

Chlorinated and Brominated Organic Pollutants in Contaminated River Sediments

E. Eljarrat¹ (✉) · D. Barceló^{1,2}

¹Department of Environmental Chemistry, IDAEA, CSIC, Jordi Girona 18–26,
 08034 Barcelona, Spain
 eeeqam@cid.csic.es

²Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la
 Universitat de Girona, Edifici Jaume Casademont, Porta A, Planta 1 –
 Despatx 13C/Pic de Peguera 15, 17003 Girona, Spain

1	Introduction	23
2	Chlorinated Organic Pollutants	24
2.1	Polychlorinated Dibenzo- <i>p</i> -dioxins (PCDDs) and Dibenzofurans (PCDFs)	24
2.2	Polychlorinated Biphenyls (PCBs)	25
2.3	Polychlorinated Naphthalenes (PCNs)	26
2.4	Polychlorinated <i>n</i> -Alkanes (PCAs)	26
3	Brominated Organic Pollutants	27
3.1	Polybrominated Diphenyl Ethers (PBDEs)	28
3.2	Hexabromocyclododecane (HBCD)	29
3.3	Polybrominated Dibenzo- <i>p</i> -dioxins (PBDDs) and Dibenzofurans (PBDFs)	29
4	Analytical Methodologies	30
4.1	Chemical Analysis	30
4.1.1	Extraction	31
4.1.2	Purification	33
4.1.3	Chromatographic Separation	34
4.1.4	Mass Spectrometric Detection	37
4.2	Bioanalytical Methods	41
4.3	Toxicity Identification Evaluation (TIE) Methods	42
5	Environmental Levels	45
5.1	Chlorinated Organic Pollutants	46
5.2	Brominated Organic Pollutants	49
6	Conclusions and Perspectives	51
	References	52

Abstract Sediments are the main abiotic reservoirs where POPs from different emission sources are accumulated. In the last few decades, a great deal of emphasis has been placed on evaluating the contamination of “classical” POPs such as PCBs, PCDDs and PCDFs. More recently, scientific interest has been focused on other groups of POPs, the so-called “emerging” POPs, such as PCAs and brominated POPs. These emerging POPs are of concern due to their toxicological properties, their capability to bioaccumulate and

their widespread and unrestricted use. Knowledge of the environmental impact of these emerging POPs is based on previous analytical work that focused on the development of sensitive and selective methodologies. Herein, an overview of current analytical methods, including different sample preparation techniques as well as the different instrumental approaches, is presented. Finally, a review of the available data concerning the occurrence of chlorinated and brominated POPs in sediments is also reported. Conclusions and future perspectives are also outlined.

Keywords Brominated flame retardants · Chlorinated paraffins · Persistent organic pollutants · Polychlorinated naphthalenes

Abbreviations

ASE	Accelerated solvent extraction
BFR	Brominated flame retardant
BSEF	Bromine Science Environmental Forum
BTIE	Bioavailable toxicity identification evaluation
CALUX	Chemically activated luciferase expression
CP	Chlorinated paraffin
DLC	Dioxin-like compound
DDT	Dichlorodiphenyltrichloroethane
dw	Dry weight
ECNI	Electron capture negative ionization
EI	Electron ionization
EROD	7-Ethoxyresorufin <i>O</i> -deethylase
GC	Gas chromatography
GCxGC	Two-dimensional gas chromatography
HBCD	Hexabromocyclododecane
HRMS	High-resolution mass spectrometry
IAC	Immunoaffinity chromatography
IT	Ion trap
K_{ow}	Octanol/water partition coefficient
LC	Liquid chromatography
LCCP	Long-chain chlorinated paraffin
LOD	Limit of Detection
LRMS	Low-resolution mass spectrometry
MAE	Microwave-assisted extraction
MCCP	Medium-chain chlorinated paraffin
MS	Mass spectrometry
MS-MS	Tandem mass spectrometry
NCI	Negative chemical ionization
PBB	Polybrominated biphenyl
PBDD	Polybrominated dibenzo- <i>p</i> -dioxin
PBDE	Polybrominated diphenyl ether
PBDF	Polybrominated dibenzofuran
PCA	Polychlorinated <i>n</i> -alkane
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo- <i>p</i> -dioxin
PCDF	Polychlorinated dibenzofuran
PCN	Polychlorinated naphthalene

PLE	Pressurized liquid extraction
POP	Persistent organic pollutant
ppb	Parts per billion
ppq	Parts per quadrillion
ppt	Parts per trillion
QqLIT	Quadrupole linear ion trap
QqQ	Triple quadrupole
SCCP	Short-chain chlorinated paraffin
SIM	Selected ion monitoring
TBBPA	Tetrabromobisphenol A
TEF	Toxic equivalent factor
TIE	Toxicity identification evaluation
ToF	Time-of-flight
WHO	World Health Organization
2,3,7,8-TCDD	2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin

1

Introduction

Persistent organic pollutants (POPs) have been shown to exhibit potentially harmful effects in man and the environment. In addition to being persistent, POPs are typically lipophilic (therefore, bioaccumulative), semivolatile and toxic. Some of these POPs have been deliberately produced by the industry for a wide variety of applications [i.e., pesticides, polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs)]. Other chemicals, such as brominated flame retardants (BFRs) are still produced in large quantities for use in electric equipment, plastics and building materials. Others are accidentally formed or eventually released as a byproduct from various activities, such as industrial or combustion processes [i.e., polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs)]. Since 1995, the international community has been working on a legally binding instrument to eliminate POPs. Different organizations initiated an assessment process, which, in December 2000, resulted in the conclusion of the text for the POPs convention. Initial action is directed at 12 POPs, including aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, PCBs and PCDDs/PCDFs. However, future obligation will be to add other POPs as new evidence becomes available.

Within classical POPs, chlorinated compounds are the most relevant, standing out are PCBs, PCDDs and PCDFs. However, among the emerging POPs, different brominated compounds have attracted the most scientific interest during the last decade. This work will focus on the most relevant chlorinated and brominated organic pollutants in river sediments.

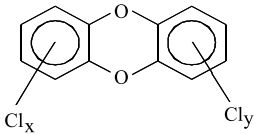
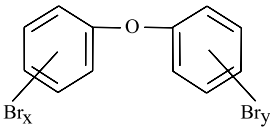
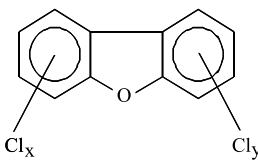
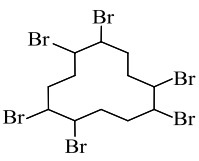
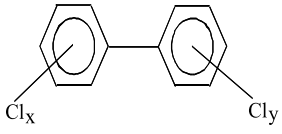
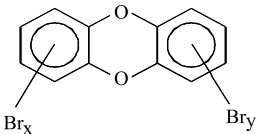
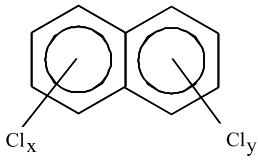
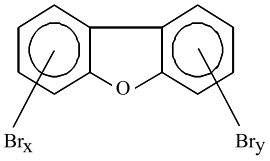
2 Chlorinated Organic Pollutants

2.1

Polychlorinated Dibenzo-*p*-dioxins (PCDDs) and Dibenzofurans (PCDFs)

PCDDs and PCDFs constitute a class of ubiquitous pollutants with aromatic structure, high chemical stability and extremely poor water solubility. They can occur in the form of 75 PCDD congeners and 135 PCDF congeners (Table 1). At present, most PCDD and PCDF sources are well characterized. These sources include chemical, thermal, photochemical and enzymatic reactions. Combustion processes, mainly incineration plants such as municipal solid waste incinerators, clinical waste incinerators and industrial waste incinerators are known to be some of the most important sources responsible for the presence of these contaminants in the environment. Soils and sedi-

Table 1 Structure of selected chlorinated and brominated POPs

Chlorinated POPs	Brominated POPs
<p>PCDDs $x + y = 1-8$ 75 congeners</p> 	<p>PBDEs $x + y = 1-10$ 209 congeners</p> 
<p>PCDFs $x + y = 1-8$ 135 congeners</p> 	<p>HBCDs 3 isomers</p> 
<p>PCBs $x + y = 1-10$ 209 congeners</p> 	<p>PBDDs $x + y = 1-8$ 75 congeners</p> 
<p>PCNs $x + y = 1-8$ 75 congeners</p> 	<p>PBDFs $x + y = 1-8$ 135 congeners</p> 

ments are the main abiotic reservoirs where PCDDs and PCDFs from different emission sources are accumulated.

It is well known that dioxins constitute a group of lipophilic, persistent, ubiquitous, and bioaccumulative environmental chemicals exhibiting a broad spectrum of biological (high toxicity) and chemical (long-range transport) effects. Dioxins have been referred to as “the most toxic man-made compounds”. The International Agency for Research on Cancer named 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) a known human carcinogen [1]. Although 210 different PCDDs and PCDFs are possible with one to eight chlorines, only 17 of these congeners are considered to be toxic. The most toxic molecules are those that contain from four to eight chlorine atoms and, in particular, those in which positions 2, 3, 7 and 8 are chlorinated. Since the individual toxicity of these compounds is different, the real toxicity of a mixture is best assessed bearing in mind the relative toxicity of the isomers with respect to the most toxic isomer, the 2,3,7,8-TCDD. There are a number of toxic equivalent factor (TEF) tables proposed by different organizations. Today, it is well accepted to use the TEFs proposed by the World Health Organization (WHO) in 1998, called WHO-TEFs [2].

2.2

Polychlorinated Biphenyls (PCBs)

PCBs are industrial contaminants formed as a result of human activities. They were widely used as industrial chemicals, particularly as dielectric fluids in electrical transformers and capacitors, hydraulic fluids, lubricating and cutting oils, and as additives in sealants, plastics, paints, copying paper, adhesives and casting agents. PCBs were commercially produced as complex mixtures from 1929 onwards under various trade names (e.g., Acelor, Aroclor, Clophen, Montar, PCBs, Sobol, Turbinol, etc.). Although uses of these ubiquitous contaminants have been banned in industrialized countries since the late 1970s, their continued presence in the environment poses considerable hazards. Their physical and chemical properties vary widely across the class, but all PCB congeners have low water solubility and low vapour pressure. They are highly soluble in non-polar solvents, oils and fats. Because of their high thermodynamic stability, all degradation mechanisms are difficult, and environmental and metabolic degradation is generally very slow. Their thermal stability and resistance to degradation contributed to their commercial usefulness, but also to their long-term environmental effects. Similar to dioxins, PCBs have accumulated in soils and sediments.

By varying the number and the position of the chlorine atoms, there are 209 theoretical PCB congeners (Table 1). Most of these have been shown to be present in the PCB mixtures that have been available on the market. Recently, interest has been focused on 12 congeners (four non-*ortho* and ten mono-*ortho*) due to their similar toxicological properties to 2,3,7,8-TCDD.

2.3

Polychlorinated Naphthalenes (PCNs)

PCNs are a group of compounds composed of two fused benzene rings (naphthalene) with one to eight chlorine substitutions. There are a total of 75 possible PCN congeners (Table 1), which have physical and chemical properties such as melting point, volatility, water solubility, octanol/water partition coefficients and bioconcentration factors, favouring their environmental persistency and bioaccumulation. These products have properties and uses similar to those of PCBs. PCN formulations have been used in industry as dielectric fluids in capacitors, transformers and cables. The production of technical PCN mixtures has ceased in many countries, but they are still found, for example, in electrical equipment. In addition, PCNs are formed and released into the environment via other processes. There are three main sources of these contaminants: (a) The use of technical PCN products manufactured in different countries under various trade-names such as Halowax (Koopers Company, USA), Seekay Wax (Imperial Chemical Industries, UK) and Nibren Wax (Bayer, Germany) accounts for most of the direct or indirect input into the global environment. (b) Commercial PCB products, such as Aroclor or Clophen, which have, at the ppm-level, PCNs as by-products. (c) PCN formation during high-temperature processes. The total world-wide production of PCNs from the above-mentioned sources has been roughly assessed at 150 000 tons (technical PCNs), plus 100 tons (technical PCBs), plus 1–10 tons (thermal formation in this century) [3].

2.4

Polychlorinated *n*-Alkanes (PCAs)

Polychlorinated *n*-alkanes (PCAs), also known as chlorinated paraffins (CPs), are a class of industrially prepared mixtures of the general formula $C_nH_{2n+2-z}Cl_z$. These mixtures have a chlorination degree between 30 and 70 wt. %, and a linear alkane chain with length of C_{10} – C_{13} (short-chain chlorinated paraffins, SCCPs), C_{14} – C_{17} (medium-chain chlorinated paraffins, MCCPs) or $C_{>17}$ (long-chain chlorinated paraffins, LCCPs). The number of theoretically possible congeners, homologues, diastereomers and enantiomers is unknown, but by far exceeds 10 000 compounds [4]. PCAs have been produced in technical formulations since the early 1930s. Because they are produced with free radical chlorination, a single PCA formulation consists of thousands of different compounds with a range of physical-chemical properties. They are used for a variety of industrial applications, including lubricating additives in plastics, adhesives, sealants, paints, cutting oil additives and flame retardants [5]. The world production of PCAs has shown a slow

growth over the last decades from ca. 230 000 tons per year in 1977–1979 [6] to ca. 300 000 tons per year in 1997 [7].

PCAs have physical and chemical properties that are similar to other high molecular weight organochlorine pollutants, such as PCBs and DDT. Water solubility was estimated based on octanol/water partition coefficient (K_{OW}) correlations, and an apparent inverse relationship between carbon chain length and water solubility was noticed with values ranging from 0.49 to 1260 $\mu\text{g/l}$ for SCCPs, from 0.029 to 14 $\mu\text{g/l}$ for MCCPs, and from 1.6×10^{-6} to 0.086 $\mu\text{g/l}$ for LCCPs [8]. The Cl substitution pattern had also significant effects on water solubility, and in contrast with known trends for chlorinated aromatic compounds, an increasing water solubility with increasing degree of chlorination up to 5 chlorines was reported [9].

Toxicity of PCAs appears to be inversely related to carbon chain length and because of this much attention has been given to SCCPs. Although PCAs generally have shown low toxicity to mammals, SCCPs have a carcinogenic potential in rats and mice [10]. However, no evidence of carcinogenicity was found for MCCPs and LCCPs. In addition, dose-response studies have shown that oral intake of SCCPs by mice results in an increase in liver weight. Moreover, in some studies, C_{10} – C_{12} CPs with 58% chlorine content caused growth inhibition and reproductive effects. Bioconcentration factors are high, reaching values of nearly 1.4×10^5 in mussels with polychlorinated dodecane with 69% chlorine content [11]. Greater bioconcentration factors were found for SCCPs, probably due to their greater water solubility. Moreover, highly chlorinated SCCPs are predicted to have the greatest bioconcentration factors because they are more hydrophobic and resistant to biotransformation than lower chlorinated PCAs, and their accumulation is not hindered by a large molecular size or extremely high K_{OW} , as observed for MCCPs and LCCPs [12].

3

Brominated Organic Pollutants

In many materials, brominated compounds have proved more suitable as flame retardants than chlorinated ones. Brominated flame retardants (BFRs) sometimes make up as much as 10–30% of the plastics used for example in the printed circuit board and housings of computers and other electrical and electronic equipment. Large-scale computerization in the 1970s and 1980s, combined with more stringent fire safety standards, resulted in rapidly growing use of these chemicals. The first BFR to be introduced included polybrominated biphenyls (PBBs), a group of compounds with the same structure as PCBs. Following an accident in the United States in 1973 (PBBs had mistakenly been sold as a feed supplement, resulting in large-scale poisoning of cattle and chickens) polybrominated

diphenyl ethers (PBDEs) assumed the position of most important category of BFRs.

3.1

Polybrominated Diphenyl Ethers (PBDEs)

Like PBBs and PCBs, this group comprises a total of 209 theoretically possible congeners (Table 1). The commercial PBDE mixtures are nominally deca-, octa-, and pentabrominated. Penta-BDE formulation consists of 41–42% tetra-BDEs (mainly BDE-47) and 44–45% penta-BDEs (predominantly BDE-99 and BDE-100), whereas deca-BDE formulation consists mainly of BDE-209 (97–98%), with a small amount of nona-BDES (0.3–3%) [13]. Some 67 000 tons were manufactured in 1999 [14], an amount rivalling PCBs at the height of their production.

Structural similarity to other environmental chemicals with known toxic effects (PCBs, PCDDs, PCDFs) could indicate that also PBDEs could be harmful to health. The acute toxicity of PBDEs is low. However, there is concern for its long-term effects on the endocrine system. Since 1994, PBDEs have been listed as compounds that can affect the regulation of thyroid and steroid hormones. Several studies indicate that commercially obtained tetra- and penta-BDE are endocrine disrupters, which can exert effects on the thyroid system. The effects of penta-BDE on thyroxine and the thyroid gland are considered to be principally due to the induction of liver enzymes, although several mechanisms may operate. The liver appears to be sensitive, and for penta-BDE, a no-observed-adverse-effect level of 1 mg/(kg bw day) has been determined, with effects evident at 2 mg/(kg bw day). Meerts et al. [15] have reported on estrogenic activities on PBDEs and hydroxylated PBDEs as determined in the human T47D breast tumor cell line stably transfected with an estrogen-responsive luciferase reporter gene construct. A commercial penta-PBDE mixture has been reported to reduce circulating thyroxine and to induce rat liver 7-ethoxyresorufin *O*-deethylase (EROD) activity in the parent animal as well as in the offspring [16].

Of the three main technical mixtures in use, the penta-BDE and octa-BDE mixtures are currently being phased out in Europe. Consequently, a shift in production of deca-BDE mixtures for these lower brominated PBDE mixtures has taken place. A recent report of The Bromine Science Environmental Forum (BSEF) estimated the total market demand for the major commercial BFRs in 2001 [17]. This shows the dominance of tetrabromobisphenol-A (TBBPA) (59% of total world usage) and the deca-mix PBDE formulation (27% of total world usage) in volume terms.

3.2

Hexabromocyclododecane (HBCD)

Recent reports also suggest that usage of hexabromocyclododecane (HBCD) is increasing [18] and that because attention is now switching to this compound which is widely used. The physical-chemical properties of HBCD (Table 1) are similar to PBDEs and other persistent organic pollutants, in fact the $\log K_{ow}$ of HBCD is 5.6 and that value places it in the optimum range for bioaccumulation [19]. Thus, HBCD is not covalently bonded to the material leading to the risk of migration out of the product during use or disposal [20]. On the basis of these properties, there is a high potential for this material to absorb to soil and sediments.

Technical 1,2,5,6,9,10-HBCD is produced industrially by addition of bromine to *cis-trans-trans*-1,5,9-cyclododecatriene, with the resulting mixture containing three predominant diastereoisomers α -, β - and γ -HBCD. Normally, the γ -isomer is the most dominant in the commercial mixtures (ranging between 75 and 89%), followed by α - and β -isomer (10–13% and 1–12%, respectively) [21, 22].

The dissimilarities in the structure of the α -, β - and γ -isomer might raise differences in polarity, dipole moment and in solubility in water; for example the solubility of α -, β - and γ -HBCD in water was 48.8, 14.7 and 2.1 $\mu\text{g/l}$, respectively. These different properties may explain the differences observed in their environmental behaviour [21]. In sediments, the stereoisomeric profile of HBCD is similar to that on commercial HBCD formulations, with γ -isomer being the most abundant stereoisomer. In contrast to sediments, the α -isomer is the most prominent stereoisomer in the majority of aquatic invertebrate and fish samples [23, 24].

3.3

Polybrominated Dibenzo-*p*-dioxins (PBDDs) and Dibenzofurans (PBDFs)

Polybrominated dibenzo-*p*-dioxins (PBDDs) and dibenzofurans (PBDFs) are the bromine homologues of PCDDs and PCDFs (Table 1). Thus, depending on the number and position of bromine atoms, there are 75 PBDD and 135 PBDF congeners. PBDDs and PBDFs occurred as trace contaminants in BFRs (e.g., PBDE or PBB) and were produced during combustion of these chemicals. They are formed when organics are incinerated in the presence of bromine. Combustion of organics in the presence of both bromine and chlorine results in the formation of mixed (i.e., bromo-, bromo/chloro- and chloro-) halogenated dibenzo-*p*-dioxins and dibenzofurans. There are 4,600 potential mixed congeners.

Incineration at sufficiently high temperatures under well-controlled conditions will destroy the BFRs, but less well-controlled incineration may result in the formation of limited quantities of PBDDs, PBDFs and bromochlorodi-

benzo-*p*-dioxins and furans [25, 26]. Different studies showed that bromine substitution appears to have a stronger toxic effect than chlorine substitution. In the chemically activated luciferase expression (CALUX) bioassay the brominated analogue of 2,3,7,8-TCDD showed equivalent activity to their chlorinated analogue [27]. Behnisch et al. [28] published a comparative study of the activity of PBDD/F congeners and their chlorinated homologues (PCDD/F). In the case of dioxins, similar potencies were obtained for the chlorinated and brominated congeners. However, significant differences were detected for the furans, with greatest activities for brominated furans.

4

Analytical Methodologies

The detection and quantification of chlorinated and brominated POPs in environmental samples requires sensitive analysis techniques. There are two main approaches for determinations, the first one focused on the congener specific analysis (chemical analysis), and bioanalytical methods that directly provide a total toxicity value. Chemical analytical methods are expensive and require sophisticated instrumentation and considerable effort in the sample preparation. However, the main advantage of chemical analysis is that it provides information on congener distributions and levels, which is very useful in identifying sources of the contaminants responsible for increased levels and risk management. Unlike chemical analysis, bioanalysis provides information on the total activity of the samples under study, without providing information on the levels of individual congeners. The main advantage of these methods is their high throughput rate and low cost. These characteristics make bioassays valuable screening techniques.

4.1

Chemical Analysis

The majority of POPs occur in very limited quantities in the natural environment [from parts per billion (ppb) to parts per quadrillion (ppq)]. To be able to detect such pollutants, therefore, it is first of all necessary to increase their concentration in a small portion of the sample collected. This process of sample preparation is often appreciably time-consuming. However, another property of pollutants of this type is more helpful to the analyst: being persistent, they are not destroyed even if the sample is subjected to fairly rough treatment. Moreover, the analytical methodologies are especially difficult due to the complexity of the mixtures of congeners: 75 PCDDs, 135 PCDFs, 209 PCBs, 75 PCNs, 209 PBDEs, etc. The different toxicity of each congener requires the development of congener-specific methods. Overcoming all these analytical problems has only been possible with the application of rigorous

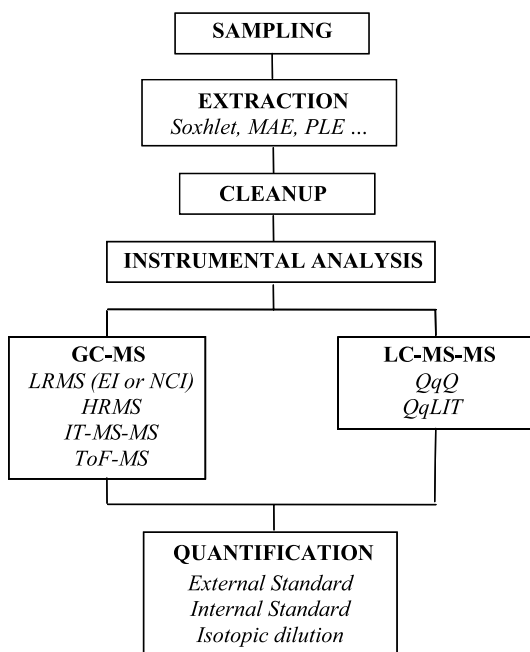


Fig. 1 Principal steps of the analytical methods used for the analysis of chlorinated and brominated POPs

cleanup schemes and by using gas chromatography (GC) coupled to mass spectrometry (MS). The cleanup steps provide a suitable removal of the bulk matrix and some interfering compounds; the GC allows an appropriate separation between the different congeners, and MS affords a sensitive and selective method of detection. An analytical protocol to determine chlorinated and brominated POPs includes the steps shown in Fig. 1. As can be seen, instrumental analysis is divided into two different options: GC-MS and liquid chromatography (LC)-MS. Whereas POPs were classically analyzed using gas chromatographic techniques, recently some liquid chromatographic methods have been introduced due to the occurrence of some emerging POPs such as HBCD, for which isomeric determination must be carried out by LC-MS.

4.1.1

Extraction

In the environmental analysis of chlorinated and brominated compounds, substantial analyte enrichment is necessary to isolate the target compounds from the matrix and to achieve the detection limits required. For trace analysis of these contaminants, Soxhlet extraction is widely accepted as a robust liquid–solid extraction technique. However, the main drawback of this tech-

nique is the fact that refluxing with cold solvent is time consuming (up to 48 hours). Furthermore, the solvent consumption is considerable (~ 300 ml), demanding evaporation of a large amount of solvent before subsequent cleanup. Over the past few years, efforts were made on the development of extraction techniques that allow efficient extraction along with reduced solvent volumes in shorter times, incorporating high levels of automation. The traditional techniques face severe competition due to the development of new techniques, such as pressurized liquid extraction (PLE) [commonly named accelerated solvent extraction (ASE)] and microwave-assisted extraction (MAE). In PLE systems, the liquid state of solvent extractant is maintained at elevated temperatures by application of a moderate pressure. Optimization of extraction conditions is facilitated as organic solvents recommended in traditional techniques can usually be used and pressure has little influence. In addition, these systems offer a high level of automation although only one extraction at a time can be conducted.

A review of the PLE applications for the extraction of moderately and non-volatile organic pollutants from a variety of solid environmental matrices has been published [29]. In this review we can find studies on the analysis of PCBs, PCDDs and PCDFs in sediments. But PLE has also been applied to the PCA determinations. PLE was tested for the determination of SCCPs in sediment cores from six lakes in Canada [30]. These samples were extracted with CH_2Cl_2 at a temperature of 100°C and at a pressure of 2000 psi for 30 min. Tomy and Stern [31] proposed a PLE method for the extraction of MCCPs in different environmental matrices. Extractions were carried out with CH_2Cl_2 /hexane (1 : 1) at a temperature of 100°C and at a pressure of 136 atm. The length of the extraction was 30 min and the volume of extract ~ 60 ml. Recoveries of MCCPs were good (79–108%), with coefficients of variation ranging from 14 to 21%. As regards brominated compounds, PLE uses have also been reported [32, 33]. In these reports, CH_2Cl_2 and hexane : CH_2Cl_2 mixtures were applied as solvent extractors. The temperature and pressure used varied from 100 to 150°C and from 1000 to 1500 psi, respectively. PBDE recoveries obtained using the PLE method are similar to those obtained using the conventional Soxhlet extraction. However, it should be pointed out that lower standard deviations were found with PLE, probably due to the automation of the system.

However, in most of the reported applications of PLE, an exhaustive cleanup of the extracts prior to injection in the chromatographic system is necessary [34, 35]. In an attempt to eliminate this time-consuming step, some authors proposed in-cell cleanup by packing the sample dispersed in an adsorbent, such as modified silica, Florisil or alumina [36–38].

On the other hand, MAE affords the opportunity of performing several simultaneous extractions with a closed-vessel system (pressurized MAE). The cell containing the sample and the liquid solvent is subjected to microwave radiation that enables instantaneous and efficient heating in the presence of

microwave-absorbing compounds. However, this technique requires further filtration to obtain the final extract. MAE has been tested by a number of laboratories for the extraction of PCDDs, PCDFs and PCBs [39, 40]. The considerable saving in extraction time (up to 1 h), solvent consumption (typical volumes are ~ 30 ml) and energy, in addition to the reduction in generated waste, has made MAE a very attractive alternative to the conventional Soxhlet procedure. MAE was also used for the determination of SCCPs in river sediment samples [41]. Maximum extraction efficiencies were obtained using 30 ml of hexane/acetone (1 : 1) as solvent extraction. Extraction time and extraction temperature were also optimized. The highest recovery for SCCPs was found at 15 min, and the recoveries increased with temperature, reaching their maxima at 115 °C. Relative standard deviations of 7 and 9% were obtained for run-to-run and day-to-day, respectively.

However, the major drawback of PLE and MAE is the high investment cost of the commercialized systems.

4.1.2 Purification

The crude extracts contain a substantial amount of interfering substances. As a consequence, subsequent cleanup and fractionation is indispensable. The cleanup step is typically based on solid-liquid adsorption chromatography in open columns using a combination of different adsorbents. Among the stationary phases currently commercially available, the most commonly used are modified silica, Florisil, alumina and different types of carbon (Amoco PX-21, Carbosphere, Carbopack). However, the whole procedure is time and labour intensive, and it represents the bottleneck of the analytical method. The development of sample-handling techniques is directed, on the one hand, toward automation, and from another, toward development of more selective adsorbents such as immunosorbents. Automated cleanup systems were developed based on the use of pressured column chromatographic procedures. This is regarded as an alternative system that offsets most of the disadvantages of the conventional cleanup methods given its capacity for processing automatically different samples simultaneously in approx. 1.5 h [42, 43]. This system was recently tested for PBDE analyses. It is based on the sequential use of multilayer silica and basic alumina adsorbents respectively, pre-packed in Teflon columns. The automated system configuration consists of a valve module, a valve drive module and a pump. The whole system is computer controlled and can be programmed as required (i.e. volume, flow rates, direction of solvent flow ...).

Immunoaffinity chromatography (IAC) is one approach that has been investigated to simplify dioxin cleanup [44–46]. Immunoaffinity columns have been generated from anti-dioxin antibodies and shown to selectively bind dioxins from samples. A monoclonal antibody column showed acceptable re-

coveries and reliable quantification for five of the most toxic dioxins and furans at the sub parts per trillion (ppt) level. When compared to classical cleanup and isolation methods, the IAC procedure was more than 20-times faster and used 100-times less organic solvents, and their selectivity was enormously enhanced. The application of IAC to PCB analysis has shown similar potential in a preliminary study [47]. Limitations of IAC include its incompatibility with high fat matrices and the lack of selectivity for all 17 toxic congeners.

4.1.3

Chromatographic Separation

The method of choice for the determination of many halogenated contaminants is GC because the volatility of these compounds allows a GC determination. The development of capillary columns in GC enables congener-specific determination of a number of these mixtures. The isomer specific elution pattern of PCDDs and PCDFs is well established for such widely used columns as DB-5/DB-5ms (methyl, 5% phenyl polysiloxane), DB-Dioxin (44% methyl, 28% phenyl, 20% cyanopropyl polysiloxane with 8% polyoxyethylene), Sil 88/SP 2331 (100% cyanopropyl polysiloxane) [48–50]. Analogous studies have been reported on the chromatographic separation of PCB congeners using different columns such as DB-5 [51] or SGE HT8 [52]. A few studies were published regarding the PBDE elution pattern [53]. De Boer et al. [54] reported that a good separation can be obtained for most PBDE congeners using 50 m columns. However, more studies are required in order to determine potential coelutions between PBDE congeners. Using the available 46 PBDE standards from mono- to decabromination, a gas chromatography relative retention time model was developed to predict retention times of the 209 individual PBDE congeners [55]. This model concludes that full congener resolution and reliable quantification of all 209 PBDEs on a single column may either require significant advances in column materials and instrumental programming, or simply may not be possible. More and more scientists have become aware of the limitations of single-column capillary GC for the determination of chlorinated and brominated POPs. The chromatographic separation of the toxic isomers from all the non-toxic ones requires the use of at least two columns with different composition and polarity. Injection on two different columns is recommended for an unambiguous determination.

BDE-209 should receive special attention because of its sensitivity for higher temperatures and the higher susceptibility for degradation in the GC system. Analyses were carried out using short GC columns (10–15 m) avoiding long exposures to elevated temperatures [56, 57]. The film thickness of the short column should preferably be 0.1–0.2 μm , again with the aim not to extend the exposure to high temperatures unnecessarily [54]. This means that

the analysis of BDE-209 should occur separately from the analysis of the rest of PBDE congeners.

In recent years, comprehensive two-dimensional chromatography (GCxGC) has been shown to be very useful for the separation of various complex samples. In GCxGC two independent GC separations are applied to the sample. The most obvious advantage of GCxGC is the large peak capacity. Because retentions in the two dimensions are almost independent, the peak capacity that can be achieved is close to the product of the peak capacities of the two individual columns [58]. Another advantage of the GCxGC system is the increase of signal to noise ratios, which leads to an improvement in detection limit. Finally, all peaks in the chromatogram are described by two time co-ordinates, which make the identification more reliable. Some studies examined the potential of GCxGC for the qualitative analysis and characterization of complex mixtures of halogenated contaminants, such as PCBs, with emphasis on the non- and mono-*ortho* PCB congeners [59, 60]. Focant et al. [61] optimized a GCxGC methodology for the determination of PCBs, PBBs and PBDEs. Using this technique, they solve most of the potential coelution problems that can arise during the simultaneous analysis of several classes of halogenated POPs.

Analysis of PCAs is a difficult task because these mixtures contain at least several thousand individual congeners. Complete separation of individual compounds cannot be accomplished by means of single capillary column GC. PCA chromatograms display a characteristic broad envelope indicative of a large number of co-eluting peaks [62]. Coelutions in PCA analyses are cases where the use of GCxGC should be rewarding. Korytár et al. [63] tested the recently introduced rapid-scanning quadrupole mass spectrometer with an electron capture negative ionization (ECNI) option as a detector for GCxGC in the analysis of PCAs. Figure 2 shows the chromatogram obtained for a mixture of polychlorinated decanes with an average chlorine content of 65 wt. %. As can be seen, the separation was much improved, though not yet complete, and an ordered structure with four parallel groups of peaks was observed. More recently, GCxGC with ECNI-time-of-flight (ToF)-MS was used to study the composition and characteristics of SCCP, MCCP and LCCP mixtures [64]. From among the six column combinations tested, DB-1 x 007-65HT was found to be the best choice. The separation of PCA congeners with the same chain length is based on the number of chlorine substituents. When mixtures of PCAs of varying chain length are analyzed, ordered structures are observed which comprise compounds having the same number of carbon plus chlorine atoms (for example, $C_{10}Cl_8$ and $C_{11}Cl_7$, or $C_{12}Cl_6$ and $C_{13}Cl_5$). With the selected column combination, PCAs which differ at least three carbon atoms in their chain length were well resolved. This enables us to distinguish between SCCPs, MCCPs and LCCPs.

HBCD can be determined by GC-MS, using similar methods developed for PBDE determinations. As apparently the response factors of the three diastereomers do not differ very much, HBCD can be quantified as total

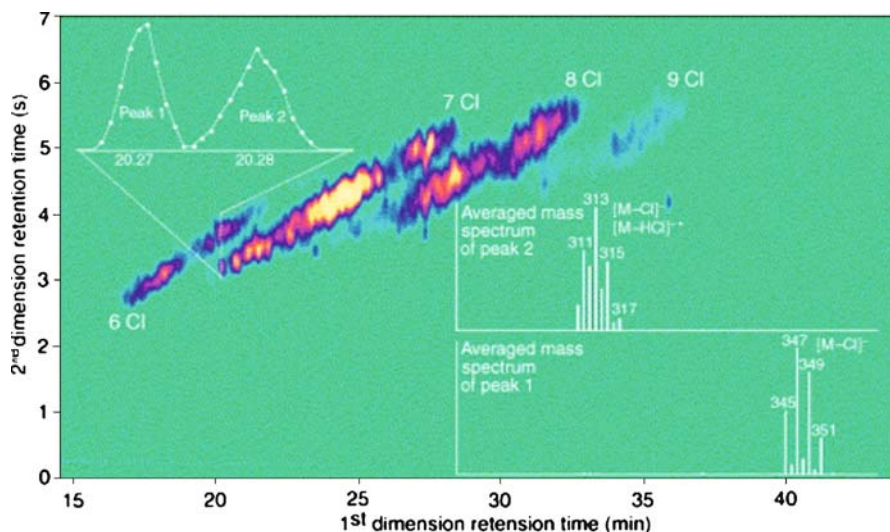


Fig. 2 Full-scan GC×GC-ECNI-MS of a mixture of polychlorinated decanes with average chlorine content of 65 wt. %. *Left-hand-side insert*: separation of hexa- and hepta-chlorinated decanes in second dimension and number of data points per peak. *Right-hand-side insert*: part of averaged mass spectra of peaks 1 and 2. Reproduced from [63]

HBCD. However, until now the different isomers have not been separated by this technique. Moreover, and because isomers of HBCD are thermally labile (it is known that HBCD decomposition takes place between 240 and 270 °C), elution from a GC column usually results in a broad and diffuse peak. In addition, a number of chromatographic peaks corresponding to different breakdown products were detected. These peaks could interfere with some BFR congeners, such as BDE-99 [65]. To solve these analytical difficulties, recent investigations were made using LC approaches. Actually, LC-MS and LC-tandem MS (MS-MS) are the best methods for measuring HBCD diastereoisomers separately in environmental samples [66, 67]. Chromatographic columns such as C18 were used in the majority of HBCD-isomers studies.

But, the α -, β - and γ -HBCD diastereoisomers are chiral and because of that must be present in the environment as enantiomeric pairs. The enantiomers have identical physicochemical properties and abiotic degradation rates, but may have different biological and toxicological properties and therefore different biotransformation rates. These transformations may result in non-racemic mixtures of the enantiomers that were industrially synthesized as racemates. A chiral chromatographic column must be used in order to obtain an enantiomeric separation. All recent studies showed a good separation using a Nucleodex β -PM (4.0 mm \times 200 mm \times 5 μ m) [68–70]. A representative chromatogram of a standard mixture of three diastereomers of HBCD

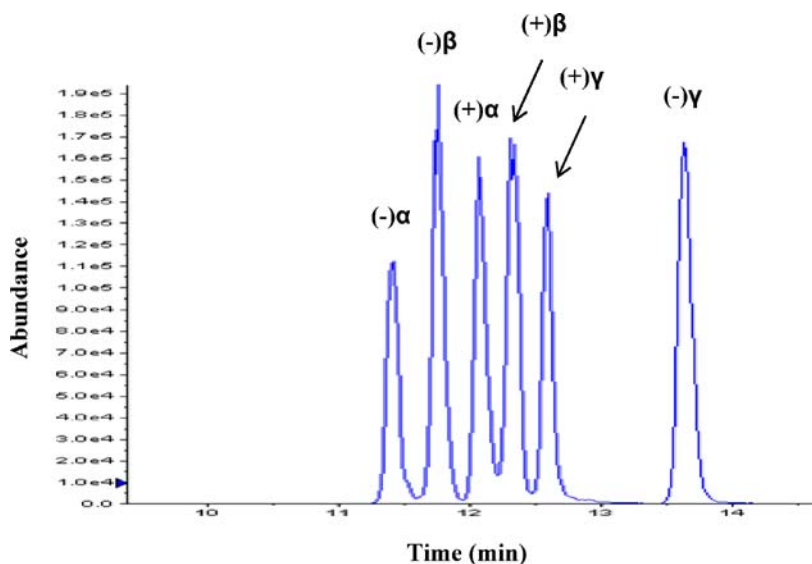


Fig. 3 Enantiomeric separation of a standard mixture of three diastereomers of HBCD (3 pairs of enantiomers) using a chiral column

that results in six peaks is illustrated in Fig. 3. The elution order of each enantiomer was assigned according to Heeb et al. [71]. Good separations were obtained for $(-)\alpha$ -, $(-)\beta$ - and $(-)\gamma$ -HBCD. Only in the case of $(+)\alpha$ - and $(+)\beta$ -, and $(+)\beta$ - and $(+)\gamma$ -HBCD separations there are some minor coelutions (at 4.9 and 4.4% of the baseline, respectively).

4.1.4

Mass Spectrometric Detection

The complexity of chlorinated and brominated POP analyses requires a comprehensive approach for their quantitative determination in environmental samples. Moreover, concentrations of some of them, such as PCDDs and PCDFs in environmental samples are decreasing [72, 73] reflecting a general decline in dioxin inputs to the environment owing to tighter controls. The safe values established in different directives and recommendations are more and more restrictive, thus analytical methodologies must be able to achieve the low detection limits now required.

The PCDD, PCDF and PCB analyses involve detection of multiple congeners at the ppt or ppq level for which isotope dilution techniques using GC-HRMS are currently recommended methods (EN Method 1948, US EPA Method 1613, US EPA Method 8290, US EPA Method 1668) [74–76]. HRMS was used operating in the electron ionization (EI) mode at a resolving power of 10 000. Under these conditions, different ions (isotopic labelled included)

were monitored in selected ion monitoring (SIM) mode. Identification of analytes was based on the following restrictive criteria: (1) retention times of chromatographic peaks must be within the appropriate chromatographic windows; (2) simultaneous responses for the two masses monitored must be obtained; (3) signal to noise ratios must be greater than 3; and (4) relative isotopic peak ratios must be within $\pm 15\%$ of the theoretical values. Once these criteria were accomplished, assignment of toxic congeners was performed by comparing the retention times with the corresponding labelled standards added as internal standards. Quantification was carried out by an isotopic dilution technique, based on the addition of labelled standards.

GC-NCI-MS and GC-EI-MS are the approaches more frequently used for PBDE analyses. Mass spectra strongly depend on the type of ionization used. NCI mass spectra of all PBDEs were dominated by the bromine ion $[\text{Br}]^-$ and did not show any molecular ion. In contrast, EI provided better structural information, giving the molecular ions and the sequential losses of bromine atoms. For NCI-MS experiments, the two ions corresponding to $m/z = 79$ and 81 ($[\text{Br}]^-$) were monitored, whereas for EI-MS experiments, the two most-abundant isotope peaks for each level of bromination, corresponding to the molecular cluster for mono- to tri-BDEs and $[\text{M}-\text{Br}_2]^+$ for tetra- to hepta-BDEs, were selected.

The main advantage of NCI-MS versus EI-MS is the lower limit of detection (LOD) afforded, but higher specificity and accuracy were obtained using EI-MS. However, both ionization modes are subjected to different types of interferences. Sediment samples are usually polluted with a variety of compounds. The general problem in analysis of complex samples is that the extract obtained by exhaustive extraction techniques typically contains a large number of matrix components, which may co-elute with the analytes and disturb the quantitative analysis. The presence of interfering substances demands either a very selective detection or tedious extract cleanup or even both. The presence of potential interferences in NCI and EI approaches was studied by some authors [77, 78]. In general, EI-MS is affected by chlorinated interferences especially PCBs. Sediments are frequently contaminated with both PBDEs and PCBs. Moreover, analytical procedures for PBDE analyses are mainly based on already available analytical methods for PCBs. Thus, purified extracts contained both PCBs and PBDEs. Few of the ions commonly used for the determination of PBDE homologue groups are isobaric with PCB homologue groups. Moreover, under the chromatographic conditions normally used there are some coelutions, i.e. hepta-CBs eluted in the same chromatographic window as tetra-BDEs.

NCI-MS eliminated chlorinated interferences but presented different brominated interferences well resolved with the EI-MS approach. When PBBs and PBDEs were simultaneously determined, some important coeluting peaks appear. One critical chromatographic pair is BDE-154 and PBB-153 which coelute in many cases [54]. PBB-153 and TBBPA can also coelute with BDE-

154 and BDE-153, respectively, when using non-polar capillary columns and hence interfere with the determination of the corresponding PBDE congeners when monitoring the bromine ions ($m/z = 79$ and 81). Moreover, naturally produced brominated compounds, such as halogenated bipyrrols, brominated phenoxyanisols can be considered as potential interferences.

It was generally believed that the MS-MS technique surpasses others in analytical specificity, but it was not widely used owing to its relatively poor sensitivity and reproducibility. QIT-MS in tandem mode has been confirmed as a low-cost alternative for the analysis of some persistent organic pollutants (POPs) such as dioxins, furans and PCBs [79, 80]. Following this trend, the feasibility of QIT-MS for the analysis of PBDE congeners in sediment samples was tested [81]. The developed method allowed the quantification by isotopic dilution technique. Moreover, the sensitivity obtained using QIT-MS was compared with NCI-MS and EI-MS. LODs ranged from 57 to 128 fg, from 62 to 621 fg, and from 0.7 to 14 pg, for NCI-MS, QIT-MS and EI-MS, respectively. Thus, similar sensitivity was observed for QIT-MS and NCI-MS techniques. Taking into account that the main disadvantage of NCI-MS is the low specificity, and that this problem is well solved by QIT-MS, IT-MS seems to be the alternative offering the best compromise between sensitivity and selectivity.

Since the availability of labelled standards for other chlorinated and brominated POPs, such as PCNs and PBDEs, similar isotope dilution techniques using GC-HRMS were developed for an accurate determination of these contaminants [82, 83].

At the moment, isotope dilution techniques using GC-HRMS are the established and recommended methods. However, other MS techniques, such as time-of-flight (ToF)-MS, are now being developed for this kind of analysis. Two complementary approaches are available in ToF-MS. One employs instruments that provide high resolution but have a moderate scan speed, the other instruments that feature a high storage speed of, typically 100–500 spectra per second but usually provide only unit mass resolution. The first instruments are interesting for high-resolution applications, whereas the second group is used in studies that apply GCxGC. Because of its non-scanning character ToF-MS is a valuable tool for fast GC because this type of instrument can be used to monitor the entire mass range in very short times with high sensitivity. Narrow bore columns are a popular choice when performing complex GC analyses. They produce excellent chromatographic resolution without incurring the penalty of excessive run time. In the field of GC-MS, the disadvantage of short run times where separated components have narrow peak widths is that the desirable data sampling rate exceeds the capability of the MS. Scanning-type mass spectrometers are inherently unsuitable for fast separations, since they are, at best, limited to only 5 or 10 data samples per second. Therefore, when the GC peak widths are below 0.5 s wide, a different type of mass detector must be used. The ToF-MS is ideally suited to this type

of analysis due to the high sampling rates that can be achieved (hundreds of mass spectra per second).

Different recent studies showed the capabilities of ToF-MS for the analysis of PCBs in different type of samples [84–88]. The ToF-MS analyses were generally achieved using an orthogonal acceleration ToF mass spectrometer. Using this state of the art technology it is possible to operate at elevated resolving power (7000). The GC-ToF-MS results were consistent with the GC-HRMS results within the 95% confidence interval limits [86]. These results are very encouraging, and show that GC-ToF-MS allows for analysis times an order of magnitude faster than GC-HRMS methods without a loss in qualitative or quantitative power.

As regards PCA determinations, while a GC-MS method based on SIM of positive ions has been reported [89], the more popular methods have relied on ECNI-LRMS. However, the major problem associated with these methods is lack of selectivity. Since Tomy et al. [90] developed a quantitative method for SCCP analyses in environmental samples based on the use of GC-ECNI-HRMS, this approach was the more frequently used, and it became the standard method for PCA determinations. Using this methodology, molecular composition could be determined by monitoring $[M-Cl]^-$ ions of specific m/z value corresponding to formula groups and by assuming that the integrated ion signals are proportional to molar concentration weighted by the number of chlorine atoms in the formula group. Formula group abundance profile generation is useful because it allows PCA concentrations to be reported according to individual formula and homologue groups.

GC-ECNI-HRMS is a very selective detection method; however, this detection method is not available at many laboratories and is too costly for routine analysis. Therefore, LRMS is also used for the quantification of PCAs. The use of LRMS increases the risk of interferences, which have to be controlled and eliminated. An improved sample cleanup removing other polychlorinated compounds is one possibility. But, disturbances might also occur when mixtures of SCCPs and MCCPs are present. Reth and Oehme [91] studied the limitations of the GC-ECNI-LRMS for the analysis of SCCPs and MCCPs. The analysis can be disturbed by mass overlap caused by congeners with the same nominal mass, but with five carbon atoms more and two chlorine atoms less, for example, $C_{11}H_{17}^{37}Cl^{35}Cl_6$ ($m/z = 395.9$) and $C_{16}H_{29}^{35}Cl_5$ ($m/z = 396.1$). This can lead to an overestimation of congener group quantity and/or total PCA concentration. The authors of the study concluded that the quantification of the most-abundant congeners (C_{11} – C_{14}) is not affected by this interference, if isotopic ratios, retention time changes and shapes of the signals are investigated. As regards sensitivity, LODs were around 1 ng/ μ l, whereas HRMS provided lower LODs (between 60 and 200 pg) [90]. However, the sensitivity of LRMS is still appropriate for the analysis of PCAs in environmental samples.

Castells et al. [92] also developed a method based on the use of GC-ECNI-LRMS. Since the $[M-Cl]^-$ and $[M-HCl]^-$ cluster ions are subject to interference from other PCA homologues, the $[HCl_2]^-$ and $[Cl_2]^-$ ions have been selected for quantification. Moreover, $[M-Cl]^-$ ions always showed lower responses than the $[HCl_2]^-$ and $[Cl_2]^-$ ions. Nevertheless, other organochlorine compounds could interfere with the determination using these common fragment ions. The authors evaluated the potential interferences between organochlorine pesticides, toxaphenes, PCBs and PCNs with SCCPs, and they concluded that no significant interferences were observed under the proposed GC-MS conditions. This is due to the fact that, under optimized ECNI conditions (methane pressure set at 1.2×10^{-4} Torr, and trap-offset voltage set at 3 V), the formation of $[HCl_2]^-$ and $[Cl_2]^-$ ions from the potential interferences was not favoured. Only some organochlorine pesticides gave a small response, and they did not coelute with SCCPs in the chromatogram. Moreover, the LOD obtained using this method is 0.20 ng, which was lower than those obtained in other studies working with ECNI-LRMS. It should be mentioned that one disadvantage of the proposed method is that obtained information was a total PCA concentration, but not the molecular composition.

The use of ECNI, however, has some disadvantages. Response factors depended strongly on the number of chlorine atoms and their position at the carbon chain. Congeners with a higher degree of chlorination have higher response factors due to a higher electron affinity. Therefore, the use of different technical mixtures as quantification standards can lead to considerable deviations in the results. Additionally, PCAs with low chlorine content are neither sensitively nor completely detected by ECNI-MS. The use of CH_2Cl_2 /methane reagent gas mixtures is described as an alternative to conventional GC-ECNI-MS, which used methane as the moderating gas [93]. Using this non-conventional reagent gas, a nearly exclusive formation of $[M+Cl]^-$ ions was observed, enhancing selectivity and sensitivity. Both interferences, PCAs themselves and other chlorinated contaminants, were solved or reduced by selecting $[M+Cl]^-$ ions, enabling the determination by LRMS. Moreover, quite similar response factors were obtained for congeners of different degrees of chlorination, allowing the use of technical mixtures as reference standards for a congener- and homologue-specific analysis. Another interesting advantage is that, in contrast to methane-ECNI, CH_2Cl_2 /methane-NICI enabled also the detection of tri- and tetrachloro congeners.

4.2

Bioanalytical Methods

Chemical analysis of contaminants in environmental matrices is essential to assess exposure concentrations of individual compounds, e.g. for (trend) monitoring and for risk assessment purposes. However, with this type of analysis alone, biological effects cannot be predicted, and mixture effects and

contributions of unknown compounds with similar modes of action to the overall effect cannot be taken into account. Therefore, several bioassays have been developed as tools to address the above-mentioned issues. These assays can be used to determine potencies of individual compounds and for measuring the total activity of complex mixtures of compounds. Bioassays can thus be used to determine total activities in sediment samples, without the necessity of knowing all compounds present that contribute to the activity.

Determination of environmental pollutants using biodetectors such as bioassays, biomarkers, enzyme immunoassays or other bioanalytical tools is a continuously growing area. Behnisch et al. [94] reviewed the principles and advantages/limitations of several bioanalytical detection methods for the screening and diagnosis of some chlorinated and brominated POPs, with special emphasis on dioxin-like compounds (DLCs). These methods are based on the ability of key biological molecules (e.g., antibodies, receptors, enzymes) to recognize a unique structural property of the DLC, or on the ability of cells or organisms to have a specific response to DLCs. Bioanalytical methods for measuring DLCs included the EROD bioassay, the AHH bioassay, the enzyme immunoassay (EIA), the reporter gene assay (e.g., CALUX), the gel retardation of AhR DNA binding (GRAB) assay, the reach receptor DELFIA assay kit, the AhR assay with radiolabelled dioxins, and the Ah-immunoassay (AhIA). For several bioanalytical dioxin tests, official methods by governmental authorities have been approved such as EPA Method 4425 (reporter gene assay) or EPA Method 4025 (immunoassay). Moreover, the minimum detection limits reported for 2,3,7,8-TCDD for the bioanalytical methods are similar to those reported for chemical analysis.

The CALUX and EROD methods were two of the most-used bioassays for measuring the dioxin-like activity of environmental samples. The EROD method measured the binding of the dioxin-like compound to the AhR and the subsequent induction of CYP1A related deethylation of 7-ethoxyresorufin to resorufin. In this bioassay, several CYP activities can be measured by using different substrates. Moreover, several cell lines are used for the EROD bioassay, e.g., the rat H4II cell line, the chicken embryo hepatocytes, cultured chicken embryo liver, etc. On the other hand, the CALUX bioassay is based on the induction of reporter genes through AhR binding and the subsequent analysis of light production by luciferase. Similar results were obtained using both bioassays; however, CALUX is faster, has a more stable response with better reproducibility and luciferase is more stable than EROD protein.

4.3

Toxicity Identification Evaluation (TIE) Methods

Assessments of the risk connected to the contamination should rely on a multidisciplinary approach based on both chemical and biological techniques and should be able to address both exposure and effects. Traditional chemical

techniques for the detection and quantification of contaminants provide detailed information regarding the presence and concentration of specific compounds. On the other hand, information often is limited to a small number of compounds that are above the limit of detection and for which analytical standards are available. Furthermore, chemical techniques do not provide any information concerning the biological activity of the contamination. This is particularly relevant when complex mixtures are present at low levels and synergistic or antagonistic effects may take place.

In order to identify compounds responsible for specific effects (i.e., endocrine disrupting or AhR ligands) observed in field studies, TIE or bioassay directed analysis approaches have increasingly been applied over the last decade. In such approaches, sensitive bioassays are used to direct the fractionation of a sample extract until its complexity is sufficiently reduced to enable identification of those compounds responsible for the activity measured in the bioassay. This strategy is based on differential extraction and fractionation methods and identification by chemical and biochemical analysis. TIE is a well-established technique having been originally developed by

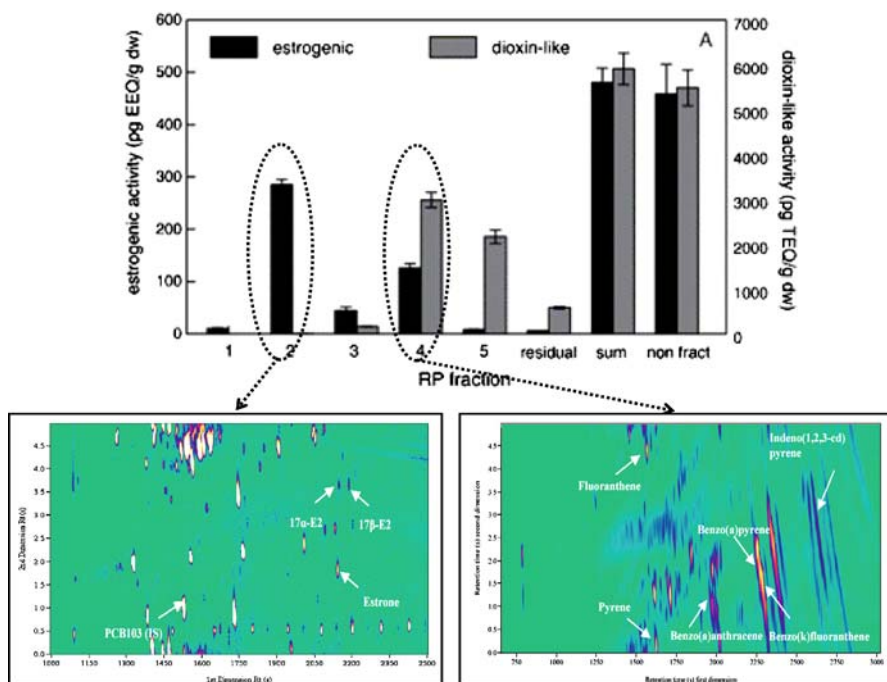


Fig. 4 Estrogenic and dioxin-like activities in fractions of sediment extract from Kierikzee harbour: fraction 2 showed characteristic ions of 17 α - and 17 β -estradiol and estrone, and fraction 4 showed characteristic ions of various polycyclic aromatic hydrocarbons. Reproduced from [95]

the U.S. Environmental Protection Agency for identifying the cause of toxicity in effluents. More recent applications include some studies related to POP contamination. For instance, Houtman et al. [95] identified estrogenic and DLCs in sediments from Zierikzee harbour with a CALUX assay-directed fractionation combined with GCxGC-ToF-MS. To reduce the complexity of the sediment extracts, total extracts were fractionated (Fig. 4). Five fractions with decreasing polarity were collected and tested for estrogenic and dioxin-like activities. Most estrogenic activity was found in fraction 2 which is known to cover the natural estrogenic hormones. To confirm the presence of natural estrogenic hormones in this fraction, GCxGC-ToF-MS was applied, and the presence of 17α -, 17β -estradiol and estrone was confirmed. Moreover, most dioxin-like activity was found in fraction 4. In this case, the presence of polycyclic aromatic hydrocarbons, which are known to have dioxin-like properties, justified the activity detected in this fraction.

In a more recent work, Puglisi et al. [96] introduced the relevance of using approaches based on non-exhaustive extraction techniques. Usually, approaches are based on total extraction of contaminants, and then, they do not take into account the importance of bioavailability and aging processes. Tenax and cyclodextrin extractions over time were carried out to determine the bioavailable fractions. Results obtained in this study showed that the adoption of a bioavailability-based assessment of contamination led to a large reduction of the toxicity signal (Fig. 5). The total extract had 70 pM; this signal was reduced to 19 pM for the cyclodextrin-assessed bioavailable fraction and to only 3 pM for the Tenax-assessed bioavailable fraction.

From this study, we can conclude that coupling of non-exhaustive extraction and bioanalyses leads to a more realistic and, generally much lower estimated risk for the toxicity of the extracts as compared to commonly

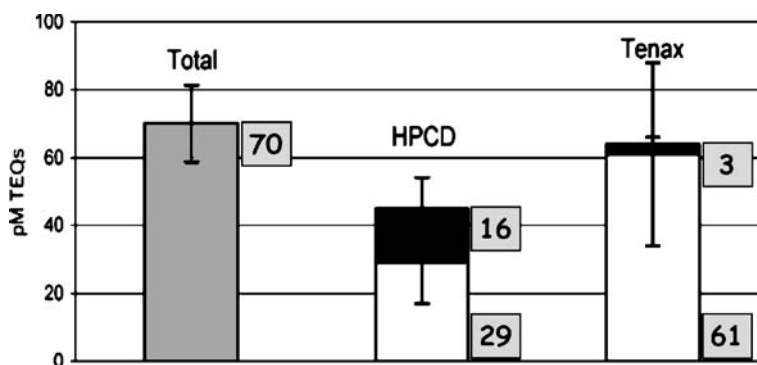


Fig. 5 Comparison between DR-CALUX signals of total, bioavailable, and residual fractions. Bioavailable and residual fractions were collected by hydroxypropyl- β -cyclodextrin (HPCD) or by Tenax extraction. Total extraction was microwave assisted. Reproduced from [96]

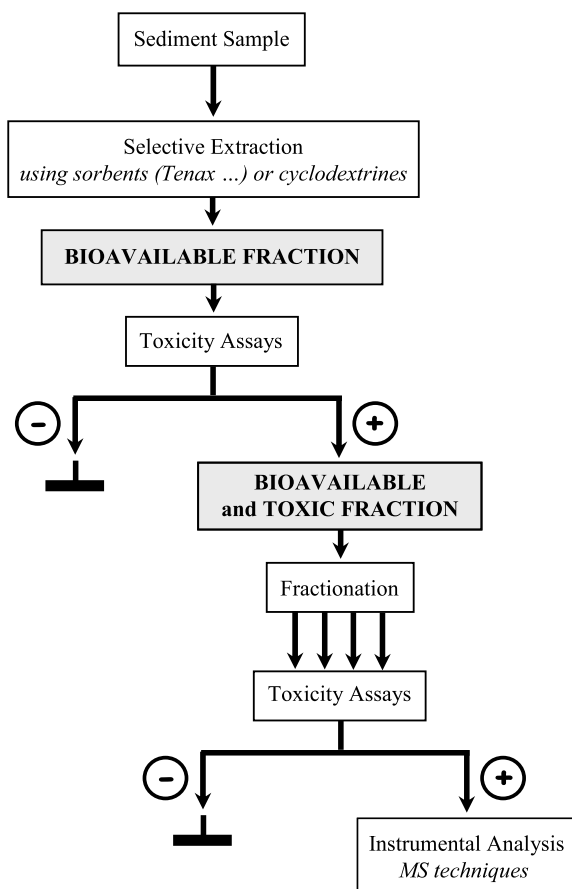


Fig. 6 Different steps to be followed in the new concept of a bioavailable toxicity identification evaluation (BTIE) method

adopted exhaustive techniques. Following these findings, we proposed the introduction of a bioavailable toxicity identification evaluation (BTIE) concept, in which the steps to be followed are similar to those of TIE methods, but following bioavailable extractions (Fig. 6).

5 Environmental Levels

The contamination of sediments may pose an unacceptable risk to aquatic organisms, which tend to bioaccumulate chlorinated and brominated POPs, and to wildlife and humans through the ingestion of contaminated fish and shellfish. During the last four decades a large amount of environmental data

has been generated for different chlorinated POPs, such as PCDDs, PCDFs and PCBs. However, for some other chlorinated POPs (e.g. PCAs) literature data are very scarce. As regards brominated POPs, during the last decade a large amount of environmental data has been generated for PBDEs. However, HBCD literature data is scarce.

5.1

Chlorinated Organic Pollutants

In the literature, the PCDD and PCDF results are given as Toxicity Equivalent Quantities (TEQs). Between the 210 PCDD and PCDF congeners, the most toxic molecules are those whose positions 2, 3, 7, 8 are chlorinated. The compounds that meet these conditions number a total of 17, 10 furans and 7 dioxins. Since the individual toxicity of these compounds is different, the real toxicity of a mixture was assessed bearing in mind the relative toxicity of the isomers with respect to the most toxic isomer, the 2,3,7,8-TCDD; a toxicity equivalence factor (TEF) equal to the unit was assigned to the 2,3,7,8-TCDD. For the toxic assessment, the 17 toxic isomers were normalized by multiplying their measured concentrations by the appropriate TEFs. The sum of these products yields the total TEQs, which express these analyte concentrations as a single number, equivalent to that of a toxicity derived exclusively from 2,3,7,8-TCDD.

In order to assess the quality of freshwater sediments, quality objectives for PCDDs and PCDFs have been formulated. Of the eight approaches available, the tissue residue-based (TRB) is the most commonly used. This method involves establishing a safe chemical concentration in sediment which results in an acceptable tissue residue in biota. A NOEC (no observed effect concentration) of 200 pg TEQ/g dw in sediment was derived, but when chronic toxicity data are scarce a safety factor of 10 is applied, which resulted in a safe sediment value of 20 pg TEQ/g dw [97]. Empirically derived sediment quality guidelines (SQGs) were developed on the basis of the associations observed between measures of adverse biological effects and the concentrations of potentially toxic substances in sediments. One set of SQGs developed includes the effects range low (ERL) and effects range median (ERM) values. The ERL value is known to be a concentration that has no harmful effect on biota. An ERL value of 50 ng/g dw was established for total PCBs in sediments [98].

A number of studies have reported PCDD and PCDF levels from sediments in North America, Europe and Asia (Table 2). Generally, the PCDD and PCDF levels in background areas ranged between < 0.1 and the safe sediment value established at 20 pg TEQ/g dw, whereas levels found in polluted areas (i.e., harbours), clearly exceeded the safe value.

Regarding the PCB data, a number of studies have reported levels expressed as total PCBs or as a sum of the seven indicator PCBs; however, the literature on the dioxin-like PCBs is very scant. The concentrations found

Table 2 Levels of PCDDs and PCDFs in sediments from different locations

Location	Levels (pg TEQ/g dw)	Year [Refs.]
<i>Background areas</i>		
11 lakes (USA)	0.1–15.6	1996 [122]
River Danube (upper Austria)	0.4–12	1993 [123]
Oder river (Germany)	0.1–17.5	1997 [124]
Orbetello lagoon (Italy)	0.4–7.3	1998 [125]
Volga riverside (Russia)	0.08–9.4	2000 [126]
Ebro river (Spain)	0.4–3.7	2001 [127]
Llobregat river (Spain)	1.8–7.7	2001 [127]
Umler estuary (UK)	14–24	1996 [128]
12 rivers (Japan)	0.02–24	1998 [129]
Han river (Korea)	0.04–4.4	2000 [130]
<i>Polluted areas</i>		
Black Rock harbour (USA)	223–250	1989 [131]
New Bedford harbour (USA)	10–761	1989 [131]
18 lakes (central Finland)	< 20–230	1990 [102]
Kymijoki river (Finland)	100–59 000	1995 [132]
Chemieharbour (The Netherlands)	434–923	1989 [133]
Laurens harbour (The Netherlands)	352–1849	1989 [133]
Frierfjorden (Norway)	6234–19 444	1989 [134]
(Japan)	1.1–150	1999 [135]

in the background areas ranged between < 0.1 and 28 ng/g dw, always below the ERL value of 50 ng/g dw. However, the levels found in the polluted areas were higher, ranging between 200 ng/g dw and 120 µg/g dw. Khim et al. [99] estimated concentrations of TEQ_{PCB} in sediments from Korea, in the range of 0.05–1.7 pg/g dw. Müller et al. [100] found a mean TEQ_{PCB} value of 20.3 pg/g dw in sediments from Germany. Eljarrat et al. [101] reported concentration levels between 0.03 and 25 pg TEQ/g dw in sediments from the Catalan coast (Spain).

PCNs were quantified in sediment samples from different locations. In sediments from Central Finland [102], the total PCN values ranged from 0.5 to 3.5 ng/g dw. Kjell et al. [103] measured PCN contamination in surface sediments collected in the Gulf of Bothnia (northern Baltic Sea). PCN concentrations were in the range of 0.1–1.9 ng/g dw. PCNs were also studied in two Mediterranean lagoons (Venice and Orbetello, Italy) [104]. The levels of the sum of mono- to octa-CN ranged from 0.03 to 1.5 ng/g dw. In a Swedish sediment study [105], the PCN results ranged between 0.6 and 304 ng/g dw. Kannan et al. [106] determined the concentrations of PCNs in sediments from the upper Detroit and lower Rouge Rivers. Levels ranged from 0.08 to 187 ng/g dw. On average, penta- and hexa-CN accounted for 60% of the total PCN concentrations in sediments. PCN-71 and 72 (1,2,4,5,6,8- and

Table 3 Levels of PCAs in sediments from different locations

Location	Levels (ng/g dw)	Year [Refs.]
Detroit River (North America)	288	1997 [90]
Canada	4.52–135	1999 [30]
Norway	330–19 400 *	2003 [136]
Besos River (Spain)	0.27–3.26	2004 [41]
North Sea	10–258	2004 [137]
Baltic Sea	209–876	2004 [137]
Lake Thun (Switzerland)	47	2008 [107]

* This results are expressed in wet weight basis

1,2,4,5,7,8-HxCN) were the most abundant in sediment from several locations, followed by PCN-59 (1,2,4,5,8-PeCN) and PCN-69 (1,2,3,5,7,8-HxCN), which collectively accounted for 20 to 30% of the total PCN concentrations.

Literature data on PCA concentration levels in sediments are very scarce (Table 3). Recently, Iozza et al. [107] studied a dated sediment core from lake Thun covering the last 120 years, to get an overview of the historical trend of PCAs. Total PCA concentrations (sum of SCCPs, MCCPs and LCCPs) showed a steep increase in the 1980s and a more-or-less stable level of 50 ng/g dw since then. Comparison of the time profiles of total PCAs, SC-

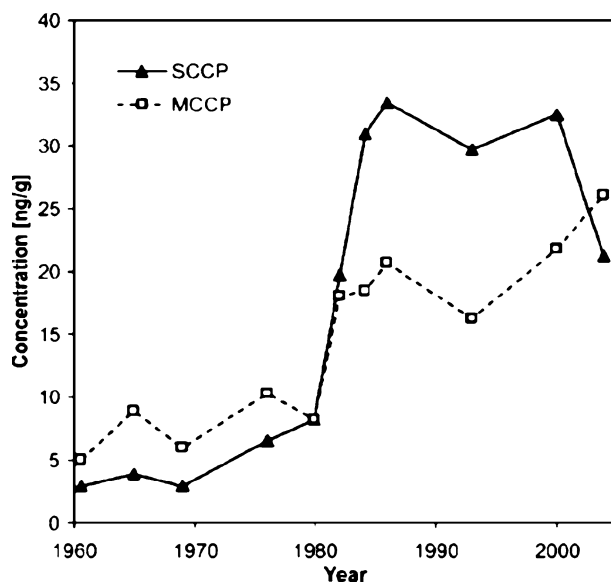


Fig. 7 Historical time trends of SCCP (solid line) and MCCP (dashed line) concentrations. Reproduced from [107]

CPs and MCCPs revealed that the rapid increase of total PCA concentrations in the 1980s is mainly caused by SCCPs, whereas MCCP levels changed much less (Fig. 7). The maximum SCCP concentration was 33 ng/g dw in 1986, and the level of the surface sediment was 21 ng/g dw of SCCPs. MCCP concentrations increased since 1965 and reached a maximum in the surface sediment (26 ng/g dw). A decrease of SCCPs and a shift to more MCCPs was observed after 2000. Future research must be planned to verify this decrease, which could be attributed to an effect of the regulations of the EU Water Framework Directive and the preceding discussions about a general ban of SCCPs.

5.2

Brominated Organic Pollutants

Several studies have been done on the concentrations and distributions of PBDEs in sediments (Table 4). The major congeners detected were BDE-47, BDE-99, BDE-100, BDE-153 and BDE-209. The concentrations of these compounds were highly variable from location to location, but in general, the concentrations of PBDEs are similar to those of the PCBs. PBDEs were determined in Swedish river sediments at 8–50 ng/g dw [57]. Similar values were found in Japanese river sediments, with concentration levels between 21 and 59 ng/g dw [108]. Sediment samples from a Spanish river collected

Table 4 Reported concentrations (expressed in ng/g dw) of PBDEs in sediment samples

Matrix (location)	Compounds	Concentration	Refs.
River sediments (Japan)	Tetra- + Penta-BDEs	21–59	[108]
Downstream of a plastic industry (Sweden)	BDE-47	490	[138]
	BDE-99	750	
	BDE-100	170	
River with textile industries (Sweden)	BDE-47 + 99 + 100	nd–9.6	[57]
	BDE-209	nd–360	
Sediment (Baltic Sea)	Sum PBDE	nd–1.1	[139]
River mouth sediments (Europe)	BDE-47	< 0.17–6.2	[140]
	BDE-99	< 0.19–7.0	
River sediments (Spain)	Sum PBDE	2–42	[65]
Marine sediments (Spain)	BDE-209	2–132	[38]
River sediments (Danube)	11 PBDEs	0.06–84	[110]
River sediments (Spain)	7 PBDEs	2.5–9.8	[111]
Sediment from estuary (The Netherlands)	13 PBDEs	262–1660	[112]
Industrialized bays (Korea)	Sum PBDE	2.03–2253	[113]
River with chemical industries (Spain)	BDE-209	up to 12 000	[114]
River sediments (Pearl river)	10 PBDEs	1.9–3580	[115]

up- and downstream from a possible point source (chemical industry), presented levels ranging from 2 to 42 ng/g dw [65]. Different marine sediments collected in Spain showed concentration levels of BDE-209 ranging between 2 and 132 ng/g dw [38]. Higher levels up to 1400 ng/g dw were found in a downstream area of a manufacturing plant in the United Kingdom [109] and at 120 ng/g dw downstream of an area with textile industries [57].

More recent studies were also focused on PBDE determinations, and specially, on deca-BDE-209 levels. Sawal et al. [110] determined 11 PBDE congeners (including BDE-209) in sediments from 32 sites along the River Danube. BDE-209 was detected in 93% of the sediment samples and contributed more than 80% to the total PBDE concentration. Total PBDE levels ranged from 0.06 to 84 ng/g dw. Labandeira et al. [111] analyzed seven PBDE congeners (including BDE-209) in sediments from the Anoia and Cardener Rivers in Spain. Total PBDE levels ranged from 2.5 to 9.8 ng/g dw. BDE-209 accounted for 60% of total PBDE. Verslycke et al. [112] determined 13 PBDE congeners (including BDE-209) in sediments from the Scheldt estuary in the Netherlands, with total PBDE concentration levels ranging from 262 to 1660 ng/g dw. Moon et al. [113] analyzed PBDEs in sediments from 111 locations in three industrialized bays in Korea. Total PBDE concentrations ranged between 2.03 and 2253 ng/g dw, and from 2.0 to 2248 ng/g dw for BDE-209. Higher values were found in sediment samples collected downstream of an industrial park in Spain, with BDE-209 values up to 12 µg/g dw [114]. Mai et al. [115] determined ten PBDE congeners (including BDE-209) in 66 surface sediment samples in the Pearl River in 2002. Profiles were again dominated by BDE-209, which ranged in concentration from 1.9 to 3580 ng/g dw.

Regarding HBCD data, some studies have reported levels expressed as total HBCD; however, the literature on the HBCD isomeric distribution is very scant. Klammer et al. [116] determined total HBCD concentrations in the North Sea surface sediments, with values ranging from < 0.2 to 6.9 ng/g dw. Lepom et al. [117] determined HBCD in 12 sediment samples collected in 2002–2005 from the German Bight. Concentrations ranged from 0.03 to 6.5 ng/g dw. Verslycke et al. [112] determined HBCD in sediments from the Scheldt estuary in the Netherlands. At the three sites sampled, concentrations were between 14 and 71 ng/g dw. Evenset et al. [118] determined HBCD in four replicate sediment cores collected in April 2001 from the deepest part of Lake Ellasjoen. HBCD was detected only in the depth interval from 1 to 2 cm (median age 1980). Only the α - and γ -isomers were detected, at 0.43 and 3.9 ng/g dw, respectively. This was an order of magnitude higher than \sum BDE in the same depth slice. Three sediment cores and six surface sediment samples from Tokyo Bay were also analyzed [119]. \sum HBCD were detected for the first time in this region at concentrations ranging from 0.06 to 2.3 ng/g dw, implying widespread contamination. HBCDs first appeared in the mid-1970s and concentrations observed in the cores have increased since then.

PBDDs and PBDFs are often formed in the process of manufacturing BFRs and from the combustion of e-waste products containing BFRs. However, there is only limited information on the environmental concentrations and human exposure of PBDDs and PBDFs. Choi et al. [120] analyzed sediment samples from industrialized areas of Japan collected in 2000. Some 2,3,7,8-tetra- to hexabrominated dioxins and furans were detected in these sediment samples, with different patterns of congeners, indicating that different sources of PBDD/Fs were present. 2,3,7,8-TeBDD was detected above the limit of quantification (LOQ) in only one sediment and 2,3,7,8-TeBDF was identified in three sediments (< 0.2 – 3.2 pg/g dw). Wang et al. [121] analyzed sediment samples collected in the vicinity of a recycling site for electronic wastes, and they found trace levels of PBFDs (0.025 – 0.92 ng/g dw) and non-detectable PBDDs.

6

Conclusions and Perspectives

The wide distribution of chlorinated and brominated POP contamination in the environment suggests that monitoring programmes should be extended to include classical POPs, such as PCDDs, PCDFs and PCBs, but also new emerging POPs, such as PCAs and BFRs. Concentrations of deca-BDE and HBCD are rapidly increasing today as the PCB and PCDD/PCDF are declining. In this respect, deca-BDE and HBCD are prime examples for emerging persistent organic contaminants.

Furthermore, brominated dioxin, as well as mixed brominated-chlorinated dioxin, data are needed in order to determine their environmental impact. However, chemical analysis of mixed halogenated dioxins is very difficult due to the large number of possible combinations (there are 4,600 potential mixed congeners). In order to achieve this goal it is necessary to develop analytical procedures that permit determination of different groups of brominated contaminants.

Thus, additional investigation is required in the chemical analysis field, but also for development of new bioassays. The bioanalytical methods offer the chemical analyst the benefits of sensitive and cost/time-effective solutions to diagnose more sufficiently all kinds of environmentally POPs. In the future, these bioanalytical tools will help us to understand more the different classes of AhR agonists/antagonists, their mechanism of toxicity and their potency in the environment. Moreover, research is also needed to develop TIE programmes for the evaluation of toxicity activities in environmental samples. The existence of unknown active compounds has been basically suggested for the explanation of higher bioassay estimates. In order to answer such open questions, as well as contribution analysis of the newly known POPs such as PBDDs and PBDFs, detailed chemical fractionation and identification of the

active compounds will be important using the state-of-the-art fractionation and analytical/bioassay techniques.

Acknowledgements This work was funded by the European Union under the Global Change and Ecosystems (FP6) Water Cycle and Soil Related Aspects: Integrated modelling of the river-sediment-soil-groundwater system; advanced tools for the management of catchment areas and river basins in the context of global change (AQUATERRA, Project number 505428), and by the Spanish Ministry of Education and Science (Project numbers CTM2005-25168-E and CEMAGUA (CGL2007-64551/HID)). This work reflects only the author's views and the European Community is not liable for any use that may be made of the information contained therein.

References

1. IARC (1997) Polychlorinated Dibenzo-*p*-dioxins and Polychlorinated Dibenzofurans; Monograph 69. International Agency for Research on Cancer, Lyon, France
2. Berg MV, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, Leeuwen FXR, Liem AKD, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T (1998) *Environ Health Perspect* 106:775
3. Falandysz J (1997) *Organohalogen Compd* 32:374
4. Shojania S (1999) *Chemosphere* 38:2125
5. Zitko V (1980) *Chemosphere* 9:139
6. Campbell I, McConnell G (1980) *Environ Sci Technol* 14:1209
7. Jansson B (1998) Presented at the Annual Meeting Marine Chemistry Working Group, Stockholm, Sweden, 1998. International Council for the Exploration of the Sea, Copenhagen, Denmark
8. Environment Canada (1993) Priority Substances List Assessment Report: Chlorinated Paraffins. En 40 215/17E. Health and Welfare Canada, Ottawa, Ontario
9. Drouillard KG, Hiebert T, Tran P, Tomy GT, Muir DCG, Friesen KJ (1998) *Environ Toxicol Chem* 17:1261
10. Bucher JR, Alison RH, Montgomery CA, Huff J, Haseman JK, Farnell D, Thompson R, Prejean JD (1987) *Fundam Appl Toxicol* 9:454
11. Renberg L, Tarkpea M, Sundström G (1986) *Ecotoxicol Environ Saf* 11:361
12. Fisk AT, Cymbalisty CD, Bergman A, Muir DCG (1996) *Environ Toxicol Chem* 15:1775
13. Alaee M, Arias P, Sjödin A, Bergman A (2003) *Environ Int* 29:683
14. Renner R (2000) *Environ Sci Technol* 34:452A
15. Meerts IATM, Letcher RJ, Hoving S, Marsh G, Bergman A, Lemmen JG, Van der Burg B, Brouwer A (2001) *Environ Health Perspect* 109:399
16. Legler J, Van den Brink CE, Brouwer A, Murk AJ, Van der Saag PT, Vethaak AD, Van der Burg B (1999) *Toxicol Sci* 48:55
17. Law RJ, Allchin CR, De Boer J, Covaci A, Herzke D, Lepom P, Morris S, Tronczynski J, De Wit CA (2006) *Chemosphere* 64:187
18. De Wit C (2002) *Chemosphere* 46:583
19. Marvin C, Tomy G, Alaee M, MacInnis G (2006) *Chemosphere* 64:268
20. Tomy G, Halldorson T, Danell R, Law K, Arsenault G, Alaee M, MacInnis G, Marvin C (2005) *Rapid Commun Mass Spectrom* 19:2819

21. Barontini F, Marsanich K, Petarca L, Cozzani V (2004) *Ind Eng Chem Res* 43:1952
22. Tomy G, Budakowsky W, Halldorson T, Whittle D, Keir M, Marvin C, MacInnis G, Alahee M (2004) *Environ Sci Technol* 38:2298
23. Covaci A, Gerecke A, Law R, Voorspoels S, Kohler M, Heeb N, Leslie H, Allchin C, De Boer J (2006) *Environ Sci Technol* 40:3679
24. Dodder N, Peck A, Kuclick J, Sander L (2006) *J Chromatogr A* 1135:36
25. Vehlow J, Bergfeldt B, Jay K, Seifert H, Wanke T, Mark FE (2000) *Water Manag Res* 18:131
26. Sakai S, Watanabe J, Honda Y, Takasuki H, Aoki I, Futamatsu M, Shiozaki K (2001) *Chemosphere* 42:519
27. Brown DJ, Overmeire IV, Goeyens L, Denison MS, Clark GC (2001) *Organohalogen Compd* 54:12
28. Behnisch PA, Hosoe K, Brouwer A, Sakai S (2001) *Organohalogen Compd* 52:49
29. Ramos L, Kristenson EM, Brinkman UAT (2002) *J Chromatogr A* 975:3
30. Tomy GT, Stern GA, Lockhart WL, Muir DCG (1999) *Environ Sci Technol* 33:2858
31. Tomy GT, Stern GA (1999) *Anal Chem* 71:4860
32. Hale RC, La Guardia MJ, Harvey E, Mainor TM (2002) *Chemosphere* 46:729
33. Quade SC, Alae M, Marvin C, Hole R, Solomon KR, Bunce NJ, Fisk AT (2003) *Organohalogen Compd* 62:327
34. Ramos L, Vreuls JJ, Brinkman UAT (2000) *J Chromatogr A* 891:275
35. Hubert A, Wenzel KD, Manz M, Weissflog L, Engewald W, Schürmann G (2000) *Anal Chem* 72:1294
36. Muller A, Bjorklund E, Van Holst C (2001) *J Chromatogr A* 925:197
37. Gomez-Ariza JL, Bujalance M, Giradles I, Velasco A, Morales E (2002) *J Chromatogr A* 946:209
38. De la Cal A, Eljarrat E, Barceló D (2003) *J Chromatogr A* 1021:165
39. Eljarrat E, Caixach J, Rivera J (1998) *Chemosphere* 36:2359
40. Fernandez AE, Ferrera ZS, Rodriguez JJS (2001) *Chromatographia* 53:375
41. Parera J, Santos FJ, Galceran MT (2004) *J Chromatogr A* 1046:19
42. Turner WE, Cash TP, DiPietro ES, Patterson DG Jr (1998) *Organohalogen Compd* 35:21
43. Eljarrat E, Sauló J, Monjonell A, Caixach J, Rivera J (2001) *Fresenius J Anal Chem* 371:983
44. Shelver WL, Larsen GL, Huwe JK (1998) *J Chromatogr B* 705:261
45. Shelver WL, Huwe JK (1999) *J Liq Chromatogr Relat Technol* 22:813
46. Huwe JK, Shelver WL, Stanker L, Patterson DG Jr, Turner WE (2001) *J Chromatogr B* 757:285
47. Concejero MA, Galve R, Herradón B, González MJ, De Frutos M (2001) *Anal Chem* 73:3119
48. Ryan RJ, Conacher HBS, Panopio LG, Lau BPY, Hardy JA, Masuda Y (1991) *J Chromatogr* 541:131
49. Bacher R, Ballschmiter K (1992) *Chromatographia* 34:137
50. Abad E, Caixach J, Rivera J (1997) *J Chromatogr A* 786:125
51. Matsumura T, Tsubota H, Ikeda Y, Chisaki Y, Ito H, Morita M (1997) *Organohalogen Compd* 31:14
52. Matsumura T, Tsubota H, Ikeda Y, Chisaki Y, Ito H, Morita M (1998) *Organohalogen Compd* 35:141
53. Ikonomou MG, Rayne S (2002) *Anal Chem* 74:5263
54. De Boer J, Allchin C, Law R, Zegers B, Boon JP (2001) *Trends Anal Chem* 20:591
55. Rayne S, Ikonomou MG (2003) *J Chromatogr A* 1016:235

56. Eljarrat E, De la Cal A, Barcelo D (2004) *Anal Bioanal Chem* 378:610
57. Sellstrom U, Kierkegaard A, De Wit C, Jansson B (1998) *Environ Toxicol Chem* 17:1065
58. Phillips J, Beens J (1999) *J Chromatogr A* 856:331
59. Korytár P, Brinkman UAT, Leonards PEG, De Boer J (2001) *Organohalogen Compd* 50:21
60. De Boer J, De Geus HJ, Brinkman UAT (2000) *Organohalogen Compd* 45:1
61. Focant JF, Sjödin A, Patterson DG Jr (2003) *J Chromatogr A* 1019:143
62. De Boer J (1999) *J Chromatogr A* 843:179
63. Korytár P, Parera J, Leonards PEG, De Boer J, Brinkman UAT (2005) *J Chromatogr A* 1067:255
64. Korytár P, Parera J, Leonards PEG, Santos FJ, De Boer J, Brinkman UAT (2005) *J Chromatogr A* 1086:71
65. Eljarrat E, De la Cal A, Raldúa D, Duran C, Barceló D (2004) *Environ Sci Technol* 38:2603
66. Budakowsky W, Tomy G (2003) *Rapid Commun Mass Spectrom* 17:1399
67. Guerra P, De la Torre A, Martínez MA, Eljarrat E, Barceló D (2008) *Rapid Commun Mass Spectrom* 22:916
68. Marvin C, MacInnis G, Alae M, Arsenault G, Tomy G (2007) *Rapid Commun Mass Spectrom* 21:1925
69. Janák K, Covaci A, Voorspoels S, Becher G (2004) *Organohalogen Compd* 66:3913
70. Guerra P, Eljarrat E, Barceló D (2008) *J Chromatogr* (in press)
71. Heeb N, Schweizer W, Kohler M, Gerecke A (2005) *Chemosphere* 61:65
72. Kjeller LO, Jones KC, Johnston AE, Rappe C (1996) *Environ Sci Technol* 30:1398
73. Eljarrat E, Caixach J, Rivera J (1999) *Environ Sci Technol* 33:2493
74. US EPA (1994) Method 1613A, Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS
75. US EPA (1998) Method 8290A, Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS)
76. US EPA (1997) Method 1668, Toxic Polychlorinated Biphenyls by isotope dilution high resolution gas chromatography/high resolution mass spectrometry
77. Eljarrat E, De la Cal A, Barceló D (2003) *J Chromatogr A* 1008:181
78. Alae M, Backus S, Cannon C (2001) *J Sep Sci* 24:465
79. Focant JF, Eppe G, De Pauw E (2001) *Chemosphere* 43:417
80. Fabrellas B, Sanz P, Larrazábal D, Abad E (2000) *Organohalogen Compd* 45:160
81. Larrazabal D, Martínez MA, Eljarrat E, Barceló D, Fabrellas B (2004) *J Mass Spectrom* 39:1168
82. Ryan JJ, Patry B (2000) *Organohalogen Compd* 47:57
83. Lebeuf M, Trottier S (2001) *Organohalogen Compd* 52:22
84. Van Bavel B, Hughes J, Davis S, Wingfors H, Lindstrom G (1999) *Organohalogen Compd* 40:293
85. Hardell L, Van Bavel B, Lindström G, Fredrikson M, Hagberg H, Nordström M, Liljegen G, Johansson B (1996) *Int J Oncol* 9:603
86. Dimandja JM, Grainger J, Patterson DG Jr (1999) *Organohalogen Compd* 40:23
87. Focant JF, De Pauw E, Grainger J, Patterson DG Jr, Dimandja JMD (2001) *Organohalogen Compd* 50:25
88. Dalluge J, Roose P, Brinkman UAT (2002) *J Chromatogr A* 970:213
89. Junk SA, Meisch HU (1993) *Fresenius J Anal Chem* 347:361

90. Tomy GT, Stern GA, Muir DCG, Fisk AT, Cymbalisty CD, Westmore JB (1997) *Anal Chem* 69:2762
91. Reth M, Oehme M (2004) *Anal Bioanal Chem* 378:1741
92. Castells P, Santos FJ, Galceran MT (2004) *Rapid Commun Mass Spectrom* 18:529
93. Zencak Z, Reth M, Oehme M (2003) *Anal Chem* 75:2487
94. Behnisch PA, Hosoe K, Sakai S (2001) *Environ Int* 27:413
95. Houtman CJ, Booij P, Jover E, Del Rio DP, Swart K, Van Velzen M, Vreuls R, Legler J, Brouwer A, Lamoree MH (2006) *Chemosphere* 65:2244
96. Puglisi E, Murk AJ, Van den Berg HJ, Grotenhuis T (2007) *Environ Toxicol Chem* 26:2122
97. Evers EHG, Laane RWPM, Groeneveld GJJ, Olie K (1996) *Organohalogen Compd* 28:117
98. Kennicutt MC, Wade TL, Presley BJ, Requejo AG, Brooks JM, Denoux GJ (1994) *Environ Sci Technol* 28:1
99. Khim JS, Kannan K, Villeneuve DL, Koh CH, Giesy GP (1999) *Environ Sci Technol* 33:4199
100. Muller L, Neugebauer F, Fromme H (1999) *Organohalogen Compd* 43:397
101. Eljarrat E, Caixach J, Rivera J, De Torres M, Ginebreda A (2001) *Environ Sci Technol* 35:3589
102. Koistinen J, Paasivirta J, Sarkka J (1990) *Chemosphere* 21:1371
103. Lundgren K, Tysklind M, Isaac R, Broman D, Van Bavel B (2002) *Environ Sci Technol* 36:5005
104. Eljarrat E, Caixach J, Jiménez B, González MJ, Rivera J (1999) *Chemosphere* 38:1901
105. Jarnberg U, Asplund L, De Wit C, Grafstrom AK, Haglund P, Jansson B, Lexén K, Strandell M, Olsson M, Jonsson B (1993) *Environ Sci Technol* 27:1364
106. Kannan K, Kober JL, Kang YS, Masunaga S, Hakanishi J, Ostaszewski A, Giesy JP (2001) *Environ Toxicol Chem* 20:1878
107. Iozza S, Müller CE, Schmid P, Bogdal C, Oehme M (2008) *Environ Sci Technol* 42:1045
108. Watanabe I, Kawano M, Tatsukawa R (1995) *Organohalogen Compd* 24:337
109. Allchin CR, Law RJ, Morris S (1999) *Environ Pollut* 105:197
110. Sawal G, Woitke P, Lepom P (2005) *Organohalogen Compd* 67:587
111. Labandeira A, Eljarrat E, Barceló D (2007) *Environ Pollut* 146:188
112. Verslycke TA, Vethaak AD, Arijs K, Janssen CR (2005) *Environ Pollut* 136:19
113. Moon HB, Kankan K, Choi M, Choi HG (2007) *Mar Pollut Bull* 54:1402
114. Eljarrat E, Labandeira A, Marsh G, Raldua D, Barceló D (2007) *Chemosphere* 69:1278
115. Mai B, Chen S, Luo X, Chen L, Yang Q, Sheng G, Peng P, Fu J, Zeng EY (2005) *Environ Sci Technol* 39:3521
116. Klamer HJC, Leonardo PEG, Lamoree MH, Villerius LA, Akerman JE, Bakker JF (2005) *Chemosphere* 58:1579
117. Lepom P, Sawal G, Theobald N, Warmbrunn-Suckrow E (2007) In: *Proc 4th International Workshop on Brominated Flame Retardants*, Amsterdam, The Netherlands, 24–27 April 2007. Unpaginated
118. Evenset A, Christensen GN, Carroll J, Zaborska A, Berger U, Herzke D, Gregor D (2007) *Environ Pollut* 146:196
119. Minh NH, Isobe T, Ueno D, Matsumoto K, Mine M, Kajiwara N, Takahashi S, Tanabe S (2007) *Environ Pollut* 148:409
120. Choi JW, Onodera J, Kitamura K, Hashimoto S, Ito H, Suzuki S, Morita M (2003) *Chemosphere* 53:637
121. Wang D, Jiang G, Cai Z (2007) *Talanta* 72:668

122. Cleverly D, Monetti M, Phillips L, Cramer P, Heit M, McCarthy S, Rourke KO, Stanley J, Winters D (1996) *Organohalogen Compd* 28:77
123. Chovanec A, Vogel WR, Lorbeer G, Moche W, Hartl W (1993) *Organohalogen Compd* 12:347
124. Dannenberg D, Andersson R, Rappe C (1997) *Mar Pollut Bull* 34:1016
125. Jiménez B, Hernández LM, González MJ, Eljarrat E, Rivera J, Fossi MC (1998) *Environ Sci Technol* 32:3853
126. Semenov SY, Zykova GV, Mikhailov GM, Smirnov VN, Finakov GG (2000) *Organohalogen Compd* 46:395
127. Eljarrat E, Caixach J, Rivera J (2001) *Water Res* 35:2799
128. Tylerr AO, Millward GE (1996) *Mar Pollut Bull* 32:397
129. Onodora S, Sugimoto M, Takagi T, Tamaka K (1998) *Organohalogen Compd* 39:355
130. Moon HB, Choi HG, Kim SS, Lee PY, Ok G (2000) *Organohalogen Compd* 46:427
131. Norwood CB, Hackett M, Pruett RJ, Butterworth BC, Williamson KJ, Naumann SM (1989) *Chemosphere* 18:553
132. Koistinen J, Paasivirta J, Suonperä M, Hyvärinen H (1995) *Environ Sci Technol* 29:2541
133. Evers EHG, Van Berghem JM, Olie K (1989) *Chemosphere* 19:459
134. Oehme M, Mano S, Brevik EM, Knutzen J (1989) *Fresenius J Anal Chem* 335:987
135. Makiya K, Ueda H, Nakano T, Suzuki N, Tanabe K, Morita M (1999) *Organohalogen Compd* 43:217
136. Borgen AR, Schlabach M, Mariussen E (2003) *Organohalogen Compd* 60:17
137. Hüttig J, Zencak Z, Oehme M (2004) *Organohalogen Compd* 66:1344
138. Sellström U, Jansson B (1995) *Chemosphere* 31:3085
139. Jonsson P, Kankaanpää H (1999) In: Perttilä M (ed) *Contaminants on the Baltic Sea sediments. Results from the 1993 ICES/HELCOM Sediment Baseline Study, 1999*
140. Van Zeijl H (1997) *Report of the results of the one-off survey DIFFCHEM. Report SIME 97/6/1-E, Oslo and Paris Commissions*

Contaminated Sediments

Kassim, T.A.; Barceló, D. (Eds.)

2009, XVI, 181 p. 36 illus., 2 illus. in color., Hardcover

ISBN: 978-3-540-88013-4