Comparison of Analytical Strategies for the Chromatographic and Mass Spectrometric Measurement of Brominated Flame Retardants: 1. Polybrominated Diphenylethers

Ronan Cariou[1], Jean-Philippe Antignac[1,*], Laurent Debrauwer[2], Daniel Maume[1], Fabrice Monteau[1], Daniel Zalko[2], Bruno le Bizec[1], and **François Andre[1]**

[1]Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Ecole Nationale Vétérinaire de Nantes (ENVN), Unité Sous Contrat INRA (Institut National de la Recherche Agronomique), BP 50707, 44307 Nantes cedex 3 and [2]UMR 1089 Xénobiotiques, Institut National de la Recherche Agronomique, BP3, 31931 Toulouse cedex 9, France

Abstract

The chromatographic and mass spectrometric (MS) behaviors of 49 polybrominated diphenylether (PBDE) homologues toward various techniques is investigated. Special attention is paid to chromatographic separation, ionization processes, and signal acquisition modes. Different liquid chromatographic (LC) separation systems and gas chromatographic (GC) temperature program parameters are studied. For LC–MS experiments, the ionization efficiencies of electrospray, atmospheric pressure chemical ionization, and atmospheric pressure photoionization (APPI) are evaluated. For GC–MS experiments, negative chemical ionization with ammonia as reagent gas as well as negative and positive electron impact (EI) ionization are studied. Thus, fragmentation pathways of PBDEs are investigated, with the main objective being to determine the sensitivity/specificity balance of each tested technique with respect to their potential respective application (parent compound focusing, metabolite identification, and screening of analogue compounds). Finally, performances of the different tested techniques are compared and evaluated in terms of detection limits on standard solutions for each homologue group. In terms of ionization, EI remains the best compromise between sensitivity and specificity with possible complementary applications in MS–MS and high-resolution MS. Nevertheless, APPI appears to be a promising alternative.

Introduction

The impact of brominated flame retardants (BFRs) on the environment and their potential risk for animal and human health is a recent but growing problem for the scientific community. Because of their low cost and high efficiency, BFRs take up a large share of the flame retardant market, 39% in 2000 (1). They are mainly represented by polybrominated diphenylethers (PBDEs), tetrabromobisphenol A (TBBP-A), and hexabromocyclododecane (HBCD). Because of their structural and physicochemical properties, similar to other lipophilic contaminants, such as dioxins or polychlorinated biphenyls, a large number of these substances has been described as persistent organic pollutants (POPs), and they are identified in various environmental compartments, including abiotic matrices (air, rain, sea and river water, sediments, and sewage sludge) (2–6) and biological matrices from oceanic, river, or terrestrial ecosystems (mollusks, crustaceans, fishes, birds, mammals, and humans) (7–12). Contamination and biomagnification through the marine ecosystem appears especially important. However, available data regarding the environmental contamination, metabolism, or toxicology of BFRs appear largely insufficient. Moreover, if a number of PBDE congeners are commercially available, some debromination (13–14) and degradation reactions (15) as well as biotransformation (metabolism) can lead to a relatively high number of compounds to be identified. Therefore, highly specific and sensitive methods are required for the identification and the quantitation of these substances in biological matrices.

PBDEs have been quantitatively determined using gas chromatography (GC) incorporating an electron capture detector (ECD) (16). PBDEs have also been quantitatively determined using GC coupled with mass spectrometry (MS). To our knowledge, only GC has been used for the separation of PBDEs, and a representative overview of the chromatographic conditions used by laboratories can be seen in the first world-wide interlaboratory study organized in 1999 to 2000 (17). GC–MS techniques include negative chemical ionization (NCI) (18–20) or electron impact (EI) (21) associated with low-resolution MS (LRMS) and high-resolution MS (HRMS). The potential of metastable atom bombardment (MAB) has been recently demonstrated on standard PBDE congeners (22). Only a few

* Author to whom correspondence should be addressed: email laberca@vet-nantes.fr.

comparative studies have been carried out (22–27), and they conclude that NCI–LRMS provides high sensitivity and can be used as a specific detection and quantitation technique for brominated compounds. EI(+) and MAB(+)HRMS provide better structural information for unambiguous identification. These techniques enable PBDEs to be quantitatively determined by isotope dilution. Nitrogen MAB ionization has a lower limit of detection than EI for tetra- and pentaBDEs. Although GC–NCI-MS or GC–EI-HRMS generally remain the most commonly used detection techniques, highly specific tandem MS (GC–EI-MS–MS) has been recently proposed for the analysis of tri- to heptaBDEs in fatty matrices (28,29).

In this context, the present study was devoted to the systematic comparison of different analytical techniques for the measurement of a wide range of PBDEs, with the further objective to use the most efficient methods with a complete extraction/purification protocol for the identification and quantitation of BFRs and their potential degradation products or metabolites (or both) in biological matrices. Certified reference compounds were used to test and optimize various LC and GC chromatographic separation conditions, to investigate the suitability of different ionization techniques, including electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionizations (APPI), NCI, and EI (both in positive or negative modes), and to compare the performances of different MS and MS–MS acquisition modes. The final objective was to propose an exhaustive overview of the PBDE behavior regarding their analysis using MS-related techniques.

Experimental

Reagents and chemicals

Analytical-grade acetic acid and high-performance liquid chromatography (HPLC)-grade methanol and toluene were purchased from Solvents Documentation Synthesis (Peypin, France). HPLC-grade acetonitrile, dichloromethane, and hexane were from Scharlau (Barcelona, Spain). *N*-Nonane (GC grade) and heptafluorobutyric anhydride were provided by Sigma (Steinheim, Germany) and *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) by Fluka (Buchs, Switzerland). Reference native (Figure 1) and ^{13}C-labelled PBDEs were purchased from Cambridge Isotope Laboratories (Andover, CA) or Wellington Laboratories (Guelph, Canada). International Union of Pure and Applied Chemisty numbers of the investigated congeners were as follows: monoBDEs (1, 2, 3), diBDEs (10, 7, 8/11, 12, 13, 15), triBDEs (30, 32, 17, 25, 28/33, 35, 37), tetraBDEs (75, 49, 71, 47, 66, 77), pentaBDEs (100, 119, 99, 116, 118, 85, 126), hexaBDEs (155, 154, 153, ^{13}C-139, 138, 166), heptaBDEs (183, 181, 190), octaBDEs (196), nonaBDEs (206), ^{12}C and ^{13}C-decaBDE (209). Some photolysis products obtained from decaBDE solution were also investigated (four corresponding to octaBDEs and the three nonaBDEs). PBDE standard solutions were prepared by tenfold dilutions in *n*-nonane and were stored in the dark at 4°C.

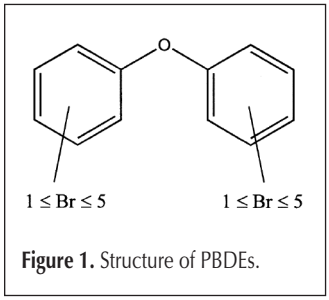

Figure 1. Structure of PBDEs.

LC–MS–MS experiments

ESI and APCI interfaces were first evaluated, in positive and negative modes, using a QuattroLC (Micromass, Manchester, UK) triple quadrupole and an LCQ Deca XP (Thermo Electron, Les Ulis, France) ion trap MS. APPI experiments were performed on the LCQ Deca XP instrument. Reversed-phase LC separations were achieved on Hypersil C_{18} stationary phase column (250 × 4.6 mm, 5 µm) from Thermo Hypersil (Les Ulis, France) using methanol–water or acetonitrile–water mixtures as elution solvents. Normal-phase separations were performed on a Hypercarb column (100 × 4.6 mm, 7 µm) from Thermo Hypersil, using a dichloromethane–hexane gradient elution. For the two systems, the flow rate was set to 1 mL/min. A Thermo Separation P4000 pump fitted with a Rheodyne injector (Rohnert Park, CA) was used for LC–MS experiments, whereas other mass spectra were obtained by direct introduction of standard solutions (1 ng/µL) using a manual syringe (5 µL/min). The nebulizer of the APPI source was set to 450°C. The heated capillary temperature was 250°C. Typical voltages used for the heated capillary and the tube lens offset were 10 V and –40 V, respectively. The ion trap analyzer was operated under automatic gain control (AGC) conditions. Other oper-

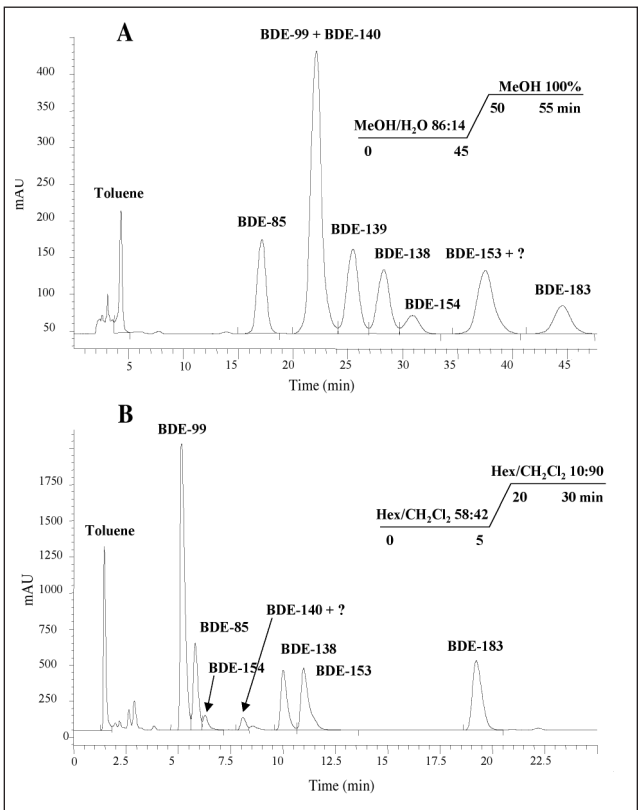

Figure 2. LC–APPI-MS separation of a mixture of penta- to heptaBDEs (85, 99, 138, 139, 140, 153, 154, and 183), using reversed phase (A) and normal phase (B).

ating parameters were adjusted in order to get the maximum signal intensity or structural information (or both) on the analyzed compounds.

GC–MS experiments

A Hewlett Packard 5890 or 6890 GC (Palo-Alto, CA) were used for NCI–LRMS, EI–HRMS, and EI–LRMS experiments on quadrupole or electromagnetic sector instrument, respectively. A Thermo Electron Trace2000 (Thermo Electron) was used for the EI–LRMS trials on ion trap instrument. Separation of PBDEs were achieved using capillary columns (15, 25, or 30-m length × 0.25-mm i.d. × 0.25-µm film thickness) coated either with a crosslinked methylsilicone stationary phase (MN-δ3, Macherey-Nagel, OV-1, Ohio-Valley) or a low bleeding diphenyl (5%)–dimethylpolysiloxane (95%) copolymer BPX5 (SGE, Courtaboeuf, France), DB-5MS (J&W Scientific, Folsom, CA), Rtx-5MS (Restek, Bellefonte, PA), and UB5-P (Interchim, Montluçon, France). Injected volumes were 1 or 2 µL in the split/splitless mode. Helium (N55) was used as carrier gas.

For NCI–LRMS experiments, a Hewlett Packard 5989A MS was used, with ammonia (NH_3) as reagent gas at a pressure of 300 Pa. Source and quadrupole temperatures were set at 250°C and 150°C, respectively. EI(+/–)–LRMS data were acquired on a VG Quattro II (Micromass, Manchester, UK) triple quadrupole MS. Source temperature was set at 220°C. Resolution of Q_1 quadrupole in the simple MS acquisition mode was set at 15, and MS–MS experiments were performed using argon as collision gas at a pressure of 0.45 µBar, with resolutions set for Q_1 and Q_3 at 12.5 and 15, respectively. Other EI–MS experiments were carried out on a Finnigan PolarisQ quadrupole ion trap MS, using inlet and source temperatures of 250°C and 230°C, respectively. The ion trap was operated in the segmented scan acquisition mode in order to frame each isotopic cluster of interest. Perfluorotributylamine (PFTBA) was used as the reference calibrating compound in all LRMS experiments. HRMS experiments (R = 7000) were performed on a SX-102A (Jeol, Tokyo, Japan) double focusing electromagnetic instrument, using perfluorokerosene as calibrating reference and to provide a "lock" mass. Source temperature was set at 230°C. All mass spectra were generated using an electronic beam energy of 70 eV.

Results and Discussion

LC–MS

The suitability of LC for separating PBDEs has been assessed on a PBDE standard mixture containing BDE-85, 99, 138, 139, 140, 153, 154, and 183 (penta- to heptaBDEs). Both reversed-phase and normal-phase separation systems were investigated. A satisfactory separation was obtained using reversed-phase LC as shown in Figure 2A. Because of the very weak solubility of highly brominated PBDEs in polar solvent systems, such mobile phases, should initially be enriched in an organic modifier (typically > 85%). This constraint serves to limit the development of an efficient gradient elution system. Using a normal-phase separation system (more compatible for the PBDE solubility), an efficient separation could be obtained with a porous graphitic carbon stationary phase and a dichloromethane–hexane gradient elution mobile phase. As shown in Figure 2B, the eight injected PBDEs led to seven well-separated peaks within a run time of 20 min.

Regarding classical ionization techniques associated with LC separation, ESI was found inefficient for several tested congeners (47, 99, 153, 183, and 209) in our operating conditions, both in positive or negative ionization mode. Taking into account the elevated K_{OW} values [up to 6 (30)] and the absence of labile protons for these compounds, these observation were expected and should be generalized for the whole PBDE homologue groups. Indeed, these results confirmed the limitations of ESI techniques for apolar compounds.

The use of positive APCI led to the formation of $[M]^{+\bullet}$ ions for the congeners studied in this work, demonstrating that the main mechanism responsible for the ionization of PBDEs in our experimental conditions was a charge exchange process (data not shown). However, the abundance of this ion remained very poor and noncompatible with realistic monitoring of PBDE in biological matrices. Under negative ionization conditions, the proton exchange process led to $[M–H]^-$ species, although the ionization efficiency was poor. Moreover, decaBDE (209) could not be detected in this mode because of the absence of the proton in this compound. In

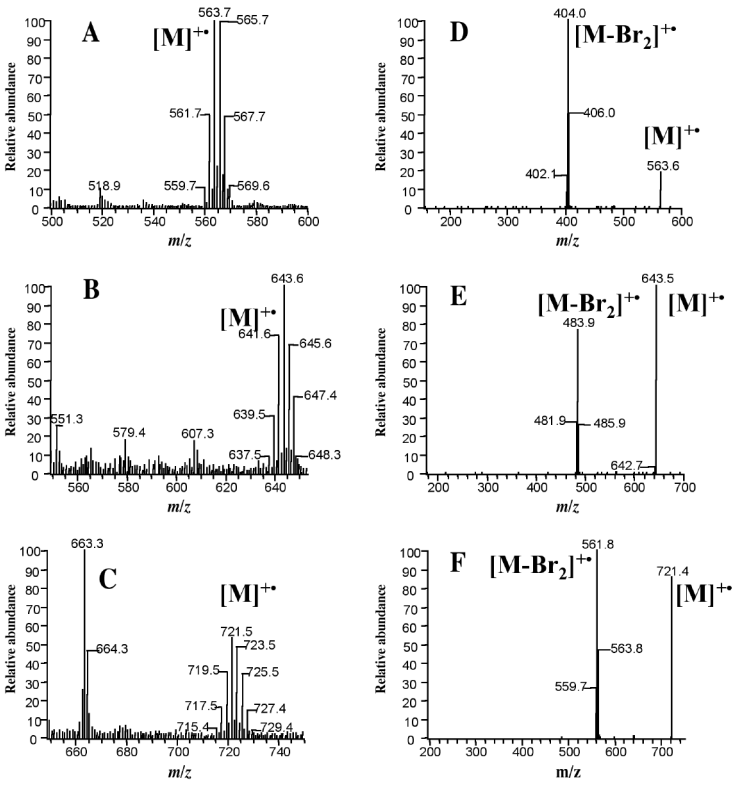

Figure 3. APPI and MS mass spectra of pentaBDE-85 (A), hexaBDE-138 (B), heptaBDE-183 (C), and the corresponding (APPI, MS–MS$[M]^{+\bullet}$) mass spectra (D, E, F, respectively).

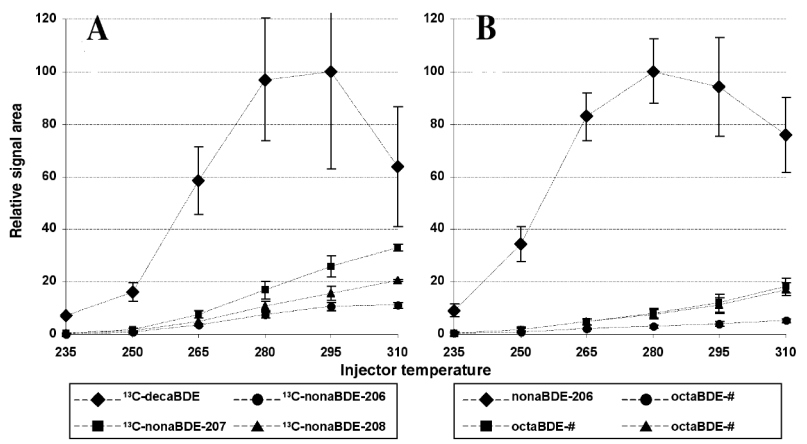

Figure 4. Influence of the temperature on the relative signal area (n = 3) of injected solutions of ^{13}C-decaBDE (A) and nonaBDE-209 (B), their three main respective degradation products also being monitored (GC–EI-MS–MS acquisition, VG Quattro II MS).

Table I. Parameters for LC–APPI-MS–MS Analysis of PBDEs (MRM Acquisition Mode)

Compounds	Diagnostic MRM transition	Excitation energy (V)	Precursor > fragment *m/z*	
			^{12}C compound	^{13}C compound
MonoBDEs	$[M]^{+\bullet}$ > $[M–CO]^{+\bullet}$	1.65	248 > 220	260 > 231
	$[M]^{+\bullet}$ > $[M–Br]^{+}$		248 > 169	260 > 181
	$[M]^{+\bullet}$ > $[M–Br\text{-}CO]^{+}$		248 > 141	260 > 152
PentaBDEs	$[M]^{+\bullet}$ > $[M–Br2]^{+\bullet}$	1.1	564 > 404	576 > 416
HexaBDEs	$[M]^{+\bullet}$ > $[M–Br2]^{+\bullet}$	1.0	644 > 484	656 > 496
HeptaBDEs	$[M]^{+\bullet}$ > $[M–Br2]^{+\bullet}$	1.0	722 > 562	734 > 574

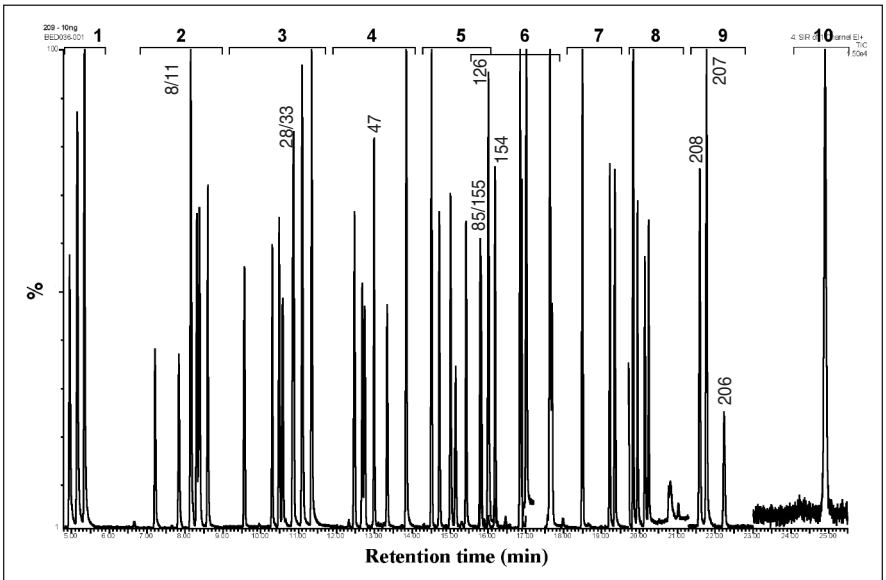

Figure 5. GC–(EI+)-MS single ion monitoring chromatograms for PBDEs. 1 to 10: mono- to decaBDE homologue groups in the cited order; GC column: UB5-Premium 15 m × 0.25 mm × 0.25 µm; temperature program: 120°C (2 min) to 280°C (10°C/min) and 320°C (20°C/min, 8 min); injector temperature: 280°C.

conclusion, neither ESI nor APCI was found to be adapted for efficient ionization of PBDEs. APPI has been recently introduced as an alternative ionization technique better suited for the analysis of apolar compounds by LC–MS (31). Thus, this technique was assessed on our reference PBDEs. For all studied congeners, APPI resulted in better ionization efficiencies than APCI. Figure 3A–3C show typical mass spectra obtained from BDE 85, 138, and 183 after APPI ionization. These spectra clearly show that $[M]^{+\bullet}$ molecular species are efficiently generated after ionization of PBDEs by 10 eV photons. As expected with low proton affinity compounds, the PBDEs studied in this work do not undergo any protonation process when ionized by means of APPI. As indicated in the MS–MS spectra presented in Figures 3D–3F, BDE-85, 138, and 183 all undergo the elimination of $[Br_2]$ as the only decomposition process occurring under resonant excitation conditions into an ion trap device. Experiments carried out on monoBDE3 showed that the loss of [CO] and $[Br]^{\bullet}$ were both observed, but that the formation of $[MBr_2]^{+\bullet}$ ions was the only fragmentation process observed from the collisional excitation of $[M]^{+\bullet}$ ions generated from PBDEs by APPI (data not shown). Using positive APPI, the achieved sensitivity was in the 200–400 pg injected for di- to pentaBDE and in the 500–1500 pg injected for hexa- to decaBDE. APPI was also assessed under negative ionization conditions. No molecular species could be observed, and the mass spectra obtained were mainly characterized by $[M–Br+O]^-$ and $[M–Br_2]^-$ species arising from dissociative resonance capture processes as recently reported by Traldi et al. (32).

From such observations, parameters to be used for the analysis of PBDEs by LC–APPI–MS are given in Table I. The analysis of PBDEs using APPI is now under further investigation, and more comprehensive results will be published later. Nevertheless, the potential of APPI for the analysis of PBDEs by LC–MS is already clearly demonstrated.

GC–MS

Chromatographic separation

The capability of GC separation for PBDEs was investigated using a mixture

of the 49 studied congeners. The 30 m OV-1 column did not allow the complete separation of PBDE congeners 8/11, 12/13 (diBDEs), 17/25, 28/33 (triBDEs), and 85/155 (penta/hexaBDEs). However, pentaBDE-85 and hexaBDE-155 could be distinguished on the basis of different fragment ions. The separation of diBDEs-12/13 and triBDEs-17/25 could be obtained with the DB-5MS or Rtx-5MS column. Using 30-m length columns, high retention times were observed for high brominated PBDEs, with a negative incidence on peak width and sensitivity. No significant improvement of peak shapes was observed with the corresponding 15-m columns (DB-5MS or equivalent UB5-P). Nevertheless, a 15-m length UB5-P column was finally selected because of the shorter analysis time and its lower cost.

The optimization of the injector temperature was carried out between 235°C and 310°C on four PBDEs, ranging from tetra- to decabrominated congeners (47, ^{13}C-139, 206, and ^{13}C-209). No significant influence of the temperature was noticed on tetraBDEs-47 and ^{13}C-hexaBDE-139, but signals of BDE-206 and ^{13}C-BDE-209 were highly affected, showing a bell-shaped curve centered around optimums between 280°C and 295°C (Figure 4). For lower temperatures, a condensation in the insert was observed, whereas thermal degradation may occur for higher temperatures, as evidenced by the appearance of signals corresponding to debrominated products for higher brominated PBDEs. Therefore, a temperature of 280°C appeared as the best compromise between these two phenomena. In order to optimize the GC separation quality, various temperature programs were tested. Using MSTFA/*n*-nonane 1:1 (v/v) as the solvent, a noticeable decrease of the chromatographic resolution of monoBDEs occurred over an initial temperature of 130°C. The effects of the final temperature on the decaBDE signal was also studied. By increasing the final temperature from 290°C to 320°C, the retention time of decaBDE was shortened by 10 min, and the peak half-width was strongly improved. Finally, a temperature program starting at 120°C (held for 2 min), increased to 280°C (10°C/min), and then further increased to 320°C (20°C/min, 8 min) was retained. A typical chromatogram obtained under these conditions is reported in Figure 5, showing retention times contained in-between 5 and 24 min. Among the 49 considered analytes, only diBDEs-8/11 and triBDE-28/33 remained undistinguishable because they were not com-

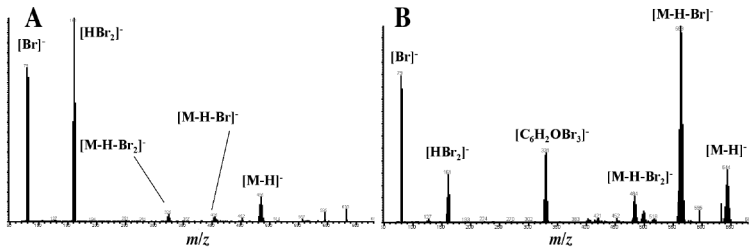

Figure 6. NCI(NH_3) and MS spectra of tetraBDE-47 (A) and hexaBDE-153 (B).

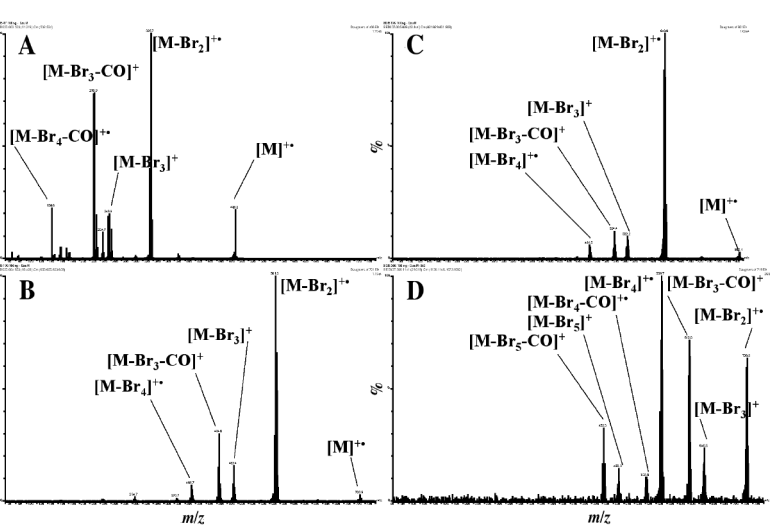

Figure 7. EI+ and MS–MS–MS $[M]^{+\bullet}$ spectra of tetraBDE-47 (A), heptaBDE-190 (B), octaBDE-196 (C), and (EI+, $MS^2[M\text{-}Br_2]^{+\bullet}$) spectra of nonaBDE206 (D). Ionization energy: 70 eV; collision energy: 60 eV; Micromass VG Quattro II MS.

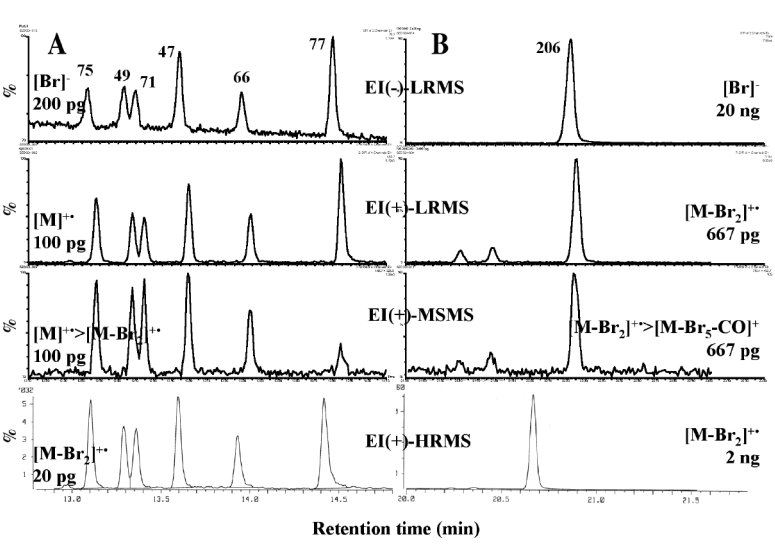

Figure 8. Ion chromatograms (SIM or MRM transition) of six tetraBDEs (A) and nonaBDE-206 (B) for the different tested GC–MS techniques. Mass spectrometers: Micromass VG Quattro II for EI(+/–)-LRMS and Jeol SX-102A for EI(+)-HRMS.

Table II. Relative Signal-to-Noise Ratios* Observed in GC–EI(+)-MS–MS for Each Studied Transition at the Optimized Collision Energy (eV) on Standard Compounds

MRM transition	OCE†		Relative s/n for each congener					
MonoBDEs			**1**	**2**	**3**			
$[M]^{+\bullet} > [M–CO]^{+\bullet}$	10		1	8	23			
$[M]^{+\bullet} > [M–Br]^{+}$	10		100	49	23			
$[M]^{+\bullet} > [M–Br–CO]^{+}$	20		27	100	100			
DiBDEs			**10**	**7**	**8/11**	**12/13**	**15**	
$[M]^{+\bullet} > [M–Br–CO]^{+}$	20		0	1	19	41	41	
$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	20		100	100	100	100		
$[M]^{+\bullet} > [M–Br_2–CO]^{+\bullet}$	35		1	2	25	46	49	
$[M–Br_2]^{+\bullet} > [M–Br_2–CO]^{+\bullet}$	13		9	10	15	27	26	
TriBDEs			**30**	**32**	**17/25**	**28/33**	**35**	**37**
$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	20		62	92	94	8	86	100
$[M]^{+\bullet} > [M–Br_3–CO]^{+}$	65		16	31	30	26	100	97
$[M–Br_2]^{+\bullet} > [M–Br_3]^{+}$	20		16	12	23	26	20	18
$[M–Br_2]^{+\bullet} > [M–Br_3–CO]^{+}$	30		100	100	100	100	59	60
TetraBDEs			**75**	**49**	**71**	**47**	**66**	**77**
$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	35		100	91	82	100	100	97
$[M]^{+\bullet} > [M–Br_3–CO]^{+}$	60		18	15	15	20	14	76
$[M–Br_2]^{+\bullet} > [M–Br_3–CO]^{+}$	30		47	57	53	41	53	21
$[M–Br_2]^{+\bullet} > [M–Br_4–CO]^{+\bullet}$	60		87	100	100	73	98	41
PentaBDEs		**100**	**119**	**99**	**116**	**118**	**85**	**126**
$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	30	100	75	96	49	76	100	100
$[M]^{+\bullet} > [M–Br_3–CO]^{+}$	70	37	25	27	12	15	15	59
$[M–Br_2]^{+\bullet} > [M–Br_3–CO]^{+}$	30	41	48	38	45	33	38	12
$[M–Br_2]^{+\bullet} > [M–Br_5–CO]^{+}$	77	76	100	100	100	100	28	12
HexaBDEs			**155**	**154**	**153**	**138**	**166**	
$[M^{+}] > [M–Br_2]^{+\bullet}$	35		100	100	100	100	100	
$[M–Br_2]^{+\bullet} > [M–Br_3–CO]^{+}$	37		40	25	19	16	48	
$[M–Br_2]^{+\bullet} > [M–Br_4]^{+\bullet}$	45		11	28	60	61	17	
$[M–Br_2]^{+\bullet} > [M–Br_5–CO]^{+}$	85		22	31	31	26	51	
HeptaBDEs			**183**	**181**	**190**			
$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	40		100	100	77			
$[M–Br_2]^{+\bullet} > [M–Br_3–CO]^{+}$	45		58	62	80			
$[M–Br_2]^{+\bullet} > [M–Br_4]^{+\bullet}$	55		59	21	37			
$[M–Br_2]^{+\bullet} > [M–Br_5–CO]^{+}$	90		77	79	100			
OctaBDEs (numbering not elucidated)			**octa#**	**octa#**	**octa#**	**octa#**	**octa#**	
$[M]^{+\bullet} > [M–Br2]^{+\bullet}$	40		100	100	100	100	100	
$[M–Br_2]^{+\bullet} > [M–Br_3]^{+}$	35		46	45	27	32	29	
$[M–Br_2]^{+\bullet} > [M–Br_3–CO]^{+}$	50		54	44	29	25	22	
$[M–Br_2]^{+\bullet} > [M–Br_5\text{-}CO]^{+}$	90		60	59	40	47	28	
NonaBDEs			**208**	**207**	**206**			
$[M–Br_2]^{+\bullet} > [M–Br_3]^{+}$	40		55	37	53			
$[M–Br_2]^{+\bullet} > [M–Br_3–CO]^{+}$	55		67	84	91			
$[M–Br_2]^{+\bullet} > [M–Br_4]^{+\bullet}$	58		80	43	81			
$[M–Br_2]^{+\bullet} > [M–Br_5–CO]^{+}$	90		100	100	100			
DecaBDE			**209**					
$[M–Br_2]^{+\bullet} > [M–Br_3]^{+}$	50		59					
$[M–Br_2]^{+\bullet} > [M–Br_4]^{+\bullet}$	60		100					
$[M–Br_2]^{+\bullet} > [M–Br_5–CO]^{+}$	95		59					
$[M–Br_2]^{+\bullet} > [M–Br_6]^{+\bullet}$	125		47					

* s/n = signal-to-noise ratio.
† OCE = optimized collision energy.

pletely resolved, as already reported by other authors (33).

CI

$NCI(NH_3)$ was tested on several tri- to heptaBDE congeners (28, 47, 99, 154, 153, and 190). As already reported (26), the $[Br]^-$ ion was systematically observed with high intensity and, consequently, can be used in screening analysis for the efficient detection of PBDEs and related brominated compounds. Some other fragments were also produced with relative intensities globally higher than those reported by other similar studies (26) (Figure 6). These ions corresponded to the $[M\text{-}H]^-$, $[M\text{-}H\text{-}Br]^-$, $[M–H–Br_2]^-$, and $[HBr_2]^-$ fragments, with ion ratios strongly different from one congener to another. A fragment ion corresponding to the loss of a ring with retention of the negative charge on the fragment containing the oxygen atom was finally observed for hexaBDE-153. This cleavage of the ether bond had already been observed for decaBDE by Björklund et al. (34) in $NCI(CH_4)$, but was never described for mono- to heptaBDEs. Monitoring the $[Br]^-$ ions in $NCI(NH_3)$ as a diagnostic signal for PBDEs led to a limit of detection (LOD) estimated at 15–35 pg injected for tri to heptaBDEs. Compared with other studies (26), these values appeared one to three orders of magnitude more elevated.

In contradiction with existing data (19,23,24,26), NCI did not appear as the most efficient technique for unambiguous identification of PBDEs, especially when measurements have to be assumed at trace levels in complex biological matrices. Indeed, the presence of interfering compound and matrix effects are especially important and disruptive in the low mass region corresponding to $[Br]^-$ ions. However, this technique should be helpful in finding approaches to screen for the presence of unknown brominated analogues or metabolites (or both).

EI

In the negative mode (EI–), only $[Br]^-$ ions were observed. With a classical 70 eV energy brought by electrons, monitoring the $[Br]^-$ ions led to LODs between 70 and 400 pg injected on single quadrupole MS for mono- to nonaBDEs, that is 5–10 times more elevated than in

NCI(NH_3). Because of this poor sensitivity and lack of structural information for unambiguous identification, the (EI–) mode was not found to be suitable for our purposes.

In the positive mode (EI+), spectra obtained for the tested di- to decaBDEs appeared very similar. The two predominant isotopic clusters corresponded to the $[M]^{+\bullet}$ and $[M–Br_2]^{+\bullet}$ ions, and the relative intensity of $[M–Br_2]^{+\bullet}$ ions increased with the number of bromine atoms, until being higher than the molecular ions over six bromine atoms. Additional losses of bromine atoms and carbon oxide (CO) were also observed. The presence of ions highly suspected to be doubly charged $[M–Br_2]^{2+}$ species were also observed for the di- to decaBDEs homologue groups. Finally, EI+ should permit efficient identification of PBDEs through the diagnostic ions $[M]^{+\bullet}$ and $[MBr_2]^{+\bullet}$ for homologue groups bearing less than five and more than six bromine atoms, respectively.

MS–MS experiments were performed on different PBDEs congeners. Fragmentation of the $[M]^{+\bullet}$ ion with a moderated collision energy (40 eV) mainly led to the loss of two bromine atoms. The $[M]^{+\bullet}$ and $[M–Br_2]^{+\bullet}$ ions also underwent consecutive loss of bromine and CO for all tested congeners, as illustrated by Figure 7. For monoBDEs, the occurrence of the CO neutral loss was observed as a weak, medium, and relatively high process, depending on the ortho, meta, or para positions of the bromine atom, respectively. More generally, the relative intensity of the $[M–Br_X–CO]^+$ ions was clearly related to the sterical hindrance near the oxygen atom, as already reported by Pirard et al. (29). As mentioned by these authors, we have also noticed that the behavior of BDE-35, 37, 77, 85, and 126 was very different compared with their respective homologue congeners. These congeners did not carry the bromine atom in the ortho position, resulting in the most elevated retention times in their homologue groups and lower ionization yields. The particular behavior of BDE-85 remains unexplained. At last, fragmentation of doubly charged $[M–Br_2]^{2+}$ ions led to the same fragments as observed for singly charged species, these fragment ions remaining doubly charged. For the 48 unlabelled PBDEs, collision energies were optimized for each fragment ion

Table III. Parameters for GC–EI-HRMS Analysis of PBDEs (SIM Acquisition mode)*

Window (min)	Homologues	Ion	^{12}C ion mass No. 1	^{12}C ion mass No. 2	^{13}C ion mass No. 1	^{13}C ion mass No. 2	PFK lock mass
4.0	monoBDEs	$[M]^{+\bullet}$	247.9837	249.9817	260.0239	262.0219	254.9856
5.2	diBDEs	$[M]^{+\bullet}$	327.8922	325.8942	339.9324	337.9344	330.9792
8.2	triBDEs	$[M]^{+\bullet}$	405.8027	407.8006	417.8429	419.8408	416.9760
11.2	tetraBDEs	$[M]^{+\bullet}$	485.7112	483.7132	497.7514	495.7534	492.9697
13.3	pentaBDEs	$[M]^{+\bullet}$	563.6216	565.6196	575.6619	577.6598	530.9665
	hexaBDEs	$[M–Br_2]^{+\bullet}$	483.6955	481.6975	495.7357	493.7377	
17.0	heptaBDEs	$[M–Br_2]^{+\bullet}$	561.6060	563.6040	573.6462	575.6442	604.9633
	octaBDEs	$[M–Br_2]^{+\bullet}$	641.5145	639.5165	653.5547	651.5567	
20.0	nonaBDEs	$[M–Br_2]^{+\bullet}$	719.4250	721.4229	731.4652	733.4632	730.9537
21.8	decaBDE	$[M–Br_2]^{+\bullet}$	799.3335	797.3355	811.3736	809.3757	804.9505

* Column: UB5 P 15 m × 0.25 mm × 0.25 µm; injector temperature: 280°C; injection volume: 2 µL; injection solvent: MSTFA–*n*-nonane (1:1); temperature program: 120°C (2 min), 280°C (10°C/min), 320°C (20°C/min; 8 min); Jeol SX-102A MS; ionization energy: 70 eV; switching rate: 50 ms; external standard: 13C-hexaBDE-139.

Table IV. Parameters for GC–EI-MS–MS Analysis of PBDEs (MRM Acquisition Mode)

Window (min)	Compounds	Diagnostic MRM transition	Collision energy (eV)	Precursor > fragment *m/z* ^{12}C compound	Precursor > fragment *m/z* ^{13}C compound
4.8–6.0	monoBDEs	$[M]^{+\bullet} > [M–Br–CO]^+$	20	248.0 > 141.1	260.0 > 152.1
		$[M]^{+\bullet} > [M–Br]^+$	10	248.0 > 169.1	260.0 > 181.1
6.0–9.0	diBDEs	$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	20	327.9 > 168.1	339.9 > 180.1
		$[M–Br_2]^{+\bullet} > [M–Br_2–CO]^{+\bullet}$	13	168.1 > 140.1	180.1 > 151.1
9.0–12.0	triBDEs	$[M–Br_2]^{+\bullet} > [M–Br_3–CO]^+$	30	246.0 > 139.1	258.0 > 150.1
		$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	20	405.8 > 246.0	417.8 > 258.0
12.0–14.2	tetraBDEs	$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	35	485.7 > 325.9	497.8 > 337.9
		$[M–Br_2]^{+\bullet} > [M-Br_4–CO]^{+\bullet}$	60	325.9 > 138.0	337.9 > 149.1
14.2–16.5	pentaBDEs	$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	30	563.6 > 403.8	575.7 > 415.8
		$[M–Br_2]^{+\bullet} > [M–Br_5–CO]^+$	77	403.8 > 137.0	415.8 > 148.1
15.6–18.1	hexaBDEs	$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	35	643.5 > 483.7	655.6 > 495.7
		$[M–Br_2]^{+\bullet} > [M–Br_5–CO]^+$	85	483.7 > 214.9	495.7 > 226.0
18.1–19.7	heptaBDEs	$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	40	721.4 > 561.6	733.5 > 573.6
		$[M–Br_2]^{+\bullet} > [M–Br_5–CO]^+$	90	561.6 > 294.9	573.6 > 305.9
19.7–21.3	octaBDEs	$[M]^{+\bullet} > [M–Br_2]^{+\bullet}$	40	801.3 > 641.5	813.4 > 653.6
		$[M–Br_2]^{+\bullet} > [M–Br_5–CO]^+$	90	641.5 > 372.8	653.6 > 383.8
21.3–23.0	nonaBDEs	$[M–Br_2]^{+\bullet} > [M–Br_5–CO]^+$	90	719.4 > 452.7	731.5 > 463.7
		$[M–Br_2]^{+\bullet} > [M–Br_3–CO]^+$	55	719.4 > 612.5	731.5 > 623.5
23.0–26.0	decaBDE	$[M–Br_2]^{+\bullet} > [M–Br_4]^{+\bullet}$	60	799.3 > 639.5	811.4 > 651.5
		$[M–Br_2]^{+\bullet} > [M–Br_3]^+$	50	799.3 > 718.4	811.4 > 730.5

* Column: UB5P 15 m × 0.25 mm × 0.25 µm; injector temperature: 280°C; injection volume: 2 µL; injection solvent: MSTFA–*n*-nonane (1:1); temperature program: 120°C (2 min), 280°C (10°C/min), 320°C (20°C/min; 8 min); Micromass VG Quattro II MS; ionization energy: 70 eV; external standard: 13C-hexaBDE-139.

candidate after selection of $[M]^{+\bullet}$ (excepted for nona- and decaBDEs) and $[M–Br_2]^{+\bullet}$ as precursor ions. The $[M]^{+\bullet} > [M–Br_2]^{+\bullet}$ transition globally appeared as the most sensitive one. Nevertheless, some exceptions could be underlined, including monoBDEs and triBDEs, for which the $[M–Br_2]^{+\bullet} > [M–Br_3–CO]^{+}$ transition appeared as the most intense one, as already reported (28). After the optimization of collision energies for each congener, a "consensual value" had to be defined for each possible transition inside each homologue group, leading to the classification of the suitability of transitions, based on the observed signal-to-noise ratios (Table II). Future investigations will concern the influence of the ionization energy on the observed mass spectra as well as the confirmation of the suitability of the selected transitions on biological extracts.

Finally, the high sensitivity and the specific structural information obtained with EI+ led to the selection of this technique for the analysis of known parent compounds. Several complementary diagnostic ion acquisition modes are possible, including LRMS and HRMS in selected ion monitoring (SIM) acquisition mode and low resolution MS–MS in MRM acquisition mode. Regarding the acquisition program details, the parameters used in the GC–EI(+)-MS (high resolution) and SIM mode are reported in Table III, allowing the analysis of tri- to decaBDEs in a single injection (masses between m and 2 m). The parameters used in GC–EI(+)-MS–MS (low resolution) and multiple reaction monitoring (MRM) mode are given in Table IV.

In GC–MS–SIM acquisition mode, LODs were estimated between 1 to 20 pg for 38 mono- to nonaBDEs, with a significant tendency to rise with the number of bromine atoms. With the same equipment (Micromass Quattro II) and the same injection conditions, GC–MS–MS (MRM acquisition) led to two to 10 times more elevated LODs. However, it is expected that this loss of sensitivity will be largely compensated for by a reduced noise on matrix samples. In the EI(+)–SIM acquisition mode and for tri- to nonaBDE's, HRMS (R = 7000) gave LODs 1 to 4 times better than LRMS. Figure 8 shows chromatograms obtained for some congeners using EI, and Table V shows the LODs obtained for each tested detection technique and for the mono- to nonaBDE homologue group. For the decaBDE, worse LODs were obtained, with 950, 3500, and 50 pg injected, respectively, for LRMS (SIM), MS–MS (MRM), and HRMS (SIM). Numerous hypothesis can be drawn to explain these poor results. Compared with other congeners, the more elevated molecular weight and the nonnegligible degradation in the injector can explain a decrease in the sensitivity. Next, degradation of the injected solution (instability in time) cannot be excluded. At last, the absence of hydrogen atoms is probably a disadvantage of this mechanism.

Table V. LODs (s/n = 3) Obtained with Reference Standard Solution of Mono- to NonaBDEs (pg on Column) with Different Analytical Techniques

Compounds	NCI–MS (NH3) HP 5989	EI(–)–MS VG Quattro II	EI(+)–MS VG Quattro II	EI(+)–MS–MS VG Quattro II	EI(+)–HRMS Jeol SX 102A
monoBDEs	nd*	200–240	1–2	5–10	nd
diBDEs	nd	100–200	1–2	2–6	nd
triBDEs	15	100–150	2–3	3–15	0.5–1
tetraBDEs	15	70–100	1–3	5–20	0.5–2
pentaBDEs	20	80–150	2–16	5–30	1-4
hexaBDEs	20	70–180	2–3	10–25	1.5–2
heptaBDEs	35	100–150	1–3	10–20	1–1.5
octaBDEs	nd	120	1.5	15	1
nonaBDEs	nd	400	20	150	10

* nd = not determined.

Conclusion

The purpose of this work was to investigate and to discuss the interest and respective advantages of different MS techniques for the detection and the identification of a wide range of PBDEs. In terms of separation, the resolution achieved by GC was (logically) clearly better than the resolution observed in LC. That is an undisputable advantage for the analysis of a complex mixture of homologous compounds. In terms of ionization, EI remained the best compromise between sensitivity and specificity, with possible applications in MS, MS–MS, and HRMS. Nevertheless, APPI appeared to be an interesting alternative and will be investigated further in the immediate future, especially with the recent introduction of ultra-performance LC. Regarding the detection technique and the acquisition mode, GC–MS–MS with triple quadrupole proved to be a good analytical choice for the analysis of PBDEs at low concentration levels, especially when associated with the MRM acquisition mode, which presents clear advantages in terms of unambiguous identification and quantitation. However, the specificity of high resolution remains necessary for the detection of ultratraces in complex biological matrices. The present work objective was to investigate the MS behavior of PBDE using various ionization and acquisition modes. It is clear that the observed fragmentation pathways and performances are given for standard reference substances as a basis of justification for a further choice depending on the application (i.e., highly sensitive detection of specific congeners or metabolic investigation). Now, the efficiency of these strategies remains to be confirmed on biological extracts. For this purpose, a specific extraction/purification procedure is under development. Future studies include applying these MS methods to the analysis of BFR degradation products and potential metabolites in several biological fluids and tissues, including human matrices.

Acknowledgments

This work was financially supported by the Agence Française de Sécurité Sani-

taire et Environnementale (Maisons, France). The authors would like to greatly thank F. Redero, P. Blaise, and T. Barbier (Thermo Electron) for kindly providing an APPI source and E. Genin (Thermo Electron) for helpful preliminary experiments.

References

1. Bromine Science and Environmental Forum. An introduction to brominated flame retardants. 19 October 2000. Bromine Science and Environmental Forum, Brussels, Belgium. http://www.ebfrip.org/download/weeeqa.pdf (accessed June 2003).
2. R.G.M. Lee, G.O. Thomas, and K.C. Jones. Atmospheric concentrations of PBDEs in Western Europe. *Organohalogen Compd.* **58:** 193–96 (2002).
3. A.F.H. ter Schure and P. Larsson. Polybrominated diphenyl ethers in precipitation in Southern Sweden (Skane, Lund). *Atmos. Environ.* **36:** 4015–22 (2002).
4. K. Öberg, K. Warman, and T. Öberg. Distribution and levels of brominated flame retardants in sewage sludge. *Chemosphere* **48:** 805–809 (2002).
5. K. Booij, B.N. Zegers, and J.P. Boon. Levels of some polybrominated diphenyl ether (PDBE) flame retardants along the Dutch coast as derived from their accumulation in SPMDs and blue mussels (*Mytilus edulis*). *Chemosphere* **46:** 683–88 (2002).
6. C.R. Allchin, R.J. Law, and S. Morris. Polybrominated diphenylethers in sediments and biota downstream of potential sources in the UK. *Environ. Pollut.* **105:** 197–207 (1999).
7. Ö. Andersson and G. Blomkvist. Polybrominated aromatic pollutants found in fish in Sweden. *Chemosphere* **10:** 1051–60 (1981).
8. I. Watanabe, T. Kashimoto, and R. Tatsukawa. Polybrominated biphenyl ethers in marine fish, shellfish and river and marine sediments in Japan. *Chemosphere.* **16:** 2389–96 (1987).
9. U. Sellström, B. Jansson, A. Kierkegaard, C. de Wit, T. Odsjö, and M. Olsson. Polybrominated diphenyl ethers (PBDE) in biological samples from the Swedish environment. *Chemosphere* **26:** 1703–18 (1993).
10. U. Sellström, A. Kierkegaard, C. de Wit, and B. Jansson. Polybrominated diphenyl ethers and hexabromocyclodecane in sediment and fish from a Swedish river. *Environ. Tox. Chem.* **17:** 1065–72 (1998).
11. D. Meironyté, K. Norén, and A. Bergman. Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972–1997. *J. Toxicol. Env. Health* **58:** 329–41 (1999).
12. C.A. de Wit. An overview of brominated flame retardants in the environment. *Chemosphere* **46:** 583–24 (2002).
13. I. Watanabe and R. Tatsukawa. Formation of brominated dibenzofurans from the photolysis of flame retardant decabromobiphenyl ether in hexane solution by UV and sun light. *Bull. Environ. Contam. Toxicol.* **39:** 953–59 (1987).
14. U. Sellström, G. Söderström, C. de Wit, and M. Tysklind. Photolytic debromination of decabromodiphenyl ether (DeBDE). *Organohalogen Compd.* **35:** 447–50 (1998).
15. L. Asplund, M. Athanasiadou, U. Eriksson, A. Sjödin, H. Börjeson, and A. Bergman. Mass spectrometric screening for organohalogen substances (OHs) in blood plasma from Baltic salmon (*Salmo salar*). *Organohalogen Compd.* **33:** 355–59 (1997).
16. L. Hovander, M. Athanasiadou, L. Asplund, S. Jensen, and E. Klasson Wehler. Extraction and cleanup methods for analysis of phenolic and neutral organohalogens in plasma. *J. Anal. Toxicol.* **24:** 696–703 (2000).
17. J. de Boer and W.P. Cofino. First world-wide interlaboratory study on polybrominated diphenyl ethers (PBDEs). *Chemosphere* **46:** 625–33 (2002).
18. K. Akutsu, H. Obana, M. Okihashi, M. Kitagawa, H. Nakazawa, Y. Matsuki, T. Makino, H. Oda, and S. Hori. GC/MS analysis of polybrominated diphenyl ethers in fish collected from the Inland Sea of Seto, Japan. *Chemosphere* **44:** 1325–33 (2001).
19. J. de Boer, C. Allchin, R. Law, B. Zegers, and J.P. Boon. Method for the analysis of polybrominated diphenylethers in sediments and biota. *Trends Anal. Chem.* **20:** 591–99 (2001).
20. P. Lepom, T. Karasyova, and G. Sawal. Occurrence of polybrominated diphenyl ethers in freshwater fish from Germany. *Organohalogen Compd.* **58:** 209–12 (2002).
21. J.K. Huwe, M. Lorentzsen, K. Thuresson, and A. Bergman. Analysis of mono- to deca-brominated diphenyl ethers in chickens at the part per billion level. *Chemosphere* **46:** 635–40 (2002).
22. M.G. Ikonomou and S. Rayne. Chromatographic and ionization properties of polybrominated diphenyl ethers using GC/high-resolution MS with metastable atom bombardment and electron impact ionization. *Anal. Chem.* **74:** 5263–5272 (2002).
23. M. Alaee, D.B. Sergeant, M.G. Ikonomou, and J.M. Luross. A gas chromatography/high-resolution mass spectrometry (GC/HRMS) method for determination of polybrominated diphenyl ethers in fish. *Chemosphere* **44:** 1489–95 (2001).
24. C. Thomsen, L. Smastuen Haug, H. Leknes, E. Lundanes, G. Becher, and G. Lindström. Comparing electron ionization high-resolution and electron capture low-resolution mass spectrometric determination of polybrominated diphenyl ethers in plasma, serum and milk. *Chemosphere* **46:** 641–48 (2002).
25. T. Hyötyläinen and K. Hartonen. Determination of brominated flame retardants in environmental samples. *Trends Anal. Chem.* **21:** 13–29 (2002).
26. E. Eljarrat, S. Lacorte, and D. Barcelo. Optimization of congener-specific analysis of 40 polybrominated diphenyl ethers by gas chromatography–mass spectrometry. *J. Mass Spectrom.* **37:** 76–84 (2002).
27. E. Eljarrat, A. de la Cal, and D. Barcelo. Potential chlorinated and brominated interferences on the polybrominated diphenyl ether determinations by gas chromatography–mass spectrometry. *J. Chromatrogr. A* **1008:** 181–92 (2003).
28. S. Crhova, M. Cerna, R. Grabic, T. Tomsej, and T. Ocelka. Polybrominated flame retardants in human adipose tissue in Czech Republic inhabitants. The pilot study. *Organohalogen Compd.* **58:** 241–44 (2002).
29. C. Pirard, E. De Pauw, and J.F. Focant. New strategy for comprehensive analysis of polybrominated diphenyl ethers, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls by gas chromatography coupled with mass spectrometry. *J. Chromatogr. A* **998:** 169–81 (2003).
30. S.A. Tittlemier, T. Halldorson, G.A. Stern, and G.T. Tomy. Vapor pressure, aqueous solubilities, and Henry's law constants of some brominated flame retardants. *Environ. Tox. Chem.* **21:** 1804–10 (2002).
31. D.B. Robb, T.R. Covey, and A.P. Bruins. Atmospheric pressure photoionization: an ionization method for liquid chromatography–mass spectrometry. *Anal. Chem.* **72:** 3653–59 (2000)
32. E. Basso, E. Marotta, R. Seraglia, M. Tubaro, and P. Traldi. On the formation of negative ions in atmospheric pressure photoionization conditions. *J. Mass Spectrom.* **38:** 1113–15 (2003).
33. S. Rayne and M.G. Ikonomou. Predicting gas chromatographic retention times for the 209 polybrominated diphenyl ether congeners. *J. Chromatogr. A* **1016:** 235–48 (2003).
34. J. Björklund, P. Tollbäck, and C. Östman. Mass spectrometric characteristics of decabromodiphenyl ether and the application of isotopic dilution in the electron capture negative ionization mode for the analysis of polybrominated diphenyl ethers. *J. Mass Spectrom.* **38:** 394–400 (2003).

Manuscript received February 4, 2005;
Revision received April 7, 2006.