Monitoring of Contamination by Non-PBDE Brominated Flame Retardants in Asian Coastal Waters Using Mussels as a Bioindicator

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Abstract—Hexabromocyclododecanes (HBCDs), 1,2-bis-(2,4,6-tribromophenoxy)ethane (BTBPE) and Decabromodiphenylethane (DBDPE) are used as alternatives for PBDEs and they have similar physicochemical properties as PBDEs. Only limited information on these non-PBDE BFRs is available, in particular, no information on environmental pollution by these BFRs in Asian coastal waters have been reported. In this regard, we investigated the contamination status of these BFRs in Asian coastal waters using mussels as a bioindicator. Concentrations of these BFRs were measured in green (Perna viridis) and blue mussels (Mytilus edulis) collected from the coastal areas in some Asian countries during 2003–2008. HBCDs, BTBPE and DBDPE were found in mussels at levels ranging <0.01–1400, <0.1–13 and <0.3–22 ng/g lipid wt, respectively. Concentrations of HBCDs and DBDPE in mussels from Japan and Korea were higher compared to those from other countries, indicating extensive usage of these non-PBDE BFRs in Japan and Korea. Higher levels of HBCDs and DBDPE than PBDEs were detected in some mussel samples from Japan. These results may indicate that PBDEs have been substituted by non-PBDE BFRs in Japan. To our knowledge, this study is the first report on comprehensive monitoring of BTBPE and DBDPE pollution in the coastal waters of Asian countries.

Keywords: mussels, hexabromocyclododecanes (HBCDs), 1,2-bis-(2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenylethane (DBDPE)

INTRODUCTION

Due to the ban on the use of two polybrominated diphenyl ethers (PBDEs) commercial mixtures, production and use of non-PBDE based brominated flame retardants (BFRs) may be increasing at present. Hexabromocyclododecanes (HBCDs), 1,2-bis-(2,4,6-tribromophenoxy)ethane (BTBPE) and Decabromodiphenylethane (DBDPE) are used as alternatives for PBDEs and they have similar physicochemical properties as PBDEs. It has been reported that these BFRs were detected in the environment and biota (Minh et al., 2007;
Stapleton et al., 2008; Gauthier et al., 2009; Shi et al., 2009). However, only limited information on these non-PBDE BFRs is available, in particular, no study on environmental pollution by these BFRs in Asian coastal waters had been carried out. In this regard, we investigated the contamination status of HBCDs, BTBPE and DBDPE as PBDEs alternative in Asian coastal waters using mussel as a bioindicator.

MATERIALS AND METHODS

Details of mussel samples

Green (Perna viridis) and blue mussels (Mytilus edulis) were collected from various locations in Cambodia, China, Hong Kong, India, Indonesia, Japan, Malaysia, the Philippines and Vietnam from 2003 to 2008 (n = 45). After biometric measurements, the whole soft tissues of mussels from each location were shucked, pooled, and homogenized. The homogenized samples were transferred into glass bottles and lyophilized. The sampling locations are shown in Fig. 1.
Chemical analysis

Analysis of PBDEs, HBCDs, BTBPE and DBDPE was performed following the procedures previously described with slight modifications (Isobe et al., 2009). Briefly, 2–3 g of lyophilized mussel sample was ground with anhydrous sodium sulfate and Soxhlet extracted with diethyl ether/hexane (3:1, v/v) for 7–8 h. An aliquot of the extract, after adding 5 ng of internal standards for PBDEs (13C12-labeled BDE-3, -15, -28, -47, -99, -154, -183, -197, -207 and -209), 10 ng of internal standards for HBCDs (13C12-labeled α-, β-, γ-HBCD), 5 ng of internal standards for BTBPE (13C12-labeled BTBPE) and 5 ng of internal standards for DBDPE (13C12-labeled DBDPE) was loaded to a gel permeation chromatography (GPC: Bio-Beads S-X3, Bio-Rad, CA, 2 cm i.d. x 50 cm) column for lipid removal. The GPC fraction containing the target compounds was concentrated and passed through 4 g of activated silica gel (Wakogel DX, Wako Pure Chemical Industries Ltd., Japan) column for clean-up and fractionation. The first fraction eluted with 80 ml of 5% dichloromethane in hexane from the silica gel column contained PBDEs, BTBPE and DBDPE, while the second fraction eluted with 100 ml of 25% dichloromethane in hexane contained HBCDs. 13C12-labeled BDE-139 was spiked to the first fraction solution prior to a gas chromatograph (GC: Agilent 7890A) equipped with a mass-selective detector (MS: Agilent 5975C) analysis for PBDEs, BTBPE and DBDPE. Quantification of PBDEs, BTBPE and DBDPE was performed using electron ionization with selective ion monitoring (EI-SIM) mode. GC columns used for quantification were DB-1 fused silica capillary (J&W Scientific Inc.) having 30 m x 0.25 mm i.d. x 0.25 μm film thickness for mono- to hepta-BDEs and BTBPE, and 15 m x 0.25 mm i.d. x 0.1 μm film thickness for octa- to deca-BDEs and DBDPE. Fourteen major congeners of PBDEs (BDE-3, -15, -28, -47, -99, -100, -153, -154, -183, -196, -197, -206, -207, and -209) were quantified in this study. All the congeners were quantified using the isotope dilution method to the corresponding
$^{13}$C$_{12}$-labeled congener.

The fraction containing HBCDs was solvent exchanged into methanol and 10 ng of $d_{18}$-labeled $\alpha$-, $\beta$-, $\gamma$-HBCD was added prior to liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) analysis as a performance standard. The diastereomeric analysis of HBCDs was performed on the basis of the reported analytical methods (Tomy et al., 2005; Isobe et al., 2009). Quantification of HBCDs was performed using an Acquity UPLC (Waters, Tokyo, Japan) ultra-performance liquid chromatograph equipped with Quattro Micro API triple-quadrupole mass spectrometer (Waters/Micromass, Tokyo, Japan). Separation of the three diastereoisomers was achieved with an Extend-C$_{18}$ column (2.1 mm i.d. $\times$ 100 mm, 1.8 $\mu$m particle size). The mobile phase consisted of milli-Q/acetonitrile/methanol (20:20:60) at 0.2 ml/min in initial condition for 1 min and then ramped to 100% methanol in 5 min, and held for 2 min. The MS/MS analysis, which was operated in negative mode of electrospray ionization (ESI), was performed in multiple reactions monitoring mode (MRM). Quantification of native HBCDs was achieved from mean value of the response of two MRM transitions (i.e., $m/z$ 640 > 81, $m/z$ 642 > 81) corrected with response of $^{13}$C$_{12}$-HBCDs (i.e., $m/z$ 652 > 81 MRM transition). HBCD isomers were quantified by isotope dilution using the corresponding $^{13}$C$_{12}$-labeled isomers.

Recoveries of $^{13}$C$_{12}$-labeled HBCDs during analytical procedure were determined using $d_{18}$-labeled isomers and the values ranged from 60 to 120%.

Procedural blanks were analyzed simultaneously with every batch of five samples to check for contamination from solvents and glassware. Lipid contents were determined by measuring the total nonvolatile solvent extractable materials on subsamples taken from the original extracts. Concentrations of analytes were expressed as ng/g lipid weight unless otherwise stated.
RESULTS AND DISCUSSION

Contamination status of PBDEs and HBCDs in Asian coastal waters

PBDEs and HBCDs were detected in almost all the mussel samples, indicating widespread contamination by these two BFRs (Fig. 2). The levels of PBDEs and HBCDs varied depending on the countries and the sampling locations, ranging from 0.66 to 420 and from <0.01 to 1400 ng/g lw, respectively. Higher concentrations of PBDEs were detected in mussels from Korea, Hong Kong and the Philippines, suggesting that the contamination is serious in developed countries and now extending to countries with emerging economies. On the other hand, the levels of PBDEs in mussel samples from Japan were low. The result may reflect the regulatory measures undertaken on the usage of PBDEs in Japan. Higher concentrations of HBCDs were found in mussels from Japan and Korea, indicating that the extent of contamination by HBCDs is intense in Asian developed countries. The levels of HBCDs in mussel samples from developing countries were low. Contamination status of PBDEs differed from those of HBCDs, reflecting the difference in the usage amounts and patterns of PBDEs and HBCDs according to the locations.

Contamination status of BTBPE and DBDPE in Asian coastal waters

BTBPE and DBDPE were detected in mussel samples from 8 out of 67 and 17 out of 67 locations, indicating that these non-PBDEs BFRs are used as PBDEs alternatives in Asia (Fig. 3). Levels of BTBPE and DBDPE ranged from <0.1 to 13 and from <0.3 to 22 ng/g lw, respectively. The highest concentration of BTBPE was detected in the mussel sample from Sihwa Lake, Korea. Higher
levels of DBDPE were detected in mussel samples from Japan and Korea than the other countries, suggesting that contamination by DBDPE is serious in developed countries as also observed in the case of HBCDs. Concentrations of BTBPE and DBDPE in most of the samples analyzed were lower than those of PBDEs and HBCDs, indicating that contamination by BTBPE and DBDPE have not yet been widespread compared to PBDEs and HBCDs. However, it can be presumed that the use of these non-PBDEs BFRs and consequent environmental contamination by these chemicals may increase in future.

Comparison between concentrations of BTBPE and penta- and octa-BDE mixtures

BTBPE is used as an alternative to penta- and octa-BDE mixtures. In this study, concentrations of BTBPE in mussel samples were compared to those of representative BDE congeners containing in penta- and octa-BDE mixtures (e.g., BDE-47, 99, 100, 153, 154 in penta-BDE mixtures and BDE-153, 183, 196, 197, 203, 206, 207 in octa-BDE mixtures) (Fig. 4). The BTBPE/Penta-BDE ratios were more than 1 in some locations, implying that PBDEs have been substituted by BTBPE in some regions of Asia.

Comparison between concentrations of DBDPE and deca-BDE mixtures

DBDPE is used as an alternative of deca-BDE mixture. Concentrations of DBDPE in mussel samples were compared to those of BDE-209 containing in deca-BDE mixture (Fig. 5). The DBDPE/BDE-209 ratios were more than 1 in some locations, implying that PBDEs have been substituted by DBDPE in some regions of Asia. In Japan, the market demand of DBDPE has exceeded that of deca-BDE mixtures since 1997 (Watanabe and Sakai, 2003). The result of this study may be a reflection of such a market demand.
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