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Separation of polybrominated diphenyl ethers in fish for compound-specific stable carbon isotope analysis

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ABSTRACT

A separation and isotopic analysis method was developed to accurately measure the stable carbon isotope ratios of polybrominated diphenyl ethers (PBDEs) with three to six substituted bromine atoms in fish samples. Sample extracts were treated with concentrated sulfuric acid to remove lipids, purified using complex silica gel column chromatography, and finally processed using alumina/silica (Al/Si) gel column chromatography. The purities of extracts were verified by gas chromatography and mass spectrometry (GC–MS) in the full-scan mode. The average recoveries of all compounds across the purification method were between 60% and 110%, with the exception of BDE-154. The stable carbon isotopic compositions of PBDEs can be measured with a standard deviation of less than 0.5‰. No significant isotopic fraction was found during the purification of the main PBDE congeners. A significant change in the stable carbon isotope ratio of BDE-47 was observed in fish carcasses compared to the original isotopic signatures, implying that PBDE stable carbon isotopic compositions can be used to trace the biotransformation of PBDEs in biota.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been widely used as additive brominated flame retardants in the past decades (Rahman et al., 2001). There are three commercial PBDE products: Penta-BDE (containing 50–60% penta-BDE congeners), Octa-BDE (containing 30–40% octa-BDE congeners), and Deca-BDE (containing ~97% deca-BDE congeners). The highest demand for technical products in the global market existed for Deca-BDE, followed by Octa-BDE and Penta-BDE (de Wit, 2002). Due to their widespread industrial use and because they are easily released into the environment, PBDEs have been detected throughout the environment (in sediment, water, air, biota, and in human beings), and comparatively high levels are often detected in aquatic biotopes (Darnerud et al., 2001). Due to environmental concerns, Penta- and Octa-BDE have been banned in the European Union, Canada, and China and have been voluntarily withdrawn from use in several U.S. states (Chen et al., 2009; Newsome et al., 2010; Zhang et al., 2011). Deca-BDE has also been phased out in Europe since 2008, and its production and use in the U.S. will be discontinued by the end of 2013 (Hess, 2009).

Generally, deca-BDE (BDE-209) is the most abundant PBDE congener in abiotic matrices such as dust and sediments (Harrad et al., 2008; Mai et al., 2005), which is consistent with industrial PBDE consumption patterns. However, the most frequently detected congeners in biota, and especially in aquatic biota, are the less brominated congeners (tri- to hexa-BDEs) (de Wit, 2002; Watanabe and Sakai, 2003). Two possible explanations have been given for this observation. The first is the difference in bioavailability between less brominated congeners and more highly brominated congeners. More highly brominated congeners are thought to have lower bioavailabilities due to their larger molecular weights and volumes (Xia et al., 2008). The other explanation is that significant amounts of the less brominated BDE congeners may be the result of biotic or abiotic debromination of more highly brominated congeners (Ross et al., 2009). In fact, a large number of studies have demonstrated the debromination of PBDE congeners in biotic and abiotic matrices (Vonderheide et al., 2008). The debromination occurring in biota is a confounding factor in the understanding of the bioaccumulation behavior of individual PBDE congeners. It is crucial to determine whether a specific PBDE congener in an organism is an accumulated contaminant from diet and/or abiotic environmental matrices or a metabolic debromination product of more highly brominated congeners. Conventional methods based on concentration and composition information cannot solve this issue.

Compound-specific isotope analysis (CSIA) can be used to study this issue. Due to isotopic effects, the fractionation of stable isotopes such as hydrogen, carbon, and nitrogen occurs during abiotic and biotic transformations of a compound, providing a unique means to identify and quantify the transformation, and sometimes even to elucidate the mechanism of the transformation (Schmidt et al., 2004). Recently, two studies reported the stable carbon isotopic compositions of PBDE

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congeners for two technical PBDE mixtures (Rosenfelder et al., 2011; Vetter et al., 2008). The results show that the stable carbon isotope ratios ($\delta^{13}C$) of BDE congeners decreased with increasing degrees of bromination. Thus, although no observable fractionation of stable isotopes occurs during the biotransformation of PBDEs, stable isotope analysis is still valuable for examining these processes because of the systemic internal variability of ^{13}C in PBDE mixtures. To date, no study has investigated the stable isotopic partitioning of PBDE congeners in biogeochemical processes.

Because of the low carbon contents in PBDE congeners, it was assumed that more than 50 ng would be required for appropriate measurement of compound-specific stable carbon isotopic compositions (Vetter et al., 2008). Therefore, large sample volumes are required to obtain sufficient amounts of compounds, which inevitably lead to complex matrices containing large amounts of lipids that must be dealt with and to interference due to co-elution. Therefore, extensive purification steps are required to attain high-purity extracts before CSIA. Although methods for purifying PBDEs, including oxidative treatment and size exclusion column chromatography, have been developed for dealing with small amounts of biota extracts (Sellström et al., 2003; Zhang et al., 2011), no information is available on the purification of PBDE from biota samples for CSIA.

The object of the present study was to develop a purification method to accurately measure the stable carbon isotope ratios of individual PBDE congeners. The purities of the extracts were verified by gas chromatography-mass spectrometry (GC–MS) in the full-scan mode and stable carbon isotope conservation was confirmed by CSIA using gas chromatography in combination with isotope ratio mass spectrometry (GC–C-IRMS). The purification and isotopic analysis method was then applied to juvenile common carp samples that were exposed to Penta-BDE to trace the biotransformation of BDE congeners. To the best of our knowledge, this report is the first of a purification method for PBDE in fish for CSIA.

2. Experimental methods

2.1. Chemicals and materials

Penta-BDE (TBDE-71X, lot 05500F16P) was acquired from Wellington Laboratories Inc. Dichloromethane (DCM) and n-hexane (Hex) were HPLC grade. DCM was obtained from CNW technologies GmbH, Germany. Hex was obtained from Honeywell, Korea. Analytical reagent grade acetone (Ace) was purchased from the Tianjing Chemical Reagent Factory, China and distilled before use. Concentrated sulfuric acid (analytical reagent grade) and anhydrous sodium sulfate (analytical reagent grade) were purchased from the Guangzhou Chemical Reagent Factory, China.

Silica gel (80-100 mesh, Qingdao Oceanic Chemical Factory, China) and neutral aluminum oxide (100-200 mesh, Jiangsu Wuxi Chemical Reagent Company, China) were Soxhlet extracted with DCM for 72 h. The neutral silica gel was heated at 170 °C for 12 h, cooled to room temperature, deactivated with 3% (w/w) distilled water, and preserved in Hex. Sulfuric acid silica gel was prepared by impregnating the deactivated neutral silica gel with 44% concentrated sulfuric acid (w/w) and was preserved in Hex. Aluminum oxide was heated at 250 °C for 12 h, cooled to ambient temperature, deactivated with 3% (w/w) distilled water, and conserved in Hex.

Two columns were prepared. The complex silica gel column (i.d. = 1.0 cm) was packed from bottom to top with 8 cm neutral silica, 8 cm sulfuric acid silica, and a 1 cm layer of anhydrous sodium sulfate. The alumina/silica (Al/Si) gel column (i.d. = 1.0 cm) consisted of 12 cm of neutral silica and 6 cm of aluminum oxide topped with a 1 cm layer of anhydrous sodium sulfate. Both columns were pre-eluted with Hex (30 mL) to remove background contaminants.

SX-3 Bio-Beads (Bio-Rad Laboratories, Hercules, CA) (40 g) were soaked in Hex/DCM (1:1, V/V) for 24 h. Gel permeation chromatography (GPC) was performed using a glass column packed with processed SX-3 Bio-Beads with an appropriate solvent reservoir. The column was eluted with 500 mL Hex/DCM (1:1, V/V) and suspended in 100 mL Hex/DCM (1:1, V/V). Silica gel solid-phase extraction (SPE) columns were obtained from Isolute, International Sorbent Technology, UK. The SPE columns were activated at 130 °C for 15 h and cooled to indoor temperature before use.

Common carp were purchased from the largest aquaria wholesale market in Guangzhou, China, and transferred to the laboratory immediately. The carp (30 fish) were exposed to the Penta-BDE mixture at a dose of 100 ± 10 μg/day/fish for 20 days. Detailed information regarding the feeding of carp in the laboratory has been provided previously (Zeng et al., 2012). The carcasses of every five fish were pooled into one sample, resulting in six composite samples. These samples were used for PBDE analysis and stable carbon isotope analysis. The control experiment was conducted during whole expose experiment and the results have been provided in details in our previous publication (Zeng et al., 2012) and were not present herein.

2.2. Clean-up and fractionation

Based on the purification of PBDEs in previous studies (de Boer Colin and Law Bart, 2001; Hyötyläinen and Hartonen, 2002), three purification methods were tested. Fig. 1 shows the development of clean-up processes for the final purification of PBDE in fish for compound-specific isotope analysis. Details about each pretreatment are described below.

The pooled fish samples were freeze-dried, weighed (approximately 50 g dry weight), and extracted with 300 mL Ace/Hex (1:1, V/V). In the first purification method, the conventional PBDE analysis method for biota was used with a minor modification (Yu et al., 2011). Briefly, the extract was subjected to GPC to remove lipids, further purified on a complex silica gel column and eluted with 30 mL of 50% DCM in Hex (by volume). The eluate was concentrated to 1 mL and separated on an SPE column. The column was eluted with Hex (3.5 mL) followed by 6.5 mL DCM/Hex (4:6, V/V) and 7 mL DCM. The last two fractions were combined and reduced to near dryness under a gentle nitrogen flow and then reconstituted with 200 μL Hex before GC–MS analysis.

In a second purification method, the extract was first treated with concentrated sulfuric acid to remove the lipids. The procedures for further clean-up were the same as those in the first method. In the third and final purification method, the extract was concentrated, subjected to solvent exchange with Hex (90 mL), and then treated with 10 mL of concentrated sulfuric acid three times. The lipid-free extract was washed with sodium sulfate solution (5%, w/w) to remove the residual sulfuric acid. After being purified on a complex silica gel column as described above, the extract was concentrated to 1 mL and cleaned up with an Al/Si column. The column was eluted with 22 mL Hex followed by 5 mL Hex and 15 mL DCM/Hex (1:1, V/V). The first 22 mL of Hex was discarded, and the last two fractions were collected and concentrated to 0.2 mL under a gentle N_2 stream.

2.3. PBDE quantification

The purified extracts for spiked blanks and spiked matrices and the Penta-BDE standards were analyzed by GC–MS (Agilent 6890 GC-5975 MS; Agilent Technology, CA) with an electron impact (EI) ion source in full-scan mode. The mass range m/z 50-800 was scanned at 1 s intervals. GC separation was performed using a DB-5 capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness). The oven temperature program was settled as follows: 70 °C (held for 1.5 min), increasing to 230 °C at 30 °C/min, increasing to 270 °C at 4 °C/min (held for 1 min), increasing to 280 °C at 2 °C/min (held for 0.5 min), and increasing at 5 °C/min to 310 °C (held for 30 min). Penta-BDE was used as a calibration standard. The individual congeners of PBDEs