PCB analyses: Samples (5–10 g) spiked with $^{13}C_{12}$-labelled internal standards were stirred with ethanolic KOH and then kept for 16 h at RT. The alkaline hydrolysates were added to water and extracted with *n*-hexane. The extracts were treated with concentrated sulphuric acid, and then purified on a silica gel column. The elute obtained with *n*-hexane was subjected to gel-permeation chromatography (GPC) using 5% cyclohexane/acetone. The fraction containing PCBs was concentrated and spiked with $^{13}C_{12}$-labelled recovery standards. The PCBs were quantified by HRGC/HRMS, and their determination was performed on an HT8-PCB column.

PBDE analyses: Samples (5–10 g) spiked with $^{13}C_{12}$-labelled internal standards were stirred with ethanolic KOH and then kept for 16 h at RT. The alkaline hydrolysates were added to water and extracted with *n*-hexane. The extracts were treated with concentrated sulphuric acid, and then purified on a silver nitrate/silica gel column. The elute obtained with *n*-hexane was subjected to GPC using acetone. The fraction containing PBDEs was concentrated and spiked with $^{13}C_{12}$-labelled recovery standards. The PBDEs were quantified by HRGC/HRMS, and their determination was performed using a DB-5HT and BP1 column.

Results and Discussion

The dioxin concentrations in the 30 products and the associated intakes are presented in Table 1. The dioxin concentrations varied significantly. Product no. 1, which was made from tiger shark liver (crude extract), had extremely high dioxin concentrations; however, most samples had levels below 10 pg-TEQ/g. These values were low compared with the dioxin concentrations in the source species. The fish oil purification processes could thus have effectively removed dioxins from the samples. Indeed, Hilbert and colleagues[6] reported that steam distillation, which is a refining process for fish oil, reduced the amounts of organochlorine contaminants, including PCBs.

The total dioxin intake from the most contaminated product reached 1,500 pg-TEQ/person/day, corresponding to 30 pg-TEQ/kg bw/day for an adult weighing 50 kg. This was about eight times higher than the tolerable daily intake (TDI) of dioxins (4 pg-TEQ/kg bw/day) set by the Japanese government in 1999[7]. The intakes from most products were under 10% of the TDI, although those of samples no. 2 and no. 3 corresponded to about 30 and 14%, respectively. The major contributors to the total TEQ were DL-PCBs, which accounted for more than 90% in the most contaminated sample. This was in agreement with previous reports on fish oil supplements[1,2,4].

We determined the dioxin concentrations in different batches of the same products and found no significant differences (Figure 1). Product no. 1 had the highest variation in dioxin concentrations between batches, although the ratio of the maximal/minimum dioxin concentrations was only about 2. The dioxin intakes from the four batches of product no. 1 ranged from 16 to 30 pg-TEQ/kg bw/day. Thus, if an individual consumed the product regularly over a long period, their daily dioxin intake would continuously exceed the TDI.

The four products with relatively high dioxin concentrations were also analyzed for PBDD/F, PXDD/F, PCB and PBDE (Table 2). PCBs and PBDEs were found in all samples, although their concentrations varied significantly between products. Two batches of product no. 1 had much higher concentrations of PCBs and PBDEs than the other products, with respective intakes of 32,000 to 57,000 ng/person/day and 480,000 to 670,000 pg/person/day. By contrast, PBDD/Fs and PXDD/Fs were detected much less often in all samples. Only one isomer, 2,3,7,8-TeBDF, was quantified in two products (nos. 3 and 6). The intakes of PBDD/Fs and PXCDD/Fs were calculated assuming that the levels of non-detected isomers were equal to half of their limits of detection (LODs). Overall, the intakes were much lower that those of dioxins. The dioxin-like toxicity of the PBDD/Fs and PXDD/Fs in fish oil supplements appeared to be negligible.

Thus, although rare, fish oil supplements may contain significantly high concentrations of dioxins, PCBs and PBDEs. Continuous monitoring of the levels of these compounds in fish oil supplements is therefore recommended.

Acknowledgements

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Table 1 Dioxin concentrations in individual fish oil supplements and associated intakes

Product no. [a]	Fish source [b]	Daily intake (g) [c]	Dioxin conc. (pg-TEQ/g) [d]			Dioxin intake [e] (pg-TEQ/person/day)
			PCDD/Fs	DL-PCBs	Total	
1	Tiger shark	3.17	37	450	480	1,500 (30)
2	Sardine	4.80	4.2	7.7	12	58 (1.2)
3	Lampern	2.84	2.6	7.8	10	28 (0.57)
4	Cod	2.00	< 0.10	8.4	8.4	17 (0.34)
5	Gulper shark	2.91	0.51	2.8	3.3	10 (0.19)
6	Tuna	1.25	< 0.10	6.5	6.6	8.3 (0.17)
7	Gulper shark	3.15	1.3	0.65	1.9	6.0 (0.12)
8	Lampern etc.	2.10	0.58	1.6	2.2	4.6 (0.092)
9	Sardine	1.53	0.17	2.4	2.5	3.8 (0.077)
10	Gulper shark	1.28	1.3	0.64	2.0	2.6 (0.051)
11	Herring, sardine	2.84	< 0.10	0.5	0.56	1.6 (0.032)
12	NS	3.60	0.11	0.22	0.32	1.2 (0.023)
13	Tuna	1.76	< 0.10	0.45	0.46	0.81 (0.016)
14	Lampern etc.	1.32	0.13	0.43	0.56	0.74 (0.015)
15	Sardine	2.70	< 0.10	0.17	0.25	0.68 (0.014)
16	NS	2.82	< 0.10	0.17	0.18	0.51 (0.010)
17	NS	2.23	< 0.10	0.12	0.20	0.45 (0.0089)
18	Sardine etc.	2.67	< 0.10	0.11	0.14	0.37 (0.0075)
19	Gulper shark	1.52	< 0.10	0.16	0.16	0.24 (0.0049)
20	Tuna, sardine	1.80	< 0.10	< 0.10	0.10	0.18 (0.0036)
21	NS	1.66	< 0.10	< 0.10	< 0.10	0.11 (0.0023)
22	NS	1.16	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
23	NS	1.76	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
24	NS	1.40	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
25	NS	1.28	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
26	Tuna etc.	3.87	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
27	Tuna etc.	2.10	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
28	Tuna etc.	2.19	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
29	NS	1.92	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
30	Tuna etc.	0.93	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)

[a] All samples except sample 4 (bottled) were capsule formulations.

[b] Unspecified fish oil content is expressed as "NS". Nine products (nos. 3, 8, 13, 14, 26, 27, 28, 29 and 30) were a mixture of fish oils and vegetable oils.

[c] Daily intakes of each product were calculated from the maximal recommended dosages on the product labels.

[d] Dioxin concentrations are presented on a whole weight basis. For encapsulated fish oil products, the entire samples, including capsules, were analyzed. The concentrations were calculated assuming that the non-detected isomers were equal to zero.

[e] Dioxin intakes in parentheses (pg-TEQ/bw kg/day) were based on a person weighing 50 kg.

Table 2 Summary of dioxins and other organohalogen compounds in the selected fish oil supplements

Product no. [a]	Dioxins [b]		PBDD/Fs+PXDD/Fs [b]		PCBs		PBDEs	
	Conc. (pg-TEQ/g)	Intake (pg-TEQ/ person/day)	Conc. (pg-TEQ/g)	Intake (pg-TEQ/ person/day)	Conc. (ng/g)	Intake (ng/ person/day)	Conc. (pg/g)	Intake (pg/ person/day)
1 (B)	510	1,600	0	0	18,000	57,000	210,000	670,000
	(510)	(1600)	(1.3)	(4.1)				
(D)	250	800	0	0	10,000	32,000	150,000	480,000
	(250)	(800)	(1.3)	(4.1)				
3 (D)	9.9	28	0.13	0.37	140	400	1,800	5,100
	(9.9)	(28)	(0.48)	(1.4)				
5	3.3	9.6	0	0	52	150	550	1,600
	(3.3)	(9.6)	(1.3)	(3.8)				
6 (D)	7.0	8.8	0.12	0.15	110	140	5,700	7,100
	(7.0)	(8.8)	(0.47)	(0.59)				

[a] Letters in parentheses indicate batch no. in Figure 1.

[b] Dioxin concentrations are presented on a whole weight basis. For encapsulated fish oil products, the entire samples, including capsules, were analyzed. The concentrations as well as intakes were calculated assuming that the non-detected isomers were equal to zero, and also calculated in parentheses assuming that non-detected isomers were equal to half of their LODs.

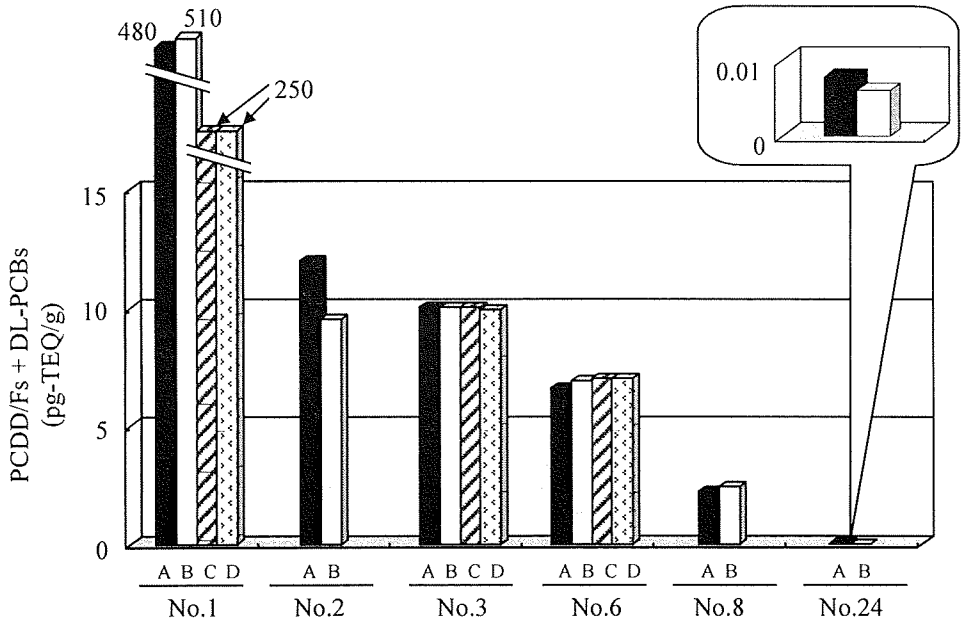

Figure 1 Batch differences in dioxin concentrations for several fish oil supplements
The same products, two to four batches, were purchased during 2002-2005. The samples designated "A" in each product are the same samples as in Table 1.

DAILY INTAKE OF BROMINATED DIOXINS AND POLYBROMINATED DIPHENYL ETHERS ESTIMATED BY MARKET BASKET STUDY

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Abstract

A market basket study of brominated dioxins and polybrominated diphenyl ethers (PBDEs) was performed to estimate daily intake levels of these compounds in Japan. We analyzed brominated dioxins and PBDEs in food mixtures from each of 13 food groups from 6 regions (Hokkaido, Tohoku, Kanto, Tyubu, Tyugoku-Shikoku and Kyushu) in Japan and calculated the daily intakes from food consumption. From the results of analyzing the brominated dioxins, only 1,2,3,4,6,7,8-HpBDF was detected in the mixture of group 4 (fats and oils) at 0.14-0.44 pg/g wb. To estimate the influence of brominated dioxins, we calculated the total TEQ per day, using TEFs of chlorinated dioxins. The mean daily intake was calculated at 0.00056 pg TEQ / kg body weight /day (assuming ND = 0). Due to the small daily consumption of fats and oils, the daily intake of brominated dioxins was at a low level. For PBDEs, the mean daily intake was calculated at 2.17ng / kg body weight /day (assuming ND = 0). Since the estimated value in this study was much less than LOAEL (1mg / kg / day), the daily intake level of PBDEs was not considered a serious problem.

Introduction

Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA), and hexabromocyclododecane (HBCD) have been widely used in plastics and textile coatings throughout the world. For PBDEs, although the usage of low brominated PBDEs has decreased, DeBDE is currently in use. PBDEs are additives to polymers such as polystyrene and are not chemically bound to the polymer. Therefore, it is considered that they are easily released into the environment from waste products. Furthermore, polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/DFs) are pollutants generated by the manufacture of brominated flame retardants (BFRs) such as brominated diphenyl ethers (PBDEs) and are formed by 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 [1]. In a recent report, PBDD/DFs and PBDEs have been detected in human adipose tissue in Japan [2]. Therefore, it is necessary to investigate levels of these brominated organic compounds in several foods and to estimate the influence they have on a daily intake level.

A market basket study is a useful method for estimating the average intake level in regions, based on a model of the average domestic diet. It is possible to provide information for the daily intake of food groups, such as rice, fruits, vegetables, fish and meat. In the present study, we analyzed brominated dioxins and PBDEs in food mixtures from each of 13 food group from 6 regions (Hokkaido, Tohoku, Kanto, Tyubu, Tyugoku-Shikoku and Kyushu) in Japan and estimated daily intake levels of brominated dioxins and PBDEs.

Materials and Methods

Sampling.

Table 1 shows the food groups analyzed in this study and their mean daily consumption for 6 regions as calculated from the data of the Japanese Nutrition Survey carried out by the Ministry of Health, Labour and Welfare. For a market basket study, 120-200 kinds of foods were purchased from markets in each of 6 regions (Hokkaido, Tohoku, Kanto, Tyubu, Tyugoku-Shikoku and Kyushu) from 2004 to 2005. These foods were divided into 13 food groups, and weighed and cooked based on the daily consumption data of each region. Then, they were blended in a food processor. The food mixtures were prepared and analyzed for groups 10, 11 and 12 (n=2) and other groups (n=1). The food mixtures were kept below –20°C until analysis.

Analytical Methods and Instrumentation.

The concentrations of PBDD/DFs and PBDEs in the food mixtures were determined using high-resolution gas chromatography / high-resolution mass spectrometry (HRGC/HRMS). The analytical conditions of HRGC/HRMS are shown in Table 2. The PBDD/DFs (tetra-octa) analytical standard was purchased from Cambridge Isotope Laboratories (MA). The PBDE analytical standard was purchased from Wellington Laboratories (Ontario). 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 3h at 130°C. Florisil (Kanto Chemicals, Tokyo) was heated for 3h at 130°C and deactivated with 1% water. Further information about analytical methods and instrumentation is described in our previous article [3].

Sample Preparation.

The analytical method for the PBDD/DFs and PBDEs was as follows. Each 50g of food mixture for the market basket study was freeze dried using a model AD 2.0ES-BC (Virtis, NY) freeze dryer. Dried samples were extracted with 10% (v/v) dichloromethane / n-hexane by accelerated solvent extractor ASE300 (Dionex, 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 mixture for group 4 was dissolved in 100ml n-hexane and purified by sulfuric and the silica gel column in the same way. The column was prewashed with 100ml n-hexane, and PBDD/DFs and PBDEs were eluted with 150ml of 10% (v/v) dichloromethane / n-hexane. The eluate was evaporated and dissolved in n-hexane. It was then loaded onto a Florisil (5 g) column. The PBDEs fraction was obtained by elution with 150 ml of n-hexane, and the successive PBDD/DFs fraction was obtained by elution with 200 ml of 60% (v/v) dichloromethane /n-hexane. The PBDEs fraction was treated with a DMSO /n-hexane partition to remove the matrix. The PBDD/DFs fraction was further loaded on an active carbon column, which in advance was washed with 50 ml of 10% (v/v) dichloromethane /n-hexane, eluted with 200 ml of toluene. Both fractions were concentrated to a final volume of approximately 50µl, and these samples were analyzed by HRGC/HRMS.

Table 1 Daily consumption of food (13 groups) in 6 regions of Japan

No.	Food group	Daily consumption (g)*	Ratio (%)*
1	Rice and rice products	360 (333 -382)	17.8 (15.6-20.0)
2	Cereals seeds and potatoes	172 (151-190)	8.5 (7.0-9.7)
3	Sugars and confectioneries	32.0 (27.7-36.3)	1.6 (1.3-1.8)
4	Fats and oils	10.8 (9.0-12.5)	0.5 (0.4-0.6)
5	Pulses	56.3 (43.9-64.2)	2.8 (2.2-3.1)
6	Fruits	135 (124-152)	6.7 (6.3-7.1)
7	Green vegetables	95.0 (81.4-112)	4.7 (4.1-5.2)
8	Other vegetables and sea weeds	203 (181-215)	10.1 (9.2-11.2)
9	Beverages	496 (390-587)	24.4 (20.4-27.4)
10	Fish and shellfish	97.7 (82.2-120)	4.8 (4.2-6.1)
11	Meat and eggs	110 (105-116)	5.4 (5.1-5.7)
12	Milk and dairy products	166 (147-194)	8.3 (7.1-9.8)
13	Other foods (seasoning)	88.4 (78.0-112)	4.4 (4.1-5.2)
	Total	2020 (1910-2150)	

*Mean and range in 6 regions obtained from the data of Japanese Nutrition Survey (the Ministry of Health, Labour and Welfare of Japan).

Results and Discussion

We analyzed brominated dioxins and PBDEs in food mixtures from each of 13 food groups from 6 regions in Japan. In our study, the LODs (Limit of Detection) of PBDD/DFs were 0.01 pg/g wb for tetra and penta, 0.05 pg/g wb for hexa, 0.1 pg/g wb for hepta and 1 pg/g wb for octa. The LODs of PBDEs were 0.1 pg/g for tetra-hepta, 0.2 pg/g for octa, 0.5 pg/g for nona and 1 pg/g for deca.

From the results of analyzing brominated dioxins, only 1,2,3,4,6,7,8-HpBDF was detected in the mixture of group 4 (fats and oils) at 0.14-0.44 pg/g wb. MoBrPCDD/DFs congeners were not detected in any food mixtures.

Table 3 shows data for the daily intakes calculated from the concentration of brominated dioxins and PBDEs in each food group. The daily intake was estimated assuming that when a congener was below the limit of detection, the concentration was either equal to zero (ND=0) or one-half of LOD. The WHO has stated that use of the same TEF values for the PBDD/PBDF or PXDD/PXDF congeners as the chlorinated analogues appears to be justified. To estimate the influence of brominated dioxins, we calculated the total TEQ per day, using the TEFs of chlorinated dioxins. The mean daily intake was calculated as 0.00056 pg TEQ /kg body weight /day on a 50kg body weight (assuming ND = 0). Due to the small daily consumption of fats and oils, the daily intake of brominated dioxins was at a low level. In the case assuming that ND = 1/2LOD, the mean daily intake was calculated as 1.58 pg TEQ /kg body weight /day. In an investigation of chlorinated dioxin by a market basket study in Japan[4], the amount of daily intake was 1.2 pg TEQ / kg body weight /day. Even if the value of PBDD/DFs is added to the amount of chlorinated dioxin exposure, it was estimated to be within Japanese TDI (4 pg TEQ / kg body weight /day).

PBDE congeners were detected in all food mixtures. The highest PBDEs concentration was found in group 4 at 2110 pg/g wb (1190 - 3090 pg/g wb), followed by group 10 at 474 pg/g wb (237 - 840 pg/g wb). On the other hand, the concentrations of PBDEs in groups 7, 8, and 9 were at low levels. In a recent market basket study in Spain[5], the highest concentration of total PBDEs (tetra-octa) was found in oils and fats (587.7 - 569.3 ng/kg wb), followed by fish and shellfish (333.9 - 325.3 ng/kg wb), meat products (109.2 - 102.4 ng/kg wb), and eggs (64.5 - 58.3 ng/kg wb). In a market basket survey of U.S. food [6], it was reported that levels of PBDEs (tri-deca) were highest in fish (median 1725 pg/g wb), then meat (median 283 pg/g wb), and daily products such as butter and margarine (median 31.5 pg/g wb). In these reports, a high concentration of PBDEs was found in fatty food groups, such as fish, oils and fats, and meat.

For PBDE, the mean daily intake was estimated as 109 ng /day (80.2 - 140 ng /day) assuming that ND = 0. The daily intakes in other countries were reported 90.5ng / day for U.K.[7] and 81.9-97.3 ng/day for Spain[5]. Although there were some differences in analyzing congeners between studies, the daily intake in this study was close to levels of these data. The daily intake contribution of PBDEs was 45.8% (group 10), 21.1% (group 4) and 8.4% (group 11). The results suggest that the most prominent source of PBDEs is attributed to fish.

The mean daily intake was calculated as 2.17 ng / kg body weight /day (1.6 - 2.8 ng / kg body weight /day) on a 50kg body weight (assuming ND = 0). In the case assuming that ND = 1/2 LOD, the daily intake was calculated at 2.22 ng /kg body weight /day. 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 [8]. Since the calculated value in this study was much less than this LOAEL value, the daily intake level of PBDEs was not considered a serious problem. However, it is important to collect more data about brominated dioxin and BFRs in food because little information is available regarding the levels of these brominated compounds.

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Table 2 Analytical conditions of HRGC/HRMS

	Column	Injection temp.	Injection type Injection volume	Oven temp.	HRMS Conditions
PBDD/DFs MoBrPCDD/DFs	DB-5 (J&W Scientific, CA) 30m, 0.25mm(i.d.), 0.1μm film	240°C	Splitless 1μl	130° C - (20° C/min) -240 ° C - (5° C/min) 320° C(7.5min)	Electron energy 38eV Filament current 750μA
PBDEs	HP-5MS(Agilent Technology, CA) 15m,0.25mm(i.d.), 0.1μm film	240°C	Splitless 1μl	120° C (2min) - (20° C/min) -200 ° C - (10° C/min) 300° C (1min)	Ion source temp. 270° C Resolution 10,000

Table 3 Daily intake of brominated dioxins and PBDEs in Japan

	Food group	Brominated dioxins pgTEQ / day		PBDEs ng / day	
		ND=0*	ND=1/2LOD**	ND=0*	ND=1/2LOD**
1	Rice and rice products	0	15.4 (11.9-19.3)	4.4 (1.2-8.4)	5.0 (2.0-8.8)
2	Cereals seeds and potatoes	0	9.1 (5.9-14.2)	3.3 (2.3-4.2)	3.6 (2.6-4.3)
3	Sugars and confectioneries	0	1.4 (1.1-1.7)	2.2 (0.7-3.8)	2.2 (0.7-3.9)
4	Fats and oils	0.028 (0.013-0.045)	0.4 (0.3-0.5)	23.3 (10.7-35.2)	23.3 (10.7-35.2)
5	Pulses	0	2.3 (1.6-3.4)	1.5 (0.5-3.0)	1.5 (0.5-3.1)
6	Fruits	0	4.8 (4.5-5.3)	1.5 (0.05-6.3)	1.7 (0.3-6.4)
7	Green vegetables	0	3.4 (2.7-4.2)	0.6 (0.06-1.3)	0.7 (0.2-1.4)
8	Other vegetables and sea weeds	0	7.4 (6.6-8.4)	0.6 (0.06-1.4)	1.0 (0.5-1.7)
9	Beverages	0	17.3 (13.6-20.5)	1.2 (0.2-2.6)	1.9 (1.0-3.2)
10	Fish and shellfish	0	3.9 (2.9-5.3)	50.8 (26.7-75.4)	50.9 (26.8-75.4)
11	Meat and eggs	0	4.3 (3.1-4.9)	8.9 (5.8-13.5)	8.9 (5.9-13.5)
12	Milk and dairy products	0	5.8 (5.1-6.8)	2.7 (0.6-4.9)	2.9 (0.9-5.1)
13	Other foods (seasoning)	0	3.8 (2.7-8.2)	7.6 (1.6-16.5)	7.6 (1.7-16.5)
	total	0.028 (0.013-0.045)	79.2 (66.7-95.1)	109 (80.2-140)	111 (82.5-142)
	Daily intake***	0.00056 (0.00026-0.0009) pgTEQ/kg/day	1.58 (1.33-1.90) pgTEQ/kg/day	2.17 (1.60-2.79) ng/kg/day	2.22 (1.65-2.84) ng/kg/day

Mean daily intakes of 6 regions are given. Values in parentheses were ranges of the daily intake in 6 regions.
*Daily intake calculated assuming that ND = zero. ** Daily intake calculated assuming that ND = 1/2LOD.
*** Daily intake calculated in the case assuming that an average body weight of a Japanese adult is 50 kg.

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Research Article

Determination of brominated flame retardants and brominated dioxins in fish collected from three regions of Japan

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The concentrations of brominated dioxins which are polybrominated dibenzo-*p*-dioxins/polybrominated dibenzofurans (PBDD/DFs) and mono-bromo polychlorinated dibenzo-*p*-dioxins/dibenzofurans, polybrominated diphenyl ethers (PBDEs) and tetrabromobisphenol A (TBBPA) were investigated in a total of 45 fish samples collected from three regions in Japan. In the brominated dioxins, 1,2,3,4,6,7,8-heptabromodibenzofuran (HpBDF) was the most abundant congener, and it was found in seven fish samples at 0.10 – 25.6 pg/g wet weight (ww). The highest concentration of 1,2,3,4,6,7,8-HpBDF was found in the pike eel. Regarding other congeners, 2,3,7,8-tetrabromodibenzo-*p*-dioxin was detected in the sea bream at 0.02 pg/g ww, and 2,3,7,8-tetrabromodibenzofuran was detected in the conger eel at 0.03 pg/g ww. 3-Bromo-2,7,8-trichlorodibenzofuran was detected in the *Sardinella zunasi* and the conger eel at 0.01 pg/g ww and 0.02 pg/g ww, respectively. Using toxic equivalency factors of chlorinated dioxins, we calculated the PBDD/DFs concentrations of these fish samples at 0.001 – 0.256 pg TEQ/g ww. PBDEs were detected in all of the fish samples. The concentrations of total PBDEs were 0.01 – 2.88 ng/g ww. The seerfish and the yellowtail containd PBDEs in high concentrations. The most dominant congener in most of the fish was 2,2′,4,4′-tetrabromo diphenyl ether. TBBPA was detected in 29 fish samples at 0.01 – 0.11 ng/g ww. The mean level of TBBPA was about one-tenth or less of the total level of PBDEs. A good correlation was obtained between total PBDEs and fat content. On the other hand, no correlation was obtained between TBBPA and fat content. The daily intakes from fish were estimated to be 0.58 ng/kg body weight (bw)/day for total PBDEs, 0.03 ng/kg bw/day for TBBPA, and 0.01 pg TEQ/kg bw/day for brominated dioxins in the case assuming that the average bw of a Japanese adult person is 50 kg and that the average fish consumption is 82 g/day. For PBDEs, the provisionally calculated value was much less than the lowest observed adverse effect level value (1 mg/kg bw/day). For brominated dioxins, the daily intake was at a very low level compared with the Japanese daily intake of polychlorinated dioxins from fish. Even if the value of PBDD/DFs is added to the amount of chlorinated dioxin exposure, it was estimated that it is less than the tolerable daily intake (4 pg TEQ/kg bw/day) in Japan.

Keywords: Accelerated solvent extraction / Fish / Polybrominated dibenzo-*p*-dioxins, dibenzofurans / Polybrominated diphenyl ethers / Tetrabromobisphenol A

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1 Introduction

Brominated flame retardants (BFRs) have been widely used in plastics and textiles because of their low cost and superiority in flame retardation. The BFRs used most frequently in Japan are tetrabromobisphenol A (TBBPA), hexabromocyclododecane, and polybrominated diphenyl ethers

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Abbreviations: **BDE-47**, 2,2′,4,4′-tetrabromo diphenyl ether; **BFRs**, brominated flame retardants; **bw**, body weight; **DeBDE** decabromodiphenyl ether; **HpBDF**, heptabromodibenzofuran; **HR**, high resolution; **PBDD/DFs**, polybrominated dibenzo-*p*-dioxins, dibenzofurans; **PBDEs**, polybrominated diphenyl ethers; **PCB**, polychlorinated biphenyl; **TBBPA**, tetrabromobisphenol A; **TEFs**, toxicity equivalency factors; **TEQ**, toxicity equivalent quantity; **ww**, wet weight

(PBDEs). In particular, nearly 32 000 tons of TBBPA were consumed in 2001, an amount which is ten times greater than the consumption of decabromodiphenyl ether (DeBDE) in Japan [1]. Although the usage of low brominated PBDEs has decreased, DeBDE is currently in use. PBDEs are additives of polymers such as polystyrene and are not chemically bound to the polymer. Therefore, they are easily released into the environment from waste products. It is predicted that the amount of waste Br from the plastics used in electrical appliances will increase until at least 2020 due to the increasing size of TV sets in Japan [2]. This prediction suggests the urgent necessity for waste management and surveillance of these brominated compounds.

Furthermore, incineration of waste containing PBDEs may result in formation of polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/DFs). PBDD/DFs are known as pollutants generated by the manufacture of BFRs such as PBDEs, and they are formed by the combustion of substances containing BFRs [3, 4]. Although the toxicity of these brominated dioxins is unclear, some studies have shown that the toxicity of 2,3,7,8-tetrabromodibenzo-*p*-dioxin is comparable to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [5]. It is presently considered appropriate to use the toxicity equivalency factors (TEFs) of chlorinated dioxins for each structurally corresponding congener of PBDD/DFs [5].

The toxicity of BFRs also remains unclear, but some studies have indicated that PBDEs have the polychlorinated biphenyl (PCB)-like toxicity, so as to affect the thyroid hormone function [6, 7] and an estrogenic potency [8, 9] after metabolic conversion. Recent reports have shown that PBDEs have a developmental neurotoxic effect in mice and rats [6, 10–12]. TBBPA was developed as a relatively nontoxic flame retardant. However, studies revealing toxic effects of TBBPA *in vivo* or *in vitro* have been reported. TBBPA has also been shown to affect thyroid hormonal activity [13, 14], induce neurotoxicity [15], and exhibit endocrine-disrupting activity [16]. On the other hand, some reports have suggested that TBBPA is not highly toxic [17, 18], and the no observed adverse effect level in toxicity study on mice was reported as 700 mg/kg body weight (bw)/day [19].

In recent decades, some congeners of PBDEs have been detected in environmental samples including sediment [20–22], atmosphere [23], soil [24], and biota [21, 25–27]. In reports regarding human exposure, PBDEs have been detected in human adipose tissue [28], blood [29], and mother's milk [30] in Japan. Although several PBDD/DFs congeners have also been detected in environmental samples such as sediment [22], biota [31], and human adipose tissue [28], information regarding PBDD/DFs is slight compared with that regarding PBDEs. TBBPA was also found in serum samples from computer technicians in Sweden [32] and in human blood plasma in ng/g lipid weight range [33].

In Japan, Koizumi *et al.* investigated human exposure to PCB and PBDEs using stored biological samples (serum, *etc.*) throughout Japan from the early 1980s to mid-1990s [34]. These data suggest that PBDEs levels in serum increased during the 15-year period and that there is a geographic diversity of PBDEs exposure levels, which is probably caused by the wide variety of PBDE sources that include industrial and environmental ones. Ohta *et al.* [35] reported that the concentration of total PBDEs in the milk of Japanese women ranged between 668 and 2840 pg/g, and suggested that there was a strong positive relationship between PBDE concentrations in human milk and the dietary intake of fish and shellfish.

On the basis of several years of monitoring PBDEs in food, it has been suggested that the main route of human exposure via food is via animals with high fat content [36–38]. For example, fatty fish, meat and dairy products are major contributors to dietary exposure, similar to the case with PCB or dioxins. Fish and shellfish showed the highest concentration and contributed the most to PBDEs intake because of their relatively high consumption. These results suggest that attention should be paid to food, especially fish and meat.

In our previous study, we reported a method for simultaneously analyzing PBDEs and PBDD/DFs in food samples using accelerated solvent extraction [39], and we determined the levels of these brominated compounds in several marine products. In the present study, we determined levels of brominated dioxins and furans, PBDEs, and TBBPA in a total of 45 fish samples purchased at food markets in Japan in the three regions of Nagoya (N region), Seto Inland Sea (S region), and Kyushu (K region) from 2004 to 2005 in order to obtain information about the differences in pollution between the regions and the species of fish. Additionally, we estimated the daily intakes of brominated dioxins, PBDEs and TBBPA by an average Japanese adult.

2 Materials and methods

2.1 Sampling

To investigate the brominated dioxins and BFRs in fish, a total of 45 fish samples were collected from three Japanese regions from October 2004 to February 2005. The regions were Nagoya (N region, a commercialized and industrialized area), Seto Inland Sea (S region, an industrialized area), and Kyushu (K region, a less industrialized area). Figure 1 shows the location of the sampling sites. Table 1 shows details of the fish samples from the three regions. In each region, 15 pooled samples were prepared. Approximately 1 kg fish per sample were purchased from the markets to prepare sufficient amounts of edible parts for analysis. The edible parts of the fish were blended in a food processor, and the samples were kept below −20°C until analysis.

Table 1. Details of fish samples from three regions

Region	Fish	Sample type	Number of fish pooled	Approx. length of fish (cm)	Approx. weight of fish (g)
N region	Barracuda	Natural	5	31	234
	Horse mackerel	Natural	5	23	226
	Mackerel	Natural	2	34	638
	Mullet	Natural	2	46	1350
	Octopus	Natural	2	–	436
	Pacific flying squid	Natural	2	40	300
	Sand borer	Natural	15	17	46
	Sea bass	Natural	1	45	1230
	Sea bream-1	Cultivated	2	31	1070
	Sea bream-2	Cultivated	1	37	918
	Sea bream-3	Cultivated	1	38	1073
	Seerfish	Natural	1	67	2680
	Yellowtail	Cultivated	1	73	3000/half
	Young bass	Natural	2	41	775
	Young seerfish	Natural	2	40	555
S region	Black rockfish	Natural	7	24	214
	Conger eel	Natural	7	38	100
	Flatfish	Natural	6	26	159
	Hemiramph	Natural	14	32	68
	Horse mackerel	Natural	11	19	64
	Octopus	Natural	3	28	209
	Oyster	Cultivated	46	8	16
	Pike eel	Natural	–	–	771/slice
	Sardinella zunasi	Natural	35	11	11
	Sea bream-1	Natural	1	39	1000
	Sea bream-2	Cultivated	2	37	750
	Shrimp	Natural	34	15	18
	Sole	Natural	4	35	253
	Tuna	Natural	–	–	602 /slice
	Young seerfish	Natural	1	52	750
K region	Banded blue sprat	Natural	191	9	5
	Barracuda	Natural	5	30	237
	Calamary	Natural	2	37	247
	Conger eel	Natural	4	52	233
	Flounder	Natural	2	39	632
	Horse mackerel	Natural	3	30	314
	Mackerel	Natural	2	30	376
	Ribbonfish	Natural	–	–	–
	Sardine	Natural	20	15	31
	Scorpionfish	Natural	8	19	128
	Sea bream	Natural	4	23	265
	Shrimp-1	–	–	–	–
	Shrimp-2	Natural	16	16	37
	Sole	Natural	2	44	464
	Surfperch	Natural	7	20	156

2.2 Analytical methods and instrumentation

The concentrations of brominated dioxins which are PBDD/DFs and mono-bromo polychlorinated dibenzo-*p*-dioxins/dibenzofurans, PBDEs, and TBBPA in the samples were determined using high-resolution GC/high-resolution MS (HRGC/HRMS). The gas chromatograph was an HP6890 (Hewlett-Packard, CA) coupled to an Autospec Ultima (Micromass, UK). The analytical conditions of HRGC/HRMS are shown in Table 2. In order to avoid decomposition of high-brominated compounds, the injection temperature in the HRGC/HRMS method for brominated dioxins and PBDEs was set at a lower temperature, 260°C. The monitoring ions used in the HRGC/HRMS method for brominated dioxins are given in Table 3 and those for PBDEs are given in Table 4. For monitoring ions of TBBPA, 528.7296 (quantification) and 556.7609 (confirmation) were used for native TBBPA, and 540.7699 was used for $^{13}C_{12}$-labeled TBBPA.

Table 2. Analytical conditions of HRGC/HRMS

Compound	Column[a)]	Injection temp.	Injection type Injection volume	Oven temp.
PBDD/DFs MoBrPCDD/DFs[b)]	DB-5 30 m, 0.25 mm (id), 0.1 μm film	260 °C	Splitless 1 μL	130 °C–(20 °C/min) –240 °C–(5 °C/min)–320 °C (7.5 min)
PBDEs	HP-5MS 15 m, 0.25 mm (id), 0.1 μm film	260 °C	Splitless 1 μL	120 °C (2 min)–(20 °C/min)–200 °C–(10 °C/min)– 300 °C (1 min)
TBBPA	DB-5 30 m, 0.25 mm (id), 0.25 μm film	280 °C	Splitless 1 μL	120 °C (1 min)–(20 °C/min)–300 °C (8 min)

HRMS conditions: electron energy: 38eV; filament current: 750 μA; ion source temp.: 270 °C; resolution: 10000
a) Agilent Technology, CA
b) mono-bromo polychlorinated dibenzo-*p*-dioxins/dibenzofurans

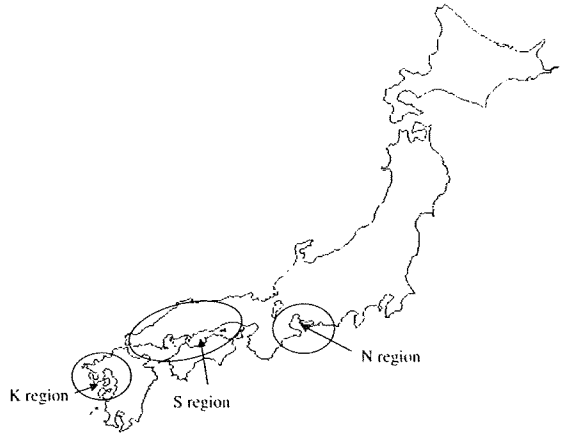

Figure 1. Location of sampling sites of Japan in this study.

The PBDD/DFs and TBBPA analytical standards were purchased from Cambridge Isotope Laboratories (MA). The PBDE analytical standards were purchased from Wellington Laboratories (Ontario). Dichloromethane, *n*-hexane, and toluene used for extraction and cleanup were of dioxin-analysis grade (Kanto Chemicals, Tokyo). DMSO was of spectrochemical analysis grade (Wako Pure Chemical Industries, Tokyo). Silica gel (Wako Pure Chemical Industries) was heated for 3 h at 130°C. Florisil (Kanto Chemicals) was heated for 3 h at 130°C and deactivated with 1% water. Further information about our analytical methods and instrumentation can be found in our previous article [39].

2.3 Sample preparation

The congeners of PBDEs and brominated dioxins were analyzed simultaneously. TBBPA was analyzed by a discrete method.

The method for analyzing PBDD/DFs and PBDEs was as follows. Each 100 g of the homogenized samples was freeze-dried using a model AD 2.0ES-BC (Virtis, NY) freeze dryer. Dried samples spiked with $^{13}C_{12}$-labeled standards were extracted with *n*-hexane by an accelerated solvent extractor ASE300 (Dionex, CA). The extraction temperature was 100°C, and the extraction 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. It was then loaded onto a Florisil (5 g) column. The PBDEs fraction was obtained by elution with 150 mL of *n*-hexane, and the successive PBDD/DFs fraction was obtained by elution with 200 mL of 60% v/v dichloromethane/*n*-hexane. The PBDEs fraction was treated by DMSO/*n*-hexane parti-

Table 3. Selected monitoring ions used in the HRGC/HRMS method for brominated dioxins

Compound	Ions (*m/z*)	
	Quantification	Confirmation
TeBDD	499.6904	497.6924
PeBDD	577.6009	579.5989
HxBDD	657.5094	655.5114
OcBDD	815.3282	813.3302
TeBDF	483.6955	481.6975
PeBDF	561.6060	563.6039
HxBDF	641.5145	639.5165
HpBDF	719.4248	721.4228
$^{13}C_{12}$-TeBDD	511.7307	–
$^{13}C_{12}$-PeBDD	589.6412	–
$^{13}C_{12}$-HxBDD	669.5496	–
$^{13}C_{12}$-OcBDD	827.3685	–
$^{13}C_{12}$-TeBDF	495.7357	–
$^{13}C_{12}$-PeBDF	573.6462	–
Mono-Br-TriCDD	365.8436	367.8410
Mono-Br-TeCDD	399.8045	401.8019
Mono-Br-PeCDD	435.7628	433.7655
Mono-Br-HxCDD	469.7237	467.7265
Mono-Br-HpCDD	503.6847	505.6819
Mono-Br-TriCDF	349.8487	351.8460
Mono-Br-TeCDF	383.8096	385.8070
$^{13}C_{12}$-Mono-Br-TeCDD	411.8448	–

TeBDD, 2,3,7,8-tetrabromodibenzo-*p*-dioxin; TeCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TeBDF, 2,3,7,8-tetrabromodibenzofuran

Table 4. Selected monitoring ions used in the HRGC/HRMS method for PBDEs

Compound	Ions (*m/z*)	
	Quantification	Confirmation
TriBDE	405.8027	407.8006
TeBDE	485.7111	483.7132
PeBDE	563.6216	565.6196
HxBDE	643.5301	641.5321
HpBDE	721.4406	723.4386
OcBDE	641.5145	639.5160
NoBDE	719.4250	721.4230
DeBDE	799.3335	797.3355
$^{13}C_{12}$-TriBDE	417.8429	–
$^{13}C_{12}$-TeBDE	497.7514	–
$^{13}C_{12}$-PeBDE	575.6619	–
$^{13}C_{12}$-HxBDE	655.5704	–
$^{13}C_{12}$-HpBDE	733.4809	–
$^{13}C_{12}$-OcBDE	653.5547	–
$^{13}C_{12}$-NoBDE	731.4652	–
$^{13}C_{12}$-DeBDE	811.3737	–

tioning to remove the matrix. The PBDD/DFs fraction was further loaded on an active carbon column, which in advance was washed with 50 mL of 10% v/v dichloromethane/*n*-hexane, and then eluted with 200 mL of toluene. Both fractions were concentrated to a final volume of approximately 50 μL, and these samples were analyzed by HRGC/HRMS (Table 2).

Simultaneously, *n*-hexane extracts from fish samples obtained by accelerated solvent extraction were evaporated under a vacuum below 40°C, and the residual compounds were measured gravimetrically as fat content.

Detection limits of brominated dioxins were 0.01 pg/g wet weight (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. Detection limits of PBDEs were 0.1 pg/g ww for tetra to octa, 0.2 pg/g ww for nona, and 1 pg/g ww for deca.

The method for analyzing TBBPA was as follows. A homogenized sample (5 g) spiked with $^{13}C_{12}$-TBBPA was extracted twice with 20 mL of methanol. The methanol extract was defatted by liquid-liquid partition with 20 mL *n*-hexane. Then, to the methanol layer, 120 mL of 5% sodium chloride solution was added and re-extracted twice with 25 mL of dichloromethane. The extract was concentrated to dryness, and then 1 mL of 1N potassium hydroxide/ethanol and 0.2 mL of diethyl sulfate were added, and the mixture was kept at 30°C for 30 min. Four milliliters of 1N potassium hydroxide/ethanol was added to the mixture, which was then maintained at 70°C for 1 h. Next, 3 mL of water was added, and the mixture was re-extracted twice with 10 mL of *n*-hexane. The *n*-hexane extract was cleaned up by Florisil mini-column chromatography using an eluant of 8 mL of 2% v/v diethyl ether/*n*-hexane. The final eluate was concentrated, re-dissolved in 20 μL of nonane with 2.5 ng of chrysene-d_{12} as a syringe spike, and subjected to measurement by HRGC/HRMS. The detection limit of TBBPA was 0.01 ng/g ww.

3 Results and discussion

Brominated dioxins (a total of 18 congeners of PBDD/DFs and mono-bromo polychlorinated dibenzo-*p*-dioxins/dibenzofurans), PBDEs (23 congeners), and TBBPA were analyzed in a total of 45 fish samples from the three regions of Nagoya (N), Seto Inland Sea (S), and Kyushu (K) in Japan. The levels of brominated dioxins, PBDEs and TBBPA in 45 fish samples from three regions are summarized in Table 5.

Brominated dioxins were only detected in eight fish samples from the S region. The most abundant congener was 1,2,3,4,6,7,8-heptabromodibenzofuran (HpBDF), and it was found in seven fish samples (conger eel, flat fish, pike eel, cultivated sea bream, natural sea bream, sole, and young seerfish) at 0.10–25.6 pg/g ww. The highest concentration of 1,2,3,4,6,7,8-HpBDF was found in the pike eel. Regarding other congeners, 2,3,7,8-tetrabromodibenzo-*p*-dioxin was detected in the sea bream at 0.02 pg/g ww, and 2,3,7,8-tetrabromodibenzofuran was detected in the conger eel at 0.03 pg/g ww. 3-Bromo-2,7,8-trichlorodibenzofuran was detected in the *Sardinella zunasi* and the conger eel at 0.01 pg/g ww and 0.02 pg/g ww, respectively. Because analytical standards of hepta- and octa-BDD/DF were difficult to be obtained commercially until three or four years ago, it was hard to analyze these compounds. Therefore, such determination data seems to be valuable. For estimation of toxicities by brominated dioxins, the World Health Organization stated that using the same TEF values for the PBDD/DF or PXDD/DF congeners as the chlorinated analogues appears to be justified [5]. Using TEFs of chlorinated dioxins, we calculated the concentrations of brominated dioxins in fish at 0.001–0.256 pg TEQ/g ww.

PBDEs were detected in all the fish samples. The concentrations of total PBDEs were 0.01–2.88 ng/g ww. The seerfish and the yellowtails contained PBDEs at high concentrations. The most dominant congener was 2,2′,4,4′-tetrabromo diphenyl ether (BDE-47) in most of the fish, as seen in Fig. 4. The means of total PBDEs were 0.75 ng/g ww (0.02–2.88 ng/g ww) for N region, 0.16 ng/g ww (0.01–0.53 ng/g ww) for S region, and 0.15 ng/g ww (0.01–0.70 ng/g ww) for K region. TBBPA was detected in 29 fish samples at 0.01–0.11 ng/g ww. The means of TBBPA were 0.01 ng/g ww (ND–0.04 ng/g ww) for N region, 0.01 ng/g ww (ND–0.10 ng/g ww) for S region, and 0.02 ng/g ww (ND–0.11 ng/g ww) for K region. The detection rates were 53.3% for both the S region and N region and 86.7% for K region. The mean level of TBBPA equaled about one tenth or less of the total PBDEs. In all the samples, the levels of PBDEs were found to be higher than those of TBBPA, despite the high industrial consumption of TBBPA. This may be related to the fact that PBDEs are bio-

Table 5. Summary of results on the pollutant levels in fish samples from three regions of Japan

Region	Fish	Fat content (%)	Total PBDE[a] ng/g ww	Brominated dioxins[b] pgTEQ/g ww	TBBPA ng/g ww
N region	Barracuda	4.50	0.53	ND	0.01
	Horse mackerel	4.72	0.66	ND	ND
	Mackerel	13.65	1.77	ND	ND
	Mullet	1.69	0.25	ND	ND
	Octopus	0.35	0.02	ND	0.03
	Pacific flying squid	1.19	0.06	ND	ND
	Sand borer	0.46	0.03	ND	ND
	Sea bass	0.72	0.33	ND	ND
	Sea bream-1	8.12	0.68	ND	0.01
	Sea bream-2	9.36	0.42	ND	0.03
	Sea bream-3	4.10	0.19	ND	0.01
	Seerfish	11.27	2.88	ND	0.04
	Yellowtail	17.28	2.81	ND	ND
	Young bass	0.98	0.18	ND	0.04
	Young seerfish	1.30	0.41	ND	0.01
	Mean	5.31	0.75	ND	0.01
S region	Black rockfish	0.50	0.12	ND	0.01
	Conger eel	12.65	0.31	0.007	0.10
	Flatfish	0.35	0.03	0.004	0.03
	Hemiramph	0.92	0.11	ND	ND
	Horse mackerel	2.28	0.12	ND	ND
	Octopus	0.26	0.02	ND	0.01
	Oyster	2.26	0.05	ND	ND
	Pike eel	3.40	0.31	0.256	ND
	Sardinella zunasi	4.53	0.53	0.001	ND
	Sea bream-1	1.10	0.05	0.017	0.02
	Sea bream-2	7.11	0.30	0.003	0.01
	Shrimp	0.49	0.01	ND	ND
	Sole	0.35	0.02	0.001	0.02
	Tuna	0.51	0.04	ND	0.01
	Young seerfish	1.91	0.41	0.002	ND
	Mean	2.57	0.16	0.019	0.01
K region	Banded blue sprat	1.82	0.09	ND	ND
	Barracuda	9.88	0.70	ND	0.04
	Calamary	0.38	0.17	ND	0.01
	Conger eel	7.52	0.11	ND	0.03
	Flounder	0.30	0.04	ND	0.02
	Horse mackerel	5.67	0.28	ND	0.02
	Mackerel	20.45	0.30	ND	0.01
	Ribbonfish	0.33	0.11	ND	ND
	Sardine	0.74	0.13	ND	0.11
	Scorpionfish	0.37	0.05	ND	0.03
	Sea bream	1.01	0.05	ND	0.02
	Shrimp-1	1.02	0.05	ND	0.01
	Shrimp-2	0.19	0.01	ND	0.02
	Sole	1.42	0.08	ND	0.01
	Surfperch	3.93	0.06	ND	0.01
	Mean	3.67	0.15	ND	0.02
	Mean of 3 region	3.85	0.35	0.006	0.02

a) Total PBDEs include tri- to deca-PBDE monomers.
b) Brominated dioxins include PBDD/DFs (tetra- to octa-brominated dibenzo-*p*-dioxins and tetra- to hepta-brominated dibenzofurans) and MoBrPCDD/DFs (monobromo-polychlorinated dibenzo-*p*-dioxins/dibenzofurans).

magnified in an organism [40, 41], although TBBPA is easily metabolized and eliminated from the organism [14, 42].

Figure 2 shows the correlation between total PBDEs and fat content in fish from N region, S region, K region and the entire three regions. Although differences in PBDE concentrations between the three regions were expected, only a correlation between PBDE concentrations and fat contents was found in each region and the entire three regions using Spearman's rank correlation. Taking it into consideration

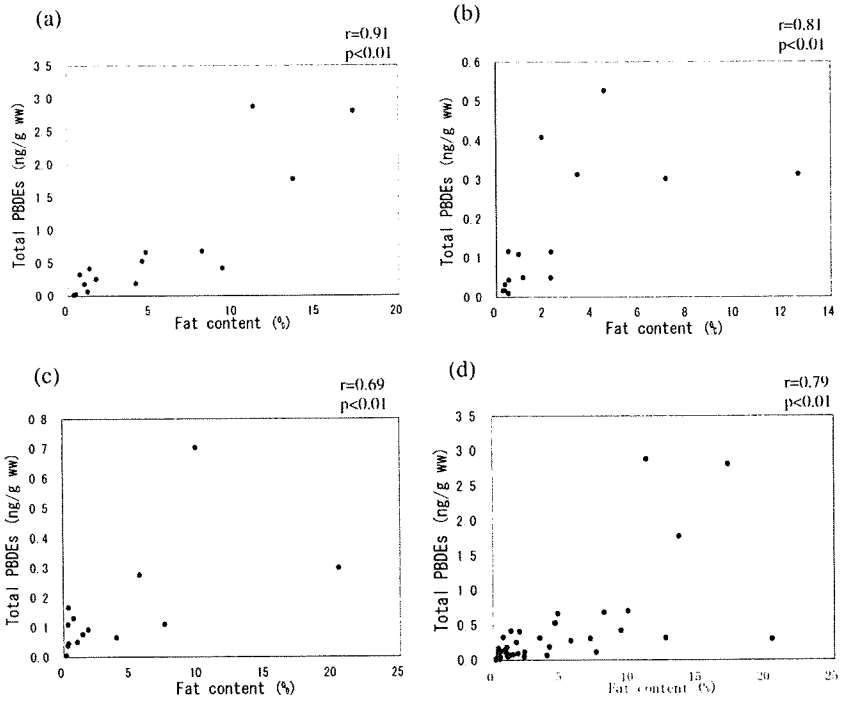

Figure 2. Correlation between total PBDEs and fat content in fish samples from (a) N region, (b) S region, (c) K region and (d) the entire three regions.

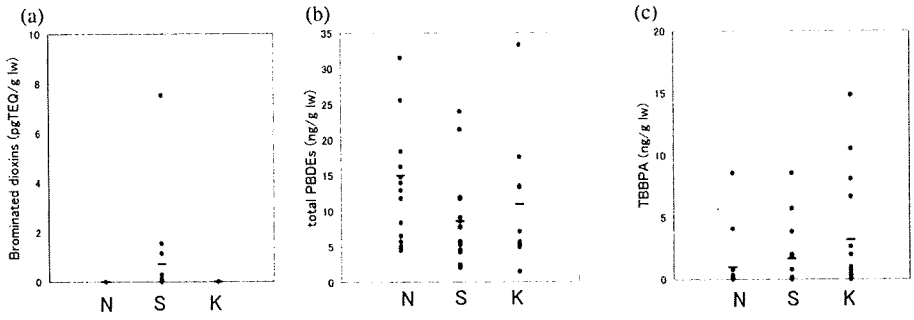

Figure 3. Levels of brominated compounds in fish samples from the three regions; bars indicate the means of concentrations in the regions: (a) Brominated dioxins; (b) total PBDEs; (c) TBBPA; N: N region, S: S region; K: K region.

that PBDEs are lipophilic compounds and ubiquitous pollutants like PCBs and organic chlorine pesticides, this result seems acceptable. As noted in some reports [35, 43], high concentrations of PBDEs were found in fish with a high fat content. On the other hand, no correlation was obtained between TBBPA and fat content in any region (data not shown). The chemical characteristics of phenolic structure and the rapid metabolic conversion of TBBPA are probably the reasons for no correlation with fat content.

Figure 3 shows a comparison of the levels of brominated compounds in fish samples from the three regions on a lipid weight basis. The mean level of total PBDEs in the fish samples from N region was higher than in the other regions. On the other hand, the mean level of TBBPA in the fish samples from K region was higher than that of fish samples from the other regions. For brominated dioxins, the congeners were detected for S region only. And 1,2,3,4,6,7,8-HpBDF was significantly detected as the most abundant congener. In a recent study, 1,2,3,4,6,7,8-HpBDF was found in the commercial flame retardant product of PBDE (octa- and deca-brominated mixtures), at concentrations ranging from 1242–4418 ng/g [44]. On the other hand, in an investigative report by the Japanese Ministry of the Environment, high concentration of 1,2,3,4,6,7,8-HpBDF was also detected in post-treatment effluent from production and processing facilities and from electrical appliance recycling facilities [45, 46]. In another investigation, the Japanese Ministry of the Environment found 1,2,3,4,6,7,8-HpBDF in the atmosphere of several urban districts in Japan [47]. It is not known whether the source of the 1,2,3,4,6,7,8-HpBDF

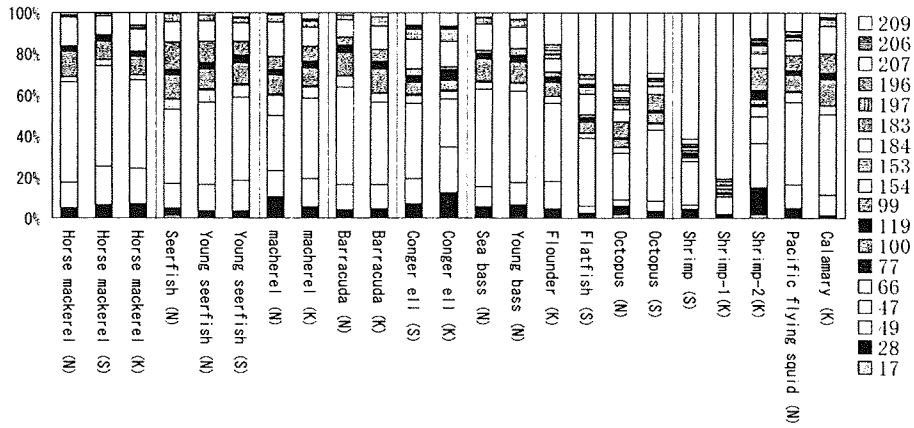

Figure 4. Congener patterns of PBDEs in fish samples. Letters in parentheses after sample names show regions; N: N region; S: S region; K: K region.

was plants, incineration of waste or some other source. However, since the S region is an industrialized area, it is possible that such contamination is caused from plant effluent. Contamination by these brominated compounds needs to be continuously monitored.

Among the data we gathered about PBDEs, the congener patterns were important information. Five congeners (BDE-28, -47, -99, -153, and -154) were detected in all of the samples. Figure 4 shows the congener patterns of PBDEs in nine species of fish. The most abundant congener was BDE-47 in most of the fish. Seerfish, mackerel, and barracuda have a very similar pattern of PBDEs. However, BDE-99 was barely found in horse mackerel, clearly different in this regard from the other fish. For all of flatfish, flounder, octopus, and shrimp, the contribution ratio of BDE-209 was relatively high. BDE-209 is the primary component of a commercial flame retardant of DeBDE. BDE-209 was a particularly significant congener in octopus and shrimp. Because of its high molecular weight, BDE-209 has been assumed to have low bioavailability and low bioaccumulatively [40]. Therefore, it was suspected that the finding of BDE-209 in shrimp and octopus was contributed by sediment particles in their gut. However, BDE-209 was also found in fish such as flounder and sea bass in Japan [43] and in organisms from both the Atlantic Ocean and the Baltic Sea [48]. These results show that its bioavailability is not negligible. Interestingly, in the pike eel in which 1,2,3,4,6,7,8,-HpBDF was detected, high levels of BDE-209, -206, and -207 were found at the relative ratio of 25, 10 and 10%, respectively. The commercial products of DeBDE are almost entirely composed of BDE-209, but there are small amounts of BDE-206 and -207 as PBDEs and of HpBDF and OBDF as brominated dioxins [44]. The congeners BDE-209, -206, and -207 are speculated to be closely related to 1,2,3,4,6,7,8-HpBDF, and we suggest that the finding of contamination in this study are related to the DeBDE commercial products.

Figure 5 shows the congener patterns of PBDEs in the sea bream samples (cultivated and natural). There were clear differences in PBDEs patterns. The ratios of BDE-47 in the cultivated sea bream were more than 50%, which were higher than those in the natural sea bream. On the other hand, the ratios of BDE-209 were remarkably higher in the natural sea bream. The concentrations of total PBDEs in the cultivatd sea bream were three times greater than those in the natural sea bream (Table 5). This difference in concentration seems to result from the difference in fat content. Feed used for cultivation is likely to be a source of the high PBDEs and fat content in cultivated fish.

The amount of daily fish consumption by an average person in Japan was estimated to be 82.2 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 bw were calculated to be 0.58 ng/kg bw/day for total PBDEs, 0.03 ng/kg bw/day for TBBPA, and 0.01 pg TEQ/kg bw/day for brominated dioxins. The lowest observed adverse effect level value suggested as reasonable for compounds or mixtures belonging to the PBDE group was 1 mg/kg bw/day [49], while the provisionally calculated value was much less than this lowest observed effect level value. For brominated dioxins, the daily intake was a very low level compared with the Japanese daily intake of polychlorinated dioxins from fish (1.33 pg TEQ/ kg bw/day) [50]. Even if the value of PBDD/DFs is added to the amount of chlorinated dioxin exposure, we estimate that it is less than the tolerable daily intake (4 pg TEQ/kg bw/day) in Japan. Based on these results, the PBDD/DFs contamination level in fish is not considered a serious problem.

4 Concluding remarks

In the present study, the levels of brominated dioxins, PBDD/DFs, PBDEs, and TBBPA were determined in the

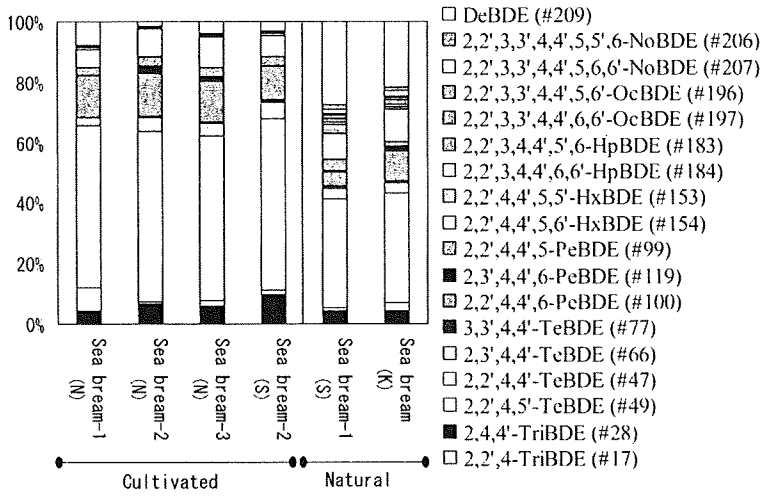

Figure 5. Congener patterns of PBDEs in cultivated and natural sea breams. Letters in parentheses after sample names show regions; N: N region; S: S region; K: K region.

fish samples from three regions in Japan, and the daily intake from fish was estimated. The levels of PBDEs were considered to depend on the fat content of fish, and the patterns of PBDEs were considered to depend on the species of fish and their feeding habits under either natural or farming conditions, rather than due to regional sources. Therefore, PBDE pollution would be now ubiquitous, which is similar to PCB pollution. For TBBPA, the detection rate was not so high compared with PBDEs. The result seems to support its low bioavailability and easy metabolization in the organism. Brominated dioxins, PBDD/DFs, were detected in the several fish in only one region and were limited in terms of what congeners were present. Therefore, it was suspected that the contaminants came from local pollution strongly affected by the regional sources.

It is important to collect more data about BFRts and brominated dioxins in food, because little information is available regarding the levels of these brominated compounds. In particular, information about hepta- and octa-BDD/DFs, octa-deca BDEs, and TBBPA is scarce in spite of the importance of their surveillance. As stated earlier, it is predicted that the amount of waste-related BFR will continue to increase. It is important to continue to perform studies on its toxicity, levels in the environment and food, and human exposure.

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The authors have declared no conflict of interest.

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PCB 118 and Aryl Hydrocarbon Receptor Immunoassays for Screening Dioxins in Retail Fish

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PCB 118 and Aryl Hydrocarbon Receptor Immunoassays for Screening Dioxins in Retail Fish

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The efficacy of a combination of two enzyme-linked immunosorbent assay (ELISA) kits was examined for screening the toxic equivalent (TEQ) concentrations of dioxins in retail fish. The coplanar PCB-EIA system, which is a competitive immunoassay specific for polychlorinated biphenyl (PCB) 118, was tested as a screening method for mono-*ortho* PCBs. The Ah immunoassay (Ah-I), which is an ELISA-based aryl hydrocarbon receptor binding assay, was analyzed for its screening ability for non-*ortho* PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs). Dilution and recovery tests using purified fish extracts revealed no major interference of the matrix in the PCB-EIA and suggested that the matrix effect was minimized in the Ah-I. Finally, the results for the fish samples (n = 20) showed a strong correlation between this method and high-resolution gas chromatography coupled to high-resolution mass spectrometry for the determination of the TEQ concentrations of mono-*ortho* PCBs (r = 0.99) and non-*ortho* PCBs and PCDD/Fs (r = 0.97). These data indicate that our method is suitable for screening retail fish to determine the TEQ concentrations of dioxins.

KEYWORDS: Dioxins; immunoassay; bioassay; Ah receptor; fish; screening

INTRODUCTION

Fishery products have been identified as the main source of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs)—collectively referred to as dioxins—in the Japanese diet (*1*, *2*). We previously carried out a nationwide survey of dioxin concentrations in various fishery products available on the Japanese market during the past few years (*3*–*5*) and found that fish often showed high toxic equivalent (TEQ) levels of dioxins. It is therefore important to develop screening methods for the determination of dioxin TEQs in retail fish in order to carry out risk assessments.

High-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC-HRMS) is generally viewed as the most reliable method for determining the TEQ concentrations of dioxins. This technique is sensitive and reproducible; however, it is also time-consuming and requires expensive instruments, which limits its capacity. A reporter-gene assay, such as the chemical-activated luciferase gene expression (CALUX) assay, is currently considered to be the best screening method for the TEQ concentrations of dioxins in food [as reviewed by Hoogenboom et al. (*6*)]. The CALUX assay detects dioxin-like compounds based on their activation of the aryl hydrocarbon receptor (AhR), which increases the expression of the luciferase reporter gene as reviewed by Behnisch et al. (*7*) and Overmeire et al. (*8*). The response for a sample containing dioxin-like compounds can be converted into 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) equivalents, which are known as CALUX-based TEQs, using a 2,3,7,8-TCDD standard curve. The CALUX assay has been applied to the detection of dioxins in fish and fishery products (*9*–*13*); however, its drawbacks include the need for cell culture, which requires skilled personnel and elaborate equipment, and the likely requirement of a license for the assay.

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