Contamination Status and Geographical Distribution of Polybrominated Diphenyl Ethers, Hexabromocyclododecanes and Polychlorinated Biphenyls in Fish and Shellfish from the Seto Inland Sea, Japan

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Abstract—Brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs) and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) were determined in fish collected in 2008 from various locations in the Seto Inland Sea, Japan. PCBs, PBDEs and HBCDs were detected in most of the fish samples analyzed in this study, indicating the ubiquitous contamination of the present coastal ecosystem. PCBs were the highest in all the samples followed by BFRs. HBCDs were higher in eastern part of the Seto inland Sea than in the western part. This result indicates that the textile industries, which are many in the nearby land area may be the possible contamination sources of HBCDs. BDE-47 was the predominant congener in all the locations. In total BDEs, BDE-15 and -28 accounted for high proportions in fish samples from Suo Nada, Japan, indicating presence of specific source(s) in that region. Significant positive correlations between $\delta^{15}N$ and concentrations of PCBs, PBDEs and HBCDs, individually were found in almost all the locations, indicating that all these compounds were biomagnified in higher trophic level organisms. In Suo Nada and Osaka Bay, trophodynamic magnification factor (TMF) of the analyzed PCB congeners increased when the $\log K_{OW}$ values were 5–7, but decreased with further increase in $\log K_{OW}$ values (7–9). This phenomenon could be due to reduced bioavailability or uptake of congeners with higher K_{OW} in fish. All HQ values for PBDEs and HBCDs were 2–3 orders of magnitude lower than 1, indicating minimal health risk but the HQ values were close to 1 for PCBs, indicating potential risk.

Keywords: brominated flame retardants, Seto Inland Sea, biomagnification, food web

INTRODUCTION

Brominated flame retardants (BFRs), including polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs), have fire-proof characteristic

and hence added to a variety of industrial and household products such as housing material, electrical and electronic appliances, personal computers, thermal insulations, textiles, etc. As the physico-chemical properties of some BFRs are similar to those of classical persistent organic pollutants (POPs), global continuous use and consequent environmental pollution and human exposure are of public concern. Ocean plays a role as a major sink for persistent anthropogenic compounds, which are transported via atmospheric and aquatic processes and accumulated in higher trophic level organisms through marine food web. Although there are some studies reporting the levels and accumulations of POPs and BFRs in marine biota, only limited information is available in the Seto Inland Sea, which is a well-known coastal fishing ground. In this study, BFRs and stable isotope ratios (δ^{15}N and δ^{13}C) in fish from the Seto Inland Sea were measured to understand their contamination status, geographical distribution, biomagnification through food web and human exposure.

MATERIALS AND METHODS

Samples

One hundred twenty fish and crustacean samples were collected from various regions of the Seto Inland Sea including Suo Nada, Iyo Nada, Harima Nada, Kii Suido and Osaka Bay, in September 2008 (Table 1). All the samples were stored in the Environmental Specimen Bank (*es*-BANK) of Ehime University at –25°C until chemical analysis.

Chemical analysis

Analysis of PCBs and BFRs were carried out following the previous report (Isobe *et al.*, 2011). Briefly, 30 g (wet weight) of muscle sample from fish, shrimps and squids were ground with anhydrous sodium sulfate and extracted using a high speed solvent extractor (SE-100). An aliquot of the extract, after spiking 5 ng of $^{13}C_{12}$-CBs and 5 ng of $^{13}C_{12}$-BDEs and 10 ng of $^{13}C_{12}$-HBCDs, was loaded and extracted through a gel permeation chromatography (GPC) column for lipid removal and then passed through an activated silica gel column for separation. $^{13}C_{12}$-BDE-139 and $^{13}C_{12}$-BDE-126, 205 were spiked as performance internal standards to the fractions containing PBDEs and PCBs prior to GC-MS quantification. The HBCDs fraction was evaporated and spiked with HBCD-d_{18} prior to LC-MS/MS analysis.

Stable isotope ratio measurement

Muscle of fish and crustaceans were used for the stable isotope analysis. All the samples were dried for 24 h at 60°C, powdered and treated with a 2:1 chloroform-methanol solution to remove lipid. Then the samples were dried and loaded into tin cups. Stable carbon and nitrogen isotopes were measured using isotope ratio mass spectrometer (IR-MS).

Table 1. Sampling locations and number of samples analyzed.

Species name	Suo Nada	Iyo Nada	Harima Nada	Kii Suido	Osaka Bay
Hairtail (*Trichiurus japonicus*)	4	4	3	3	4
Red Sea bream (*Pagrus major*)	4	4	3	3	4
Large-scaled grinner (*Saurida macrolepis*)	4	4	2	3	4
Japanese spanish mackerel (*Scomberomorus niphonius*)	3	NA	NA	3	4
Jack mackerel (*Trachurus japonicus*)	4	4	2	3	4
Cuttlefish (*Sepia* (*Platysepia*) *esculenta*)	4	2	2	3	4
Velvet shrimp (*Metapenaeopsis barbata*)	2	NA	2	3	3
Anchovy (*Engraulis japonicus*)	4	NA	NA	NA	NA
Whitebait (*Schindleria praematura*)	3	NA	NA	NA	4
Bobtail squid (*Euprymna morsei*)	4	NA	NA	NA	NA

NA: not available.

Trophic level calculations

Stable isotope values were calculated using the following equation

$$\delta^{15}X = \{(R_{\text{sample}}/R_{\text{standard}}) - 1\} \times 1000 \qquad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio of ^{13}C/^{12}C or ^{15}N/^{14}N. Pee Dee belemnite (PDB) limestone carbonate and atmospheric nitrogen (N_2) were used as the standards for carbon and nitrogen isotope ratios, respectively. We assume that zooplankton (almost 100 percent copepods) represent trophic level 2.0, because they are the primary herbivores feeding on phytoplankton. For the samples from other trophic levels, we used the relationship by Post *et al.*, (2002) (altered):

$$\text{TL} = [\delta^{15}N_{\text{sample}} - \{\delta^{15}N_{\text{base2}} \times \alpha + \delta^{15}N_{\text{base1}} \times (1 - \alpha)\}]/3.3 + 1 \qquad (2)$$

$$\alpha = \{\delta^{13}C_{\text{sample}} - 1.0(\text{TL} - 1) - \delta^{13}C_{\text{base1}}\}/(\delta^{13}C_{\text{base2}} - \delta^{13}C_{\text{base1}}) \qquad (3)$$

where $\text{TL}_{\text{consumer}}$ is the trophic level and α is the degree of dependence.
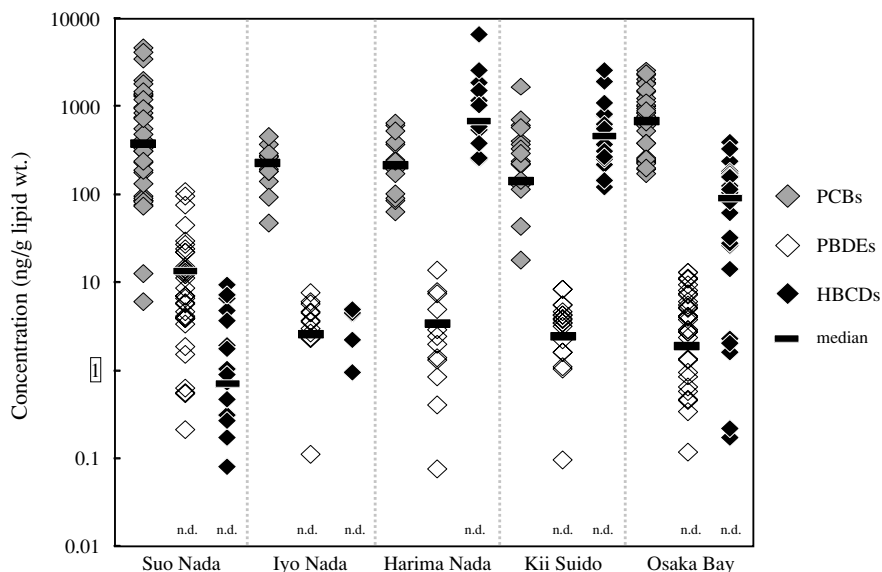
Fig. 1. Concentrations PCBs, PBDEs and HBCDs in fish and shellfish from the Seto Inland Sea, Japan.

Bioaccumulation parameters

Trophic magnification factors (TMFs), which are the markers of cumulative bioaccumulation across the food web, were determined from the log-linear regression between the base-10 logarithm ($\log_{10}$) of the lipid equivalent concentration in biota (C_B) and trophic level (TL):

$$\log C_B = a \times TL + b \qquad (4)$$

where *a* and *b* are the empirical slope and *y*-intercept, respectively. TMFs were calculated as the antilog of the slope (*a*)

$$TMF = 10^a. \qquad (5)$$

RESULTS AND DISCUSSION

Contamination status

Comparison of concentration

PCBs, PBDEs and HBCDs were detected in most of the fish and crustacean samples analyzed in this study, indicating ubiquitous contamination by these organohalogen compounds in the Seto Inland Sea coastal ecosystem (Fig. 1). Among the analytes, PCBs concentrations were the highest in almost all the fish samples, which could be attributed to the wide past usage and spillage from the
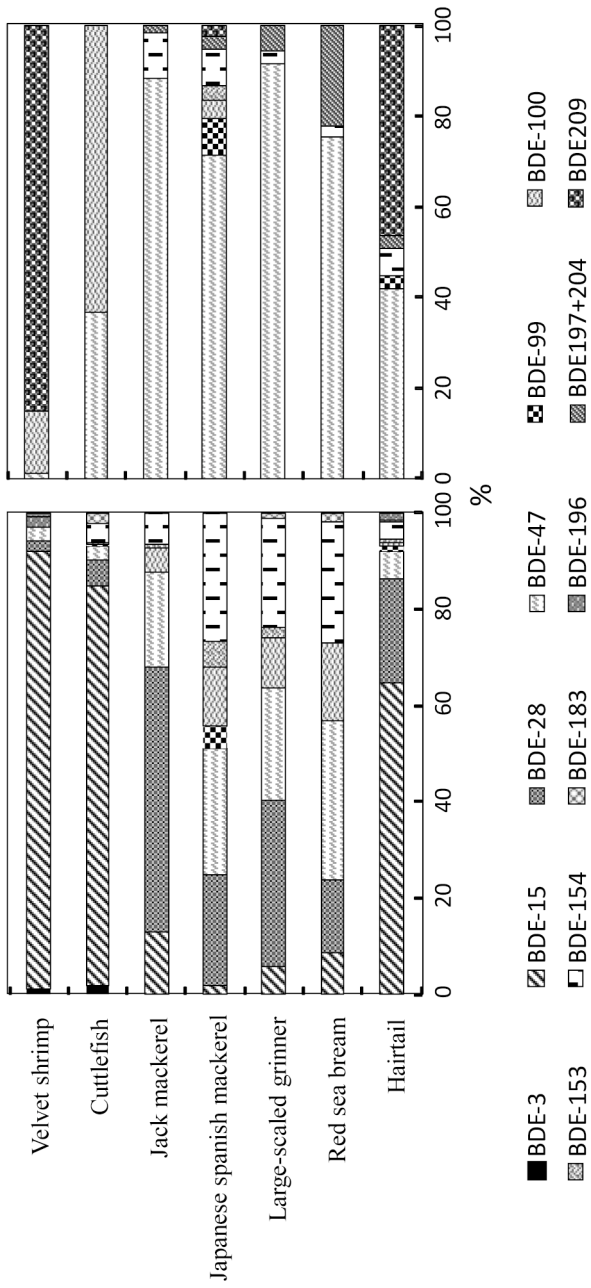
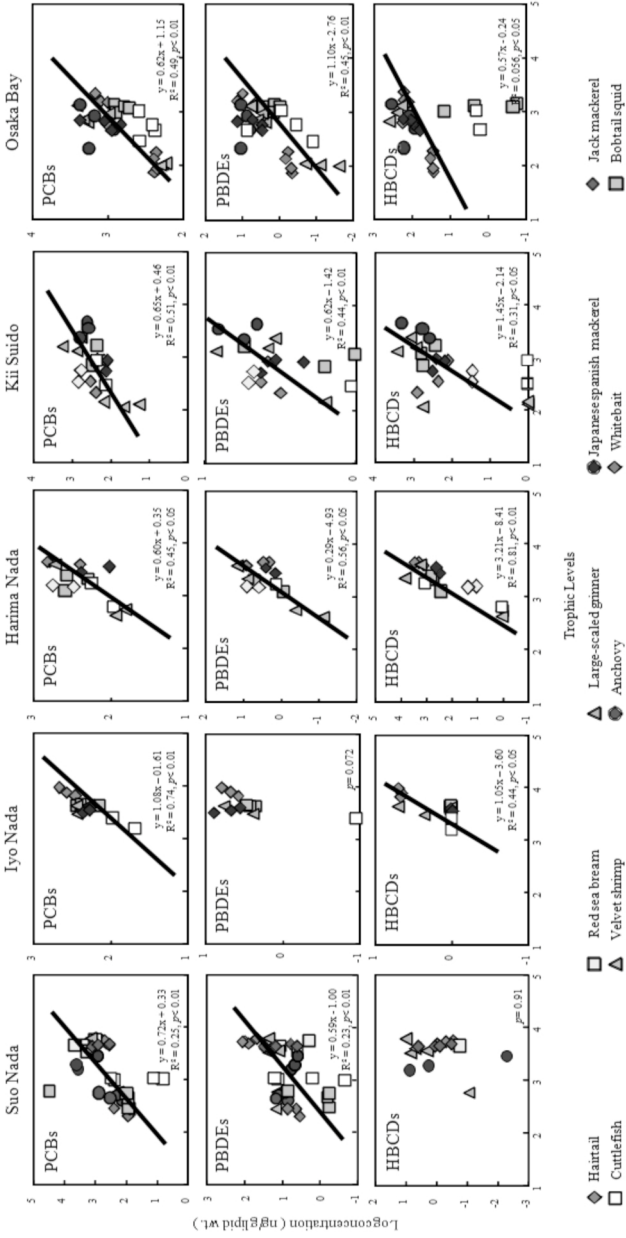Fig. 2. Congener patterns of PBDEs in fish and shellfish from Suo Nada and Osaka Bay, Japan.

Fig. 3.   Relationship between trophic levels (TLs) and concentrations of contaminants in fish and shellfish from Seto Inland Sea, Japan.
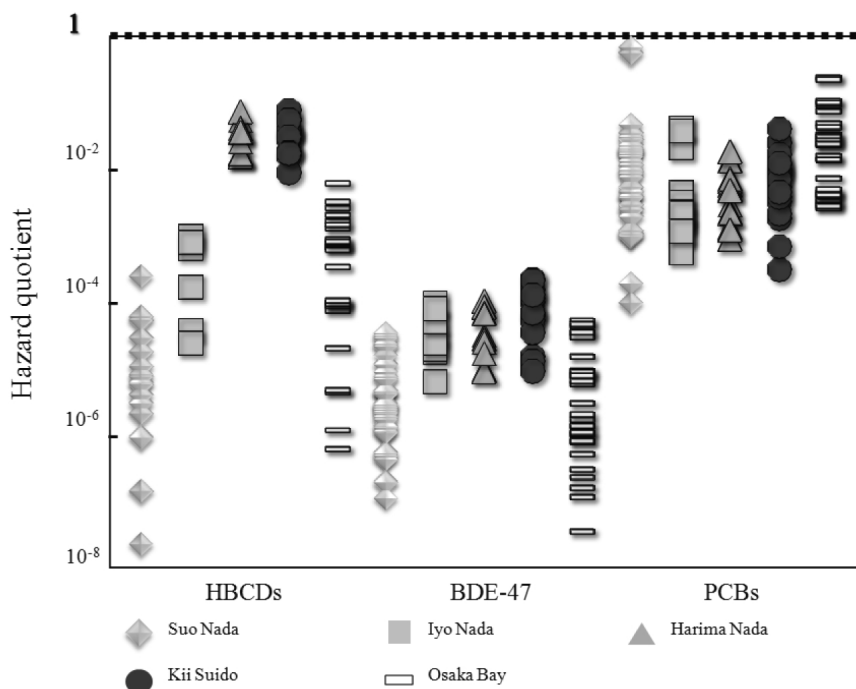
Fig. 4. Hazard Quotient values of HBCDs, BDE-47 and PCBs in fish and shellfish from Seto Inland Sea, Japan.

stockpiles of PCBs in Japan. Among the study locations, significant difference was found between Osaka Bay, a heavily industrialized and densely populated embayment, and other areas except for Suo Nada, indicating that PCBs levels are associated with human and industrial activities. PBDEs levels in Suo Nada were the highest, possibly reflecting the extensive production and usage of PBDEs in the industrial area in the Suo Nada coast (Akutsu *et al*., 2001). On the other hand, concentrations of HBCDs were higher in eastern part of the sea than in western part. The same pattern was observed in the previous monitoring survey using mussel as a bioindicator (Ueno *et al*., 2010). This result indicates that fiber and textile industries, which are many in the nearby land area may be the possible contamination sources of HBCDs (Ministry of Economy Trade and Industry, 2005).

Isomer pattern

BDE-47 was the predominant congener in all the locations, whereas BDE-15 and -28 were detected in fish from Suo Nada (Fig. 2). This specific isomer pattern in Suo Nada may indicate the use of specific PBDE technical mixtures in this area (Akutsu *et al*., 2001). Among the three HBCD isomers (α-, β- and γ-HBCD), α-HBCD was prevalent in all the species, while β and γ-isomers were

below detection limits in most of the samples (Fig. 2). The prevalence of α-HBCD has already been observed in aquatic biota like fish, shrimp and mussels (Morris *et al*., 2004; Tomy *et al*., 2004; Ueno *et al*., 2006; Ramu *et al*., 2007).

Biomagnification through the food web

Significant positive correlations between $\delta^{15}N$ and lipid normalized concentrations of PCBs, PBDEs and HBCDs were found in almost all locations, indicating that these compounds are biomagnified in higher trophic level organisms (Fig. 3). PBDEs in Iyo Nada and HBCDs in Suo Nada, however, did not show clear correlations. This result indicates that both PBDEs and HBCDs are in use at present but less persistent compared to PCBs.

Relationships between trophodynamic magnification factors (TMFs) calculated from the slope of the regression line and concentrations of each isomer were examined. In Suo Nada and Osaka Bay, TMF values of the analyzed PCB congeners increased with increasing $\log K_{OW}$ (5–7), and then decreased with increasing $\log K_{OW}$ (7–9). This phenomenon is consistent with the previous report, which concluded that the PCB congeners with $\log K_{OW}$ greater than 7 showed less TMFs because of their reduced bioavailabilities (Fisk *et al*., 1998). BDE-28 showed higher TMF values than PCB congeners which have similar $\log K_{OW}$, implying debromination of BDE-47 to BDE-28.

Human exposure

Daily intake of PCBs, PBDEs and HBCDs through fish consumption was calculated from the concentrations of analytes, average body weight of Japanese (60 kg) (Ministry of Health Labor and Welfare, 2008) and Japanese fish consumption (about 100 g/day) (Ministry of Agriculture Forestry and Fisheries, 2006), and compared with threshold reference values reported by the USEPA (United States Environmental Protection Agency, 1997) [BDE47; 0.1, BDE99; 0.1, BDE153; 0.2, PCBs; 1 (unit: μg/kg bw-day)] and the ATSDR (Agency for Toxic Substances and Disease Registry, 2008) [HBCDs; 0.2 (unit: μg/kg bw-day)], for hazard quotients (HQ; Fig. 4) (Chen *et al*., 2009).

$$HQ = \text{concentration} \times \text{intake/body weight} \times \text{reference dose.}$$

All HQ values for PBDEs and HBCDs were 2-3 orders of magnitude lower than 1, indicating minimal health risk. On the other hand, HQ values for PCBs were close to 1 implying that PCBs exposure may be of concern for population consuming a large amount of fish. Therefore, continuous monitoring and proper regulation should be implemented on the production, use and disposal of these chemicals to reduce human exposure and toxic implications.

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