POLYBROMINATED DIPHENYL ETHERS IN FISH AND SHELLFISH FROM TOKYO BAY ESTUARIES

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Abstract

Concentrations of polybrominated diphenyl ethers (PBDEs) in fish and shellfish from Tokyo Bay estuaries were analyzed for health risks from the daily intakes, and PBDEs and dioxin levels in fish tissues were compared. The concentrations of total PBDEs in fish and shellfish ranged from 0.17 to 1.9 ng/g on a wet weight basis. The order of concentration of total PBDEs was short-necked clam < marbled sole < sea bass < striped mullet < conger eel. The major congeners in all samples were BDE-47, BDE-49, BDE-99, BDE-100, and BDE-154. The highest daily intakes of BDE-47, BDE-99 and BDE-153 were estimated to be 0.0014, 0.0002, and 0.0001 μg/kg bw/day, respectively, without cooking. Considering the LOAEL or NOAEL of these congeners, the PBDE levels in fish and shellfish from Tokyo Bay estuaries were not regarded as a serious health risk, even if ingested. Concerning the distribution of PBDEs in fish tissues, the highest level was present in fat body, followed by the liver, spleen and digestive tract, thus confirming that the PBDEs are highly lipophilic. The concentration of BDE-47 was significantly correlated with that of PCB-118, 2,3,7,8-TCDD, and 2,3,7,8-TCDF in each tissue of sea bass.

Introduction

The annual consumption of polybrominated diphenyl ethers (PBDEs) used as brominated flame retardants (BFRs) in Japan, increased rapidly from 1986, reached a peak in 1990, and then decreased in 2004. PBDEs consumption in 1990 was 12,100 tons (DecaBDE 10,000 tons, Octa BDE 1,100 tons TetraBDE 1,000 tons), while that in 2004 was only 2,000 tons of DecaBDE (BDE-209). Changing the kinds of BFRs used results in zero consumption of TetraBDE and OctaBDE after 1991 and 2003, respectively. However, comparing the historical use and the temporal trends of PBDEs in the sediment cores from Tokyo Bay, the levels of PBDEs increased from 1992 to 1999, and the major congener during the whole period was BDE209, indicating a lag of ~10 years between peak use and deposition in the sediments.[1] The study of biomagnification of PBDEs in Tokyo Bay suggested that the congeners with a logK_{ow} value less than 7 showed positive correlations between bioconcentration factor (BCF) and logK_{ow} value. These congeners tended to accumulate from a lower to higher trophic level through the food web.[2] Currently, there is little data on PBDE levels of fish species and shellfish from Tokyo Bay. Our objective was to collect data regarding the levels of PBDEs in fish and shellfish from Tokyo Bay to estimate the health risk from the daily intake.

Materials and Methods

Sampling For analysis of PBDE levels in fish and shellfish, sea bass, striped mullet, conger eel and marbled sole were collected from the estuaries of Tokyo Bay (the Sumida River, the Tama River and the area of northwest of Tokyo International Airport) and short-necked clams were collected from the tidal flat area of Tokyo Bay during 2005 and 2006. The edible parts of the fish and shellfish in each sample were analyzed and homogenized. To investigate PBDE distribution in fish tissues, muscle tissue, liver, kidney, fat body (adipose tissue), digestive tract, spleen, and gonad were isolated from sea bass and striped mullet collected in 2004.

Standards and Reagent All analyses were performed using the isotope dilution method. Internal ^{13}C-labeled standards (BDE Nos. 28, 47, 99, 153, 154, 183), recovery standard and calibration standard solutions were purchased from Wellington Laboratories. Organic solvents (toluene, n-hexane, dichloromethane, acetone and ethyl alcohol), potassium hydroxide, sodium sulfate and sulfuric acid were purchased from Wako Pure Chemical Industries. Multi-layer silica gel column was obtained from Sigma-Aldrich Co. and active carbon-dispersed silica gel reversible column was obtained from Kanto Chemical Co. Inc.

Sample Preparation A total of 1-50g of sample was weighed, spiked with a ^{13}C-labeled internal standard, saponified with potassium hydroxide and ethyl alcohol for 8-12hr, and successively extracted three times with n-hexane. The n-hexane extracts were treated with sulfuric acid until no color was visible in the sulfuric acid layer, and concentrated. Multi-layer silica gel column was eluted with 200 mL of n-hexane to rinse, and the

extract was then loaded to the column and it was eluted first with 200 mL of n-hexane for the dioxin fraction and then with 200 mL of 10% dichloromethane/n-hexane (v/v) for the PBDE fraction. The PBDE fraction was concentrated and subjected to the active carbon-dispersed silica gel column without solvent conditioning and analytes were eluted with 50 mL of n-hexane to remove the interference of PBDEs, and then with 200mL of 25% dichloromethane/n-hexane (v/v) for the PBDE fraction.

HRGC/HRMS Analysis Gas chromatographic mass spectrometric analyses of PBDEs and dioxins were accomplished using a Micromass Autospec Ultima magnetic sector high resolution mass spectrometer equipped with a Hewlett Packard 6890 PLUS gas chromatograph. Chromatographic separations were achieved using a DB-5 HT column for PBDEs, a BPX-DXN column for PCDD/Fs, and an HT-8 column for Co-PCBs.

Results and Discussion

PBDE levels in fish and shellfish in Tokyo Bay estuaries

The average concentrations of total PBDEs in fish and shellfish ranged from 0.17 to 1.9 ng/g (ppb) on a wet weight basis. (Table 1) These PBDE levels were higher than those levels of marine products purchased from markets and marine fish species collected.[3,4] The order of concentration of total PBDEs was short-necked clam < marbled sole < sea bass < striped mullet < conger eel, in relation to lipid content, whereas the order of PBDE concentration on a lipid weight basis was conger eel < short-necked clam < striped mullet < sea bass < marbled sole, which seemed to reflect the trophic level of the food web in Tokyo Bay, with the exception of marbled sole, which feed on benthos such as polychaetes.[5] The major congeners in all samples were tetraBDE-47, tetraBDE-49, pentaBDE-99, pentaBDE-100, and hexaBDE-154. The other congeners (BDE-71, 77, 85, 138) were determined to be below detection level. The profile of PBDE congeners in fish species was of similar pattern but was different from the profile in short-necked clam. The contribution of BDE-183 to short-necked clam was higher than that in fish species. It was considered that the profile of PBDEs in short-necked clam was attributed to that in seawater and tidal flat. (Figure1) The amount of daily consumption of fish in Tokyo was estimated to be 75.6 g by National Nutrition Survey.[6] In the case of conger eel, the daily intake of total PBDEs was estimated to be 0.0029μg/kg bw/day assuming an average body weight of 50 kg, without cooking, this value was considerably less than the lowest observed adverse effect level (LOAEL) of 1 mg/kg bw based on thyroid hormone effects.[7] And the highest daily intakes of BDE-47, BDE-99, and BDE-153 were estimated to be 0.0014, 0.0002, and 0.0001 μg/kg bw/day, respectively. Those values were less than the no observed adverse effect level (NOAEL) for BDE-47 of 0.7 mg/kg and for BDE-153 of 0.45 mg/kg based on effects in spontaneous behavior, LOAEL for BDE-99 of 0.06mg based on disruption of neurobehavioral development and NOAEL for BDE-99 of 0.06mg based on permanent effects on the rat male reproductive system.[8,9,10] Based on these results, the level of PBDEs in fish and shellfish from Tokyo Bay is not regarded as a serious health effect even if ingested. However, it is necessary to continue to monitoring the level of PBDEs in order to evaluate the potential risk to humans, because of its ubiquitous detection in the environment and bioaccumulation in fish and shellfish.

Table1. Average concentrations of PBDEs in fish and shellfish from Tokyo Bay estuaries (pg/g wet base)

congener	striped mullet (Bora n=8) lipid content 4.1%	sea bass (Suzuki n=8) lipid content 3.0%	conger eel (Anago n=8) lipid content 10.4%	marbled sole (Makogarei n=8) lipid content 0.8%	short-necked clam (Asari n=6) lipid content 1.0%
BDE-28	74	69	87	12	6
BDE-49	75	121	223	28	17
BDE-47	794	483	928	210	70
BDE-66	15	37	65	10	5
BDE-100	90	61	116	19	7
BDE-119	15	31	60	8	4
BDE-99	34	47	132	16	14
BDE-154	78	114	202	38	19
BDE-153	24	32	68	15	13
BDE-183	3	1	2	1	18
ΣPBDEs	1,202	995	1,885	357	173
lipid base	29,497	33,745	18,081	47,607	18,223

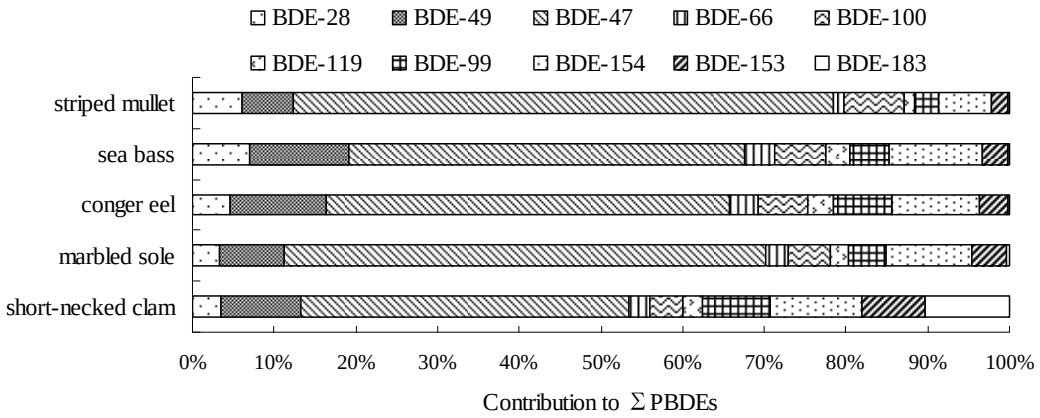

Figure 1. Comparison of PBDE congeners in fish and shellfish from Tokyo Bay estuaries

Distribution of PBDEs in fish tissues

The distribution of PBDE levels in fish tissues (sea bass and striped mullet, but there was no fat body in the analyzed striped mullet) was studied. The percent distribution of PBDE congeners in each fish tissue was approximately of similar pattern, and the major congeners were BDE-47 (38-52%), BDE-154 (11-13%), BDE-100 (8-12%), BDE-49 (7-8%), and BDE-99 (6-7%), except for low contribution of BDE-47 in sea bass A liver. (Figure 2) Compared with the concentration of BDE-47 in each tissue, the highest level was present in fat body surrounding the digestive tract, followed by the liver, spleen, and digestive tract. (Figure 3) It was confirmed that BDE-47 tends to accumulate in highly lipophilic tissues, and the high accumulation characteristics of PBDE was shown in the liver and spleen with the exception of low concentration of BDE-47 in striped mullet spleen. Further examination is necessary to explain the difference of the BDE-47 concentrations in the spleen of striped mullet and sea bass, although there was a similar report on the metabolism and distribution of ^{14}C-BDE-47 in an autoradiography study on pike exposed to ^{14}C-BDE47.[11] The concentration of BDE-47 was significantly correlated with that of PCB-118, 2,3,7,8-TCDD, and 2,3,7,8-TCDF in each tissue of sea bass. (Figure 4) It is necessary to consider that BDE-47 is formed by debromination of other PBDE congeners, [12] however the accumulation behavior of BDE-47 seem to be similar to that of these compounds in fish tissues from Tokyo Bay estuary.

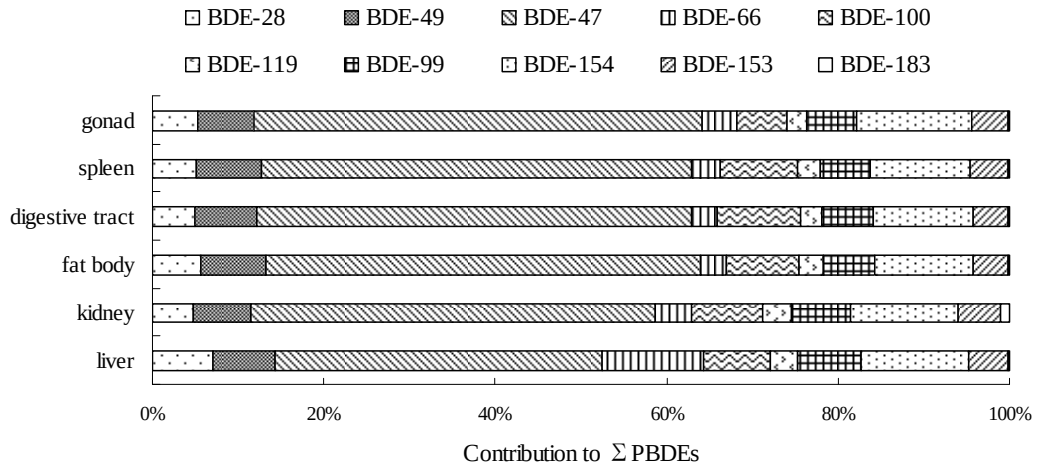

Figure 2. Profile of PBDE congeners in sea bass tissues from Tokyo Bay estuaries

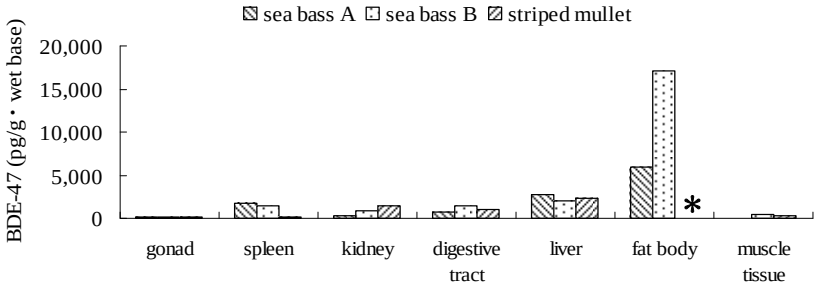

Figure 3. Distribution of BDE-47 in fish tissues from Tokyo Bay estuaries
(* Fat body was not available in the analyzed striped mullet)

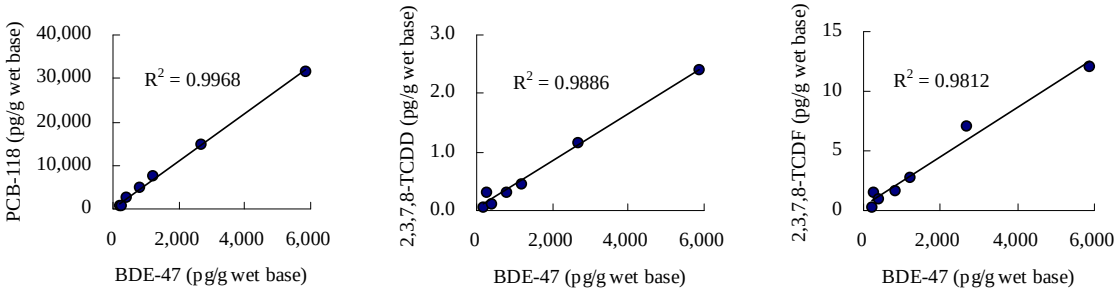

Figure 4. Relationship of the concentrations between BDE-47 and PCB-118, 2,3,7,8-TCDD, 2,3,7,8-TCDF in sea bass tissues

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