural conditions of the region to be monitored and includes

fixed-station sampling, stratified random sampling, stratified fixed sampling, or sediment cores. PCDD/Fs and dl-PCBs accumulate in the organic carbon fraction of the sediment. Therefore, sediments containing a large proportion of fine material are preferable for organic contaminant monitoring. Sediment samples consisting of coarse sandy material may require sieving (OSPAR, 2010b). Because sediments may often display a rather heterogeneous distribution of contaminants and organic carbon content, pooling of a number of subsamples from a specific area per sampling location is recommended.

3.3 Lipid and organic carbon content

The lipid content of biota samples should be determined to allow normalization of PCDD/F concentrations to lipids. Suitable methods have been described for the chemical analysis of PBDEs (Webster *et al.*, 2009) and include the methods by Bligh and Dyer (1959; modified by Hanson and Olley (1963)) and Smedes (1999). Extractable lipid determinations may be used as well, particularly for small sample sizes and high lipid contents. For both biota and sediment samples, dry weight should also be determined for normalization purposes, for instance by drying at 105°C until constant weight.

Total organic carbon (TOC) should be determined for characterization of sediments and for use as a possible normalization parameter (Schumacher, 2002; Leach *et al.*, 2008).

4 Analytical methods

Prior to systematic monitoring, an initial screening for PCDD/Fs and dl-PCBs may be undertaken in the area under consideration. Several methods are available for screening purposes. In particular, bioassays and fast semi-quantitative screening methods, based on gas chromatography (GC)–mass spectrometry (MS), can give an indication of the extent of the contamination (Schrock *et al.*, 2009).

For a full quantitative analysis of individual congeners, state-of-the-art GC with high-resolution MS (HRMS) is the preferred analytical method. Comprehensive multidimensional gas chromatography (GCxGC) could be an alternative for PCDD/F and dl-PCB analysis, even in combination with electron capture detection (ECD), because this will offer enough sensitivity and the selectivity will exceed that of single-column GC analysis, if a proper orthogonal combination of columns is selected (Haglund *et al.*, 2008). GCxGC can also be used in combination with low-resolution (LR) MS or time-of-flight (ToF)-MS (Skoczynska *et al.*, 2008). Modern GC tandem mass spectrometry (GC-MS-MS) systems based on ion traps and triple stage quadrupoles also provide the necessary selectivity and sensitivity (Malavia *et al.*, 2008; Onwudili *et al.*, 2011).

Examples of suitable methods for the analysis of PCDD/Fs and dl-PCBs in biota and sediment samples are given in Figures 2 and 3, respectively.

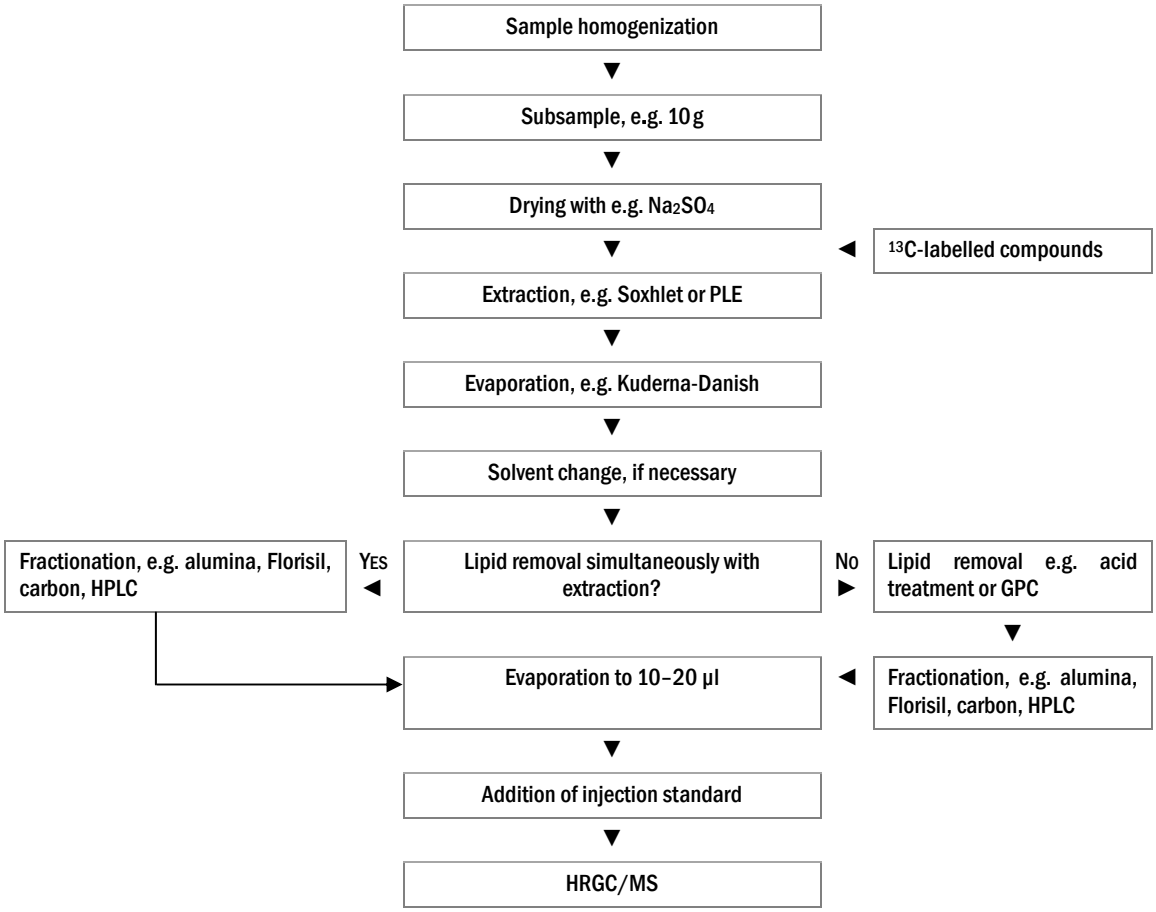

Figure 2. Example of an analytical method for the determination of PCDD/Fs and dl-PCBs in biota.

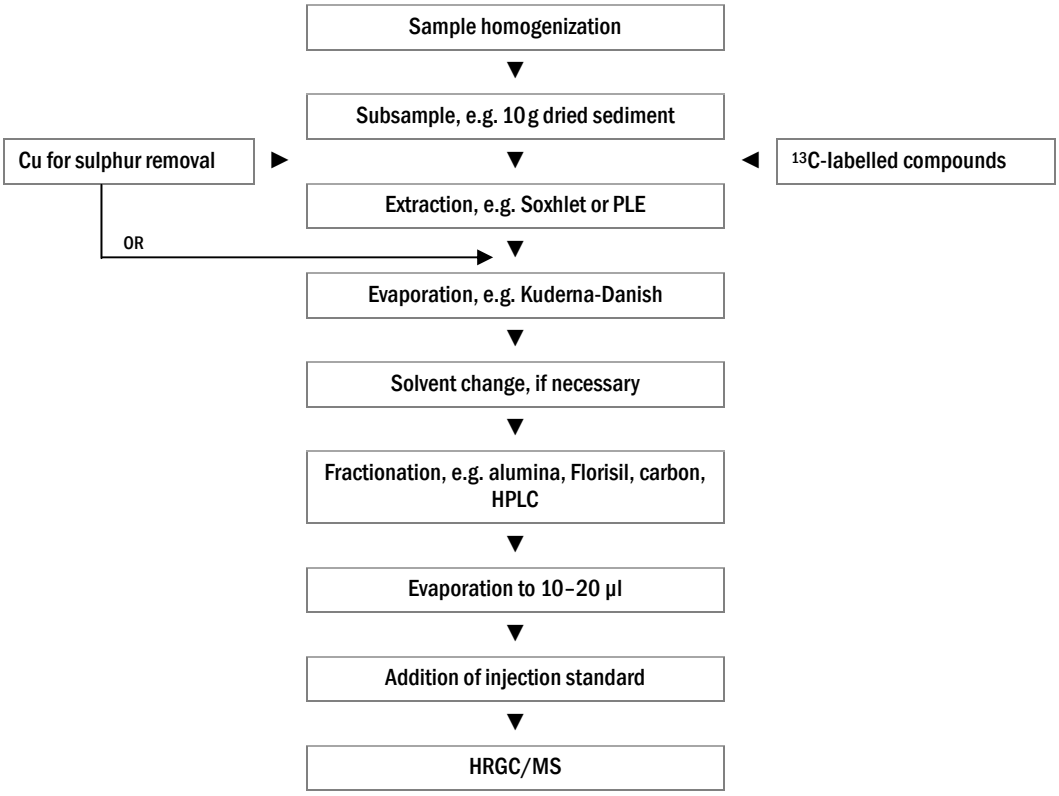

Figure 3. Example of an analytical method for the determination of PCDD/Fs and dl-PCBs in sediment.

4.1 Preparatory steps

In all analytical steps, it is essential to avoid contamination. Reagents should be of high purity or precleaned by solvent extraction. All solvents used must be checked for presence of residues of target or interfering compounds (e.g. polychlorinated diphenyl ethers). The purity of analytical standards should be checked. Reusable glassware should be rinsed with solvent, disassembled, washed with a detergent solution, and further rinsed with ultrapure 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). New glassware should be used from time to time, because scratched glassware has more active sites. Glassware that has contained fatty samples should be cleaned with enzymatic detergents.

PCDD/Fs and dl-PCBs are often determined by isotope dilution, using GC-HRMS. Less costly alternative detection methods are described in Section 5.4. ^{13}C-labelled standards of selected congeners to be analysed are added prior to extraction of the samples. These internal standards correct for recovery losses, to some extent. ^{13}C-labelled standards are commercially available for all individual congeners. The minimum number of internal standards to be used for the quantification of PCDD/Fs congeners is given in Table 2. Details on spike procedures are given by e.g. USEPA (1994).

Table 2. Native and minimum number of isotopically labelled PCDD/Fs to be used for calibration.

Substance	PCDD-Homologues		PCDF-Homologues	
	Native	$^{13}C_{12}$-labelled	Native	$^{13}C_{12}$-labelled
Tetra-CDD/F	2,3,7,8	2,3,7,8	2,3,7,8	2,3,7,8
Penta-CDD/F	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 2,3,4,7,8
Hexa-CDD/F	1,2,3,4,7,8 1,2,3,6,7,8 1,2,3,7,8,9	1,2,3,6,7,8	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
Hepta-CDD/F	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
Octa-CDD/F	1,2,3,4,6,7,8,9	1,2,3,4,6,7,8,9	1,2,3,4,6,7,8,9	1,2,3,4,6,7,8,9

4.2 Extraction

The sample intake used for the extraction must be sufficient to fulfil the requirements with respect to sensitivity. For instance, 10 g of dried sediment is typically used to measure accurately PCDD/Fs and dl-PCBs by GC-HRMS (USEPA, 1994). For biota, even higher sample amounts (up to 50 g) may be required. The samples are typically dried, for instance using sodium sulphate, and, as described above, spiked with the ^{13}C-labelled standards. Wet sediment samples have also been extracted using toluene in a Soxhlet-Dean-Stark system (Lamparski and Nestrick, 1989; Kjeller and Rappe, 1998).

For extraction of solid samples, Soxhlet is widely accepted as a robust liquid–solid extraction technique. Immediately prior to use, the Soxhlet apparatus should be pre-cleaned with e.g. dichloromethane : hexane (1 : 1 v/v) for approximately 3 h (USEPA, 1994). The sample should be rehomogenized prior to transfer to a glass Soxhlet thimble. Soxhlet extraction of biota samples proceeds for 18–24h using, e.g. dichloromethane:hexane (1:1 v/v; USEPA, 1994; Liu *et al.*, 2006). The USEPA method 1613B recommends to Soxhlet extract sediment samples with toluene for 16–24 h (USEPA, 1994). Other typical solvents for Soxhlet extraction in the literature are dichloromethane (Koh *et al.*, 2004) and toluene : acetone (4 : 1 v/v; Stern *et al.*, 2005), applicable to both biota and sediment samples. To remove sulphur compounds from sediment samples, copper granules can be either mixed with sediment during extraction or added to the extract. Sulphur can also be removed by gel permeation chromatography, see Section 4.3.

More recently, pressurized liquid extraction (PLE) has become a common and faster alternative to Soxhlet extraction (Focant *et al.*, 2004; Antunes *et al.*, 2008; Kishida *et al.*, 2010). 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 filled with an appropriate solvent (e.g. toluene, dichloromethane) and heated (e.g. 100–150°C) up to a pressure of 140 bars. Several extraction cycles are recommended (n = 2–3).

To reduce analysis time further, PLE can be combined with inline clean-up procedures. For biota samples, sulphuric acid impregnated silica has been used as a fat retainer (Björklund *et al.*, 2006). Proper fat–fat retainer ratios are important to avoid fat remaining in the sample extract, and the solvent should be selected accordingly. Sulphur removal from sediments can also be achieved as part of the inline clean-up (Chuang *et al.*, 2009).

4.3 Clean-up

The extracts are concentrated using suitable evaporation devices, e.g. rotary evaporation, Turbovap, Syncore, or Kuderna-Danish. The risk of cross-contamination is fairly high for rotary evaporation, therefore the evaporator should be precleaned, e.g. by 100 ml of clean solvent.

Because of the very low levels of PCDD/Fs in environmental samples, the elimination of interferences is essential, both with regard to matrix effects and coeluting compounds. Often, a sequence of clean-up steps will be required. The European research project DIFFERENCE recommended at least three clean-up or fractionation steps to ensure that sufficiently clean extracts are obtained (Van Loco *et al.*, 2004). These steps should ensure the removal of bulk co-extracted material, such as lipids and other natural organic matter, the removal of polar interferences and the fractionation of the analytes according to planarity.

Concentrated sulphuric acid is commonly applied for bulk matrix removal (including lipids), either in combination with 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 silica gel, potassium silicate, anhydrous Na_2SO_4, and silica gel (impregnated with sulphuric acid). The column is eluted with hexane. Ready-to-use, multilayer clean-up columns are also available commercially. Alternatively, approximately 30–100 g of sulphuric acid impregnated silica gel can be added to the extract, while stirring for 2–3 h. The treatment with sulphuric acid impregnated silica requires strict safety procedures, because 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 clean-up step using concentrated sulphuric acid might be applied after GPC to remove residual lipids from samples with high lipid contents.

Open column adsorption chromatographic techniques (preferably with Florisil or alumina columns) are frequently used for removal of polar compounds, followed by planarity fractionation using e.g. carbon columns, resulting in clean dl-PCB and/or PCDD/Fs fractions for HRGC-MS analysis. The most common solvents used in this step are hexane and/or dichloromethane and toluene. If the extracts are to be cleaned by adsorption chromatography, on e.g. silica gel, a solvent change to a non-polar solvent is essential.

High-performance liquid chromatography (HPLC) can also be used for planarity fractionation of the extracts, using two columns in series. Nitrophenylpropylsilica (Nucleosil, 5 µm particles, 250 × 4.6 mm) and 2-(1-pyrenyl)ethyldimethylsilylated silica (PYE; Cosmosil, 5 µm particles, 150 × 4.6 mm) columns allow the separation according to the number of aromatic rings and planarity, i.e. non-*ortho* and mono-*ortho* PCBs can be separated from each other, as well as from PCDD/Fs (Bandh *et al.*, 1996). Coupled with a fraction collector, the use of an HPLC system allows the automatic clean-up of a considerable number of samples. Alternatively, HPLC systems equipped with porous graphitized carbon can be used. Column dimensions are e.g. 50 × 4.7 mm, and care has to be taken that the column is not overloaded. Similar to PYE columns, they will separate non-*ortho* PCBs from other PCBs and from PCDD/Fs. Fully automated cleanup systems are also available commercially (e.g. PowerPrep system).

4.4 Concentration

After clean-up, a solvent of low volatility (e.g. an n-alkane in the nonane to tetradecane range) should be added as keeper, which will allow the evaporation of the extracts to near dryness, i.e. 10–20 µl. An injection standard should be added just before injection to evaluate the recovery of labelled internal standards. For example, $^{13}C_{12}$-1,2,3,4-TCDD can be used for recovery determinations of TCDD/Fs and PeCDD/Fs internal standards, whereas $^{13}C_{12}$-1,2,3,7,8,9-HxCDD can be used for recovery determinations of HxCDD/Fs, HpCDD/Fs, and OCDD/F internal standards. Additional $^{13}C_{12}$-non-2,3,7,8-PCDD/Fs injection standards will be suitable as well.

5 Instrumental analysis

PCDD/F concentrations of environmental samples are commonly monitored using HRGC/HRMS, but low resolution mass spectrometry (LRMS) may be a suitable alternative if the required minimum performance criteria are met.

5.1 GC analysis

GC analysis should be optimized with regard to separation and sensitivity. Gas chromatographic separation of isomers must be sufficient (<25% peak-to-peak between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF). Fishman *et al.* (2007, 2011) provided a comprehensive review of GC columns available for dioxin analysis. Generally 50–60 m, 5% diphenyl 95% dimethylpolysiloxane columns are a common choice. However, these columns could exhibit multiple coelutions for both PCBs and PCDD/Fs (Reiner *et al.*, 2006), depending on the matrix to be analysed. For complete separation of all seventeen 2,3,7,8-PCDD/Fs, DB-5 type columns have been combined with more polar cyanopropyl columns (Fishman *et al.*, 2011).

The use of an RTx-Dioxin 2 column has been reported as a suitable alternative to DB-5 columns. Combining this phase with reduced inner diameter and film thickness (for example, a 40 m × 0.18 mm × 0.18 μm) allowed the analysis of the seventeen PCDD/F congeners in 40 min, fulfilling QA/QC requirements and providing sufficient selectivity, especially for 2,3,7,8-TCDD and 2,3,7,8-TCDF (Robinson *et al.*, 2004; Reiner *et al.*, 2006; Cochran *et al.*, 2007a, 2007b). However, Cochran *et al.* (2007a, 2007b) reported on coelutions on this column, the most significant one being that of 1,2,3,6,7- and 1,2,3,7,8-PeCDDs. Promising results with few coelutions have been obtained for Si-Arylene GC stationary phases (Fishman *et al.*, 2011).

Potential interferences for dl-PCBs on a commonly used GC-column are summarized in Table 3 (Reiner *et al.*, 2006). Complete separation can be achieved by multiple analyses on columns of different polarity, but has also been demonstrated for relevant PCB congeners on one column, e.g. on an SGE HT8-PCB capillary column (Larsen *et al.*, 1995). A full separation of all PCB congeners is also possible using GCxGC (Haglund *et al.*, 2008; Skoczynska *et al.*, 2008).

Table 3. Possible interferences for selected dl-PCBs 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 analyte concentrations in the sample and the sensitivity of the instrumental analysis. In HRGC/HRMS analysis, 1–2 μl are common injection volumes. With PTV, injection volumes of up to 50 μl can be achieved. This may significantly lower detection limits, which is particularly helpful for LRMS techniques. Helium is usually used as carrier gas. The

initial oven temperature is linked to the keeper solvent and the injection technique and should ensure that no peak discrimination occurs.

5.2 Compound identification

The individual PCDD/Fs, dl-PCBs, or labelled compounds are identified by comparing the GC retention time and ion abundance ratio of two exact masses monitored (Tables 4 and 5) 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 dl-PCB, PCDD/F, or labelled compound in a standard, blank, or sample:

- The signal for the two exact masses specified in Tables 4 and 5 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 4 and 5 has to be within ±15% of the theoretical value displayed in Table 6.
- The relative retention time of a native PCDD/F and dl-PCB has to be within a time window of ±0.003, based on the retention time of the corresponding $^{13}C_{12}$-labelled standard. The relative retention time of congeners for which there are no labelled analogues has to be within ±0.002.

If interferences preclude identification, a new aliquot should be extracted, cleaned up further, and analysed again. If interferences cannot be removed, the data should be flagged to indicate that results are maximum concentrations.

5.3 GC-HRMS quantification

The HRMS system should be operated at a minimum resolution of 10 000 throughout all runs, and resolution should be checked regularly during the sequence of runs. Quantitative analysis is performed using selected ion monitoring (SIM), in one of the following ways:

- For the PCDD/Fs and dl-PCBs for which labelled analogues have been added to the sample, the GC/MS system is calibrated, and the concentration of each compound is determined using the isotope dilution technique.
- For the PCDD/Fs and dl-PCBs for which labelled analogues are not added to the sample (Table 2), 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 five calibration points. Mass drift correction is mandatory, usually based on a lock-mass m/z of perfluorokerosene (PFK) or perfluorotributylamine (PFTBA, FC43).

Table 4. Masses for the detection and quantification of PCDD/Fs.

	Dibenzofurans		Dibenzo-p-dioxins	
Substance	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 5. Masses for the detection and quantification of PCBs.

Homologue group	Native CBs	$^{13}C_{12}$-labeled CBs
Tetra-CB	289.9223	301.9626
	291.9194	303.9597
Penta-CB	325.8804	337.9207
	327.8775	339.9177
Hexa-CB	359.8415	371.8817
	361.8385	373.8788
Hepta-CB	393.8025	405.8427
	395.7995	407.8398

Table 6. Tolerance limits of isotope ratios for PCDD/Fs and dl-PCBs.

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

5.4 GC-LRMS and GC-MS/MS

LRMS has also been applied to the analysis of PCDD/Fs and/or dl-PCBs. Limits of detection are higher than those obtained with HRMS detectors, but can be compensated by e.g. larger injection volumes. Identification can be less clear than for HRMS analysis, and a very efficient extract clean-up is important to avoid interferences. A technique commonly applied is GC-LRMS using ion trap mass analysers working in tandem mode (Eppe *et al.*, 2004; Focant *et al.*, 2005; Malavia *et al.*, 2008). Table 7 provides information on precursor and product ions obtained by GC-ion trap MS. GC-LRMS (quadrupole) can be an option for dl-PCBs in particular; however, the maintenance of the instrument could be time consuming (e.g. frequent cleaning of the ion source). Triple-quadrupole instruments are becoming a common technique and offer a suitable alternative to GC-ion trap MS (March *et al.*, 2000; Onwudili *et al.*, 2011).

Schrock *et al.* (2009) implemented a method capable of detecting the lowest calibration point of the USEPA Method 1613B (USEPA, 1994) corresponding to sample concentrations of 1 pg g^{-1} for tetra-chlorinated congeners, 5 pg g^{-1} for penta-, hexa-, and hepta-chlorinated congeners, and 10 pg g^{-1} for octa-chlorinated congeners, for a sample intake of 10 g sediment when samples were sufficiently cleaned. Chromatograms generated by LRMS were quantified using the same identification and quantification criteria, achieving a resolution of 45% between 2,3,7,8-TCDD and its closest eluting isomer. The USEPA method 8280A is also focused on the analysis of PCDD/Fs by low resolution MS (USEPA, 1996). This method can be applied if the expected concentrations of the PCDD/Fs are above 1, 2.5, or 5.0 µg kg^{-1} for tetra-, penta-, hexa-, hepta-, and octa-chlorinated congeners, respectively. Detection limits comparable with those of HRMS analyses have recently been demonstrated for environmental samples analysed on a triple-quadrupole MS (Onwudili *et al*., 2011).

Table 7. Precursor ions and product ions for the determination of PCDD/Fs and dl-PCBs by HRGC-ion trap tandem MS.

Target Compounds	Native		$^{13}C_{12}$-labelled	
	Precursor Ion (m/z)	Product Ions (m/z)	Precursor Ion (m/z)	Product Ions (m/z)
Tetra-CDD	322 (M + 2)	257 + 259	334 (M +2)	268 + 270
Penta-CDD	356 (M + 2)	291 + 293	368 (M +2)	302 + 304
Hexa-CDD	390 (M + 2)	325 + 327	402 (M +2)	336 + 338
Hepta-CDD	424 (M + 2)	359 + 361	436 (M +2)	370 + 372
OctaCDD	460 (M + 4)	395 + 397	472 (M +4)	406 + 408
Tetra-CDF	306 (M + 2)	241 + 243	318 (M +2)	252 + 254
Penta-CDF	340 (M + 2)	275 + 277	352 (M +2)	286 + 288
Hexa-CDF	374 (M + 2)	309 + 311	386 (M +2)	320 + 322
Hepta-CDF	408 (M + 2)	343 + 345	420 (M +2)	354 + 356
Octa-CDF	444 (M + 4)	379 + 381		
CB–81, –77	292 (M + 2)	220 + 222	304 (M +2)	232 + 234
CB–105, –114, –118, –123, –126	326 (M + 2)	254 + 256	338 (M +2)	266 + 268
CB–156, –157, –167, –169	360 (M + 2)	288 + 290	372 (M +2)	300 + 302
CB–189	394 (M + 2)	322 + 324	406 (M +2)	334 + 336

6 Quality assurance and quality control

The analytical method requires low detection limits, usually in the pg g^{-1}-range, for both PCDD/Fs and dl-PCB congeners (OSPAR, 2005) and should meet the requirements for LoQ and measurement uncertainty specified in the monitoring programme. 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. The recovery of the individual internal standards added prior to extraction should be between 60 and 120%.

All sample series should include procedural blanks and measurements of certified/laboratory reference materials. Blanks should be as low as possible, at least below 20% of the lowest concentration of interest. Certified reference materials should be analysed regularly, although only few are available for the determination of PCDD/Fs and dl-PCBs in biota, as summarized by de Boer and McGovern (2001). Examples for biota include certified reference materials from Cambridge Isotope Laboratories (EDF-2524, -2525, -2526, and -5463 with PCDD/Fs and dl-PCBs at varying concentrations), Wellington Laboratories (WMF-01), and the National Research Council of Canada (CARP-2). Examples of certified reference materials for sediment include a marine sediment from the National Institute of Standards and Technology (NIST 1944) and lake sediments from Wellington Laboratories (WMS-01) and Environment Canada (DX-1,-2,-3).

The laboratory should regularly prove its competence 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).

7 Screening methods based on bioassays

EC (2002) suggested bioassays as screening tools for monitoring PCDD/Fs and dl-PCBs in foodstuffs, with the requirement to meet the criteria given in Table 8. In environmental monitoring, screening tools might be useful in, for instance, selecting suitable sampling sites. Hurst *et al.* (2004) also emphasized that monitoring programmes were moving towards effect-based monitoring, with biological relevance becoming more important. The tool must be capable of rapid, inexpensive, and high-throughput screening, producing interpretable and meaningful results (Hurst *et al.*, 2004).

Table 8. Quality criteria for screening and verification methods (EC, 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 (DR-CALUX or DR-lux) assay is mechanism-specific and utilizes the interaction with the Ah Receptor (AhR). However, this cell line is not compound-specific and produces a response with all compounds capable of interactions with the AhR. EC (2002) demands that the TEQ-values determined by bioassays should be the sum of PCDD/Fs and dl-PCBs in the sample. However, 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, polycyclic aromatic hydrocarbons), or compounds antagonising the AhR (e.g. di-*ortho*-substituted PCBs).

These deviations from the results of chemical analysis were also considered as an advantage by Hurst *et al.* (2004), because the assay allows a more accurate assessment of the true potency of dioxin-like toxicity present in the samples. To obtain specific responses to PCDD/Fs and dl-PCBs 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.

8 Safety

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

9 Data reporting

For biota analysis, results are typically reported in pg g^{-1} wet weight (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).

For sediment analysis, results are typically reported in pg g^{-1} dry weight (dw), equivalent to ng kg^{-1} dw. The water and organic carbon content of the samples should be reported as well, the latter being used for normalizing purposes.

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

10 References

Antunes, P., Viana, P., Vinhas, T., Capelo, J. L., Rivera, J., and Gaspar, E. M. S. M. 2008. Optimization of pressurized liquid extraction (PLE) of dioxin-furans and dioxin-like PCBs from environmental samples. Talanta, 75: 916–925.

Bandh, C., Ishaq, R., Broman, D., Näf, C., Rönquist-Nii, Y., and Zebühr, Y. 1996. Separation for subsequent analysis of PCBs, PCDD/Fs, and PAHs according to aromaticity and planarity using a two-dimensional HPLC system. Environmental Science and Technology, 30: 214–219.

Björklund, E., Sporring, S., Wiberg, K., Haglund, P., and von Holst, C. 2006. New strategies for extraction and clean-up of persistent organic pollutants from food and feed samples using selective pressurized liquid extraction. Trends in Analytical Chemistry, 25: 318–325.

Bligh, E. G., and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37: 911–917.

Chuang, J. C., Van Emon, J. M., and Schrock, M. E. 2009. High-throughput screening of dioxins in sediment and soil using selective pressurized liquid extraction with immunochemical detection. Chemosphere, 77: 1217–1223.

Cochran, J., Dorman, F., Stidsen, G., Reese, S., MacPherson, K., Kolic, T., Reiner, E., *et al*. 2007a. Retention time profiling for all 136 tetra- through octa-chlorinated dioxins and furans on a unique, low-bleed, thermally-stable gas chromatography column. Organohalogen Compounds, 69: 477–480.

Cochran, J., Dorman, F., Stidsen, G., Reese, S., MacPherson, K., Kolic, T., Reiner, E., *et al*. 2007b. Retention times and coelutions for all 136 tetra- through octa-chlorinated dioxins and furans on a unique, low-bleed thermally-stable gas chromatography column. Organohalogen Compounds, 69: 1146–1149.

Daelemans, F. F., Mehlum F., and Schepens, P. J. C. 1992. Polychlorinated biphenyls in two species of Arctic seabirds from the Svalbard Area. B. Environmental Contamination and Toxicology, 48: 828–834.

de Boer, J. 1988. Chlorobiphenyls in bound and non-bound lipids of fishes; comparison of different extraction methods. Chemosphere, 17: 1803–1810.

de Boer, J. 2001. Fresh materials. *In* Reference materials for chemical analysis: certification, availability and proper usage, pp. 121–127. Ed. by M. Stoeppler, W. R. Wolf, and P. J. Jenks. J. Wiley and Sons, New York, USA.

de Boer, J., and McGovern, E. 2001. Certified reference materials for organic contaminants for use in monitoring of the aquatic environment. Trends in Analytical Chemistry, 20: 140–159.

de Boer, J., and Smedes, F. 1997. Effects of storage conditions of biological reference materials on the contents of organochlorine compounds and mercury. Marine Pollution Bulletin, 35: 93–108.

Dyke, P. H., Foan, C., and Fiedler, H. 2003. PCB and PAH releases from power stations and waste incineration processes in the UK. Chemosphere, 50: 469–480.

EC. 2002. Commission Directive 2002/69/EC of 26 July 2002. Laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs. Official Journal of the European Commission, L 209: 5–14, 06.08.2002, Brussels, Belgium.

Eppe, G., Focant, J-F., Pirard, C., and De Pauw, E. 2004. PTV-LV-GC/MS/MS as screening and complementary method to HRMS for the monitoring of dioxin levels in food and feed. Talanta, 63: 1135–1146.

Fishman, V. N., Martin G. D., and Lamparski, L. L. 2007. Comparison of a variety of gas chromatographic columns with different polarities for the separation of chlorinated dibenzo-*p*-dioxins and dibenzofurans by high-resolution mass spectrometry. Journal of Chromatography A, 1139: 285–300.

Fishman, V. N., Martin, G. D., and Wilken, M. 2011. Retention time profiling of all 136 tetra- through octa-chlorinated dibenzo-*p*-dioxins and dibenzofurans on a variety of Si-Arylene gas chromatographic stationary phases. Chemosphere, 84: 913–922.

Focant, J-F., Picard, C., and De Pauw, E. 2004. Automated sample preparation-fractionation for the measurement of dioxins and related compounds in biological matrices: a review. Talanta, 63: 1101–1113.

Focant, J-F., Picard, C., Eppe, G., and De Pauw, E. 2005. Recent advances in mass spectrometric measurement of dioxins. Journal of Chromatography A, 1067: 265–275.

Haglund, P., Korytár, P., Danielsson, C., Diaz, J., Wiberg, K., Leonards, P., Brinkman, U. A. T., *et al.* 2008. GCxGC-ECD: a promising method for determination of dioxins and dioxin-like PCBs in food and feed. Analytical and Bioanalytical Chemistry, 390: 1815–1827.

Hanson, S. W. F., and Olley, J. 1963. Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. Biochemistry Journal, 89(3): 101–102.

Hurst, M. R., Balaam, J., Chan-Man, Y. L., Thain, J. E., and Thomas, K. V. 2004. Determination of dioxin and dioxin-like compounds in sediments from UK estuaries using a bio-analytical approach: chemical-activated luciferase expression (CALUX) assay. Marine Pollution Bulletin, 49: 548–658.

Kishida, M., Maekawa, T., and Bandow, H. 2010. Effect of extraction temperature on pressurized liquid extraction of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls from a sediment sample using polar and non-polar solvents. Analytica Chimica Acta, 659: 186–193.

Kjeller, L-O., and Rappe, C. 1998. Extraction of a quality assurance sediment sample for polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F). Fresenius Journal of Analytical Chemistry, 361: 811–816.

Koh, C-H., Khim, J. S., and Kannan, K. 2004. Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) and 2, 3, 7, 8-TCDD equivalents (TEQs) in sediment from the Hyeongsan River, Korea. Environmental Pollution, 132(3): 489–501.

Lamparski, L. L., and Nestrick, T. J. 1989. Novel extraction device for the determination of chlorinated dibenzo-*p*-dioxins (CDDs) and dibenzofurans (CDFs) in matrices containing water. Chemosphere, 19(1–6): 27–31.

Larsen, B., Cont, M., Montanarella, L., and Platzner, N. 1995. Enhanced selectivity in the analysis of chlorobiphenyls on a carborane phenylmethylsiloxane copolymer gas chromatography phase (HT-8). Journal of Chromatography A, 708: 115–129.

Leach, C. J., Wagner, T., Jones, M., Juggins, S., and Stevenson, A. C. 2008. Rapid determination of total organic carbon concentration in marine sediments using Fourier transform near-infrared spectroscopy (FT-NIRS). Organic Geochemistry, 39(8): 910–914.

Liu, H., Zhang, Q., Song, M., Jiang, G., and Cai, Z. 2006. Method development for the analysis of polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins and dibenzo-furans in single extract of sediment samples. Talanta, 70: 20–25.

Malavia, J., Santos, F. J., and Galceran, M. T. 2008. Comparison of gas chromatography-ion trap tandem mass spectrometry systems for the determination of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls. Journal of Chromatography A, 1186: 302–311.

March, R. E., Splendore, M., Reiner, E. J., Mercer, R. S., Plomley, J. B., Waddell, D. S., and MacPherson, K. A. 2000. A comparison of three mass spectrometric methods for the determination of dioxins/furans. International Journal of Mass Spectrometry, 194: 235–246.

Onwudili, J. A., Hajizadeh, Y., Zainal, S., Upton, J., and Williams, P. T. 2011. Application of low-temperature CP-Sil 88 column for the isomeric analysis of toxic 2378-substituted PCDD/Fs in incinerator flyash and sewage sludge using a triple quadrupole GC-MS/MS. Talanta, 87: 143–151.

OSPAR. 2005. Background document on dioxins. Hazardous Substances Series. OSPAR Commission, update 2005, London, UK.

OSPAR. 2010a. JAMP Guidelines for Monitoring Contaminants in Biota. OSPAR Commission. Monitoring guidelines, ref. no. 1999-2, revised in 2010, London, UK. 98 pp.

OSPAR. 2010b. JAMP Guidelines for Monitoring Contaminants in Sediments. OSPAR Commission. Monitoring guidelines, ref. no. 2002-16, revised in 2010, London, UK. 84 pp.

Pandelova, M., Henkelmann, B., Roots, O., Simm, M., Järv, L., Benfenati, E., and Schramm, K-W. 2008. Levels of PCDD/F and dioxin-like PCB in Baltic fish of different age and gender. Chemosphere, 71: 369–378.

Reiner, E. J., Clement, R. E., Okey, A. B., and Marvin, C. H. 2006. Advances in analytical techniques for polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and dioxin-like PCBs. Analytical and Bioanalytical Chemistry, 386: 791–806.

Robinson, C., Blow, P., and Dorman, F. 2004. Rapid dioxin analysis using accelerated solvent extraction (ASE), multi-column sample cleanup and Rtx-Dioxin2 gas chromatography column. Organohalogen Compounds, 66: 100–105.

Schumacher, B. 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments. Ecological Risk Assessment Support Center. US Environmental Protection Agency. NCEA-C-1282; EMASC-001.

Schrock, M., Dindal, A., and Billets, S. 2009. Evaluation of alternative approaches for screening contaminated sediments and soils for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans. Journal of Environmental Management, 90: 1289–1295.

Skoczynska, E., Korytár, P., and de Boer, J. 2008. Maximizing chromatographic information from environmental extracts by GCxGC-ToF-MS. Environmental Science and Technology, 42: 6611–6618.

Smedes, F. 1999. Determination of total lipid using non-chlorinated solvents. Analyst, 124: 1711–1718.

Stern, G. A., Braekevelt, T. W., Helm, P. A., Bidleman, T. F., Outdidge, P. M., Lockhart, W. L., McNeeley, R., *et al*. 2005. Modern and historical fluxes of halogenated organic contaminants to a lake in the Canadian Arctic, as determined from annually laminated sediment cores. Science of the Total Environment, 342: 223–243.

USEPA. 1994. Tetra- through Octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS. Method 1613. Revision B. US Environmental Protection Agency. Office of Water, Engineering and Analysis Division (4304), October 1994, Washington DC, USA.

USEPA. 1996. The analysis of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans by high resolution gas chromatography/low resolution mass spectrometry (HRGC/LRMS). Method 8280A. Revision 1. US Environmental Protection Agency, December 1996, Washington DC, USA.

Van den Berg, M., Birnbaum, L. S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., *et al.* 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Sciences, 93: 223–241.

Van Leeuwen, S. P. J., Leonards, P. E. G., Traag, W. A., Hoogenboom, L. A. P., and de Boer, J. 2007. Polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls in fish from the Netherlands: concentrations, profiles and comparison with DR CALUX bioassay results. Analytical and Bioanalytical Chemistry, 216: 1352–1356.

Van Loco, J., Van Leeuwen, S., Roos, P., Carbonelle, S., de Boer, J., Goeyens, L., and Beernaert, H. 2004. The international validation of bio and chemical screening methods for dioxins and dioxin-like PCBs: The DIFFERENCE project round 1 and 2. Talanta, 63: 1169–1182.

Verta, M., Salo, S., Korhonen, M., Assmuth, T., Kiviranta, H., Koistinen, J., Ruokojärvi, P., *et al.* 2007. Dioxin concentrations in sediments of the Baltic Sea – a survey of existing data. Chemosphere, 67: 1762–1775.

Webster, L., Tronczynski, J., Bersuder, P., Vorkamp, K., and Lepom, P. 2009. Determination of polybrominated diphenyl ethers (PBDEs) in sediment and biota. ICES Techniques in Marine Environmental Sciences, 46. 16 pp.

Wells, D. E., and de Boer, J. 2006. Evaluation of the quality of measurement of organochlorine contaminants in the marine environment: the QUASIMEME experience. Trends in Analytical Chemistry, 25: 350–363.

11 Author contact information

Katrin Vorkamp
Aarhus University
Department of Environmental Sciences
Frederiksborgvej 399
4000 Roskilde, Denmark
kvo@dmu.dk

Patrick Roose
Management Unit of the North Sea Mathematical Models
3de en 23ste Linieregimentsplein
8400 Oostende, Belgium
patrick.roose@mumm.ac.be

Philippe Bersuder
Centre for Environment, Fisheries and Aquaculture Sciences (Cefas)
Pakefield Road
Lowestoft,
Suffolk NR33 0HT, UK
philippe.bersuder@cefas.co.uk

Lynda Webster
Marine Scotland–Science
PO Box 101
375 Victoria Road
Aberdeen AB11 9DB, UK
lynda.webster@scotland.gsi.gov.uk

Peter Lepom
Federal Environment Agency
Laboratory for Water Analysis
Bismarckplatz 1
14193 Berlin, Germany
peter.lepom@uba.de

Catherine Munschy
Ifremer
Rue de l'Ile d'Yeu
BP21105
44311 Nantes Cedex 03, France
cmunschy@ifremer.fr

Rossana Bossi
Aarhus University
Department of Environmental Sciences
Frederiksborgvej 399
4000 Roskilde, Denmark
rbo@dmu.dk

Jacek Tronczynski
Ifremer
Rue de l'Ile d'Yeu
BP 21105
44311 Nantes Cedex 03, France
jacek.tronczynski@ifremer.fr

Jacob de Boer
VU University
De Boelelaan 1087
1081 HV Amsterdam, The Netherlands
Jacob.de.Boer@ivm.vu.nl

12 Abbreviations

AhR	Ah Receptor
BCF	bioconcentration factor
[]CDD	chlorodibenzo-*p*-dioxin, prefixed with tetra (T), penta (Pe), hexa (Hx), hepta (Hp), or octa (O)
[]CDF	chlorodibenzofuran, prefixed as above
dl-PCB	dioxin-like polychlorinated biphenyl
DR-CALUX, DR-lux	dioxin-responsive chemically activated luciferase
dw	dry weight
ECD	electron capture detection
GC	gas chromatography
GCxGC	comprehensive multidimensional gas chromatography
GC-MS-MS	GC tandem mass spectrometry
GPC	gel permeation chromatography
HpCDD	heptachlorodibenzo-*p*-dioxin
HPLC	high-performance liquid chromatography
HRGC	high-resolution gas chromatography
HRMS	high-resolution mass spectrometry
HxCDD	hexachlorodibenzo-*p*-dioxin
LoQ	limit of quantification
LRGC	low-resolution gas chromatography
LRMS	low-resolution mass spectrometry
MS	mass spectrometry
m/z	mass/charge
OCDD	octachlorodibenzo-*p*-dioxin
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PeCDD	pentachlorodibenzo-*p*-dioxin
PFK	perfluorokerosene
PFTBA	perfluorotributylamine
PLE	pressurized liquid extraction
PTV	programmed temperature vaporizing
PYE	2-(1-pyrenyl)ethyldimethylsilylated silica
SIM	selected ion monitoring
S/N	signal-to-noise ratio
Soxhlet	a liquid–solid extraction technique
TCDD	tetrachlorodibenzo-*p*-dioxin
TEF	toxicity equivalency factor
TEQ	tetrachlorodibenzo-*p*-dioxin equivalent
TOC	total organic carbon
ToF	time-of-flight
ww	wet weight