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Analytical Aspects for Determination of Polybrominated Diphenyl Ethers in Environmental Samples

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Abstract: Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants (BFRs) which 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 and observed increasing temporal trends in the environment and in human biota. Here are briefly reviewed scientific issues related to analytical aspects for determination of PBDEs in environmental samples.

Keywords: polybrominated diphenyl ethers, BFRs, analytical methodologies, toxicological aspects.

General information concerning polybrominated diphenyl ethers

Flame retardants are materials that inhibit or resist the spread of fire that are added to polymers which are used in plastics, textiles, electronic

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circuitry or other materials.[1,2] 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.[2] The most used brominated flame retardants (BFRs) are polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), tetrabromobisphenol-A (TBBP-A) and polybrominated biphenyls (PBBs).

Chlorinated and brominated flame retardants under thermal degradation conditions release hydrogen chloride and hydrogen bromide. These reaction products react with highly reactive H• and HO• radicals present in the flame resulting in the formation of inactive molecules and of Cl• or Br• radicals. The halogen radical has much lower energy than H• and HO•, 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.[3,4]

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 appli-ance 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 world market demand of PBDEs as reported last time in 2003 is different for each continent, the most important demand being for Deca-BDE mixture in America and Asia as presented in Figure 1.[5]

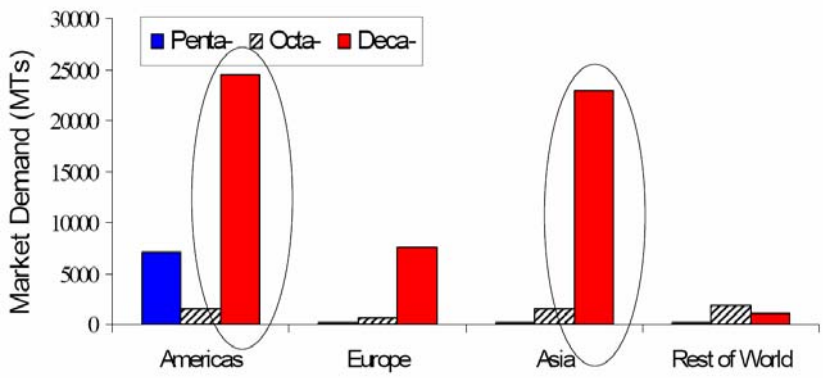

Figure 1. PBDE commercial mixture world market demand as reported in 2003 (source: www.bsef.com).

The European Union has banned since August 2004, the use of Penta- and Octa-BDE technical mixtures, and since July 2008 has banned the use of Deca-BDE mixture. In Sweeden the use of Deca-BDE mixture is banned since January 2007. In U.S., only California has banned the use, by the end

of 2008, of Penta- and Octa-BDE mixtures and the production of Deca-BDE mixture was also banned in July 2008 and other U.S. states are in the phase out legislation for PBDEs.

Analytical methodologies

The environmental detection of brominated flame retardants (BFRs) in a wide variety of matrixes has spurred scientific investigation in all disciplines. Despite the fact that several studies reported an increasing time trend in different environmental matrices and in humans, it is remarkable that so far, no standard analytical procedures have been set for these analytes. In general, the methods used for the determination of PBDEs 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.[6-10] Some basic steps of the PBDE determination are sample pre-treatment, extraction, clean-up and instrumental analysis.

Sample pre-treatment

Even if pre-treatment of the sample matrix is not always required, various procedures for this step are used depending on the employed extraction method. For solid samples (sediment, soil, dust, biological tissues), sample pre-treatment involves usually water depletion of the matrix. 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. Water evaporation below 0 °C under vacuum conditions, so called freeze-drying can also serve for sample drying.[11]

Extraction

For sensitive determination of BFRs, an efficient extraction step is mandatory prior to the chromatographic determination. Extraction techniques generally mimic those for other halogenated organic compounds, such as organochlorine pesticides or polychlorinated byphenyls (PCBs). The extraction procedure of the analytes is dependent on the sample matrix and thus different methods are used for solid or liquid samples. The extraction efficiency depends on few major factors: the analyte solubility in the extraction solvent, the accessibility of the extraction solvent to the matrix, the extraction time and eventually the extraction temperature. A schematic representation for several extraction procedures applied as function of the sample type is presented in Figure 2.

For solid samples, 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. However, other extraction techniques may also be applied including ultra-sonication, extraction of BFRs with organic solvents from a chromatographic column filled with homogenized sample, accelerated solvent extraction (ASE) or microwave assisted extraction (MAE). The main disadvantage for the use of ultra-sonication is that lower extraction recoveries compared with Soxhlet extraction were obtained.[6] Despite of its simplicity, extraction of BFRs with organic solvents from a chromatographic column filled with homogenized sample uses large volumes of organic solvents that have to be further evaporated and disposed

off.[12] The other mentioned extraction techniques, ASE or MAE, are currently applied on BFRs analysis and although higher costs which are involved compared with previous mentioned extraction methods, these techniques have the advantage of lower solvent consumption, which makes the long-term costs lower and the procedures more environmentally friendly.[6,8,10]

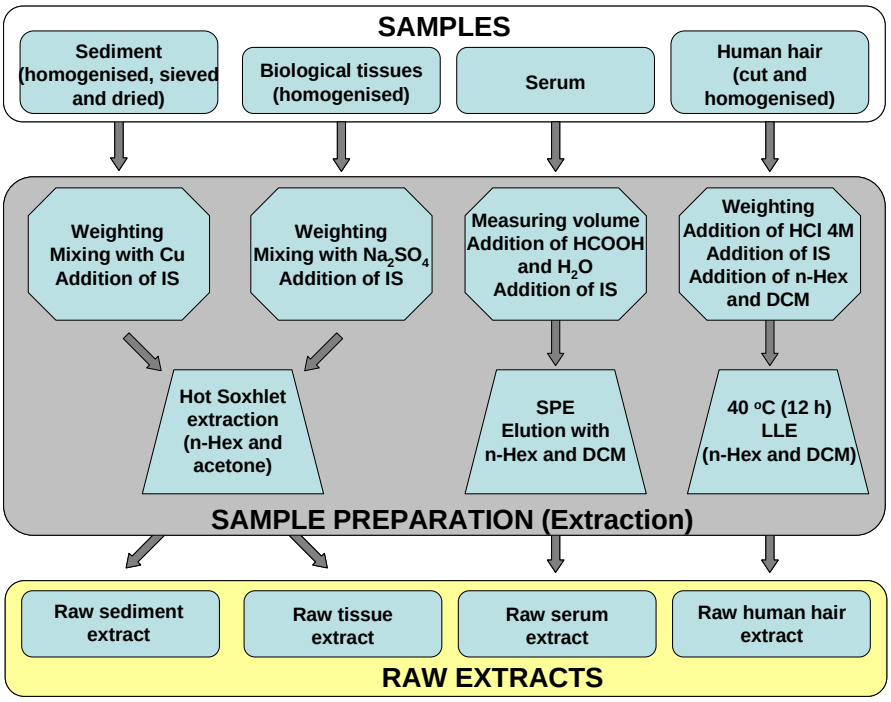

Figure 2. Workflow of the extraction procedures.

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

Clean-up

Because of 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 instrumental analysis of PBDEs. The clean-up techniques for BFRs analysis, including PBDEs, were recently reviewed in the literature.[6,8] A schematic representation for several clean-up procedures applied as function of the raw sample extract type and also depending of the co-extracted interferences and their influences on the instrumental chromatographic analysis is presented in Figure 3.

The most important interferences which have to be removed prior to instrumental analysis are sulfur (the case of sediment, sewage sludge or soil samples) or co-extracted lipids (usually the case of biological samples). For removal of elemental sulfur, the most frequently used methods are treatments of either the sample or the sample extract with copper powder or by gel permeation chromatography (GPC). GPC or either adsorption chromatography on selected sorbents may also be used for lipids removal in case of biological samples extracts. The most used destructive treatment in the PBDE analysis is the sulfuric acid treatment since they are stable under strong acid conditions. The simplest approach consists of dispersion of sulfuric acid onto the surface of activated silica gel which results in an adsorbent which can be easily loaded into a column. The use of acidified silica is very atractive because reduces the sample handling and solvent consumption and increases the sample throughput.

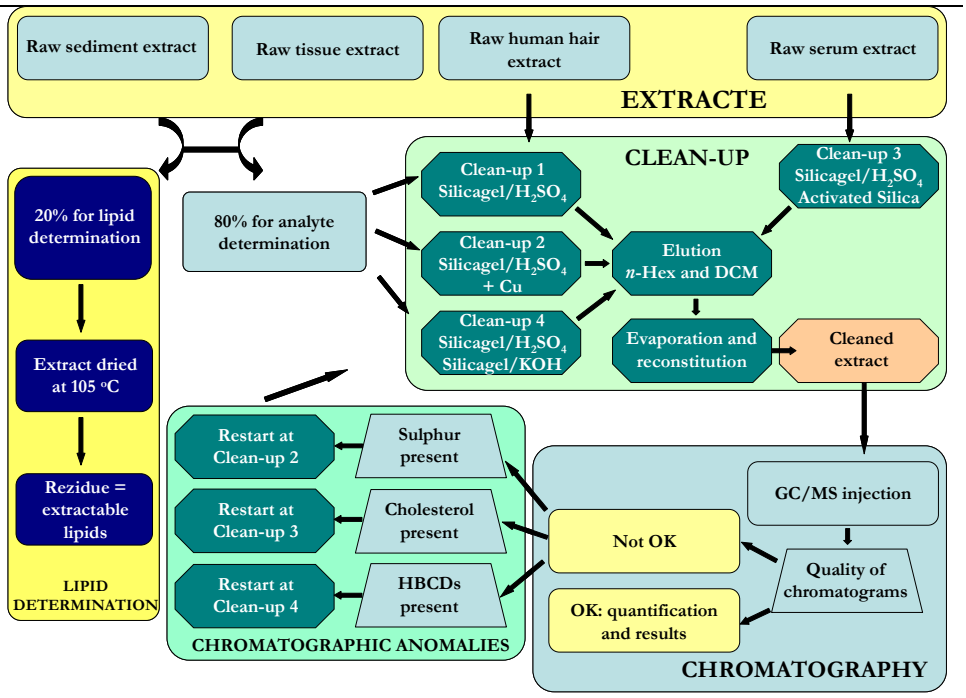

Figure 3. Flow chart of the various clean-up procedures.

Gas-chromatographic analysis of PBDEs

Gas-chromatography has become a routinely applied methodology for the analysis of BFRs. However, it has to be emphasized that because of their physico-chemical properties (like vapour pressure or polarity) and also of their stability, the instrumental analysis approach has to be treated differently for some specific PBDEs. Thermal degradation (through debromination) into GC system is the main criteria in choosing the optimum conditions for instrumental analysis of PBDEs especially when higher brominated BDEs analysis is addressed.[13]

In order to perform an accurate and optimal determination of PBDEs using a GC technique, two steps have to be considered: the sample injection and the separation of the analytes form potentially coeluting compounds through the capillary column.

While various injection methods were reported, for the analysis of PBDEs the most common are split/splitless, on-column and programmable temperature vaporization (PTV) injections. All mentioned methods possess advantages and disadvantages that derive primarily from their availability, price, acceptable detection limits and discrimination of congeners on the basis of molecular weight. Because of their relatively low levels in environmental samples, splitless injection techniques were preferred to split injections, but special precautions should be taken in order to minimize thermal degradation of higher molecular weight PBDEs. An attractive way to reduce thermal degradation of PBDEs in injection system is to apply on-column injection.[14] One of the main disadvantages of this injection technique is that the sample preparation step should lead to very clean sample extracts so that potential interferences does not rich the capillary column leading to additional problems. However, even if splitless injections were the most commonly applied, over the past years it was shown through several studies, that, if used properly, the PTV injector may lead to a minimal degradation of labile PBDEs and therefore seems to be the best approach in case of their analysis.[8,13,14]

In general, single-capillary GC column may offer sufficient resolution for individual PBDE congeners to be determined and finally to estimate their profile in various samples. 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 ($\leq$ 0.25 mm). The use of narrow bore capillary columns (internal diameter = 0.1 mm) may also lead to a good resolution.[15] Prior of using a certain capillary column it has to be tested first for possible coelutions of target compounds, internal standards and other compounds present in the

sample. 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. Selected GC elution conditions for BDE 209 for the case of different conventional GC setups compared to a low pressure (LP)-GC system are presented in Figure 4.[13] It was shown that higher sensitivity may be achieved for fast elution conditions applied on a LP-GC system. Therefore, short residence time in GC combined with low elution temperature of BDE 209 (< 295 ^{o}C), has lead to its minimal thermal degradation.[13] Besides minimal thermal degradation, the analysis of BDE 209 using LP-GC system was optimized to be performed in a minimal run-time of 6.5 min.

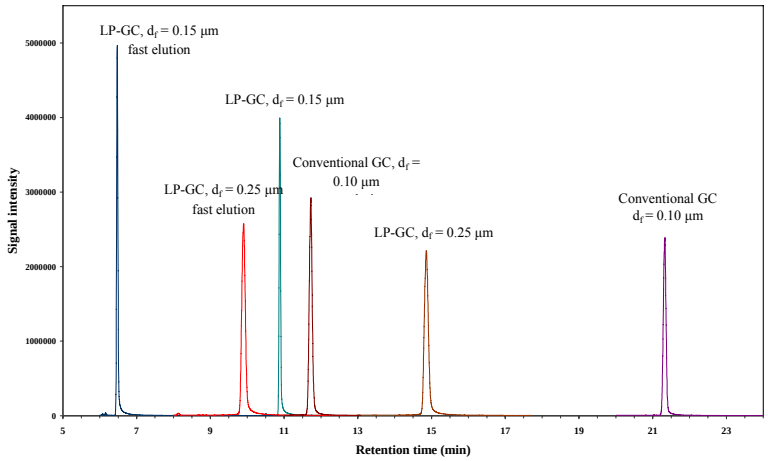

Figure 4. Selected GC elution conditions for BDE 209 for the case of different conventional GC setups compared to different elution conditions for LP-GC system.[13]

Mass spectrometric detection for PBDEs

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. In case of using EI-MS for PBDE analysis, the major ions formed are $[M]^+$ and $[M\text{-}2Br]^+$ which can be used for their identification and quantification.[16] The main potential interferences which may harm accuracy in determining PBDEs originate from chlorinated compounds, such as PCBs. For example, the nominal masses corresponding to ions monitored for di-BDEs and penta-CBs (*m/z* = 326), and also for tetra-BDEs and hepta-CBs (*m/z* = 396) are the same. In case of di-BDEs, their exact masses are 325.8942, and for penta-CBs are 325.8804, and therefore a resolution power of 24,000 is needed to separate them.[8] The use of LR-EI-MS (with quadrupoles as mass analyzer) is not routinely applied 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 groups, 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 $[M–xH–yBr]^-$ is increasing and therefore it can be used for the monitoring of each homologue group in place of the non-specific bromide ions.[17]

Acknowledgements

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