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ANALYTICAL METHODS FOR TRACE LEVELS OF POLYBROMINATED DIPHENYL ETHERS (PBDEs)

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Introduction

Polybrominated diphenyl ethers (PBDEs) are emerging as a novel group of global environmental contaminants. Their wide distribution in the environment and exponential increase in human tissues have recently caused increasing concern among the scientists, environmental experts, and government agencies [1].

PBDEs are mainly used in flame-retardants with the function of decomposing at a temperature above 50 ^{o}C and liberating bromine atoms. These bromine atoms are effective reducing agents and free radical inhibitors such that they quench oxidation reactions [2]. Due to this excellent flame retardant function, the use of PBDEs as noncovalently bound additives in many synthetic polymers has grown with increasing use of plastic resins. Nowadays PBDEs are widely used in the electronic industry and in the manufacture of wood materials, plastics, polyurethere foam, and textiles. Products containing PBDEs include electronic parts, TV and computer sets, sofa, foam-based cushion seats in vehicles and aircraft, furniture, and carpets.

PBDEs are structurally similar to polychlorinated biphenyls (PCBs), although PBDEs are generally more polar than PCBs because of the presence of an oxygen atom and the resulting asymmetry about the horizontal axis. A PBDE molecule can have between one and ten bromines. A congener is a PBDE with a specific bromine substitution pattern.

Depending on the number and positions of the bromine atoms on the two phenyl rings, there are total 209 possible PBDE congeners. Most of the PBDE congeners found in commercial flame-retardants are lipophilic, bioaccumulating, and biomagnified. These industrial chemicals are considered to be environmentally persistent organic pollutants, as they tend to concentrate at nonpolar surfaces of soil particles and in living organisms, and degrade very slowly in the environment. Scientists in Sweden, Japan, Holland, German, Canada, and the United States have found the PBDEs residues in air, water, sediments, wildlife (marine mammals, fish, and bird eggs) and humans (breast milk, serum, and adipose tissue) [3, 4].

Being lipophilic and metabolically resistant, the PBDEs share many properties with organochlorine pesticides (e.g., DDT), polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-p-dioxins and furans. Preliminary toxicity investigations show that PBDEs may induce immunosuppression and chloracne, disrupt the endocrine system, affect learning and memory functions, and cause cancers [5]. PBDEs also have a strong affinity for fat and build up in the bodies of both animals and humans from before birth until death. Swedish scientists reported that between 1972 and 1998 levels of PBDEs in breast milk had increased 40 times [6]. Based on evidence that PBDE level is growing exponentially in Swedish women, the European Union has decided to ban two PBDE formulations, octa-BDE and penta-BDE, by July 1, 2003 and intends to ban a third formulation, deca-BDE, by 2006 [1]. The United States is the world's largest PBDE consumer and it has been reported that North American mothers have breast-milk PBDE levels at least 40 times higher than the highest concentrations found in Swedish mothers. Alerted by this situation the United States has recently initiated substantial research on the distribution of PBDEs contamination.

It is essential to have a sensitive and accurate analytical method for the determination of trace level PBDEs, when investigating PBDE contamination. Currently there is no standard EPA analytical method or other comprehensive analytical technique available for PBDEs analysis. Among all 209 possible PBDE congeners, 16 are the most representative and common contaminants. A literature search found that only some of these 16 congeners have been investigated. Gas chromatography-halogen selective electrolytic conductivity detector (GC/HSECD) has been used for analysis of three PBDE congeners [7] but this method lacks the ability to confirm the actual structure. Regular gas chromatography / mass spectrometry (GC/MS) methods have been proposed, but successful analysis of the fully brominated deca-BDE has not been reported due to the difficulty that its high molecular weight of 959 amu exceeds the scan range of the mass spectrometer. Liquid chromatography / mass spectrometry (LC/MS) method can extend the ion monitoring range into higher mass area but its sensitivity is much lower. The objective of this study was to develop a pair of comprehensive and reliable analytical methods, which can meet different needs for the determination of any target PBDE congener with high sensitivity and accuracy.

Methods and Materials

Selection of Target PBDE Congeners: Since not all 209 PBDE congeners have commercial analytical standards available and it is not necessary to test 209 congeners all at once, sixteen of most common PBDE congeners of interest were selected as target PBDEs in this study. The selected 16 congeners were designed to represent most groups of PBDE congeners with bromine atoms ranged from minimum 1 to maximum 10. This broad representative ensures that the developed analytical method is suitable for the determination of any PBDE congeners as needed.

Selection of Instrumentation: In order to develop a high efficiency and low cost PBDE analytical method, a work horse regular low resolution gas chromatography / mass spectrometry (GC/MS) method was tested. To obtain high sensitivity in some cases for detecting extremely low-level PBDE residues, a high-resolution gas chromatography / high-resolution mass spectrometry (HRGC/HRMS) method was also developed. These two analytical approaches provided powerful tools and wide-range choice for different analytical needs and applications.

Standards and Reagents: Analytical standards including sixteen PBDE target congeners, four carbon-13 PBDE standards, and decachlorobiphenyl, were all obtained from Wellington Laboratories (Ontario, Canada). High-purity solvents of dichloromethane (DCM) and acetone were obtained from Fisher (Pittsburgh, PA). In the GC/MS method, three carbon-13 labeled PBDE compounds were used as internal standards and decachlorobiphenyl as surrogate. In the HRGC/HRMS method, four carbon-13 labeled PBDE compounds were used as internal standards and surrogates. The five-point standard calibration curves were prepared at ranges of 0.05 - 1.0 ng /µL in GC/MS method and 0.5 - 200 pg/µL in HRGC/HRMS approach.

Sample Matrix and Extraction: Both aqueous and soil samples were investigated. One liter of water sample was extracted with 3 portions of 60 mL DCM. For the soil matrix, 30 gram sample for GC/MS or 10 gram sample for HRGC/HRMS were extracted by soxhlet recycle with 1:1 of acetone and DCM. The final extract volumes were 1000 µL in DCM for GC/MS method, and 10 µL in nonane by solvent-exchange process for HRGC/HRMS approach.

HRGC/HRMS Instrumentation: The analysis was performed on Micromass Autospec M series magnetic sector instrument interfaced with Hewlet Packard 6890 GC and CTC A200SE autosampler. The separation was performed by a Rtx-5MS 20 meter column with 0.32 mm i.d. and 0.25-µm film thickness. The injector was operated in splitless mode at 270 ^{o}C with 1.0 µL injection volume. The GC oven ramp was 120 ^{o}C for 2 min and increased to 180 ^{o}C at rate of 10 ^{o}C/min, then increased to 320 ^{o}C at rate of 6 ^{o}C/min and hold for 15

min. The helium flow rate was 1.0 mL/min. Selective -ion monitoring mode (SIM) was used to enhance the sensitivity. Two ions from each analyte and internal standard were monitored for determination.

GC/MS Instrumentation: The analysis was performed on HP 6890 GC / HP 5970 MS with HP 7683 autosampler. The separation was performed by a Rtx-5MS 15 meter column with 0.25 mm i.d. and 0.25-µm film thickness. The injector was operated in splitless mode at 275 ^{o}C with 2.0-µL injection volumes. The GC oven ramp was 100 ^{o}C for 0.5 min and increased to 320 ^{o}C at rate of 8 ^{o}C/min, then hold for 10 min. The helium flow rate was 1.2 mL/min. Selective -ion monitoring mode (SIM) was used for increasing sensitivity. Three ions from each analyte and internal standard were monitored for determination.

Quantification: Calibrations of the GC/MS and HRGC/HRMC were performed by injecting the standard solutions that containing the target analytes at five different concentrations over the calibration range. The internal standard solution was added to each standard in the same concentration as the samples. The concentration of the target analyte in the sample was then determined by comparing the integrated area of the target analyte peak in selected ion chromatogram to that of the internal standard.

Method Precision, Accuracy, and Sensitivity: Precision of the analytical method was determined for each analyte by analyzing extracts of seven replicate matrix spikes. The mean and standard deviation were then calculated for each analyte over the seven replicate analyses. Accuracy of the analytical method was demonstrated by analyzing matrix spike samples for both water and soil sample matrices. Three target PBDEs was assigned as spike compounds in GC/MS method, while all 16-target congeners were monitored as matrix spike compounds in HRGC/HRMS method. The spiked solution was added into the matrix spike samples at three different concentration levels, each in duplicates, before the sample extraction. After HRGC/HRMS or GC/MC analysis and by comparing the testing results of both un-spiked and spiked samples, the method accuracy was obtained in the form of matrix spike percentage recoveries. Sensitivity of the analytical method was evaluated based on both method reporting limit and method detection limit (MDL). The reporting limit was the lowest concentration point on the standard calibration curve. MDL was evaluated by spiking seven replicates of blank matrix with all target analytes at a concentration five times the estimated method detection limit. After extraction, instrumental analysis, and quantification, the standard deviation S of the seven replicates for each analyte compound was calculated. The method detection limit (MDL) was obtained by MDL = $t_{(n=1,\ 1-\forall=0.99)}$ *S,* where $t_{(n=1,\ 1-\forall=0.99)}$ is the Students' value appropriate for a 99% confidence level and *S* is the standard deviation estimated with n-1 degrees of freedom.

Results and Discussion

The MDL results of both HRGC/HRMS and GC/MS methods for aqueous and soil sample matrixes are given in Table 1. Since both BDE47 and BDE 99 exist as impurities in some other PBDE standards, the effect is obvious at low concentration level and raises the MDLs of both BDE 47 and BDE 99 in the method detection limit study by HRGC/HRMS. Other researchers, who obtained the PBDE standards from the same source, also observed this phenomenon.

The results of Table 1 showed that two complementary analytical methods for monitoring PBDE congeners were successfully developed. The HRGC/HRMS method was aimed at the detection of extremely low, *ppq* level PBDE residues in aqueous sample or *ppt* level in soil and biota samples. The regular GC/MS method was designed as an economic, low cost approach for the determination of trace level of PBDE contaminations

at concentration ranges above *ppt* level in aqueous sample or *ppb* level in soil and biota samples. While both methods were developed choosing 16 representative PBDE congeners as targets, they are ready to be adapted for the analysis of any PBDE compound among all 209 possible congeners and isomers, as long as the analytical standard is available. When combined with proper clean-up procedures, which have been successfully used in dioxin and PCB analysis, these analytical methods provide powerful tools for PBDE monitoring from a variety of sample matrixes, including waste water, sediment, house dust, fish, milk, and other biota samples.

Table 1. Target Compounds and Method Detection limits

Analyte	PBDE Full Name	HRGC/HRMS method		GC/MS method	
	Sample matrix:	aqueous	soil	aqueous	soil
BDE-3	4-Bromodiphenyl ether	34 *ppq*	4.1 *ppt*	6.5 *ppt*	0.12 *ppb*
BDE-7	2,4-Dibromodiphenyl ether	2.7 *ppq*	0.3 *ppt*	4.4 *ppt*	0.14 *ppb*
BDE-15	4,4'-Dibromodiphenyl ether	2.9 *ppq*	0.8 *ppt*	4.5 *ppt*	0.10 *ppb*
BDE-17	2,2',4-Tribromodiphenyl ether	3.0 *ppq*	0.9 *ppt*	3.2 *ppt*	0.11 *ppb*
BDE-28	2,4,4'-Tribromodiphenyl ether	3.4 *ppq*	0.6 *ppt*	4.1 *ppt*	0.10 *ppb*
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether	125 *ppq*	7.4 *ppt*	2.8 *ppt*	0.10 *ppb*
BDE-66	2,3',4,4'-Tetrabromodiphenyl ether	4.6 *ppq*	0.2 *ppt*	9.1 *ppt*	0.10 *ppb*
BDE-77	3,3',4,4'-Tetrabromodiphenyl ether	5.6 *ppq*	0.3 *ppt*	4.9 *ppt*	0.09 *ppb*
BDE-85	2,2',3,4,4'-Pentabromodiphenyl ether	1.7 *ppq*	0.8 *ppt*	7.0 *ppt*	0.14 *ppb*
BDE-99	2,2',4,4',5-Pentabromodiphenyl ether	50 *ppq*	10 *ppt*	5.2 *ppt*	0.20 *ppb*
BDE-100	2,2',4,4',6-Pentabromodiphenyl ether	9.8 *ppq*	1.0 *ppt*	7.0 *ppt*	0.19 *ppb*
BDE-138	2,2',3,4,4',5'-Hexabromodiphenyl ether	1.9 *ppq*	0.7 *ppt*	5.1 *ppt*	0.20 *ppb*
BDE-153	2,2',4,4',5,5'-Hexabromodiphenyl ether	2.3 *ppq*	0.8 *ppt*	8.7 *ppt*	0.16 *ppb*
BDE-154	2,2',4,4',5,6'-Hexabromodiphenyl ether	2.3 *ppq*	0.9 *ppt*	6.7 *ppt*	0.17 *ppb*
BDE-183	2,2',3,4,4',5',6-Heptabromodiphenyl ether	3.5 *ppq*	0.8 *ppt*	6.1 *ppt*	0.16 *ppb*
BDE-209	Decabromodiphenyl ether	4.0 *ppq*	3.1 *ppt*	36 *ppt*	0.68 *ppb*

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