DETERMINATION OF POLYBROMINATED DIBENZO-*p*-DIOXINS, Co-PXBS AND BROMINATED FLAME RETARDANT IN FISH

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Abstract

The levels of brominated flame retardants (BFRs) and related compounds were determined in market fish from Japan. We collected 12 fish samples from 3 regions (Tyubu, Tyugoku-Shikoku and Kyushu) in Japan and analyzed brominated dioxins, polychlorinated/ brominated biphenyls (Co-PXBs), polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs) to determine levels of contamination. We found the brominated dioxins 2, 3, 7, 8 - TeBDD and 2, 3, 7, 8 - TeBDF in conger eels, but not at serious levels (total concentrations ranged from 0.009 to 0.015 pgTEQ/g ww). Mono-bromo polychlorinated dibenzo-*p*-dioxins/dibenzofurans (MoBrPCDD/DFs) and Co-PXBs were not detected in any samples. We detected PBDE congeners in all samples at 0.016 - 0.818 ng/g ww. The highest PBDEs concentration was found in conger eels and mackerel. For PBBs, we detected tetra-hexa brominated compounds in 9 samples at 0.105 - 2.24 pg/g ww, and the detected congeners were 2,2',5,5'-TeBB (#52), 2,2',4,5'-TeBB (#49), 2,2',4,5,5'-PeBB (#101), 2,2',4,4',6,6'-HxBB (#155) and 2,2',4,4',5,5'-HxBB (#153). The highest concentration was found in conger eels and mackerel, the same as the case with PBDEs. The most abundant congener was #155, followed by #52.

Introduction

Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs) have been widely used in plastics and textile coatings throughout the world. The major commercial products made with PBDEs were penta-BDE, octa-BDE and deca-BDE products. In Japan, although the usage of low brominated PBDEs has decreased, deca-BDE is currently in use. PBDEs are additives to polymers such as polystyrene and are not chemically bound to the polymer. Therefore, they are easily released into the environment from waste products. For PBBs, the commercial products are mixtures contain hexa-BB, octa-BB, nona-BB and deca-BB. Products made with PBBs have not been produced in Japan, but PBBs have been detected in environment samples in Japan[1)]. It is suspected that the contaminant came from imported products or impurities of other BFRs. Furthermore, *de novo* synthetic compounds related to BFRs, such as polybrominated dibenzo-*p*-dioxins, dibenzofurans (PBDD/DFs) and coplanar polychlorinated/brominated biphenyls (Co-PXBs) have been founded in market fish[2)3)] and human samples[4) 5)]. PBDD/DFs are pollutants generated by the manufacture of brominated flame retardants (BFRs) such as brominated diphenyl ethers (PBDEs) and are formed by the combustion of substances containing BFRs. Although the toxicity of these brominated dioxins is unclear, some studies have shown that the toxicity of 2, 3, 7, 8-TBDD is comparable to that of 2, 3, 7, 8-TCDD [6)]. Co-PXBs may also be formed from BFRs and have toxicity similar to that of Co-PCBs due to their similarity of structures.

It is important that we investigate levels of these brominated organic compounds in foods and estimate the influence on humans. Fish samples should take precedence over other food samples because they seem to be major contributors to dietary exposure of persistent compounds. In the present study, we analyzed brominated dioxins, Co-PXBs, PBDEs and PBBs in fish samples from 3 regions (Tyubu, Tyugoku-Shikoku and Kyushu) in Japan.

Materials and Methods

Sampling.

Table 1 shows fish samples analyzed in this study and their details. The fish samples were purchased from fish markets in each of 3 regions (Tyubu, Tyugoku-Shikoku and Kyushu) in Japan from 2007 to 2008. The edible parts of fish samples were blended using a food processor. The food mixtures were kept below -20˚C until analysis.

Analytical Methods and Instrumentation.

The PBDD/DFs (tetra-octa) and Co-PXBs (4'-Br-2,3',4,5-TeCB, 4'-Br-2,3,3',4-TeCB, 4'-Br-3,3',4,5-TeCB, 4'-Br-2,3,3',4,5-PeCB, 4'-Br-3,3',4,5,5'-PeCB, 3',4',5'-Br-3,4-DiCB) analytical standards were purchased from Cambridge Isotope Laboratories (Cambridge, MA). The PBDEs (tri-deca) analytical standards were purchased from Wellington Laboratories (Guelph, Ontario). The PBBs (tri-deca) analytical standards were purchased from Wellington Laboratories and AccuStandard, Inc. (New Haven, CT). Dichloromethane, *n*-hexane and toluene used for extraction and cleanup were of dioxins analysis grade (Kanto Chemicals, Tokyo). Silica gel (Wako Pure Chemical Industries, Ltd., Tokyo) was heated for 3 h at 130˚C. Florisil (Kanto Chemicals) was heated for 3 h at 130˚C and deactivated with 1% water.

The concentrations of brominated dioxins, Co-PXBs, PBDEs and PBBs in fish samples were determined using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). The analytical conditions of HRGC/HRMS are shown in Table 2. For measurements of PBDEs, PBBs and Co-PXBs, SLB-5MS was used for the GC column, and satisfactory separations and intensities were obtained. For measurements of brominated dioxins, especially for high brominates congeners, satisfactory intensities were obtained using DB-5.

Table 1 Details of fish samples

No.	Fish	Place of production	Sample type	Number of fish pooled	Approx. length of fish (cm)	Approx. weight of fish (g)
1	sea bream-1	Tyubu	nature	1	42	1250
2	sea bream-2	Tyubu	nature	1	43	1300
3	sea bream-3	Tyugoku-Shikoku	nature	1	43	1060
4	sea bream-4	Kyushu	nature	2	33	664
5	conger eel-1	Tyubu	nature	7	35	102
6	conger eel-2	Tyugoku-Shikoku	nature	9	43	120
7	horse mackerel	Kyushu	nature	4	32	360
8	sand borer	Tyugoku-Shikoku	nature	10	22	89
9	mackerel	Kyushu	nature	3	34	573
10	sardine	Kyushu	nature	28	16	47.5
11	shrimp	Kyushu	nature	58	9.3	9.7
12	flatfish	Tyugoku-Shikoku	nature	3	28	313

Table 2 Analytical conditions of HRGC/HRMS

	Column	Injection temp.	Injection type /volume	Oven temp.	HRMS conditions
PBDD/DFs MoBrPCDD/DFs	DB-5 (Agilent) 30 m, 0.25 mm (i.d.), 0.1 μm film	280˚C	Splitless 1 μL	130˚C - (20˚C/min) - 240˚C - (5˚C/min) 320˚C (7.5 min)	Electron energy 38eV Filament current 750 μA Ion source temp. 270˚ C Resolution 10,000
PBDEs	SLB-5MS (SUPELCO) 30 m, 0.25 mm (i.d.), 0.1 μm film	260˚C	Splitless 1 μL	125˚C (1 min) - (20˚C/min) - 200˚C - (10˚C/min) - 330˚C (5.2 min)	
PBBs	SLB-5MS (SUPELCO) 30 m, 0.25 mm (i.d.), 0.1 μm film	280˚C	Splitless 1 μL	120˚C (1 min) - (20˚C /min) - 300˚C (8 min)	
Co-PXBs	SLB-5MS (SUPELCO) 30 m, 0.25 mm (i.d.), 0.1 μm film	280˚C	Splitless 1 μL	120˚C (1 min) - (20˚C/min) -200˚C (10 min) - (10˚C/min) - 330˚ C (2 min)	

Sample Preparation.

We analyzed the brominated dioxins, Co-PXBs, PBDEs and PBBs simultaneously using accelerated solvent extraction (ASE). Each 50 g sample was freeze dried using a model AD 2.0ES-BC (Virtis, Gardiner, NY) freeze dryer, and then dried samples were extracted with 10% (v/v) dichloromethane/*n*-hexane using an accelerated solvent extractor ASE300 (Dionex, Sunnyvale, CA). The temperature of extraction was 100˚C; the time was 10 min. Extracts were treated with sulfuric acid three times and applied to a silica gel column. The column was prewashed with 100 mL *n*-hexane, and PBDD/DFs and PBDEs were eluted with 150 mL of 10% (v/v) dichloromethane/*n*-hexane. The eluate was evaporated and dissolved in *n*-hexane. and then loaded onto a Florisil (5 g) column. The PBDEs, PBBs and Co-PXBs were obtained by elution with 150 mL of *n*-hexane (fraction 1), and the PBDD/DFs fraction was obtained by elution with 200 mL of 60% (v/v) dichloromethane/*n*-hexane (fraction 2). The fraction 1 was treated with a DMSO/*n*-hexane partition to remove the matrix and then concentrated to a final volume of approximately 25 µL. The fraction 2 was loaded on an active carbon column, which in advance had been washed with 50mL of 10% (v/v) dichloromethane/*n*-hexane, eluted with 200 mL of toluene. The fractions were concentrated to a final volume of approximately 15 µL, and these samples were analyzed by HRGC/HRMS.

Results and Discussion

We analyzed brominated dioxins (a total of 18 congeners of PBDD/DFs and MoBrPCDD/DFs), Co-PXBs (7 congeners), PBDEs (23 congeners) and PBBs (18 congeners) in 12 fish from 3 regions in Japan. In our study, the limits of detection (LODs) of PBDD/DFs were 0.01 pg/g ww for tetra and penta, 0.05 pg/g ww for hexa, 0.1 pg/g ww for hepta and 1 pg/g ww for octa. The LODs of PBDEs were 0.1 pg/g ww for tetra-hepta, 0.2 pg/g ww for octa, 0.5 pg/g ww for nona and 1 pg/g ww for deca. TheLODs of Co-PXBs were 0.05 pg/g ww. The LODs of PBBs were 0.1 pg/g ww for tri-penta, 0.2 pg/g ww for hepta-nona and 0.5pg/g ww for deca.

Table 3 shows analyzed data for the total concentrations of brominated dioxins, Co-PXBs PBDEs and PBBs from fish samples in this study.

PBDD/DFs were detected in two samples of conger eel: 2,3,7,8-TeBDF was detected in conger eel-1 at 0.09 pg/g ww, and 2,3,7,8-TeBDD and 2,3,7,8-TeBDF were detected in conger eel-2 at 0.01pg/g ww and 0.05 pg/g ww, respectively. MoBrPCDD/DFs congeners were not detected in any fish. Regarding the estimation of toxicities by brominated dioxins, the World Health Organization stated that using the same TEF values for PBDD/DF or PXDD/DF congeners as the chlorinated analogues appears to be justified. Using TEFs of chlorinated dioxins, we calculated the concentrations of total brominated dioxins in these fish to be 0.009 - 0.015 pgTEQ/g ww. For Co-PXBs, it is reasonable to calculate TEQ values using TEFs of Co-PCBs in the same way as brominated dioxins. However, no Co-PXB congeners were detected from fish samples in this study.

The amount of daily fish consumption by an average Japanese person was estimated to be 82 g in an investigation conducted by the Ministry of Health, Labour and Welfare of Japan. Under this assumption, the daily intakes from fish in the case of 50 kg of body weight were calculated to be 0.015-0.025 pgTEQ/kg/day, estimated to be within Japanese TDI (4 pg TEQ/kg body weight/day).

PBDE congeners were detected in all samples at 0.016 - 0.818 ng/g ww. The most abundant congeners were #49, #154 and #209 (Figure 1). The highest PBDEs concentration was found in conger eel-1 at 0.818 ng/g ww, followed by mackerel at 0.617 ng/g ww and conger eel-2 at 0.414 ng/g ww. In our previous reports[3)], a high concentration of PBDEs was found in fatty fish, and these fish had a higher fat content compared with other fish.

For PBBs, tetra-hexa brominated compounds were detected in 9 samples at 0.105-2.24 pg/g ww. The highest concentration was found in conger eels and mackerel, the same as the case with PBDEs. The levels of total PBBs were much lower than total PBDEs, however. The detected congeners were 2,2',5,5'-TeBB (#52), 2,2',4,5'-TeBB (#49), 2,2',4,5,5'-PeBB (#101), 2,2',4,4',6,6'-HxBB (#155) and 2,2',4,4',5,5'-HxBB (#153). The most abundant congener was #155, followed by #52 (Figure 2). In the reports about PBBs in Japan, #153 was mainly detected in incinerable waste, bulky waste, automobile shredder residue and office dust, and it was suspected that the imported PBBs or products containing PBBs were the sources[1)]. Interestingly, the conger patterns of PBBs in fish seem to be different from those of environment samples. More information about food or environment samples must be collected.

In a recent report, the lowest observed adverse effect level (LOAEL) value suggested as reasonable for compounds or mixtures belonging to the PBDE group was 1mg / kg body weight / day [7)]. For PBBs, it was suggested that the total daily intake should be less than 0.15 µg/kg/day, extrapolating from a no observed adverse effect level (NOAEL) of a positive carcinogenicity study, using an uncertainty (safety) factor of 1000[8)]. The estimated intake values from fish with maximum concentration of PBDEs and PBBs in this study were 1.34 ng/kg/day and 3.67 pg/kg/day, respectively (assuming daily fish consumption is 82 g). Compared with these values, the levels of these brominated compounds in fish were not considered a serious problem. However, it is important to collect more data about brominated dioxins and BFRs in food, because little information is available regarding the levels of these brominated compounds.

Table 3 Daily intake of brominated dioxins and PBDEs in Japan

No.	Fish	Fat content (%)	Brominated dioxins (pgTEQ/g ww)	Total Co-PXBs pg/g ww	Total PBDEs ng/g ww	Total PBBs pg/g ww
1	sea bream-1	0.48	ND	ND	0.100	0.230
2	sea bream-2	2.77	ND	ND	0.247	0.813
3	sea bream-3	0.42	ND	ND	0.018	ND
4	sea bream-4	0.19	ND	ND	0.016	0.105
5	conger eel-1	11.8	0.009	ND	0.818	2.24
6	conger eel-2	9.88	0.015	ND	0.406	1.83
7	horse mackerel	4.88	ND	ND	0.334	1.43
8	sand borer	0.60	ND	ND	0.095	0.299
9	mackerel	12.2	ND	ND	0.617	1.98
10	sardine	1.73	ND	ND	0.167	0.827
11	shrimp	0.12	ND	ND	0.033	ND
12	flatfish	1.10	ND	ND	0.044	ND

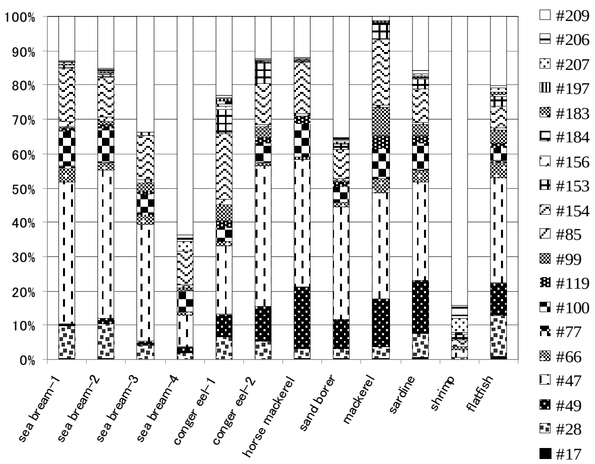

Figure 1 Congener patterns of PBDEs in fish

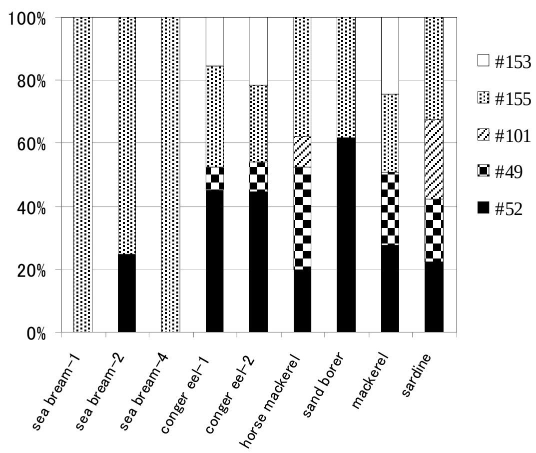

Figure 2 Congener patterns of PBBs in fish

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