Priority lists for persistent organic pollutants and emerging contaminants based on their relative toxic potency in environmental samples

E. Eljarrat, D. Barceló

In recent decades, a great deal of emphasis has been placed on evaluating the toxic potency of dioxin in different environmental samples. The commonly named dioxin-like compounds (DLCs), such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs), were studied to determine their relative toxic potency. Recently, some studies have indicated that another group of contaminants, the polycyclic aromatic hydrocarbons (PAHs), can dominate estimates of toxic equivalent quantity (TEQ) for samples containing PAHs and DLCs. Other emerging contaminants, such as brominated flame retardants (BFRs), especially polybrominated diphenyl ethers (PBDEs), also exhibit dioxin-like activities. The knowledge of the relative contribution of each contaminant to the total dioxin-like activity associated with environmental samples could aid in identifying the most important contributory pollutants. In this paper, an overview of current data for these estimates is presented.

Keywords: BFRs; PAHs; PBDEs; PCBs; PCNs; PCDDs; PCDFs; Sediment; Sludge; TEQ

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1. Introduction

Environmental samples are usually polluted with a variety of compounds and thus represent complex situations in terms of toxicity assessment. Although sediments may be contaminated with a large number of potentially toxic chemicals, their toxicity is usually caused by only a small proportion of these. Valid identification of the proportion of toxicants within the sediment mixture directly contributing most to the overall toxicity would therefore greatly help to reduce the pollutant-monitoring effort required to track toxicity problems effectively in a rapid, cost-efficient manner.

Persistent organic pollutants (POPs) (see Table 1 for a list of abbreviations and acronyms used) have been shown to exhibit potentially harmful effects in man and the environment. In addition to being persistent, POPs are typically lipophilic (therefore, bioaccumulative), semi-volatile and toxic. Some POPs have been produced deliberately by industry for a wide variety of applications (i.e., pesticides, PCBs, PCNs). Others are formed accidentally or eventually released as a by-product of various activities, such as industrial or combustion processes (i.e., PCDDs, PCDFs, PAHs).

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 being taken on 12 POPs (Table 2).

In recent decades, a great deal of emphasis has been placed on evaluating the dioxin-like toxicity of different POPs. Risk assessment of dioxin is based on the concept of toxic equivalency factor (TEF) [1]. The root cause of many damaging effects is that dioxins bind effectively to a

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Table 1. List of abbreviations and acronyms

AHH	Aryl hydrocarbon hydroxylase	MS-MS	Tandem mass spectrometry
ASE	Accelerated solvent extraction	NCI	Negative chemical ionization
Ant	Anthracene	PAH	Polycyclic aromatic hydrocarbon
BA	Benzo[a]anthracene	PBB	Polybrominated biphenyl
BAP	Benzo[a]pyrene	PBDE	Polybrominated diphenyl ether
BbF	Benzo[b]fluoranthene	PBDD	Polybrominated dibenzo-*p*-dioxin
BFR	Brominated flame retardant	PBDF	Polybrominated dibenzofuran
BkF	Benzo[k]fluoranthene	PBN	Polybrominated naphthalene
CALUX	Chemically activated luciferase expression	PCB	Polychlorinated biphenyl
Chr	Chrysene	PCDD	Polychlorinated dibenzo-*p*-dioxin
DBA	Dibenzo[a,h]anthracene	PCDF	Polychlorinated dibenzofuran
DLC	Dioxin-like compound	PCN	Polychlorinated naphthalene
EI	Electron ionization	PLE	Pressurized liquid extraction
EROD	7-ethoxyresorufin *O*-deethylase	POP	Persistent organic pollutant
HRGC	High-resolution gas chromatography	REP	Relative potency
HRMS	High-resolution mass spectrometry	SIM	Selected ion monitoring
IdP	Indeno[1,2,3-c,d]pyrene	TEF	Toxic equivalent factor
IT	Ion trap	TEQ	Toxic equivalent quantity
MAE	Microwave-assisted extraction	ToF	Time of flight
MS	Mass spectrometry	WHO	World Health Organization

specific receptor, a protein in the cytoplasm of the cell, referred to as the Ah receptor (AhR) (Fig. 1). The binding of a dioxin to this receptor triggers a chain of reactions, the end result of which is that the receptor binds to a DNA sequence. One of the possible binding sites is in the regulatory region for the gene CYP 1A1, which holds the ''blueprints'' for an enzyme of the cytochrome P450 type. Particularly in the liver, an intake of dioxins powerfully induces (increasing production or release) of this enzyme. However, the AhR can link up with many different sequences along the strands of DNA and thus influences the synthesis of some 20 proteins, at least. The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) is one of the compounds that is attracted most strongly to the AhR and that is therefore toxic at the lowest doses. Several other compounds have a similar structure, but generally do not bind quite as strongly to the AhR as 2378-TCDD, and larger doses therefore have to be given to achieve a comparable effect.

Table 2. Comparative lists of POPs selected for environmental and toxicological studies

POPs selected at the Stockholm Convention (2001)	POPs with an assigned TEF or REP	Emerging POPs
Aldrin		
Chlordane		
DDT		
Dieldrin		
Endrin		
Heptachlor		
Hexachlorobenzene		
Mirex		
Toxaphene		
PCBs	PCBs	
PCDDs/PCDFs	PCDDs/PCDFs	
	PCNs	
	PBDEs	PBDEs
	PBDDs/PBDFs	PBDDs/PBDFs
	PBBs	PBBs
	PAHs	

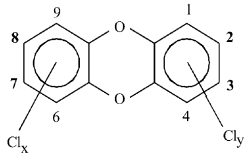
The criteria for including a compound in the TEF scheme and therefore adding it to the list of dioxin-like compounds (DLCs) were: (a) sharing certain structural relationships to the PCDDs/Fs (Table 3); (b) binding to the AhR; (c) eliciting AhR-mediated biochemical and toxic responses; and, (d) persistent and accumulating in the food chain.

A large number of halogenated compounds meet the criteria for inclusion in the TEF concept and could contribute to the total dioxin-like toxicity in environmental samples. These include any or all of the following classes of polychlorinated compounds: PCDDs, PCDFs, PCBs, PCNs, diphenyl ethers, diphenyl toluenes, phenoxy anisoles, biphenyl anisoles, xanthenes, xanthones, anthracenes, fluorenes, dihydroanthracenes, biphenyl methanes, phenylxylylethanes, dibenzothiphenes, quaterphenyls, quaterphenyl ethers and biphenylenes [2]. In addition, brominated and chloro-bromo-substituted analogues of these compounds have been found in the environment.

Different studies have indicated that some of these DLCs can dominate TEQ estimates. It is questioned to what extent these compounds contribute to the total dioxin-like toxicity of environmental mixtures in relation to e.g. humans and wildlife. The relative order of potency, along with the wide distribution in the environment, suggests that certain DLCs should be considered in assessments of the total toxicities present in environmental samples. The objective of this research was to identify the most hazardous pollutants responsible for the toxicity of sediments and sludge. This approach would have clear implications for both risk

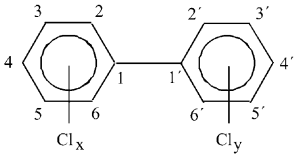
Table 3. Structures of polychlorinated and polybrominated compounds selected in this study

Polychlorinated dibenzo-*p*-dioxins (PCDDs)



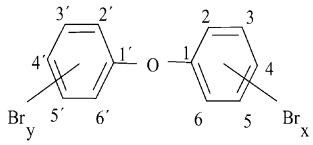
x + y = 1-8 (75 congeners)

Polychlorinated biphenyls (PCBs)



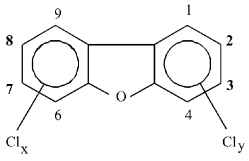
x + y = 1-10 (209 congeners)

Polybrominated diphenylethers (PBDEs)



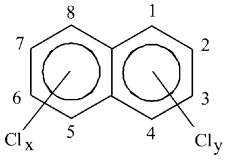
x + y = 1-10 (209 congeners)

Polychlorinated dibenzofurans (PCDFs)



x + y = 1-8 (135 congeners)

Polychlorinated naphthalenes (PCNs)



x + y = 1-8 (75 congeners)

assessment and remediation strategies, making them more efficient by focusing on the most important pollutants identified. The knowledge of the order of potency, as well as of the environmental levels, of PCDDs, PCDFs, PCBs, PCNs, BFRs (especially PBDEs), and PAHs was reviewed.

2. TEFs

The use of TEFs to provide a simple, single number that is indicative of overall toxicity of a sample containing a mixture of dioxins and DLCs is well established. TEFs are essentially weighting factors, by which the toxicity of a mixture of congeners is compared to that of 2378-TCDD. TEFs are based upon a number of endpoints, from chronic in vivo toxicity to in vitro toxicity with the former having the greatest importance in determining overall TEF. The total potency of a mixture can be expressed in 2378-TCDD TEQ concentration where

$$\mathrm{TEQ} = \Sigma\{\mathrm{compound}_1 \times \mathrm{TEF}_1 + \ldots + \mathrm{compound}_n \times \mathrm{TEF}_n\}$$

Until recently, the scheme of TEF values agreed by the NATO [3] had become widely accepted as the standard system, although other schemes have been used. This scheme is often known as the International TEF scheme, sometimes denoted I-TEF. There have been a number of efforts over recent years to extend the concept and methodology of TEF schemes to include other classes of DLCs. Recently, special attention has been focused on a few select PCB congeners (dioxin-like PCBs). An important development was the updating of the system of TEFs by an expert group convened by the World Health Organization (WHO) in 1997 [4] (Table 4).

The dioxin-like potency of a single compound is expressed as relative potency (REP). TEFs are consensus values based on REPs across multiple species and/or endpoints. Although consensus values for the relative potencies of the most active PCDDs, PCDFs and PCBs have been established, the database of relative potency values for other DLCs is currently limited. Next, different REP values from the literature are reviewed.

2.1. PCNs

The toxicological significance of the PCNs is unknown. Some of the PCN congeners have been tested for 7-ethoxyresorufin *O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) with enzyme-induction assays [5]. Some of them are highly active and, as a result, have an assigned REP. Nevertheless, other potentially very toxic PCN congeners do not have an assigned REP.

Biological activity information was studied by Villeneuve et al. [6] and consisted of the REPs for 20

individual PCNs using the H4IIE-luc *in-vitro* assay. Structure-activity relationships were observed in terms of both the degree of chlorination and the positions of chlorine substitutions. Hexa-CNs, exhibiting REPs around 10^{-3}, were the most potent congeners tested. Penta-CNs were also rather potent, yielding REPs between 10^{-3} and 10^{-7}. Tetra-, tri-, di- and mono-CNs were less active. REPs for the active congeners were similar to those for some PCBs.

2.2. PAHs

As observed in different studies, PAHs with two or three aromatic rings do not induce, or are hardly capable of inducing, EROD activity, suggesting that PAHs with one or two ring structures in general do not meet the structural requirements to bind to the AhR. However, different REP schemes have been developed for other PAHs [7–9] (Table 5), and the REP values derived vary depending upon the type of bioassay systems, end points and calculation methods for relative potencies.

Willett et al. [7] determined the induction potency of PAHs with respect to 2378-TCDD in rat hepatoma H4IIE cells, and the induction-derived TEFs with respect to TCDD were in the range of 0.000025 for benzo[a]anthracene (BA) and 0.00478 for benzo[k]-fluoranthene (BkF).

Clemons et al. [8] examined the ability of PAHs to induce AhR-mediated luciferase activity in mouse

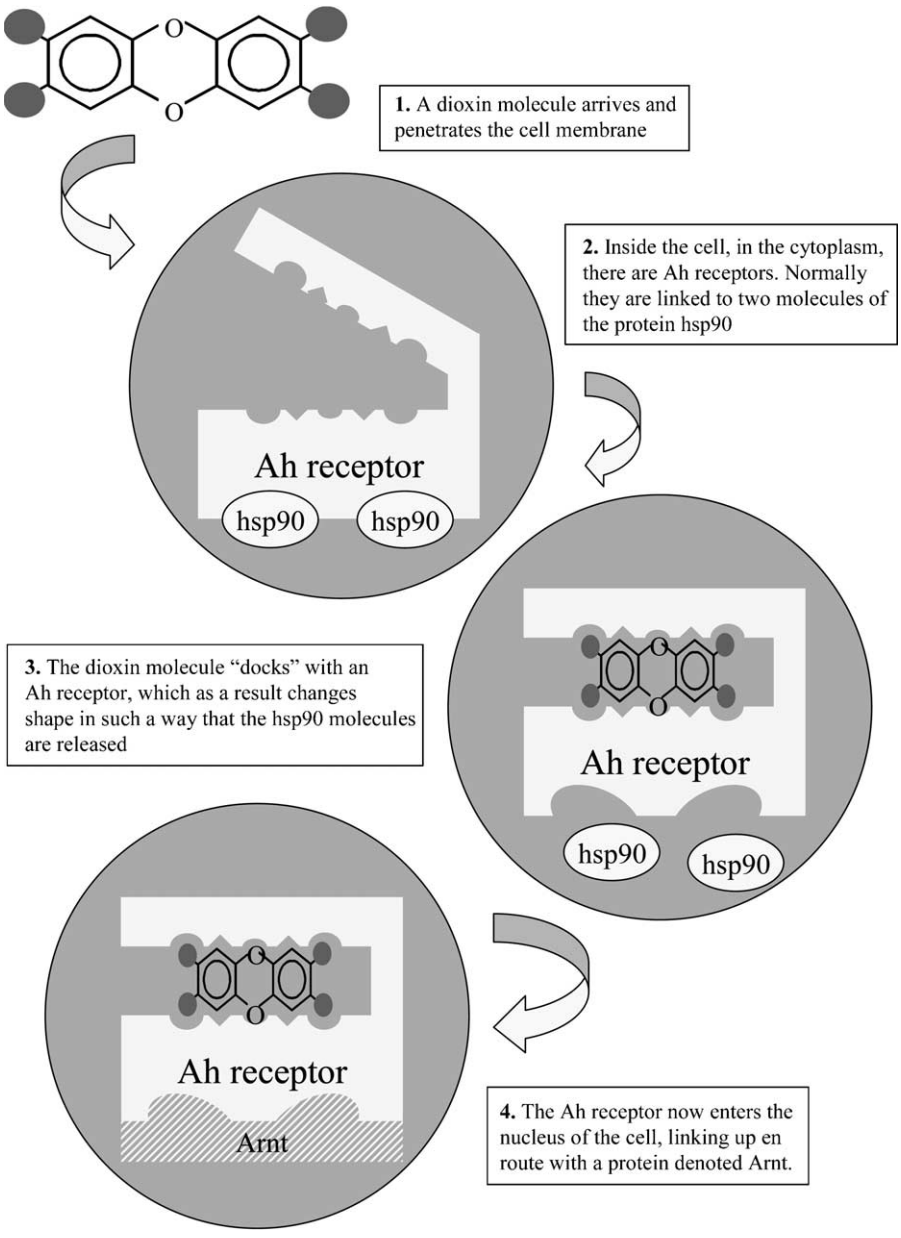


Figure 1. Scheme of dioxin molecules and Ah receptors (AhRs). What happens in the cell?

Table 4. Toxic Equivalent Factors established by the WHO (WHO-TEFs) for dioxins and dioxin-like PCBs [4]

PCDD Congener	WHO-TEF	PCDF Congener	WHO-TEF	PCB Congener	WHO-TEF
2,3,7,8-TCDD	1	2,3,7,8-TCDF	0.1	*Non-ortho*	
12,3,7,8-PeCDD	1	12,3,7,8-PeCDF	0.05	PCB#81	0.0005
123478-HxCDD	0.1	23478-PeCDF	0.5	PCB#77	0.0005
123678-HxCDD	0.1	123478-HxCDF	0.01	PCB#126	0.1
12,3,7,89-HxCDD	0.1	123678-HxCDF	0.1	PCB#169	0.01
1234678-HpCDD	0.01	234678-HxCDF	0.1	*Mono-ortho*	
OCDD	0.0001	12,3,7,89-HxCDF	0.1	PCB#105	0.0001
		1234678-HpCDF	0.01	PCB#114	0.0005
		1234789-HpCDF	0.01	PCB#118	0.0001
		OCDF	0.0001	PCB#123	0.0001
				PCB#156	0.0005
				PCB#157	0.0005
				PCB#167	0.00001
				PCB#189	0.0001

hepatoma cells, and found REPs between 0.00001 for BA or benzo[a]pyrene (BAP) and 0.05 for BkF or dibenzo[a,h]anthracene (DBA).

Klimm et al. [9] determined REPs for PAHs by comparing the induction of EROD activity by PAH standards with those of a 2378-TCDD standard. The REPs established were in the range of 0.000027 for BA and 0.00038 for benzo[b]fluoranthene (BbF).

In these three studies, BkF was found to be the most potent PAH. But, discrepancies were found for other PAHs, the biggest being REPs derived for chrysene (Chr), a PAH that is prevalent in many environmental matrices.

The dioxin-like potency of individual PAHs was similar to the potencies reported for a range of other AhR-active environmental contaminants. Some PAHs had REPs in the range of 10^{-4} to 10^{-6}. This was similar to the range of REPs reported for penta-CNs and slightly less than that reported for hexa-CNs [6].

2.3. BFRs

Although interference with the AhR has been reported for BFRs [10], the current knowledge of toxicological actions of BFRs is rather limited. Some 17 PBDEs have been tested with the chemically activated luciferase expression (CALUX) bioassay [11,12] as well as with the EROD assay [13] for their dioxin-like potency (Table 6). Seven of these PBDEs were able to activate the AhR in an agonistic and antagonistic way. The REP values were several magnitude lower than 2378-TCDD: 7.1×10^{-7} for tetraBDE#47, 5.9×10^{-6} for pentaBDE#99 and 4.3×10^{-6} for hexaBDE#153.

Different studies have shown that bromine substitution appears to have a stronger effect than chlorine substitution. In the CALUX assay, the brominated analogue of TCDD and dioxin-like polybrominated

Table 5. Relative potency (REP) schemes for PAHs

Compound	Willett [7]	Clemons [8]	Klimm [9]
Benzo[k]fluoranthene (BkF)	0.00478	0.05	0.00029
Benzo[a]pyrene (BAP)	0.00035	0.00001	0.0003
Benzo[b]fluoranthene (BbF)	0.00253		0.00038
Chrysene (Chr)	0.0002	0.01	
Benzo[a]anthracene (BA)	2.5E-05	0.00001	2.7E-05
Indeno[1,2,3-c,d]pyrene (IdP)	0.0011		8.6E-05
Dibenzo[a,h]anthracene (DBA)	0.00203	0.05	7.8E-05
Anthracene (Ant)		0.0001	

Table 6. Relative potency (REP) calculated for some BFRs

Compound	REP	Reference
PBDEs		
BDE#47	7.1×10^{-7}	[11,12]
BDE#77	0.0032	[13]
BDE#99	5.9×10^{-6}	[11,12]
BDE#100	2.4×10^{-5}	[13]
BDE3119	3.5×10^{-5}	[13]
BDE#126	0.0024	[13]
BDE#153	4.3×10^{-6}	[11,12]
PBBs		
PBB#77	0.08	[15]
PBB#169	0.021	[15]
PBDDs		
2378-TBDD	0.65	[15]
1378-TBDD	0.013	[46]
12378-PBDD	0.3	[15]
PBDFs		
2378-TBDF	0.7	[15]
12378-PeBDF	0.5	[15]
23478-PeBDF	0.21	[15]
123478-HxBDF	0.002	[46]
Mixed brominated-chlorinated dioxins		
2monoBr-378triCDD	0.94	[15]
8monoBr-2378triCDD	0.65	[46]
23-diBr-78diCDD	0.69	[15]
37-diBr-28diCDD	0.68	[46]
1monoBr-2378tetraCDD	0.6	[15]

biphenyls (PBBs) showed equivalent, or in the case of the PBB compounds, greater activity than their chlorinated analogues [14]. For example, PBB#77 caused EROD activity eight times higher than PCB#77 [2].

Behnisch et al. [15] published a comparative study of the activity of PBDD/F congeners and their chlorinated homologues (PCDD/F). In the case of dioxins, similar REPs were obtained for the chlorinated and brominated congeners. However, significant differences were detected for the furans. For example, 2378-TBDF presented a REP value of 0.7 whereas the WHO-TEF of 2378-TCDF was 0.1; 12378-PeBDF showed a REP value of 0.5 whereas the WHO-TEF of 12378-PCDF was set at 0.05. Interestingly, studies with polybrominated naphthalenes (PBNs) have reported that PBNs are more potent than PCNs [16,17].

3. Analytical approaches

The analytical methodologies for POP analyses are especially difficult because of the complexity of the mixtures of congeners (210 PCDDs/Fs, 209 PCBs, 75 PCNs, 209 PBDEs, etc.) and to the low detection limits required (ppb to ppq). Moreover, time-consuming sample-preparation steps are needed because of the presence of a large number of interfering compounds. Overcoming these analytical problems has only been possible with the application of rigorous clean-up schemes and the use of high-resolution gas chromatography (HRGC) coupled to mass spectrometry (MS). An analytical protocol to determine dioxins and related compounds included the steps showed in Fig. 2. The clean-up steps provide a suitable removal of the bulk matrix and some interfering compounds; HRGC allows an appropriate separation between the different congeners; and, MS affords a sensitive, selective method of detection. Finally, an isotopic dilution technique based on the use of standards labeled with ^{13}C provides reliable quantification needed for accurate determination of such analytes.

3.1. Extraction

For trace analysis of dioxins and DLCs, Soxhlet extraction is widely accepted as robust. However, the main drawback of this technique is that it is time consuming (up to 48 hours). Furthermore, solvent consumption is considerable (~300 mL), demanding evaporation of a large amount of solvent before subsequent clean-up. In recent years, efforts have been made to develop extraction techniques with reduced solvent volumes, shorter times, and high levels of automation. Microwave-assisted extraction (MAE) [18,19] and pressurized liquid extraction (PLE) (commonly named accelerated solvent extraction (ASE)) [20,21] have been tested by a number of laboratories for the extraction of PCDDs, PCDFs and PCBs. The considerable saving in extraction time (reducing it to 1 hour) and the low solvent consumption (typical volumes are ~30 mL) have made MAE and PLE very attractive alternatives to conventional Soxhlet procedure.

3.2. Purification

The clean-up step is typically based on solid-liquid adsorption chromatography in open columns using a combination of different adsorbents (silica, Florisil, alumina and different types of carbon). However, the whole procedure is time- and labor-consuming, and it represents the bottleneck in the analytical method. The development of sample-handling techniques is directed towards automation. Recently, automated clean-up systems have been developed based on the use of

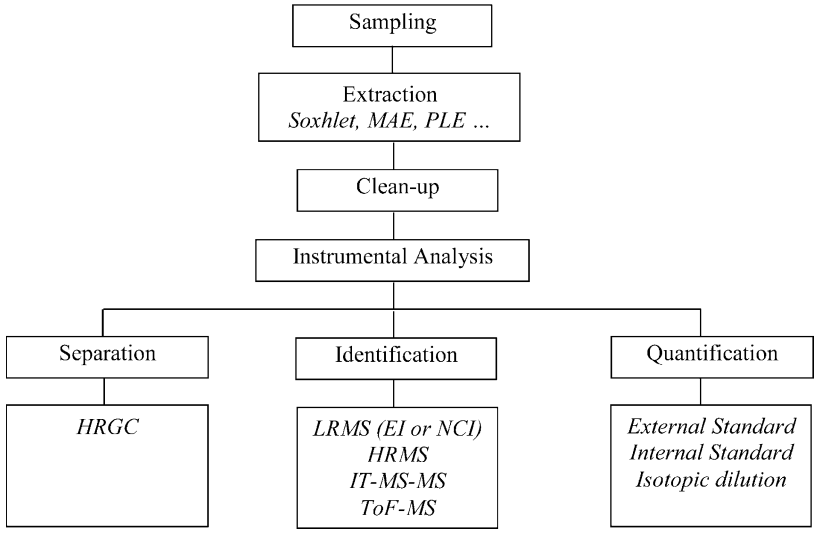


Figure 2. Prinicipal steps in the analytical methods used for the analysis of dioxins and related compounds.

pressurized column chromatographic procedures, with capacity for automatically processing different samples simultaneously in about 1.5 hours [22,23].

3.3. Instrumental analysis

Dioxin and dioxin-like PCB analyses involve detection of multiple congeners at the ppt or ppq level, for which isotope dilution techniques using HRGC-HRMS are currently recommended methods (US EPA Method 1613, US EPA Method 8290, US EPA Method 1668) [24–26].

Conventionally, HRMS was used, operating in the electron ionization (EI) mode (electron energy 38 eV) at a resolving power of 10,000. Under these conditions, different ions (isotopic labeled included) were monitored in SIM (selective ion monitoring) mode. Quantification was carried out by an isotopic dilution technique. Similar methodologies were applied to the PAH and PCN determinations [27].

Recently, new MS approaches have been applied to the analysis of dioxins and related compounds - ion trap (IT)-MS-MS and the time of flight (ToF)-MS. The advantage of IT-MS systems is the much lower price, reducing analysis costs. However, the sensitivity of IT-MS instruments is considerably lower than that of HRMS instruments. The limit of detection for IT-MS systems for TCDD can be assumed to be in the range 100–300 fg, whereas modern HRMS instruments have a LOD (limit of detection) of about 3 fg [28]. Another disadvantage of IT-MS is the low reproducibility of quantification. Excessive interfering ions coexisting with dioxins in the trap cause space-charge effects and lead the analysis to irreproducible quantification. Ionization conditions are meaningful parameters for reproducibility. The voltage, the current and the temperature of the chamber are parameters for optimizing ionization conditions. In spite of these drawbacks, an MS-MS method for the ultra-trace detection and quantification of dioxins using isotopic dilution technique is now possible. Different studies have used IT-MS-MS as an alternative to HRMS for PCDD/F [29,30] and PCB [31] analyses.

Other recent studies have shown the capabilities of ToF-MS for analysis of PCBs in different type of samples [32,33]. The ToF-MS results were consistent with the HRMS results. The tri- to hepta-CB instrumental LODs were estimated to be 1 pg, whereas the LOD was established as 4 pg based on deca-CB [32]. These LODs were at least an order of magnitude better than those found with conventional quadrupole systems operating in the full scan mode. These results are very encouraging, and show that ToF-MS allows for analysis times an order of magnitude faster than HRMS methods without loss of qualitative or quantitative power.

Several methods for qualitative and quantitative analysis of PBDEs have been developed involving GC-negative chemical ionization (NCI)-MS, or GC-EI-MS. There has been a comparative study of the congener-specific analysis of 40 different PBDEs by the two approaches, NCI and EI [34]. NCI is the most frequently used ionization mode for determination of brominated compounds. This technique offers greater sensitivity than EI but is less selective, since only bromine can be monitored. Furthermore, it does not allow quantification by isotopic dilution technique whereas EI does, making the analysis more reliable at trace levels.

4. Environmental levels and contribution to the total toxicity

The contribution of each contaminant to the total toxicity of environmental samples was found to depend on the relative order of potency along with the contamination levels in the environment. Dioxins are the most potent contaminants. However, their levels in sediments and sludge were lower than those of other POPs, such as PCBs, PCNs, PBDEs or PAHs. For this reason, the contributions to toxicity were greater for less potent contaminants with higher concentrations.

DLCs, such PCDDs, PCDFs, PCBs, and PCN, enter the environment from a number of potential sources. These hydrophobic chemicals are highly persistent in the environment and have a strong affinity with sediments. A number of studies have reported PCDD, PCDF and PCB levels from sediments in North America, Europe and Asia. Generally, the PCDD and PCDF levels in background areas ranged between <0.1 pg TEQ/g and the safe sediment value established at 20 pg TEQ/g [35], whereas levels found in polluted areas clearly exceeded the safe value. Regarding PCB data, a number of studies have reported levels expressed as total PCBs or as a sum of seven indicator PCBs; however, the literature on the dioxin-like PCBs is very scant.

Some investigations into relative abundances based on TEQs between PCDDs/Fs and dioxin-like PCBs in sediment samples have been carried out. A study of the contribution of PCDDs/Fs and dioxin-like PCBs in northwest Mediterranean sediments (Catalonia, Spain) has been carried out [36]. The general trend is that PCBs are found at higher concentrations than the PCDDs/Fs in the same sample (at least, one order of magnitude higher). However, the percentage contribution to total TEQ from PCBs can be significant, but it is usually lower than the contribution of PCDDs/Fs. The values of $TEQ_{PCDD/F}$ and TEQ_{PCB} were in the ranges 0.4–39.2 pg/g and 0.03–24.8 pg/g, respectively. The contribution of PCDDs and PCDFs in the total TEQ was in the range 68–99% for 15 samples out of the 18 analyzed (Fig. 3). In general, the contribution of mono-*ortho* PCBs to TEQ_{PCB} was important (90–100%) whereas the contribution of non-*ortho* PCB was very low ($<10\%$). PCB congeners 156 and 118 were the predominant contributors to TEQ_{PCBs} in sediments.

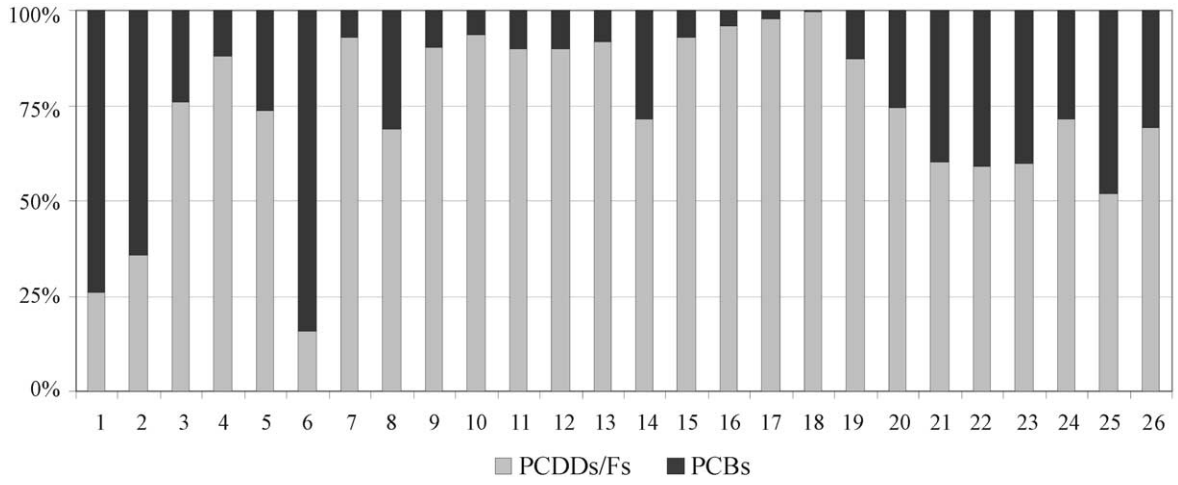


Figure 3. Percentage contribution to the total WHO-TEQ from dioxin-like PCBs and PCDD/Fs. (Samples 1-18 = sediment samples; samples 19-26 = sludge samples).

These findings were consistent with those obtained by Yao et al. [37], who found that PCDD/Fs contributed to more than 90% of the total TEQ throughout the sediment samples from Tokyo Bay.

Another study regarding the dioxin-like toxic potency of harbor sediments along the North Sea coast of The Netherlands was published recently [38]. The analysis of 20 sediment extracts showed that $TEQ_{PCDD/F}$ values were in the range 1.8–39.6 pg/g, whereas TEQ_{PCB} values were in the range 0.3–6.2 pg/g. On average, PCDDs and PCDFs accounted for 89% of the toxicity and dioxin-like PCBs for 11%. Interestingly, these samples were also analyzed using a CALUX bioassay. Dioxins and PCBs accounted only for 56% of the CALUX activity, indicating that other contaminants with a dioxin-like mode of action were present in these samples. A full scan determination indicated the presence of many chlorinated compounds, and specially some tetra-, penta-, hexa- and octa-CNs.

It should be pointed that a complete characterization of TEQ values demands measurement of not only PCDDs and PCDFs, but also dioxin-like PCBs. For some samples, the $TEQ_{PCDD/F}$ value was below the safe sediment value, but the total TEQ for the same sediment exceeded this safe value set at 20 pg TEQ/g [35].

The contamination of PCDDs and DLCs in sewage sludge is well documented. Analytical results have been reported from different industrialized countries. Moreover, different studies showed that PCDD, PCDF and PCB levels have declined since the 1980s. The lower contamination values reflect a general decline in dioxin input into the environment, as a result of tighter controls on pentachlorophenol use and disposal [39]. Contemporary sludge samples presented levels below 100 pg TEQ/g.

Regarding the contribution of PCDDs/Fs and dioxin-like PCBs in sewage sludge, eight samples from rural, urban and industrial waste water treatment plants in Catalonia (Spain) have been analyzed [40]. The WHO-$TEQ_{PCDD/F}$ values for these samples were in the range 4.9–20.8 pg/g, whereas WHO-TEQ_{PCB} levels were in the range 1.9–6.6 pg/g. The WHO-TEQ_{PCB} contribution varied (13–50%, Fig. 3), suggesting that the PCB contribution on the toxicity of the samples must be taken into account. Moreover, in all cases, the largest contribution to WHO-TEQ_{PCB} came from the non-*ortho* PCBs (62–91%), followed by the mono-*ortho* PCBs (9–38%). PCB congeners 126, 118 and 156 were the predominant contributors to WHO-TEQ_{PCBs} in sludge samples.

The available PCN REPs were applied to literature-derived data on concentrations of PCN congeners in environmental mixtures to assess the potential contribution of PCNs to total TEQs in environmentally weathered complex mixtures. This calculation is not meant to include all TEQ contributions due to PCNs because there are other PCN congeners present in environmental samples for which REPs are not available. In sediments collected near the site of a former chlor-alkali plant, TEQ_{PCN} (5.16 pg/g) contributed up to 59% of the total TEQ calculated, including PCDDs/Fs, PCBs and PCNs (8.81 pg/g) [41].

Kannan et al. [42] studied the relative contributions of PCDDs/Fs, PCBs and PCNs to the concentrations of TEQs in sediments from the Detroit and Rouge rivers (Michigan, USA). The total TEQs were in the range 5.7–31 pg/g. The PCNs contributed the greatest TEQs (42–84% of the total), followed by PCDFs (8–39%), PCDDs (5–16%), and then PCBs (2–3%).

Similar results were obtained by Marvin et al. [43] when analyzing Detroit river suspended sediments. PCDDs/Fs, dioxin-like PCBs and PCNs were determined in these samples. $TEQ_{PCDD/F}$ values exceeded those for PCBs, with $TEQ_{PCDD/F}$+TEQ_{PCB} values in the range

2.3–306 pg/g. However, TEQ_{PCN} was in the range 73–3300 pg/g. These data indicate that PCNs represent a significant contribution to dioxin-like activity. Thus, it may be important to consider PCNs when characterizing the overall dioxin-like potency of sediment samples.

There have been a few studies on concentrations and distributions of PBDEs in sediments and sludge (Table 7). 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.

Using the REPs proposed by Meerts et al. [11] and Murk et al. [12] for the BDE#47 and BDE#99, estimates of TEQ_{PBDE} were in the range 0.004–0.04 pg/g for European river sediments. The TEQ_{PBDE} values increased for sediments downstream of plastics factory (4.78 pg/g).

Regarding sludge samples, TEQ_{PBDE} values were in the range 0.31–0.77 pg/g. However, these TEQ_{PBDE} were lower than $TEQ_{PCDD/F}$ normally found in sediment and sludge samples. It should be pointed out that only two PBDE congeners were used for this TEQ_{PBDE} calculations. Moreover, different studies have suggested that the risks associated with PBDEs could be caused by their contamination at trace levels with PBDDs, PBDFs or PBBs. An additional concern associated with the use of PBDEs as BFRs is that improper incineration of these compounds can result in the production of PBDDs, PBDFs and PBBs [44]. Thus, further research is necessary to understand the impact of these brominated DLCs on potency in environmental samples.

Recently, some studies have reported a large contribution of PAHs to dioxin-like activity in sediments. Kannan et al. [45] calculated TEQ_{PAH} in sediments from Tokyo Bay, using TEFs proposed by Willett et al. [7]. They found that TEQ_{PAH} were 5–50 times greater than $TEQ_{PCDD/F}$, and that TEQ_{PAH} ranged from 11.5 pg/g d.w. to 1.8 ng/g d.w. They also observed that BbF accounted for approximately 43% of the TEQ_{PAH}, followed by BkF and indeno[1,2,3-c,d]pyrene (IdP) at 33% and 20%, respectively. However, using the same TEF scheme, TEQ_{PAH} values ranged from 4.6 pg/g d.w. to 2.0 ng/g d.w. have been found in northwest Mediterranean sediments (Catalonia, Spain) [36]. These TEQ_{PAH} were 10–240 times greater than $TEQ_{PCDD/F}$, and BkF was the major contributor to TEQ_{PAH} (35–63%) followed by BbF (23–36%). It is important to notice that these estimated TEQ_{PAH} values clearly exceeded the safe sediment value established at 20 pg TEQ/g, with some values in the ng/g order of magnitude.

5. Conclusions

Summarizing all the available data on contributions to the total toxicity, we can conclude that the most important contributory pollutants in sediment and sludge samples are PAHs > PCNs > PCDDs/Fs > PCBs > PBDEs. However, it should be pointed out that estimates discussed here are only as good as the REP values upon which they are based. Although consensus values for the relative potencies of the most active PCDDs, PCDFs and PCBs have been established [4], the database of REP values for other DLCs is currently limited. Therefore, further research looking at the toxicological effects of DLCs is required to establish a consensus on TEF values of different PCNs, PBDEs or PAHs. Once these TEFs have been established, future research should focus on the toxic potencies of each contaminant in different environmental matrices.

Consensus on the establishment of TEFs has been observed with the changes recommended by the WHO to the TEFs for PCDDs and PCDFs. The changes involved

Table 7. Reported concentrations of PBDEs in sediment and sludge samples

Matrix (Location)	Compounds	Concentration	Reference
Sediment			
River sediments (Japan)	Tetra + Penta-BDEs	21–59 ng/g	[47]
Downstream of a plastics factory (Sweden)	BDE-47	490 ng/g	[48]
	BDE-99	750 ng/g	
	BDE-100	170 ng/g	
River with textile industries (Sweden)	BDE-47 + 99 + 100	nd-9.6 ng/g	[49]
	BDE-209	nd-360 ng/g	
Sediment (Baltic Sea)	Sum PBDE	nd-1.1 ng/g	[50]
River mouth sediments (Europe)	BDE-47	< 0.17–6.2 ng/g	[51]
	BDE-99	< 0.19–7.0 ng/g	
Sewage Sludge			
Digested sludge (Gothenburg, Sweden)	Sum PBDE	20–30 ng/g	[52]
Sewage sludge (Germany)	Sum PBDE	0.4–15 ng/g	[53]
Digested sludge (Stockholm, Sweden)	BDE-47	39–91 ng/g	[54]
	BDE-99	48–120 ng/g	
	BDE-100	11–28 ng/g	
	BDE-209	140–350 ng/g	

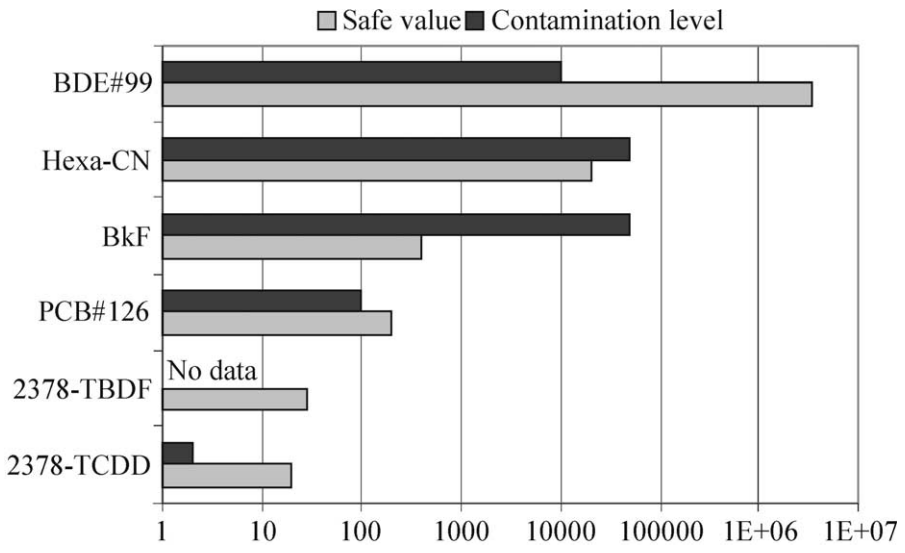


Figure 4. Comparison between the concentration levels needed to reach the safe sediment value and the concentration levels normally found in sediment samples (values expressed in pg/g).

an increase in the TEF for 12378-PeCDD from 0.5 to 1 and decreases from 0.001 to 0.0001 for OCDF and OCDD. These changes have significant implications for regulators, who have relied heavily on the I-TEF scheme in setting and monitoring limits and exposure to these compounds. In general, TEQs calculated using the WHO scheme for sludge samples showed substantial decreases (up to 70%) because of the predominance of higher chlorinated congeners.

The relative order of potency, along with the wide distribution of PAH, PCN or PBDE contamination in the environment, suggests that monitoring programs should be extended to include these persistent substances over and above the PCDDs, PCDFs and PCBs that are regularly analyzed at present.

Quality objectives for TEQs have been formulated in order to assess the quality of freshwater and coastal sediments, resulting in a safe sediment value of 20 pg TEQ/g [35]. Fig. 4 shows the concentration levels of each contaminant group needed to reach this safe value. These levels were calculated using the most potent congener of each contaminant group. Moreover, the concentration levels normally found in different sediment samples were depicted. As can be seen, the monitoring of PCDDs, PCDFs and PCBs is important, but other contaminants, such as PCNs, and particularly PAHs, need further control. Furthermore, data are needed on brominated dioxins as well as mixed brominated-chlorinated dioxins in order to determine their environmental impact. However, chemical analysis of mixed halogenated dioxins is very difficult because of the large number of possible combinations (there are 4600 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.

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