11. BROMINATED FLAME RETARDANTS: ANALYTICAL, TOXICOLOGICAL AND ENVIRONMENTAL ASPECTS

ADRIAN COVACI[1*1] AND ALIN C. DIRTU[1,2]
[1]*Toxicological Centre, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium*
[2]*Department of Inorganic and Analytical Chemistry, "Al. I. Cuza" University of Iassy, Carol I Bvd. No 11, 700506 Iassy, Romania*

Abstract. Brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs) and tetrabromobisphenol-A (TBBP-A), have routinely been added to consumer products for several decades in a successful effort to reduce fire-related injury and property damage. Recently, concern for this emerging class of chemicals has risen because of their occurrence in the environment and in human biota. Here we briefly review scientific issues related to analytical, toxicological and environmental aspects of these BFRs and discuss data gaps.

1. General Information Concerning Flame Retardants

Flame retardants are materials that inhibit or resist the spread of fire that are added to polymers which are used in plastics, textiles, electronic circuitry or other materials (WHO/ICPS, 1994, 1997). The different classes of flame retardants include naturally occurring substances (asbestos), synthetic inorganic materials, such as antimony oxides, aluminium hydroxide, magnesium hydroxide, and borates, organic phosphate esters with or without halogens and chlorinated and brominated organic compounds (WHO/ICPS, 1997). The most used brominated flame retardants (BFRs) are polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), tetrabromobisphenol-A (TBBP-A) and polybrominated biphenyls (PBBs).

Flame retardants act by different mechanisms depending on the respective chemical class: some compounds break down through endothermic processes when subjected to high temperatures (e.g., aluminium and magnesium hydroxides), while other compounds act as diluents of fuel or of the gas phase and thus lowering the combustible portion of the material (calc, calcium carbonate or inert gases, most often carbon dioxide or water). Another way to stop the flame spreading is to create a thermal insulation barrier between the burning and unburned parts of a material (e.g., intumescent additives).

Chlorinated and brominated flame retardants under thermal degradation conditions release hydrogen chloride and hydrogen bromide. These reaction products react with highly reactive $H\bullet$ and $HO\bullet$ radicals present in the flame resulting in the formation of inactive molecules and of $Cl\bullet$ or $Br\bullet$ radicals. The halogen radical has

*Corresponding author. E-mail: adrian.covaci@ua.ac.be

C. Popescu et al. (eds.), *Applications of Mass Spectrometry in Life Safety.*

much lower energy than $H\bullet$ and $HO\bullet$, and therefore much lower potential to propagate the radical oxidation reaction and therefore the flame.

Despite of their benefits for reducing fire-related injury and property damage, growing concern for BFRs has risen because of their occurrence and persistence in the environment, biota and humans, in a similar way to other persistent organic pollutants (de Wit, 2002; Birnbaum and Staskal, 2004).

2. Brominated Flame Retardants: Uses and Production Levels

2.1. POLYBROMINATED DIPHENYL ETHERS

PBDEs are flame retardant additives which are used in a wide array of household products in concentrations up to 30% by weight, typically between 2% and 6%. They are structurally related to polychlorinated biphenyls (PCBs) and are produced commercially as mixtures. However, PBDE mixtures contain fewer congeners than the commercial PCB mixtures.

The three commercially mixtures of PBDEs are Penta-BDE, Octa-BDE and Deca-BDE according to the number of bromine atoms in the dominating congeners of the mixtures. The three PBDE mixtures have different applications:

- Penta-BDE mixture is primarily used in foams, such as seat cushions and other household upholstered furniture, as well as in rigid insulation.
- Octa-BDE is used in high impact plastic products, such as housing for fax machines and computers, automobile trim, telephone handsets and kitchen appliance casings.
- Deca-BDE is used in plastics, such as wire and cable insulation, adhesives, coatings and textile coatings. Typical end products include housing for television sets, computers, audiotape cassettes stereos and other electronics. Deca-BDE is also used as a fabric treatment and coating on carpets and draperies, but it is not used in clothing.

The European Union has banned since August 2004, the use of Penta- and Octa-BDE technical mixtures, but the use of Deca-BDE mixture is unrestricted following favorable risk assessment in May 2004 (BSEF, 2007). In U.S., only California has banned the use, by the end of 2008, of Penta- and Octa-BDE mixtures and other U.S. states are in the phase out legislation for PBDEs.

2.2. HEXABROMOCYCLODODECANE

Structurally, HBCD is a cyclic aliphatic ring consisting in twelve carbon atoms with six bromine atoms tied to the ring. The commercial HBCD consists in a mixture of the α–, β– and γ–HBCD diastereomers, with the γ–HBCD isomer being dominant ($\geq$70%). With a worldwide production of 16,700 t in 2001, HBCD is the third most widely used BFR in the world and on the second place in the European

Union. HBCD is considered to be a high-production-volume chemical and a priority pollutant under the "Existing Substance Regulation" of the European Chemicals Bureau. It is mostly used in extruded (XPS) and expanded (EPS) polystyrene foams, but also is used as insulation material in construction industry. HBCD is highly efficient so that very low levels are required to reach the desired flame retardancy. Typical HBCD levels in EPS are 0.7% and in XPS 2.5%. At present, HBCD is the only suitable flame retardant for these applications. Other uses of HBCD are upholstered furniture, automobile interior textiles, car cushions and insulation blocks in trucks, packaging material, video cassette recorder housing and electric and electronic equipment.

In Europe and the United States, HBCD is not subject to regulatory restriction, whereas in Japan it is classified as a type I monitoring substance together with PBDEs and TBBP-A.

2.3. TETRABROMOBISPHENOL-A (TBBP-A)

With a global production of 170,000 t in 2004 (BSEF, 2007), TBBP-A is the largest used BFR. TBBP-A is mainly used as reactive BFR in laminates for printed wiring boards which are commonly used in electronic devices. Additionally, TBBP-A is used as an additive BFR in acrilonitrile-butadiene-styrene (ABS) polymers plastic housings, but it is also used as an intermediate in the production of other BFRs, such as TBBP-A derivatives and brominated epoxy oligomers. Following favorable risk assessments, the use of TBBP-A is not restricted in any country (BSEF, 2007).

2.4. OTHER BROMINATED FLAME RETARDANTS

There are in total a number of 75 different types of BFRs. Besides the above mentioned compounds, the most important group of BFRs according to its impact on environment are polybrominated biphenyls (PBBs). As a BFR, PBB was used in epoxy and phenolic resins, industrial plastics, such as high-impact polystyrene. Combusted PBB-containing materials may produce highly toxic brominated dioxins and furans. Since they have been found to be persistent, bioaccumulative toxins, being also classified as potential carcinogens, most of the production of PBBs was ceased in 1970s (BSEF, 2007).

3. Analytical Methodologies

Due to the observed increasing temporal trends in humans or biota, BFRs are being determined in a growing number of laboratories. Analytical methods for the determination of BFRs have shown a rapid development and they were in most of the cases based on protocols previously established for persistent organic pollutants

(POPs), such as organochlorine pesticides, PCBs or polychlorinated dioxins and furans (PCDD/Fs). Even if different properties of BFRs, such as polarity or vapor pressure, suggest that different procedures should be applied for their analysis from environmental samples, some common approaches can still be underlined depending also on the type of sample or the detection method (de Boer et al., 2001; Covaci et al., 2003, 2007; de Boer et al., 2007). Indeed, due to particular physico-chemical properties, the determination of individual HBCD diastereomers and TBBP-A may require specific analytical approaches.

In general, the methods used for the determination of BFRs in different matrices are very sensitive and thus able to detect extremely low amounts of these compounds. The methods described in the literature have been recently reviewed (Covaci et al., 2003; Stapleton, 2006; Covaci et al., 2007). Some basic steps of the BFR determination are sample pre-treatment, extraction, clean-up and instrumental analysis. However, being present in all environmental compartments, laboratory contamination during each analysis step can easily occur.

3.1. SAMPLE PRE-TREATMENT

Various sample pre-treatments are used depending on the employed extraction method. For solid samples (sediment, soil, dust, biological tissues), sample pre-treatment involves usually the drying of the sample. Dry samples are more effectively homogenized, allowing accurate sub-sampling for parallel analyses for other determinants (e.g., organic carbon). In addition, storage and transport may be easier. The absence of water in the samples avoids laborious extraction with separation funnels and makes the sample matrix more accessible to organic solvents. As an alternative to drying through evaporation, several methods can be applied for water binding and the most easily to perform is chemical drying by grinding of the sample with anhydrous Na_2SO_4. Freeze-drying (water evaporation below 0°C under vacuum conditions) can also serve for sample drying (Smedes and de Boer, 1997).

3.2. EXTRACTION

The extraction procedure of the analytes is dependent on the sample matrix and thus different methods are used for solid or liquid samples.

In the case of solid samples, including soil, sediment, sewage sludge, adsorbent materials used for air sampling and biological samples (tissues, eggs), the extraction efficiency depends on few major factors: the analyte solubility in the extraction solvent, the accessibility of the extraction solvent to the matrix and the extraction time. The use of non-polar organic solvents is advantageous because of the high solubility of most BFRs, but there is a low accessibility of such solvents to the inner part of biotic tissues or to the sediment/sewage sludge organic matter (containing many polar groups like amines, phenols and carboxylic acids). Therefore, the use

of binary solvent mixtures (combination of an non-polar and polar solvent) proved to be the ideal solution in terms of obtaining the best recoveries for extraction of BFRs from the solid samples (de Boer et al., 2001).

An attractive extraction technique, due to its general robustness and low cost, is the Soxhlet liquid-solid extraction. The Soxhlet extraction may be also performed in a classical way or in an automated way, so-called hot Soxhlet. In the later case, the extraction solvent is distilled into the extraction chamber, which is heated below the boiling point of the solvent keeping in this way the sample in permanent contact with hot, but no boiling solvent, thus accelerating the desorption and finally the extraction process.

Other extraction methods are also applied including ultra-sonication, used for the extraction of PBDEs from polyurethane foams used for air sampling or from high-impact polystyrene, but lower extraction recoveries compared with Soxhlet extraction were obtained (Covaci et al., 2003). Extraction of BFRs with organic solvents from a chromatographic column filled with homogenized sample has been used, but despite of its simplicity, the method uses large volumes of organic solvents that have to be further evaporated and disposed off (Manchester-Neesvig et al., 2001). Other new extraction techniques, such as accelerated solvent extraction (ASE) or microwave assisted extraction (MAE), are currently applied on BFRs analysis (Covaci et al., 2003) and although higher costs which are involved compared with Soxhlet extraction, these techniques have the advantage of lower solvent consumption, which makes the long-term costs lower and the procedures more environmentally friendly.

In the case of liquid samples, liquid-liquid extraction (LLE) was applied by using a binary mixture of solvents for BFRs analysis in river and seawater samples, but also from human milk or serum. Solid phase extraction (SPE) has been used mostly for the analysis of neutral type compounds from human serum or milk, but also for the analysis of phenolic BFRs. In this later case, an additional clean-up step is necessary due to higher amounts of co-extracted lipids.

3.3. CLEAN-UP

The non-selective nature of the exhaustive extraction procedures and the complexity of the sample matrices result in complex extracts that require further purification prior to the gas or liquid chromatographic separation of BFRs. The clean-up techniques for BFR analysis were recently reviewed by Covaci et al. (2003, 2007). Depending on the type of sample analyzed, the extracts may contain sulfur that has to be removed (the case of sediment, sewage sludge or soil samples) or high concentrations of lipids (usually the case of biological samples). Similar to other organic contaminants, the BFR concentrations are usually lipid-normalized and therefore, the lipid content has to be determined. This can be done gravimetrically before clean-up, determined on a separate sample aliquot a total lipid determination or on separate aliquots by enzymatic tests (for serum and plasma samples).

For abiotic samples, elemental sulfur can be removed by simple treatments of either the sample or the sample extract with copper powder or by gel permeation chromatography (GPC). There are also other procedures, including treatment with $AgNO_3$-modified silica or with tetrabutylammonium sulfide, but these techniques are less frequently used.

The lipids contained in biological samples extracts may be eliminated using either destructive or non-destructive methods. The common non-destructive methods of lipids removal are GPC and adsorption chromatography on selected sorbents. However, for complex matrices, two serially connected GPC columns or GPC followed by further clean-up by adsorption chromatography are often required for complete fat removal and/or isolation of BFRs from other organohalogenated compounds. For adsorption chromatography, silica gel, alumina and Florisil with different degrees of activation have been widely used for lipid removal.

Similarly to other organohalogenated compounds, the most used destructive treatment in the BFR analysis is the sulfuric acid treatment since they are stable under strong acid conditions. The simplest approach consists of direct addition of the acid to the sample extract dissolved in *n*-hexane. However, this treatment requires several sequential LLE and centrifugation steps, which results in a multi-step and time-consuming procedure. The dispersion of sulfuric acid onto the surface of activated silica gel results in an adsorbent which can be easily loaded into a column. The use of acidified silica avoids the emulsion problems of the LLE approach, reduces the sample handling and solvent consumption and increases the sample throughput. Silica gel can also be modified with alcoholic NaOH or KOH, but such treatment may cause losses of Br atoms from highly brominated BFRs, such as HBCDs, PBBs and PBDEs (de Boer et al., 2001).

3.4. INSTRUMENTAL ANALYSIS

Because of their physico-chemical properties (like vapor pressure or polarity), gas-chromatography has become a standard analytical separation method employed for the analysis of PBDEs, while a different approach may require the analysis of HBCDs and to some extent TBBP-A, for which LC-based separation techniques are often used.

3.4.1. *Gas-Chromatographic Analysis of BFRs*

An important step for the accurate and optimal determination of compounds with relatively high boiling points, such as PBDEs, is the sample injection into the gas chromatographic (GC) system. Various injection methods were reported, but the most common are split/splitless injection, on-column injection and programmable temperature vaporization injection. All mentioned methods possess advantages and disadvantages that derive primarily from their availability, price, acceptable detection methods and discrimination of congeners on the basis of molecular weight.

As a consequence of their selective uptake, metabolism or degradation, the profiles of BFRs in samples are commonly estimated by determining of selected individual congeners. This means that single-capillary column GC may offer sufficient resolution for a congener specific BFR determination. Therefore, in order to achieve enough separation between BDE congeners particularly and possible interferences, there is a need for using sufficiently long columns (30–50 m) and small diameters (≤0.25 mm). Good resolution may also be obtained by using narrow bore columns (internal diameter = 0.1 mm) (Covaci et al., 2002). Prior of using a certain capillary column for a specific type compound analysis, it has to be tested first for possible co-elutions of target compounds, internal standards and other compounds present in the sample, especially if electron capture detector is used. The impact of the co-elutions depends on the sample clean-up, pollutant load in the sample, chromatographic system and detection method. Several co-elutions are reported in the literature for BFR analysis (Figure 1), but most of them can be solved by MS detection either in selective ion monitoring of full scans (reviewed by Covaci et al., 2003).

The characteristics of the GC system significantly influence the PBDE determination by GC-MS. If not selected properly, the column brand, type of retention gap, press-fit connector and stationary phase as well as column length and injection technique may have a very strong influence on the accuracy and precision of the PBDE analysis (Björklund et al., 2004). By selecting an erroneous GC set-up,

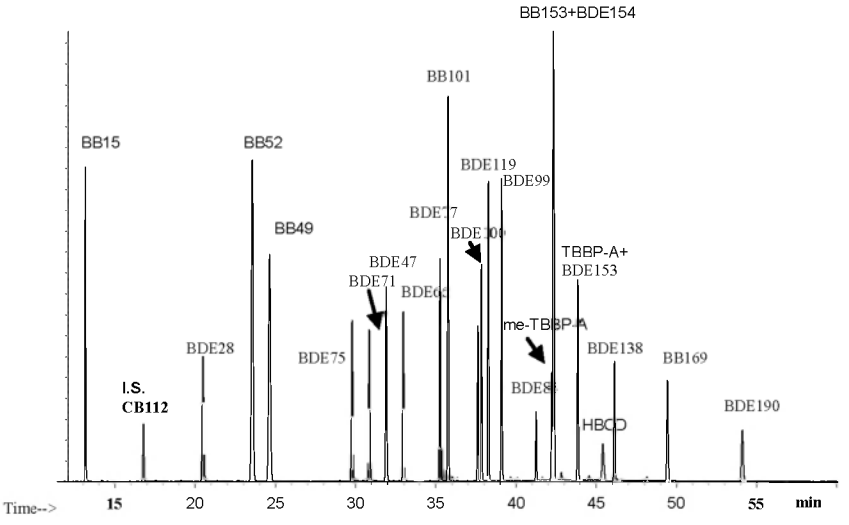

Figure 1. ECNI-MS chromatogram of a standard mixture of BFRs on a 50 m × 0.2 mm × 0.25 µm CP-Sil 8 column (1 µl injected in pulsed splitless, injector temperature: 275°C, oven program: 90°C (3 min), with 30°C/min to 210°C (20 min), with 5°C/min to 315°C) (From Covaci et al., 2003)

the yield of nona- and deca-BDE congeners can be reduced to zero and the precision of the determination of congeners with more than five bromine atoms can be strongly decreased. An overview on GC-MS parameters used in the analysis of PBDEs, TBBP-A and other BFRs is presented in Table 1.

The use of comprehensive two-dimensional gas chromatography (GC×GC) has been evaluated also for the analysis of PBDEs. It was previously shown that even on the most efficient stationary phases, single dimension GC cannot separate all or nearly all PBDE congeners. From the 125 BDE congeners that were tested, 55 congeners were involved in 22 co-elutions for the most efficient phase, DB-XLB (Korytár et al., 2005a). As a consequence, Korytár et al. (2005b) evaluated six column combinations for the GC × GC separation of PBDEs with μ-ECD or time of flight-mass spectrometry (TOF-MS) as detectors. They concluded that a DB-1 × 007-65HT (Quadrex) combination was the most suitable because of: various reasons including the highest number of PBDE congeners separated, the least decomposition of higher brominated congeners, and the most suitable maximum operating temperature. With this set-up, there were only 17 co-eluting pairs involving 35 congeners. Since other BFRs or MeO-BDEs may be present in environmental samples, it was shown that the second dimension column improves the separation of Me-TBBP-A, TBBP-A, BB 169, 6-OH-BDE 47 and 6-MeO-BDE 47, while most of other compounds were already separated in the first dimension (Figure 2).

A different approach has to be used in the case of BDE 209 analysis because of its sensitivity for higher temperatures and the higher susceptibility for degradation in the GC system. The GC column should be relatively short (10–15 m) to reduce as much as possible the residence time (de Boer et al., 2001). Based on the ability of the low pressure-GC (LP-GC) technique to elute compounds at lower temperatures than conventional GC techniques, BDE 209 was analyzed at temperatures below the degradability limit. The low elution temperature of BDE 209, combined with its short residence time in GC has lead to minimal thermal degradation (Dirtu et al., 2007).

The analysis of more polar BFRs, especially phenolic-type compounds, such as TBBP-A, would require derivatization prior to injection into the GC system. This procedure involves a more complex sample preparation procedure compared to LC methods, but the methods limits of quantification obtained by GC analysis are more appropriate for measuring contaminants like TBBP-A presents at lower concentrations in biota compared to PBDEs and HBCDs due to its lower bioaccumulation potential.

TABLE 1. Overview of GC-MS Parameters Used for the Analysis of PBDEs, TBBP-A and Other BFRs

Compound	Column	Dimensions	Injection mode	Source temp (°C)	Derivatisation	Ionisation	Instrument	Ion	References
PBDEs	VF5-MS (Factor Four, Varian)	55 m × 0.25 mm × 0.25 µm	Splitless	220	–	EI	IT	M^+ and $[M–2Br]^+$	(Gómara et al., 2006)
PBDEs	DB-5 ms (J&W Scientific)	15–30 m × 0.25 mm × 0.25 µm	Splitless	250	–	EI	QTrap	M^+ and $[M–2Br]^+$	(Wang et al., 2005)
PBDEs	CP-Sil8 CB low bleed (Chrom-Pack)	30 m × 0.25 mm × 0.25 µm	Splitless	250	–	EI	IT	BDE 47: 486-324,326,328; BDE 100:566-404,406; BDE 154:644-482,484,486	(Salgado-Petinal et al., 2006)
PBDEs	DB-1 (J&W Scientific) × 007-65HT (Quadrex)	30 m × 0.25 mm × 0.25 µm × 1.0 m × 0.1 mm × 0.10 µm	Splitless	–	–	–	µ-ECD	–	(Korytár et al., 2005b)
PBDEs	DB-1 (J&W Scientific) × HT-8 (SGE)	15 m × 0.25 mm × 0.25 µm × 1.2 m × 0.1 mm × 0.1 µm	Splitless	250	–	EI	TOF	Scan	(Focant et al., 2004)

(Continued)

(Continues)

DeBDethane	DB-5 ms (J&W Scientific)	12 m × 0.25 mm × 0.12 µm	Splitless	220	–	ECNI	Q	79, 81	(Kierkegaard et al., 2004)
DeBDethane	DB-5 ms (J&W Scientific)	14 m × 0.25 mm × 0.10 µm	on-column	260	–	EI	HRMS	$[C_6Br_5CH_2]^+$: 482,484,486,488 $[C_{12}Br_{10}C_2H_4]^+$: 969,971,973	(Kierkegaard et al., 2004)
DeBDethane	DB-5 ms (J&W Scientific)	15 m × 0.25 mm × 0.25 µm	Splitless	–	–	–	ECD	n.a.	(Kierkegaard et al., 2004)
TBBP-A	UB5-P (Interchim)	15 m × 0.25 mm × 0.25 µm	Splitless	230	MSTFA	EI	HRMS	682.8509, 684.8489	(Cariou et al., 2005)
TBBP-A	DB-5 ms (J&W Scientific)	30 m × 0.25 mm × 0.1 µm	Splitless	275	methyl chloroformate	EI	HRMS	556.7608, 554.7629	(Berger et al., 2004)

IT – Ion trap, Q – Quadrupole, TOF – Time-of-flight

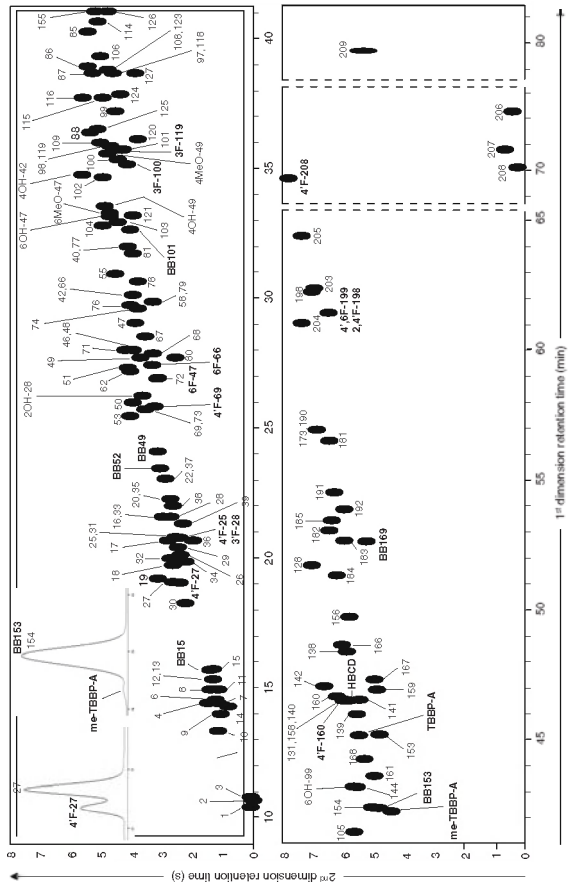

Figure 2. Overlaid GC×GC–μECD chromatograms on DB-1×007-65HT column combination of (●) BDEs, (●) fluorinated BDEs, (●) other brominated flame retardants and (●) BDE metabolites. Temperature programme: 90°C (2 min), at 20°C/min to 190°C, then at 2°C/min to 325°C (5 min). Modulation period, 8 s; constant flow of helium carrier gas, 1.2 ml/min [From Korytar et al., 2005b]

3.4.2. *Detectors*

The most widely used detectors for the BFR determination are mass spectrometers, classified into low-resolution (LR) or high-resolution (HR) mass spectrometric (MS) instruments. The LR-MS instruments are operated either in electron impact (EI) or electron capture negative ionization (ECNI) mode. For specific applications, electron capture detectors (ECD) have been also used, but for the use of such detector, the possibility of interferences to occur is very high.

In case of PBDE analysis using EI-MS, the major ions formed are M^+ and the $[M\text{-}2Br]^+$ which can be used for their identification and quantification (Sellström, 1999) allowing also their determination in the presence of possible co-eluted compounds (such as PCBs). Because of these fragments formed, the use of LR-EI-MS provides a higher selectivity compared to LR-ECNI-MS. However, the use of LR-EI-MS (with quadrupoles as mass analyzer) is not routinely used for the PBDE analysis because of its relatively low sensitivity, especially when measuring BDE congeners with more than six bromine atoms.

In contrast to EI, ECNI is a "soft" ionization technique that takes advantage of the interactions between thermal energy electrons and electrophilic molecules, such as PBDEs. In ECNI, the low-energy electrons (thermal electrons) generated by interactions between a high-energy electron beam and a moderating or reagent gas, react with the analytes to form negative ions. The electron energy should be very low to facilitate electron capture, and the specific energy required for electron capture depends on the molecular structure of the analyte. Therefore, ECNI-MS is usually preferred for the analysis of PBDEs, because it is more selective towards aromatic brominated compounds.

Compared to LR-EI-MS, the use of LR-ECNI-MS for PBDE analysis is less selective because of monitoring of the bromide ions $[Br]^-$ for all homologue group, but instead is a much sensitive method with one order of magnitude lower limits of detection. Therefore, this technique proves to be suitable for the analysis of low-concentration samples such as human serum and plasma. However, selectivity can be retained when using LR-ECNI-MS under optimized conditions. Optimizing of the electron energy, emission current, source temperature and system pressure it was noticed that relative abundance of the molecular fragment $[M\text{-}x\text{H}\text{-}y\text{Br}]^-$ is increasing and therefore it can be used for the monitoring of each homologue group in place of the nonspecific bromide ions (Ackerman et al., 2005).

The use of GC-MS (operated in EI or in ECNI mode) was also applied for the analysis of other BFRs, such as HBCD, in fish samples (Roosens et al., 2008). The results were compared to those obtained by LC-MS. A good agreement has been found between the different analysis techniques. The GC-MS methods do not allow having individual isomer data, but they give a very good estimation of the total HBCD concentrations. In parallel with the "traditionally" monitored peak at m/z = 79, other ions, such as the $[M\text{-}Br]^-$ ions at m/z = 561, could be also monitored, but this is accompanied by a decrease in sensitivity as it can be seen in the full scan ECNI-MS spectra of ^{12}C-α-HBCD (Figure 3). This procedure

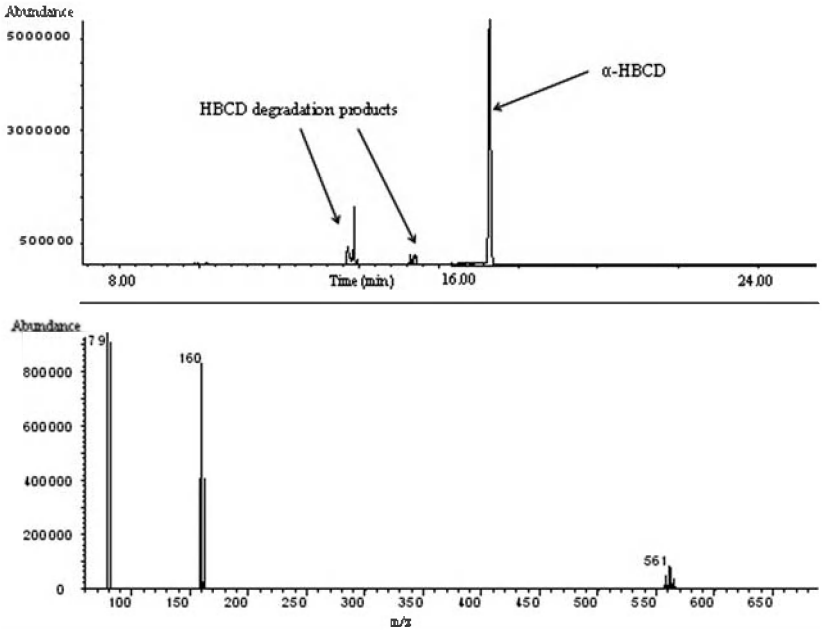

Figure 3. GC–ECNI/MS total ion chromatogram and full scan mass spectra of α–HBCD standard (conc. 1 ng/μl in iso-octane)

allows the use of ^{13}C-α-HBCD as internal standard and thus improving greatly the quality of GC-MS measurements. However, due to the 50–100 loss in sensitivity when other ions than m/z = 79 are used, this method can be employed only to samples with high concentrations of HBCDs (Roosens et al., 2008).

The use of ECD for BFR analysis was applied only when concentrations were relatively high (Allchin et al., 1999; Manchester-Neesvig et al., 2001). Its relatively good sensitivity for compounds with four or more bromine atoms, combined with its relatively low purchase and maintenance cost, could make it very attractive to be used in this field, but despite of such advantages, several drawbacks have reduced its application area. One problem is unequal responses for the different congeners caused by the influence on the detector sensitivity of the substitution pattern of the rings (Sellström, 1999). Furthermore, ECD is known for being linear only over a limited concentration range and also it is known for its lack of selectivity.

3.4.3. *Liquid-Chromatography Analysis of BFRs*

Despite the limited chromatographic resolving power of LC, methods employing LC/MS and LC/MS-MS offer very good results for the analysis of some BFRs, such as HBCDs and TBBP-A. These methods have been applied due to a number of inconveniences recorded for GC analysis of compounds such HBCD

or TBBP-A. At temperatures higher than 160°C, the interconversion of HBCD diastereomers make impossible the GC determination of HBCD as single isomers and moreover, HBCD degrades at temperatures above 240°C and partially breakdown in dirty GC systems. For TBBP-A, because of its higher polarity compared to other BFRs generated by the presence of HO groups, LC-MS methods were also intensively applied. The use of GC-based methods for this compound would require the derivatization of the hydroxyl groups, which makes sample preparation a much complex process compared with sample preparation for LC analysis. However, the method limits of quantification are much lower in the case of GC analysis, being possible using such methodology the monitoring of TBBP-A concentrations in environmental compartments in which the exposure is at a normal level. An overview of LC-MS parameters used for in the analysis of HBCDs, TBBP-A and other BFRs is presented in Table 2.

The analysis of PBDEs by atmospheric pressure photoionization (APPI) and LC/MS-MS was also tested (Debrauwer et al., 2005). It is known that PBDEs do not ionize well using the most traditional LC/MS-MS methods, electrospray ionization (ESI) or atmospheric chemical ionization. Using APPI, PBDEs ionize well in both negative (higher sensitivity for penta- through deca-BDE congeners) and positive (higher sensitivity for di- through penta-BDE congeners) modes, depending on the degree of bromine substitution. APPI is a softer ionization technique compared to electron impact (EI) since M^+ ions are the most intense ions produced by the interaction of PBDEs with dopants charged by photons and MRM in the MS-MS system follows the M^+ to $[M\text{-}Br_2]^+$ transition. The most intense ions in EI are the $[M\text{-}Br_2]^+$ ions.

For HBCD isomer-specific analysis, reversed-phase LC coupled to ESI or atmospheric pressure chemical ionization (APCI-MS) is a versatile tool for its determination in environmental samples. However, the use of LC-ESI-MS/MS results in better performances than LC–APCI-MS/MS when a single MRM for the transition $[M–H]^-$ (*m/z* 640.6) → $[Br]^-$ (*m/z* 79) is used (Budakowski and Tomy, 2003).

Column selectivity towards HBCD diastereomers was evaluated for C_{30} and C_{18} stationary phases under different mobile phase conditions and column temperatures. The HBCD elution order was dependent on the shape selectivity of the stationary phase and the mobile phase composition (Figure 4). Greater resolution, on columns with reduced shape selectivity, of β-HBCD and γ-HBCD was achieved with the use of an acetonitrile/water (compared with a methanol/water) mobile phase composition (Dodder et al., 2006).

TABLE 2. Overview of LC-MS Parameters Used for in the Analysis of BFRs

Compound	Column	Dimensions	Mobile phase (gradient)	Flow (ml/min)	Mobile phase modifiers	Ionisation	Instrument	Ion	Source temp (°C)	References
HBCDs	Luna C_{18} (Phenomenex)	150 × 2 mm; 5 µm	AcN:MeOH: H_2O (y)	0.2	Ammonium acetate	ESI	IT or Q	640.7	160	(Morris et al., 2004)
HBCDs	Genesis C_{18} (Chromatogr. specialties)	50 × 2.1 mm; 4 µm	MeOH:H_2O (y)	0.3	–	ESI	QqQ	MRM (640.6 => 79)	500	(Budakowski and Tomy, 2003)
HBCDs	Symmetry C_{18} (Waters)	150 × 2.1; 5 µm	AcN:MeOH: H_2O (y)	0.25	–	ESI	QqQ	MRM (640.6 => 79)		(Janak et al., 2005)
HBCDs	Symmetry C_{18} (Waters)	150 × 2.1 mm; 3.5 µm	AcN:MeOH: H_2O (y)	0.25	Acetic acid	ESI	QqQ	MRM (640.6 => 7 9)	–	(Cariou et al., 2005)
PBDEs	Ultrabase RP_{18} (SFCC)	250 × 2 mm; 5 µm	MeOH:Toluene:H_2O (n)	0.2	–	APPI	QTrap	Scan	n.a.	(Debrauwer et al., 2005)
TBBP-A	Nucleodur 100-C_8 (Interchim)	250 × 4 mm; 5 µm	AcN:H_2O (y)	1[a]	Acetic acid	APPI	QTrap	Scan	n.a.	(Debrauwer et al., 2005)
TBBP-A	Luna C_{18} (Phenomenex)	150 × 2 mm; 5 µm	AcN:H_2O (y)	0.25	Ammonium acetate	ESI	Q	540.9	150	(Morris et al., 2004)

(Continued)

(Continued)

TBBP-A	Ace 3 C_{18} (Advanced chromatography technologies)	150 × 2.1 mm; 3.0 µm	MeOH:H_2O (y)	0.2	Ammonium acetate	ESI	TOF	Scan (230–550)	130	(Berger et al., 2004)
TBPP-A	Genesis C_{18} (Chromatographic specialties)	150 × 2.1 mm; 4 µm	MeOH:H_2O (y)	0.2	–	ESI	QqQ	MRM (543 => 81)	130	(Chu et al., 2005)

[a]Post-column splitting 1:10; IT – ion trap, Q – quadrupole, QqQ – triple quadrupole; TOF – time-of-flight

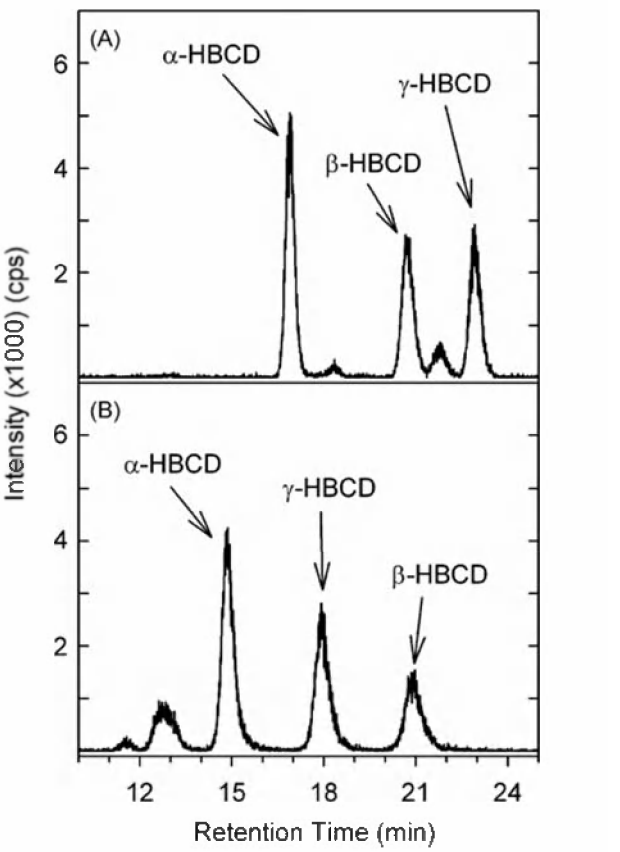

Figure 4. Atmospheric particle-phase MRM chromatograms. The same sample was analyzed using an Eclipse C18 column (A) and a Carotenoid C30 column (B), both 250 × 4.6 mm. In both cases, the mobile phase was 90% methanol, 10% water (From Dodder et al., 2006)

Although TBBP-A is the most widely used BFR, this compound is not frequently measured, due to its lower concentrations in biota compared to PBDEs and HBCDs and due to its lower bioaccumulation potential. TBBP-A is also more polar than PBDEs and HBCDs, which demands therefore more complicated methods for a proper determination. Acidification and derivatization are compulsory before GC analysis can be carried out, while LC has the advantage that no derivatization step is required (de Boer and Wells, 2006). The LC separation of TBBP-A from other composunds and matrix components is greatly dependent on the mobile phase used. For example, one-third higher response with methanol instead of acetonitrile was reported (Chu et al., 2005). For optimized chromatographic separation and/or ionization response, mobile phase additives, such as formic acid, tris(hydroxymethyl)aminomethane and ammonium acetate, are often used. However, while the two former products were found to give a decreased

ESI-response, ammonium acetate significantly increased the response. Furthermore, using a methanol mobile phase alone resulted in a more stable detector baseline and thus a lower LOQ. Therefore, using methanol and water as a mobile phase can be more advantageous for the quantitative analysis of TBBP-A.

3.5. QUALITY ASSURANCE/QUALITY CONTROL

Quality assurance (QA) is a set of procedures, which include the quality control (QC) activities, and which are undertaken to confirm the quality of obtained data. As a general rule, approximately 15% of the analysis time should be spent on the QA procedures. To assure sufficient quality, a number of measures should be taken during the pre-analysis quality control (or validation) and in-process quality control. These measures can be divided into three major areas: calibrants, analytical procedure control, system performance/long-term stability.

A proper dilution (working with volumes sufficiently large to minimize the accuracy errors) and storage of calibration standards (usually cool, dark place) should be employed in such way that after regular checking the weight loss is maximum 2% for a 6–9 month period. Quantification procedures based on internal standard addition (the use of ^{13}C-labelled compounds is recommended) should be addressed to compensate for the losses throughout the analytical procedure, combined with the use of syringe standards for inter-injection fluctuations compensation.

Because all essential steps of analytical procedure are matrix-specific, the analyte recovery, the use of procedural blanks and the determination of limits of detection and quantification should be performed for each compound and matrix to be investigated.

The analytical characteristics of the method should be also considered as an internal quality control by determining the following parameters: repeatability (same operating conditions over a short time), intermediate precision (within-laboratory variation), reproducibility (precision between laboratories) and accuracy (estimated through the use of certified reference materials). The external quality control is usually assessed through the participation in interlaboratory tests which facilitates the evaluation and assessment of the overall method performance.

4. Toxicity

Although several BFRs are found in quantifiable levels in wildlife and in humans and have been extensively investigated in the last decade, we are still lacking information on the health effects caused by these compounds (Birnbaum et al., 2004; McDonald, 2002).

Generally, HBCD, TBBP-A and PBDEs are absorbed from the gastrointestinal tract and accumulate in fatty tissues. It seems that they don't cause immediate symptoms from acute toxicity at average doses, but their health effects from

chronic exposure are of more concern, especially when they are related to the exposure of developing infants and wildlife. However, based on the available data, it is known that BFRs are associated with several health effects in animal studies, including neurobehavioral toxicity, thyroid hormone disruption and possibly cancer, only for some PBDE congeners. Even if limited information is published in this field, there is some evidence that BFRs can cause developmental effects (Darnerud, 2003), endocrine disruption (McDonald, 2002; Darnerud, 2003), immunotoxicity (Birnbaum et al., 2004; Darnerud, 2003), reproductive and long term effects, including second generation effects (Birnbaum et al., 2004; Kuriyama et al., 2005). For PBDEs and TBBP-A, there is some evidence available for estrogenic activity (Meerts et al., 2001; Legler and Brouwer, 2003), but more studies have to be undertaken to determine if low dose exposures have estrogenic activity in humans or other species. The penta-BDE congeners have been shown to cause toxicity at lower doses than the octa- through deca-BDE congeners (Darnerud, 2003). There are no data on the relative toxicity of the different HBCD isomers or TBBPA derivatives (Birnbaum et al., 2004). Furthermore, there are no data on the toxicity of exposure to BFR mixtures.

One case in history caused the removal from the market in the early 1970s of a class of flame retardants, namely PBBs because of the poisonings in Michigan by mixing a bag containing a commercial PBB mixture, into animal feed. This resulted into long-term impacts on the health of exposed farm families (Dunckel, 1975).

5. Environmental Levels

Despite of their societal benefits, BFRs seem to migrate from the products in which they are used and entering the environment and people. An increasing numbers of papers, including several reviews, have been published in the last decades and it was shown that BFRs may be measured now in a lot of variety of samples, including air, water, fish, birds, marine mammals and humans. In many cases, it was revealed that the concentrations of these compounds are increasing over time.

5.1. TEMPORAL TRENDS

5.1.1. *Aquatic Environment*

Most data related to the BFR levels presented in the literature focuses on the aquatic environment. A good example of showing time trends in aquatic environment species was underlined by Hites (2004) based on the determination of PBDE concentrations in tissues of marine mammals (seals and porpoises). One data series is composed by samples from the Canadian Arctic, in which low PBDE levels less than 5 ng/g lipid weight (lw) (Ikonomou et al., 2002), while the second series various species all over the world. A significant increasing of ΣPBDE levels

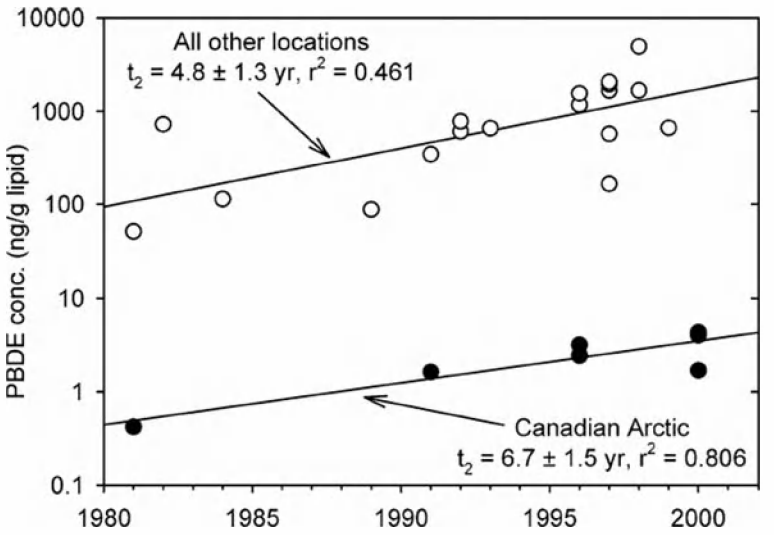

Figure 5. ΣPBDE concentrations in marine mammals (in ng/g lipid weight) shown as a function of the year in which the samples were collected. The bottom line with filled symbols represents samples from the Canadian Arctic (Ikonomou et al., 2002) and the top line with open symbols is for all other samples. The regressions for the two data sets are shown separately; the doubling times of the types of samples are not significantly different (From Hites, 2004)

with the time could be observed for each series (Figure 5). For the Arctic samples, a doubling in the PBDE concentration over a 7 year-period was calculated, while for the rest of the samples, a doubling time of 5 years was estimated (Hites, 2004).

Similar to other organohalogenated pollutants, the BFR levels found in adult marine mammals are significantly higher compared to juvenile animals showing that they are able to bioaccumulate BFRs with time (Law et al., 2003, 2006).

Except few studies, when fish samples are analyzed, the BFR concentrations did not vary systematically with the sampling date either in Europe, in North America or for both combined (Hites, 2004) indicating that temporal trends can not be assessed using such samples. However, a recent study (Batterman et al., 2007) on trends of four PBDE congeners in fish species from Great Lakes, showed large increases in concentrations of PBDEs that started in the early to mid-1980s with fairly consistent doubling times (generally 2–4 years, except in Lake Erie smelt where levels increased very slowly). Furthermore, concentrations and trends show differences by congener, fish species and lake. It was also shown that in fish samples collected recently, the accumulation rates are slowing and concentrations of penta- and hexa-BDE congeners in trout from Lakes Ontario and Michigan and smelt from Lake Ontario started to decrease in the mid-1990s.

Other studies focused on determination of PBDEs from marine sediment cores, showed also time trends for these compounds in collected samples. In sediment cores taken from Baltic Sea (Nylund et al., 1992), a doubling in the PBDE concentration over a 3–4 years period was shown compared to sediment core samples from Drammenfjord, Norway, where a doubling time of approximately 3 years was estimated until the mid-1980s at which time the concentrations seem to have leveled off (Zegers et al., 2003).

Temporal trends for HBCDs were also investigated (Figure 6) through studies applied on different bird eggs (guillemot eggs from the Baltic Sea, peregrine falcon eggs from Greenland or other marine bird eggs from Norway) and also marine mammals (sea lions from USA). HBCD measured in individual and pooled archived guillemot eggs from the Baltic Sea indicated an increase in concentrations between 1969 and 1995 and this increase has leveled-off between 1995 and 2001 when concentrations of HBCDs seem to have stabilized, whereas PBDE concentrations were decreasing (Sellström et al., 2003). A clear and remarkably consistent increasing of HBCD levels since 1983 was reported for the bird egg sampled in the Northern Norway (Knudsen et al., 2005). In another study, Stapleton et al. (2006b) pointed to an exponential increase in the HBCD concentrations with a doubling time of approximately 2 years in California sea lions stranded between 1993 and 2003 (Figure 6). It is unclear at this time why HBCD concentrations increase in the sea lions, while PBDE levels were highly variable without a significant

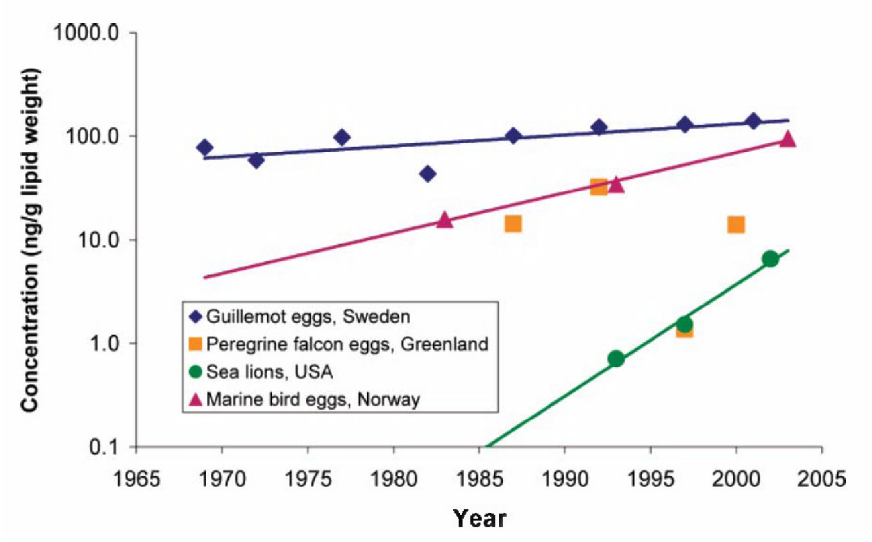

Figure 6. Temporal trends of HBCDs in different bird eggs and in sea lions. Exponential regressions of the complete HBCD concentration data vs. time were significant ($p < 0.05$) for the guillemot eggs, the marine birds eggs and the sea lions data. The peregrine falcon data did not show a significant change over the observed time period (From Covaci et al., 2006)

temporal trend. In male juvenile gray seals from the Baltic Sea sampled in the 1990s, HBCD concentrations were higher compared to those sampled between 1980 and 1985 (Roos et al., 2001).

5.1.2. *Terrestrial Environment*

In contrast to aquatic species, no time trends could be established for HBCDs in terrestrial birds, such as peregrine falcon and sparrowhawk tissues from the UK sampled between 1973 and 2002, due to a low detection frequency and biased sampling (de Boer et al., 2004) or in peregrine falcon eggs from South Greenland sampled between 1986 and 2003 (Vorkamp et al., 2005). Interestingly, for the latter species, a 10% increase per year in the PBDE levels has been observed throughout the investigated time period.

In summary, time trends are not clear yet as the data obtained so far showed either an increasing or no significant trend (Covaci et al., 2006). However, there are no indications available that industry's measures to limit emissions of HBCDs at production and handling sites have led to decreasing concentrations in the environment on a global scale. No study has found parallel time trends for HBCDs and PBDEs. This likely reflects the different regional production and application history for these two BFRs.

5.2. GEOGRAPHICAL TRENDS

When available data regarding the environmental levels of BFRs has to be related to the geographical position of the samples taken into study, few general remarks may be deducted:

(a) Concentrations of BFRs in general (HBCD in particular (Covaci et al., 2006) are often elevated by at least one order of magnitude in the vicinity of plants either producing or using these compounds.
(b) Detection of BFRs in air samples from remote sites, strongly suggest that these compounds undergo long-range transport. Although the influence of possible local sources cannot be ruled out completely, the human activities of these areas are probably not sufficient to explain the environmental levels observed.
(c) In general, the different continental market demands seem to be reflected in different environmental residue levels.

5.2.1. *Aquatic Environment*

When fish samples are analyzed, a high variability of BFR concentrations is recorded, even for the same species, suggesting that these concentrations are related to the proximity of the feeding grounds to BFR sources. These results indicate that fish samples may be used as local indicators of exposure to BFRs. In general, the concentrations of PBDEs in fishes from Europe are significantly

lower than in fishes from North America, the arithmetic and geometric averages for ΣPBDEs are 120 and 50 ng/g lipid for the European fishes, respectively, and 1,050 and 310 ng/g for the North American fishes, respectively (Hites, 2004). Contrarily, HBCD concentrations found in fish (Tommy et al., 2004), dolphins (Peck et al., 2005) and sea lions (Stapleton et al., 2006b) from the North American environment appear to be lower than levels in similar samples from Europe (Janák et al., 2005; Morris et al., 2004; Zegers et al., 2005).

The idea of local monitoring of BFR exposure through fish contamination is sustained by the results of the study performed by Voorspoels et al. (2003). A variety of suspected BFR sources present in the Western Scheldt estuary, such as BFR manufacturing plant, Antwerp harbor or textile industry, was pointed by measuring eight PBDE congeners concentrations in samples of biota, including crab, shrimp, starfish, benthic fish and gadoid fish from the Scheldt Estuary and afterwards compared to those in samples from the Belgian North Sea beyond the mouth of the estuary. Concentrations observed in the Scheldt Estuary samples were up to 30 times higher than in those from the Belgian North Sea, with an increasing gradient towards Antwerp.

5.2.2. *Terrestrial Environment*

Different biota samples have been used to study the geographical distribution of BFRs and other persistent organic pollutants in terrestrial environment, but sometimes it is difficult or expensive to sample these materials. Tree bark has been used successfully as a passive sampler to monitor such contaminants being easy and inexpensive to sample and having a high lipid content and large surface area. Such matrix was used to determine the BFR levels in 29 locations from USA and furthermore, the PBDE levels from US environment were compared with those from few European and Asiatic countries (Zhu and Hites, 2006). The average ΣPBDE concentrations from Italy (13 ng/g lw), Germany (72 ng/g lw) and South Korea (140 ng/g lw) were found to be comparable to those from the northern part of the US and Canada (median concentrations of 83 ng/g lw). For the samples from Germany and Italy, BDE-183, which is a marker of Octa-BDE, contributed around 30% to the total PBDE load, indicating more of the Octa-BDE commercial product was used in Europe than in North America. This is consistent with the known PBDE market demand; in 2001, the ratio of the demand for the Penta- to Octa-BDE was 1:4 in Europe, but it was 5:1 in North America (BSEF, 2007).

Indoor dust analysis was also used as a monitoring tool for BFR contamination, but compared to tree bark samples which are used for external monitoring, dust samples are suitable to determine the in-house exposure to such compounds and finally, human exposure to BFRs. If the use of tree bark samples did not show a very high variability of the data, this is not the case of indoor dust samples. Indeed most of the samples felt within a relatively small range, but some samples were significantly more contaminated (Harrad et al., 2006; Harrad et al., 2007; Wilford et al., 2005). A comparison study regarding the levels of PBDEs in indoor dust samples form several countries (Canada, New Zealand, United Kingdom and

United States) was performed by Harrad et al. (2007) and the median concentrations found for the main BDE congeners and ΣPBDE are presented in Table 3. The results show that North American dusts are contaminated by both Deca- and Penta-BDE commercial formulations, UK dusts are contaminated predominantly by Deca-BDE. The Octa-BDE formulation appears of minimal importance in accordance with available market demand figures. An interesting idea suggested by this study was that despite the commercial PBDE formulations have never been manufactured in nor imported into New Zealand, their presence in dusts from that country suggests international trade in PBDE-containing goods is an important pathway effecting their global distribution.

Reports on BFR concentrations from terrestrial biota samples are generally scarce, compared to aquatic species, with most data being available for top-predator species. de Boer et al. (2004) showed that most samples from predatory water birds had no detectable BDE 209 concentrations, while samples from terrestrial birds of prey had a relative high number of positive results. The extremely low water solubility of BDE 209, the large size of this molecule, combined with a very low uptake and a possible fast metabolism in fish may be explanations for this phenomenon. BDE 209 in dust and other fine particles can apparently be taken up by small terrestrial animals, which are prey for some birds of prey. Terrestrial birds of prey were also intensively studied in order to monitor the contamination with PBDEs (Voorspoels et al., 2006a; Jaspers et al., 2006).

5.3. BIOMAGNIFICATION THROUGH THE FOOD CHAIN

According to above presented data regarding BFRs, it is obvious that these compounds are chemically and biologically persistent and furthermore lipophilic, which results in their bioaccumulation in fatty tissues of organisms and enrichment throughout food chains (Law et al., 2003). As a consequence of bio-magnification, increasing concentrations of PBDEs can be found with increasing trophic level, leading to highest concentrations in top-predators (Law et al., 2006).

Until this date, only few studies have discussed biomagnification of BFRs and they were oriented toward aquatic biota. Based on biomagnification models used by Broman et al. (1992) and Rolff et al. (1993), biomagnification of a persistent substance is independent on the concentration of the substance at the base of the food chain, but it is dependent on the species position in the food chain. Therefore, Burreau et al. (2000) conducted a study on the biomagnification of PBDEs in food chains from the Baltic Sea and the Northern Atlantic Sea and showed that biomagnification occurred similarly, meaning that the ratio between a prey and its predator is the same in spite of different concentrations. The lipid-normalized levels of the major congeners (BDEs 47, 99, and 100) were up to two times higher in large herring than in zooplankton, whereas the levels in salmon were again 2–3 times higher than in large herring.

TABLE 3. Summary of Concentrations (ng/g) of Selected PBDE Congeners in Indoor Dust Samples From Different Countries

		Median concentrations (ng/g)										
Location	**N**	**BDE 28**	**BDE 47**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154**	**BDE 183**	**BDE 207**	**BDE 209**	**ΣPBDEs[a]**	**Reference**
Toronto, Canada	–[b]	4.1	140	330	65	43	39	9.0	29	560	970	(Harrad et al., 2008)
Wellington, New Zealand	–[c]	0.65	24	51	8.9	5.4	5.1	–	–	–	–	(Harrad et al., 2008)
Birmingham, UK	–[d]	0.53	13	23	4.2	5.2	3.3	13	57	2800	3000	(Harrad et al., 2008)
Amarillo and Austin, TX, US	–[e]	14	410	820	160	110	89	16	71	1300	4000	(Harrad et al., 2008)
Ottawa, Canada, n = 68	68	3.0	300	430	73	49	37	19	–	630	1800	(Wilford et al., 2006)
Various regions, UK	10	0.35	24.8	44	–	23	–	–	–	7100	–	(Santillo et al., 2003)
Newcastle, UK	10	–	22	28	4	5	3	–	–	10000	10000	(Sjödin et al., 2006)
Various locations, US	17	14.8	644	676	119	64.4	72.8	17.6	19.1	1350	4250	(Stapleton et al., 2005)
Various locations, Romania	18	–	2.5	2.6	0.4	2.2	0.4	–	–	482	490	(Dirtu and Covaci, unpublished data)

[a]Sum of PBDEs 28, 47, 49, 66, 99, 100, 153, 154, 183, 196, 197, 203, and 209.
[b]Ten samples analysed for tri-hexa BDEs; seven samples analysed for tri-deca BDEs.
[c]Twenty samples analysed for tri-hexa BDEs; hepta-deca BDEs not analysed.
[d]Twenty eight samples analysed for tri-hexa BDEs (eight reported previously [Harrad et al., 2006]); sixteen samples analysed for tri-deca BDEs.
[e]Twenty samples analysed for tri-hexa BDEs; seventeen samples analysed for tri-deca BDEs.

It was also shown that the major biomagnification step in the aquatic food chain occurs from fish to marine mammals based on the fact that the lipid normalized PBDE levels in blubber and liver were similar and generally higher with more than one order of magnitude compared to levels found in invertebrates and fish (Law et al., 2003). Additionally, Muir et al. (2006) demonstrated that some PBDE congeners show substantial biomagnification from seals to polar bears with biomagnification factors ranging from 3.9 to 71.

The biomagnification potential of the most commonly reported PBDE-congeners was recently assessed in three small terrestrial food chains (Voorspoels et al., 2007a). To achieve this, PBDE data on passerines and rodents were combined with previously published data on terrestrial predators from the same area, i.e., birds of prey (Voorspoels et al., 2006a; Jaspers et al., 2006) and red fox (Voorspoels et al., 2006b). Because birds of prey occupy top positions in the food chain, it is most likely that biomagnification of persistent and lipophilic PBDEs through their preys would be substantial. Therefore, BMFs (the ratio of lipid normalized concentrations in the same tissues (if available) of predator and prey) were calculated by authors using the median concentrations. The BMF values for PBDEs in buzzard and sparrowhawk ranged from 2 to 34, depending on the congener, thus evidencing biomagnification. All PBDE congeners that could be determined in both prey and predator were biomagnified (BMF > 1). Surprisingly, no biomagnification could be observed from rodents to foxes. The median levels of sum PBDEs measured in fox were even lower than those in rodents. This was not in accordance with the biomagnification hypothesis and the observations made for BMFs in birds.

5.4. HUMAN EXPOSURE TO BFRS

A large number of samples from people (including human tissues, serum and milk) have been analyzed for PBDEs and, it could be concluded that the PBDE concentrations have increased in people by a factor of ~100 during the last 30 years (Hites, 2004). The regression of these data as a function of year is good, despite the disparate sample types, the different continents of origin, and the various congeners measured (Figure 7). This analysis shows that samples coming from North America are always above the regression line (in recent years by a factor of >10) and that the Japanese samples are usually below the regression line (by a factor of ~5) showing that the results are related to the exposure level of the general population from studied areas.

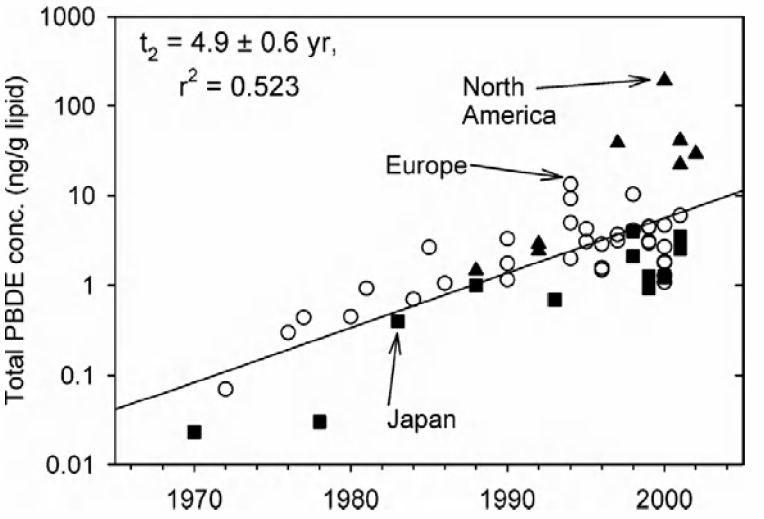

Figure 7. Total PBDE concentrations (ΣPBDE) in human blood, milk and tissue (in ng/g lipid) shown as a function of the year in which the samples were taken. The three symbol types indicate the location from which the samples were collected. The overall regression is shown (From Hites, 2004)

Considering that BFRs are used in plastics or other materials which end up in consumer electronics, it would be expected that workers involved in assembling or disassembling of these products would have an increased level of some BFRs in their blood. Indeed, occupational exposure has been reported to result from the repair and maintenance of computers (Jakobsson et al., 2002), dismantling electronics (Sjödin et al., 1999; Bi et al., 2007) and recycling printed circuit boards (Thuresson et al., 2002), commercial Deca-BDE flame retarded rubber manufacture and handling electric cables using the same rubber (Thuresson et al., 2005). Furthermore, different PBDE profiles were observed comparing occupationally exposed (with higher levels of PBDEs with 7–10 bromine atoms) and non-occupationally exposed populations (with higher levels of PBDEs with 3–6 bromine atoms) (reviewed by Sjödin et al., 2003).

Another pathway for human exposure to BFRs is the dietary intake (Voorspoels et al., 2007b). Due to the lipophilic nature of these chemicals, BFRs are mostly found in lipid-rich food of animal origin, such as meat, fish and diary products, which are a part of our daily diet. It has been shown that food, and more in particular food of animal origin, is responsible for more than 90% of the average human intake of polychlorinated biphenyls (PCBs) (Liem et al., 2000) and therefore for BFRs, this should be similar.

Acknowledgments

Dr. Adrian Covaci acknowledges the financially support by a postdoctoral fellowship from the Research Scientific Foundation-Flanders (FWO). The organizers of the 1st workshop on "*Applications of Mass Spectrometry in Life Safety* Conference", Herculane, Romania, 23–27 September 2007 and the NATO programme "Science for Peace" are acknowledged for inviting Adrian Covaci as platform speaker.

References

1. Ackerman LK, Wilson GR, Simonich SL. Quantitative analysis of 39 polybrominated diphenyl ethers by isotope dilution GC/low-resolution MS. *Anal Chem* **2005**, 77, 1979–1987.
2. Allchin CR, Law RJ, Morris S. Polybrominated diphenyl ethers in sediments and biota downstream of potential sources in the UK. *Environ Pollut* **1999**, 105, 197–207.
3. Batterman S, Chernyak S, Gwynn E, Cantonwine D, Jia C, Begnoche L, Hickey JP. Trends of brominated diphenyl ethers in fresh and archived Great Lakes fish (1979–2005). *Chemosphere* **2007**, 69, 444–457.
4. Berger U, Herzke D, Sandanger TM. Two trace analytical methods for determination of hydroxylated PCBs and other halogenated phenolic compounds in eggs from Norwegian birds of prey. *Anal Chem* **2004**, 76, 441–447.
5. Bi X, Thomas GO, Jones KC, Qu W, Sheng G, Martin FL, Fu J. Exposure of electronics dismantling workers to polybrominated diphenyl ethers, polychlorinated biphenyls, and organochlorine pesticides in South China. *Environ Sci Technol* **2007**, 41, 5647–5653.
6. Birnbaum LS, Staskal DF. Brominated flame retardants: Cause for concern? *Environ Health Perspect* **2004**, 112, 9–17.
7. Björklund J, Tollbäck P, Hiarne C, Dyremarck E, östman C. Influence of the injection technique and the column system on gas chromatographic determination of polybrominated diphenyl ethers. *J Chromatogr A* **2004**, 1041, 201–210.
8. Broman D, Näf C, Rolff C, Zebühr Y, Fry B, Hobbie J. Using ratios of stable nitrogen isotopes to estimate bioaccumulation and flux of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in 2 food-chains from the Northern Baltic. *Environ Toxicol Chem* **1992**, 11, 331–345.
9. Bromine Science and Environmental Forum (BSEF). Website: http://www.bsef.com last accessed 15th December 2007.
10. Budakowski W, Tomy G. Congener-specific analysis of hexabromocyclododecane by high-performance liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* **2003**, 17, 1399–1403.
11. Burreau S, Zebühr Y, Ishaq R, Broman D. Comparison of biomagnification of PBDEs in food chains from the Baltic Sea and the Northern Atlantic Sea. *Organohalogen Compd* **2000**, 47, 253–255.
12. Cariou R, Antignac JP, Marchand P, Berrebi A, Zalko D, Andre F, Le Bizec B. New multiresidue analytical method dedicated to trace level measurement of brominated flame retardants in human biological matrices. *J Chromatogr A* **2005**, 1100, 144–153.
13. Chu SG, Haffner GD, Letcher RJ. Simultaneous determination of tetrabromobisphenol A, tetrachlorobisphenol A, bisphenol A and other halogenated analogues in sediment and sludge by high performance liquid chromatography-electrospray tandem mass spectrometry. *J Chromatogr A* **2005**, 1097, 25–30.

14. Covaci A, de Boer J, Ryan JJ, Voorspoels S, Schepens P. Determination of polybrominated diphenyl ethers and polychlorinated biphenyls in human adipose tissue by large-volume injection-narrow-bore capillary gas chromatography/electron impact low-resolution mass spectrometry. *Anal Chem* **2002**, 74, 790–798.
15. Covaci A, Voorspoels S, de Boer J. Determination of brominated flame retardants, with emphasis on polybrominated diphenyl ethers (PBDEs) in environmental and human samples–a review. *Environ Int* **2003**, 29, 735–756.
16. Covaci A, Gerecke AC, Law RJ, Voorspoels S, Kohler M, Heeb NV, Leslie H, Allchin CR, de Boer J. Hexabromocyclododecanes (HBCDs) in the environment and humans: A review. *Environ Sci Technol* **2006**, 40, 3679–3688.
17. Covaci A, Voorspoels S, Ramos L, Neels H, Blust R. Recent developments in the analysis of brominated flame retardants and brominated natural compounds. *J Chromatogr A* **2007**, 1153, 145–171.
18. Darnerud PO. Toxic effects of brominated flame retardants in man and wildlife. *Environ Int* **2003**, 29, 841–853.
19. de Boer J, Allchin C, Law R, Zegers B, Booij JP. Method for the analysis of polybrominated
diphenyl ethers in sediments in biota. *Trends Anal Chem* **2001**, 20, 591–599.
20. de Boer J, Leslie LA, Leonards PEG, Bersuder P, Morris S, Allchin CR. Screening and time trend study of decabromodiphenylether and hexabromocyclododecane in birds. In: Proceedings of the Third International Workshop on Brominated Flame Retardants BFR 2004, Toronto, Canada, June 6–9, **2004**, pp. 125–128.
21. de Boer J, Wells DE. Pitfalls in the analysis of brominated flame retardants in environmental, human and food samples–including results of three international interlaboratory studies. *Trends Anal Chem* **2006**, 25, 364–372.
22. de Wit C. An overview of brominated flame retardants in the environment. *Chemosphere* **2002**, 46, 583–624.
23. Debrauwer L, Riu A, Jouahri M, Rathahao E, Jouanin I, Antignac JP, Cariou R, Le Bizec B, Zalco D. Probing new approaches using atmospheric pressure photo ionization for the analysis of brominated flame retardants and their related degradation products by liquid chromatography–mass spectrometry. *J Chromatogr A* **2005**, 1082, 98–109.
24. Dirtu AC, Ravindra K, Roosens L, van Grieken R, Neels H, Blust R, Covaci A. Fast analysis of decabrominated diphenyl ether using low-pressure gas chromatography–electron-capture negative ionization mass spectrometry. *J Chromatogr A* **2008**, 1186, 295–301.
25. Dodder NG, Peck AM, Kucklick JR, Sander LC, Analysis of hexabromocyclododecane diastereomers and enantiomers by liquid chromatography/tandem mass spectrometry: Chromatographic selectivity and ionization matrix effects. *J Chromatogr A* **2006**, 1135, 36–42.
26. Dunckel AE. An updating on the polybrominated biphenyl disaster in Michigan. *J Am Vet Med Assoc* **1975**, 167, 838–841.
27. Focant JF, Sjödin A, Turner WE, Patterson Jr. DG. Measurement of selected polybrominated diphenyl ethers, polybrominated and polychlorinated biphenyls, and organochlorine pesti-cides in human serum and milk using comprehensive two-dimensional gas chromatography isotope dilution time-of-flight mass spectrometry. Anal Chem 2004, 76, 6313–6319.
28. Gómara B, Herrero L, Bordajandi LR, González MJ. Quantitative analysis of polybrominated diphenyl ethers in adipose tissue, human serum and foodstuff samples by gas chromatography with ion trap tandem mass spectrometry and isotope dilution, Rapid Commun Mass Spectrom 2006, 20, 69–76.
29. Harrad S, Hazrati S, Ibarra C. Concentrations of polybrominated diphenyl ethers in indoor air and dust and polychlorinated biphenyls in indoor air in Birmingham, UK: Implications for human exposure. *Environ Sci Technol* **2006**, 40, 4633–8.
30. Harrad S, Ibarra C, Diamond M, Melymuk L, Robson M, Douwes J, Roosens L, Dirtu AC, Covaci A. Polybrominated diphenyl ethers in domestic indoor dust from Canada, New Zealand, United Kingdom and United States. *Environ Int* **2008**, in press.

31. Hites RA. Polybrominated diphenyl ethers in the environment and in people: A meta-analysis of concentrations. *Environ Sci Technol* **2004**, 38, 945–956.
32. Ikonomou MG, Rayne S, Addison RF. Exponential increases of the brominated flame retardants, polybrominated diphenyl ethers, in the Canadian Arctic from 1981 to 2000 *Environ Sci Technol* **2002**, 36, 1886–1892.
33. Jakobsson K, Thuresson K, Rylander L, Sjödin A, Hagmar L, Bergman A. Exposure to polybrominated diphenyl ethers and tetrabromobisphenol A among computer technicians. *Chemosphere* **2002**, 46, 709–716.
34. Janak K, Covaci A, Voorspoels S, Becher G. Hexabromocyclododecane in marine species from the Western Scheldt Estuary: Diastereomer- and enantiomer-specific accumulation. *Environ Sci Technol* **2005**, 39, 1987–1994.
35. Jaspers VLB, Covaci A, Voorspoels S, Dauwe T, Eens M, Schepens P. Brominated flame retardants and organochlorine pollutants in aquatic and terrestrial predatory birds of Belgium: Levels, patterns, tissue distribution and condition factors. *Environ Pollut* **2006**, 139, 340–352.
36. Kierkegaard A, Björklund J, Friden U. Identification of the flame retardant decabromodiphenyl ethane in the environment. *Environ Sci Technol* **2004**, 38, 3247–3254.
37. Knudsen LB, Gabrielsen GW, Verreault J, Barrett R, Skare JU, Polder A, Lie E. Temporal trends of brominated flame retardants, cyclododeca-1,5,9-triene and mercury in eggs of four sea bird species from Northern Norway and Svalbard; Report 942/205; Norwegian Pollution Control Authority, Oslo, **2005.**
38. Korytár P, Covaci A, de Boer J, Gelbin A, Brinkman UATh. Retention-time database of 126 polybrominated diphenyl ether congeners and two Bromkal technical mixtures on seven capillary gas chromatographic columns. *J Chromatogr A* **2005a**, 1065, 239–251.
39. Korytár P, Covaci A, Leonards P E G, de Boer J, Brinkman UATh. Comprehensive two-dimensional gas chromatography of polybrominated diphenyl ethers. *J Chromatogr A* **2005b**, 1100, 20–31.
40. Kuriyama SN, Talsness CE, Grote K, Chahoud I. Developmental exposure to low dose BDE 99. Effects on male fertility and neurobehavior in rat offspring. *Environ Health Perspect* **2005**, 113, 149–154.
41. Law RJ, Alaee M, Allchin C, Boon JP, Lebeuf M, Lepom P, Stern GA. Levels and trends of Polybrominated diphenylethers and other brominated flame retardants in wildlife. *Environ Int* **2003**, 29, 757–770.
42. Law RJ, Allchin CR, de Boer J, Covaci A, Herzke D, Lepom P, Morris S, Tronczynski J, de Wit CA. Levels and trends of brominated flame retardants in the European environment. *Chemosphere* **2006**, 64, 187–208.
43. Legler J, Brouwer A. Are brominated flame retardants endocrine disruptors? *Environ Int* **2003**, 29, 879–885.
44. Liem AKD, Fürst P, Rappe C. Exposure of populations to dioxins and related compounds. *Food Addit Contam* **2000**, 17, 241–259.
45. Manchester-Neesvig JB, Valters K, Sonzogni WC. Comparison of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in Lake Michigan salmonids. *Environ Sci Technol* **2001**, 35, 1072–1077.
46. McDonald TA. A perspective on the potential health risks of PBDEs. *Chemosphere* **2002**, 46, 745–755.
47. Meerts I, Letcher RJ, Hoving S, Marsh G, Bergman A, Lemmen J, van der Burg B, Brouwer A. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and poly-brominated bisphenol A compounds. *Environ Health Perspect* **2001**, 109, 399–407.
48. Morris S, Allchin CR, Zegers BN, Haftka JJH, Boon JP, Belpaire C, Leonards PEG, Van Leeuwen SPJ, de Boer J. Distribution and fate of HBCD and TBBP-A flame retardants in North Sea estuaries and aquatic food webs. *Environ Sci Technol* **2004**, 38, 5497–5504.

49. Muir DCG, Backus S, Derocher A, Dietz R, Evans TJ, Gabrielsen GW, Nagny J, Norstrom R, Sonne C, Stirling I, Taylor MK, Letcher RJ. Brominated flame retardants in polar bears (*Ursus maritimus*) from Alaska, the Canadian Arctic, East Greenland, and Svalbard. *Environ Sci Technol* **2006**, 40, 449–455.
50. Nylund K, Asplund L, Jansson B, Jonsson P. Analysis of some polyhalogenated organic pollutants in sediment and sewage-sludge. *Chemosphere* **1992**, 24, 1721–1730.
51. Peck AM, Tuerk KJS, Keller J, Kucklick JR, Schantz MM. Hexabromocyclododecane diastereomers and enantiomers in white-sided dolphin blubber and liver tissue. *Organohalogen Compd* **2005**, 67, 1259–1262.
52. Rolff C, Broman D, Näf C, Zebühr Y. Potential biomagnification of PCDD/Fs–new possibilities for quantitative assessment using stable-isotope trophic position, *Chemosphere* **1993**, 27, 461–468.
53. Roos A, Nylund K, Haggberg L, Asplund L, Bergman A, Olsson M. Brominated flame retardants (BFR) in young Grey Seal Males (*Halicoerus grypus*) from the Baltic Sea. Proceedings of the Second International Workshop on Brominated Flame Retardants, BFR 2001, Stockholm, Sweden, 14–16 May, **2001,** 337–341.
54. Roosens L, Dirtu AC, Goemans G, Belpaire C, Gheorghe A, Neels H, Blust R, Covaci A. Brominated flame retardants and organochlorine contaminants in fish from the Scheldt River, Belgium. *Environ Int* **2008**, doi: 10.1016/j.envint.2008.02.009.
55. Salgado-Petinal C, Garcia-Chao M, Llompart M, Garcia-Jares C, Cela R. Headspace solid-phase microextraction gas chromatography tandem mass spectrometry for the determination of brominated flame retardants in environmental solid samples. *Anal Bioanal Chem* **2006**, 385, 637–347.
56. Santillo D, Labunska I, Davidson H, Johnston P, Strutt M, Knowles O. Consuming chemicals: hazardous chemicals in house dust as an indicator of chemical exposure in the home: Part I–UK. Greenpeace Research Laboratories Technical Note 01/2003; **2003**.
57. Sellström U. Determination of some polybrominated flame retardants in biota, sediment and sewage sludge. PhD Thesis. University of Stockholm, Sweden, **1999**.
58. Sellström U, Bignert A, Kierkegaard A, Haggberg L, de Wit CA, Olsson M, Jansson B. Temporal trend studies on tetra and pentabrominated diphenyl ethers and hexabromocyclododecane in guillemot egg from the Baltic Sea. *Environ Sci Technol* **2003,** 37, 5496–5501.
59. Sjödin A, Hagmar L, Klasson-Wehler E, Kronholm-Diab K, Jakobsson E, Bergman A. Flame retardant exposure: Polybrominated diphenyl ethers in blood from Swedish workers. *Environ Health Perspect* **1999**, 107, 643–648.
60. Sjödin A, Patterson DG, Bergman A. A review on human exposure to brominated flame retardants–particularly polybrominated diphenyl ethers. *Environ Int* **2003**, 29, 829–839.
61. Sjödin A, Päpke O, Focant J-F, Jones RS, Pless-Mulloli T, Leontjew Toms L-M. Concentration of polybrominated diphenyl ethers (PBDEs) in household dust from various countries-is dust a major source of human exposure? *Organohalogen Compd* **2006**, 68, 2181–2185.
62. Smedes F, de Boer J. Determination of PCBs in sediments–analytical methods. *Trends Anal Chem* **1997**, 16, 503–517.
63. Stapleton HM, Dodder NG, Offenberg JH, Schantz MM, Wise SA. Polybrominated diphenyl ethers in house dust and clothes dryer lint. *Environ Sci Technol* **2005**, 39, 925–931.
64. Stapleton HM. Instrumental methods and challenges in quantifying polybrominated diphenyl ethers in environmental extracts: a review. *Anal Bioanal Chem* **2006a**, 386, 807–817.
65. Stapleton HM, Dodder NG, Kucklick JR, Reddy CM, Schantz MM, Becker PR, Gulland F, Porter BJ, Wise SA. Determination of HBCD, PBDEs and MeO-BDEs in California sea lions (*Zalophus californianus*) stranded between 1993 and 2003. *Mar Pollut Bull* **2006b**, 52, 522–531.

66. Thuresson K, Jakobsson K, Hagmar L, Englyst V, Bergman Å. Work related exposure to bro-minated flame retardants when recycling metals from printed circuit boards. *Organohalogen Compd* **2002**, 58, 249–252.
67. Thuresson K, Bergman A, Jakobsson K. Occupational exposure to commercial decabromodiphenyl ether in workers manufacturing or handling flame-retarded rubber. *Environ Sci Technol* **2005**, 39, 1980–1986.
68. Voorspoels S, Covaci A, Schepens P. Polybrominated diphenyl ethers in marine species from the Belgian North Sea and the Western Scheldt estuary: levels, profiles and distribution. *Environ Sci Technol* **2003**, 37, 4348–4357.
69. Voorspoels S, Covaci A, Lepom P, Jaspers VLB, Schepens P. Levels and distribution of polybrominated diphenyl ethers in various tissues of birds of prey. *Environ Pollut* **2006a**, 144, 218–227.
70. Voorspoels S, Covaci A, Lepom P, Escutenaire S, Schepens P. Remarkable findings concerning PBDEs in the terrestrial toppredator red fox (*Vulpes vulpes). Environ Sci Technol* **2006b**, 40, 2937–2943.
71. Voorspoels S, Covaci A, Jaspers VLB, Neels H, Schepens P. Biomagnification of PBDEs in three small terrestrial food chains. *Environ Sci Technol* **2007a**, 41, 411–416.
72. Voorspoels S, Covaci A, Neels H, Schepens P. Dietary PBDE intake: A market basket study in Belgium. *Environ Int* **2007b**, 33, 93–97.
73. Vorkamp K, Thomsen M, Falk K, Leslie H, Møller S, Sørensen PB. Temporal development of brominated flame retardants in peregrine Falcon (*Falco peregrinus*) eggs from South Greenland (1986–2003). *Environ Sci Technol* **2005**, 39, 8199–8206.
74. Wang D, Cai Z, Jiang G, Wong MH, Wong WK. Gas chromatography/ion trap mass spectrometry applied for the determination of polybrominated diphenyl ethers in soil. *Rapid Commun Mass Spectrom* **2005**, 19, 83–88.
75. WHO/ICPS. Environmental Health Criteria 162: Brominated Diphenyl Ethers. Geneva: World Health Organization; 1994.
76. WHO/ICPS. Environmental Health Criteria 192: Flame Retardants–General introduction. Geneva: World Health Organization; 1997.
77. Wilford BH, Shoeib M, Harner T, Zhu J, Jones KC. Polybrominated diphenyl ethers in indoor dust in Ottawa, Canada: implications for sources and exposure. *Environ Sci Technol* **2005**, 39, 7027–7035.
78. Zegers BN, Lewis WE, Booji K, Smittenberg,RH, Boer W, de Boer J, Boon JP. Levels of polybrominated diphenyl ether flame retardants in sediment cores from Western Europe. *Environ Sci Technol* **2003**, 37, 3803–3807.
79. Zegers BN, Mets A, van Bommel R, Minkenberg C, Hamers T, Kamstra JH, Pierce G, Reid B, Patterson T, Boon JP. Levels of hexabromocyclododecane in harbour porpoises and common dolphins from Western European Seas, with evidence for stereoisomer-specific biotransformation by cytochrome P450. *Environ Sci Technol* **2005**, 39, 2095–2100.
80. Zhu L, Hites RA. Brominated flame retardants in tree bark from North America. *Environ Sci Technol* **2006**, 40, 3711–3716.