Levels of polybrominated diphenyl-ethers and polybrominated dioxins in fish, total diet study food groups, and Japanese meals

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Introduction

Since they were found in mother's milk and blood in several studies [1-3], the polybrominated diphenyl-ethers (PBDEs) and other polybrominated flame-retardants (BFRs) that are used in plastics, electrical appliances, and textiles have been recognized as ubiquitous pollutants. BFRs are precursors of polybrominated dibenzo-*p*-dioxins/ polybrominated dibenzofurans (PBDD/Fs). Recently, 2,3,7,8-TBDD/Fs and PBDEs have been detected in adipose tissue and blood in Japanese people[4]. Food is naturally suspected. However, there is very few information on food contamination with those brominated compounds in Japan . Therefore, we measured the levels of PBDEs and PBDD/Fs in various fish samples, meal samples, and total diet study (TDS) food groups and estimated Japanese people's dietary intake of PBDD/Fs and PBDEs.

Methods and Materials

Fish samples: Nine fish samples (grunt, horse mackerel, thread-sail filefish, mackerel, pacific saury, razor-shell, sardine, sea bream, and young yellowtail) were purchased from grocery stores in Fukuoka during 2002~2003.

Meal samples: Samples weighing from 1565 to 3151 g/day were collected from six persons for 2~3 days, homogenized, and frozen before analysis.

Total diet study (TDS) *samples*: In Japan, total diet studies are carried out annually as a program of National Nutrition Survey. There, all foods that Japanese eat daily are classified to 14 TDS food groups as seen in Table 1. One sample per one group was prepared except for groups X, XI and XII. The preparation was as follows: several typical foods in each food group were chosen and sampled according to the amounts consumed by an average adult in Fukuoka district in the latest survey[5]. In each food group, foods were either cooked (boiled, grilled or roasted) beforehand or kept raw (such as sashimi), just as they were served for meal. Then all of the foods in each food group were mixed together. For groups X, XI, and XII, two samples (e.g. Xa and Xb) per group were prepared, using different foodstuffs from the respective groups. Group X IV (water) was not analyzed in this study.

One hundred grams of a fish sample or a TDS sample, or 500 g of a meal sample homogenate were freeze-dried and a ^{13}C-labeled PBDD/F and PBDE mixture was added as a clean spike. The sample was then extracted with hexane using an accerrelated solvent extractor

(ASE 300,Dionex, USA) under the conditions of 100 °C, 1,500 psi. Each concentrated extract solution was treated with sulfuric acid and then cleaned using silica-gel column chromatography with 150 mL of 10% dichloromethane (DCM) in hexane as the eluate, and then using Floridil column chromatography with 150 mL of hexane (PBDE fraction) and 200 mL of 60% DCM/hexane (PBDD/F fraction). The PBDE fraction was cleaned using DMSO/hexane partitioning. The PBDD/F fraction was further cleaned using activated carbon chromatography with 50 mL of 10% DCM/hexane and 200 mL of toluene (PBDD/F fraction). The cleaned PBDD/F and PBDE fractions were concentrated and dissolved in 25 µL of nonane with $^{13}C_{12}$-OCDD or $^{13}C_{12}$-2,2',3,4,4',6-HxBDE as a syringe spike, respectively.

Table 1 Average composition of total diet of average person in Fukuoka

Group	Foods in group	Av. wt, g/day	% (by wt) of total diet
I	Rice and rice products	409.0	25.6
II	Grains, seeds and potatoes	192.8	12.1
III	Sugar and confectionaries	32.6	2.0
IV	Oils	15.2	1.0
V	Legume and legume products	73.2	4.6
VI	Fruits	113.9	7.1
VII	Carrots and green leafy vegetables	86.9	5.4
VIII	White leafy vegetables, mushrooms, and seaweeds	184.6	11.5
IX	Seasonings and beverages	172.2	10.8
X	Fish and fish products	82.1	5.1
XI	Meat and eggs	107.9	6.8
XII	Milk and milk products	122.5	7.7
XIII	Other processed foods	5.6	0.4
XIV	Water		
	Total	1598.5	100.0

Analysis of PBDD/Fs and PBDEs by HRGC/HRMS

GC6890 (Agilent, USA) /Autospec Ultima (Micromass, USA) was used at a resolution of >10,000 for PBDD/Fs. GC6890/MS5973 (Agilent) was used for PBDEs. The columns were a DB-5 (J&W, USA) (0.25 mm i.d. × 30 m, film thickness 0.1 µm) for the PBDD/Fs and a HP-5MS (Agilent, USA) (0.25 mm i.d. × 15 m, film thickness 0.1 µm) for the PBDEs.

Results and Discussion

(1) Analysis of PBDD/Fs and PBDEs

In this survey, no PBDD/Fs were detected in any of the fish or food samples. The limits of detection (LODs) were 0.01 (0.003 for meal samples) pg/g for tetra- and penta-BDD/F and 0.05 (0.005 for meal samples) pg/g for HexaBDD/F.

By contrast, PBDEs were found at 79~547 pg/g in eight of the nine fish samples; the exception was thread-sail filefish. The PBDE levels in mackerel, sea bream, and horse mackerel, which are favorites in the Japanese diet, were particularly high at 547, 503, and 472 pg/g, respectively. The major isomers in these samples were 2,2',4,4'-TeBDE (IUPAC No.#47)(avg. 39.3%), 2,2',4,5'-TeBDE (#49)(avg,16.3%), 2,2',4,4',6-PeBDE (#100) (avg,13.4%), and 2,2',4,4',5,6'-HxBDE (#154)(avg.13.7%)(Fig. 1).

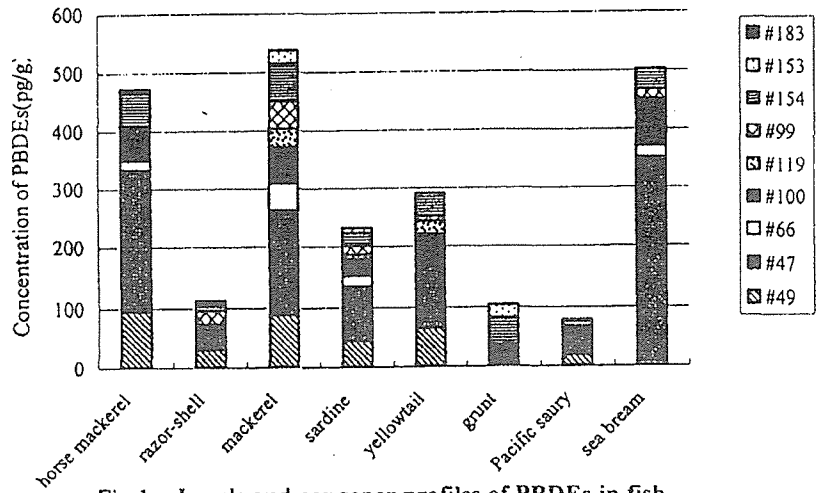

Fig.1 Levels and congener profiles of PBDEs in fish

In the meal samples collected from six individuals, PBDEs were found at 3.4~81.1 pg/g, and the major contributors were #47 (avg. 37.3%), #99 (2,2'4,4'5-PentaBDE, avg. 22.1%), #100 (avg. 11.2%), and #49 (avg. 8.7%) (Table 2, Fig.2).

On the other hand, among the 13 food group samples in the TDS, PBDEs were found in groups IV (Oil), X (Fish), XI (Meat & Eggs), and XII (Milk & milk products) at the concentrations of 122, 1259 (avg.), 64.7(avg.) and 8.6pg/g(avg.), respectively (Fig.3). In the other groups PBDEs were below detection limit. By multiplying PBDE concentrations by consumption amounts of food groups in Table 1, daily intake of PBDEs can be calculated. In result, our data suggest that more than 90% of total PBDE intake by Japanese people derives from group X.

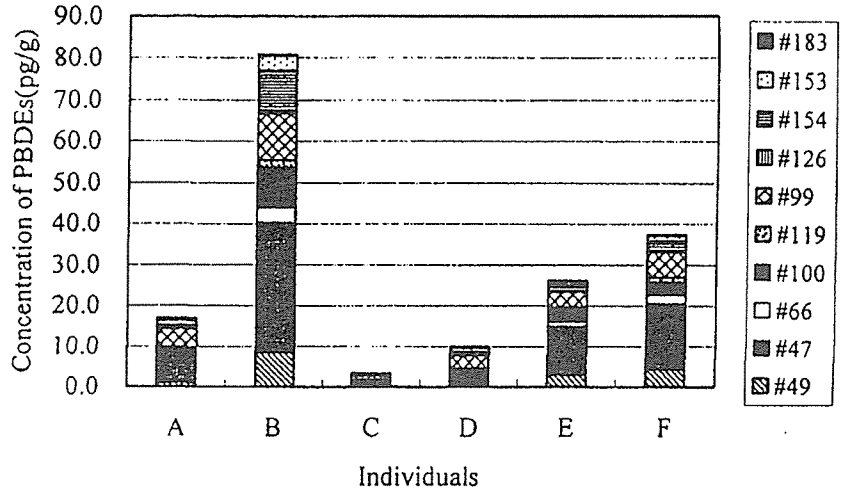

Fig.2 Levels and congener profiles of PBDEs in the meal samples collected from 6 persons

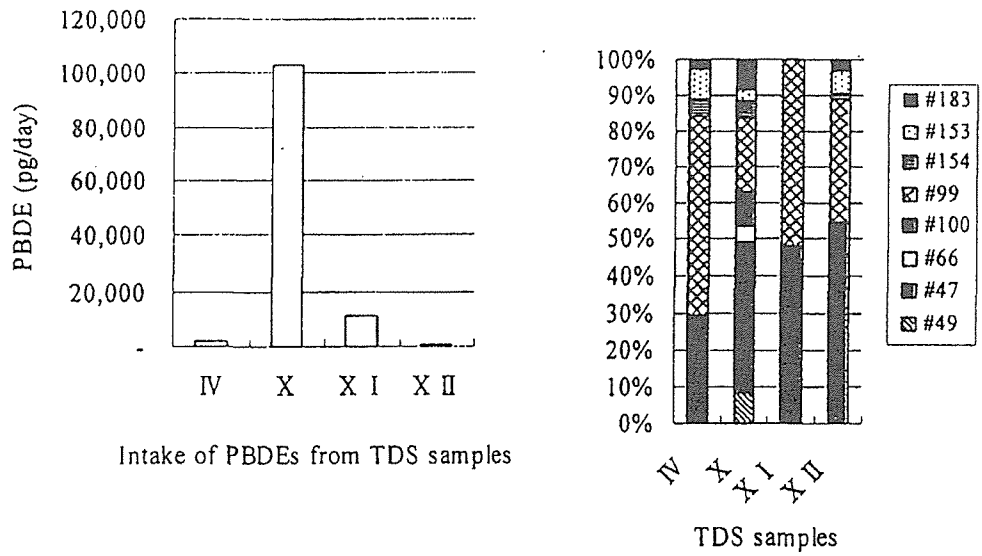

Fig.3 Daily intake and congener profiles of PBDEs in the TDS samples

Within the group X , congeners #47, #99, and #100 contributed an average of 44.3, 22.3, and 10.5%, respectively. Conversely, in groups IV and XI, penta-brominated congener #99 contributed more than tetra-brominated congener #47.

The concentration ratios between congener #99 and congener #47 in the meal samples and the group X are somewhat different from those in the raw fish samples. The PBDE congener profiles in the raw fish in this study are similar to those in raw Michigan salmon[6]. This is analogous to the phenomenon that lower chlorinated PCB congeners are more frequently found than higher ones in raw fish. We further speculate that not only various backgrounds of fish samples (i.e., fish age, cultured or not) but also certain cooking or processing procedures might have affected the isomer profile of PBDEs in the meal samples and the semi-cooked TDS sample (group X).

(2) Estimation of dietary intake of PBDEs and PBDD/Fs by Japanese people

As seen in Table 3, even after taking the intake of PCDD/Fs[7] into account, the total TEQs under the two conditions that are ND=0 and ND=1/2 × LOD were 1.24 and 1.59 pg TEQ/ kg b. w. /day, respectively. These TEQs are less than TDI (4pg TEQ /kg b. w. /day) set in the Japanese law. The contribution of PBDEs and PBDD/Fs to the total TEQ was 5.3% at ND=0 and 19.3% at ND=1/2 × LOD. Therefore, PBDD/Fs and PBDEs have not so significant influence on the total TEQ at present. Nevertheless, given the huge volume of industrial wastes containing BFRs, the levels of these brominated contaminants in food should be continually monitored.

Acknowledgment

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Table 2 Levels of PBDEs in the meal samples and daily intake of PBDEs by the six persons

PBDE congener		A	B	C	D	E	F	Average
Tetra-brominated	#49	1,2	8,6	0,2	0,4	3,0	4,4	3,0
	#71	ND	ND	ND	ND	ND	ND	0,0
	#47	5,2	31,6	1,3	2,8	12,0	16,1	11,5
	#66	0,3	3,8	ND	0,2	1,3	2,1	1,3
	#77	ND	0,3	ND	ND	ND	ND	0,0
Penta-brominated	#100	2,9	9,5	0,3	1,0	2,8	3,3	3,3
	#119	0,4	2,0	ND	0,3	0,6	1,1	0,7
	#99	4,5	11,1	1,1	3,2	3,6	6,0	4,9
	#85	ND	ND	ND	ND	ND	ND	0,0
	#126	ND	0,3	ND	ND	ND	ND	0,0
Hexa-brominated	#154	0,9	10,1	ND	0,9	1,7	2,8	2,7
	#153	1,2	3,6	0,3	0,9	0,9	1,3	1,4
	#138	ND	ND	ND	ND	ND	ND	0,0
Hepta-brominated	#183	0,5	0,3	0,3	0,3	0,3	0,3	0,3
Total (pg/g)		17,0	81,1	3,4	9,9	26,2	37,4	29,2
Total weight of meal (g)		1565	2623	3151	2123	2392	2016	2312
daily intake of PBDEs(ng)		26,6	212,7	10,8	21,1	62,8	75,5	68,2
TEQ* pg/kg b.w./day (ND=0)		0,0254	0,2438	0,0055	0,0159	0,0441	0,0562	0,065
TEQ pg/kg b.w./day (ND=1/2xLOD**)		0,0419	0,2438	0,0416	0,0401	0,0704	0,0812	0,087

*TEQ was caliculated with the relative EROD activities (0.0032 for #77, 0.00024 for #100, 0.00035 for #119, 0.0024 for #126, 0.000048 for #153) by Chen *et al.*[8].

** LOD was 0.2pg/g for each PBDE congener.

Table 3 Estimated average dietary intake of PBDEs, PBDD/Fs and PCDD/Fs by Japanese persons

	pgTEQ/kg.b.w./day			
	ND=0	(%*)	ND=1/2 xLOD	(%*)
PBDE	0,065	5.3	0,087	(5.5)
PBDD/Fs**	0,000	(0.0)	0,218	(13.8)
PCDD/Fs	1,170	(94.7)	1,280	(80.8)
Total	1,235		1,585	

(%*):contribution

**:The TEFs[9] assigned to PCDD/Fs were tentatively used for PBDD/Fs.

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Determination of Polybrominated Diphenyl Ethers and Polybrominated Dibenzo-*p*-dioxins/Dibenzofurans in Marine Products

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Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants in plastics and textile coatings, and these compounds have been recognized as ubiquitous environmental contaminants. Furthermore, it is considered a serious problem that polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/DFs), having toxicities similar to those of chlorinated dioxins, are generated by the manufacture of brominated flame retardants (BFRs) such as PBDEs, and formed by the combustion of substances containing BFRs. Several congeners of PBDD/DFs and PBDEs have been detected in the adipose tissue of the Japanese. Although food is suspected as an exposure source, little information is available regarding the levels of these brominated compounds in food, as compared with information regarding dioxin or polychlorinated biphenyls. It is necessary to investigate the levels of these brominated organic compounds in various foods and to estimate their influence in the case of human exposure. We developed an efficient method of analyzing PBDEs and PBDD/DFs contents in food samples using accelerated solvent extraction and determined the concentrations in several marine products such as raw fish, processed foods, and seaweed purchased in Japan. A recovery test ($n = 5$) using the method and involving dried fish showed acceptable recoveries of 57.7−78.5% (RSD 5.4−15.9%) for PBDEs and 50.0−56.4% (RSD 1.5−7.9%) for PBDD/DFs. In the analysis of marine product samples, several congeners of PBDEs were detected in raw fish, processed fish, and seaweed; the highest concentration of ΣPBDEs was detected in yellowtail (1161pg/g whole basis), followed by mackerel (553.5pg/g whole basis). The most dominant congener present in these marine samples was 2,2′,4,4′-tetraBDE (#47).

KEYWORDS: Polybrominated diphenyl ethers (PBDEs); polybrominated dibenzo-*p*-dioxins; dibenzofurans (PBDDs/DFs); levels in food; marine products; accelerated solvent extraction (ASE)

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are flame retardants, which have been used worldwide in plastics and textile coatings. In Japan, the domestic use of PBDEs reached its peak in 1990 (12100 tons), subsequently decreasing to 2800 tons by 2000 (*1*). The use of PBDEs will be soon replaced by the use of tetrabromobisphenol A (TBBPA), but the demand for total brominated flame retardants (BFRs) remains extensive. PBDEs are additives of polymers such as polystyrene and are not chemically bound to the polymer. Therefore, they are easily released into the environment. The toxicity of PBDEs remains unclear, but some studies have indicated the dioxin- or polychlorinated biphenyls-like toxicity of PBDEs, activating the aryl hydrocarbon receptor signal transduction pathway (*2*, *3*), affecting thyroid hormone function (*4*) and estrogenic potency (*5*). Recent reports have shown that PBDEs have a developmental neurotoxic effect in mice or rats (*4*, *6*−*8*). Furthermore, the thermal formation of polybrominated dibenzo-*p*-dioxins/dibenzofurans (PBDD/DFs) from BFRs such as PBDEs or TBBPA is considered a serious problem (*9*, *10*). Although the toxicity of these brominated dioxins is also unclear, some studies have shown that the toxicity of 2,3,7,8-TBDD is comparable to that of 2,3,7,8-TCDD (*11*). Because the international toxic equivalency factors (TEFs) have not been determined for PBDD/DFs, it is presently considered appropriate to use the TEFs of chlorinated dioxins for corresponding congeners of PBDD/DFs (*11*).

In recent decades, some congeners of PBDEs have been detected in environmental samples taken throughout the world, including sediment (*12*−*14*), atmosphere (*15*), soil (*16*), and biota (*13*, *17*−*19*). These compounds have been recognized as ubiquitous environmental contaminants because of their bioaccumulative characteristics in the food chain. Above all, tetra-

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bromodiphenyl ethers (tetraBDEs) and pentabromodiphenyl ethers (pentaBDEs) were considered to have a high bioaccumulation potential (*20*). In a recent report, some congeners of PBDE were detected in certain Arctic animals (*21*). These results show that the presence of PBDEs has reached the Arctic and that there were differences of levels and patterns of accumulation among species, which is considered to be due to differences in PBDE metabolism and accumulation. There are interesting reports regarding human exposure of PBDEs, showing up in human adipose tissue (*22*), blood (*23*), and mother's milk (*24*). Ohta et al. (*25*) 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 dietary intake of fish and shellfish. Although information regarding PBDD/DFs is slight as compared with that regarding PBDEs, several congeners have also been detected in environmental samples such as sediment (*14*). Especially, determination in biota (*26*) and human adipose tissue (*22*) is rare throughout the world. On the other hand, there are some studies concerning naturally occurring derivatives of PBDDs. In these reports, the derivatives have been shown to be produced by cyanobacteria in marine sponges (*27*, *28*).

It is important to collect more detailed data regarding the levels of contamination in food, animals, and human tissue in order to clarify the behavior of brominated organic compounds in metabolism and bioaccumulation and to estimate human risk in terms of these results. In the present study, we aim to develop an efficient method of simultaneously analyzing PBDEs and PBDD/DFs in food samples using accelerated solvent extraction (ASE). After the validation of this method, we determined the levels of these brominated compounds in several marine products (raw fishes and shellfishes, processed fishes, and seaweed) purchased in Japan.

MATERIALS AND METHODS

Analytical Methods and Instrumentation. The PBDD/DFs analytical standard (tetra-hexa) was purchased from Cambridge Isotope Laboratories (MA). A standard solution (500 ng/mL) of the mixture was prepared in our laboratory. It contained the following PBDD/DFs congeners: 2,3,7,8-tetraBDD, 1,2,3,7,8-pentaBDD, 1,2,3,4,7,8-hexaBDD, 1,2,3,6,7,8-hexaBDD, 1,2,3,7,8,9-hexaBDD, 2,3,7,8-tetraBDF, 1,2,3,7,8-pentaBDF, 2,3,4,7,8-pentaBDF, and 1,2,3,4,7,8-hexaBDF in native PBDD/DFs mixture; $^{13}C_{12}$-2,3,7,8-tetraBDD, $^{13}C_{12}$-1,2,3,7,8-pentaBDD, $^{13}C_{12}$-1,2,3,6,7,8-hexaBDD, $^{13}C_{12}$-1,2,3,7,8,9-hexaBDD, $^{13}C_{12}$-2,3,7,8-tetraBDF, $^{13}C_{12}$-1,2,3,7,8-pentaBDF, and $^{13}C_{12}$-2,3,4,7,8-pentaBDF in $^{13}C_{12}$-labeled PBDD/DFs mixture. The PBDE analytical standard was purchased from Wellington Laboratories (Ontario, Canada). It contained the following PBDE congeners: 4-monoBDE (#3), 2,4-diBDE (#7), 4,4′-diBDE (#15), 2,2′,4′-triBDE (#17), 2,4,4′-triBDE (#28), 2,2′,4,4′-tetraBDE (#47), 2,2′,4,5′-tetraBDE (#49), 2,3′,4,4′-tetraBDE (#66), 2,3′,4′,6-tetraBDE (#71), 3,3′,4,4′-tetraBDE (#77), 2,2′,3,4,4′-pentaBDE (#85), 2,2′,4,4′,5-pentaBDE (#99), 2,2′,4,4′,6-pentaBDE (#100), 2,3′,4,4′,6-pentaBDE (#119), 3,3′,4,4′,5-pentaBDE (#126), 2,2′,3,4,4′,5′-hexaBDE (#138), 2,2′,4,4′,5,5′-hexaBDE (#153), 2,2′,4,4′,5,6′-hexaBDE (#154), 2,2′,3,4,4′,5′,6-heptaBDE (#183), and decaBDE (#209). The mixture also contained the following $^{13}C_{12}$-labeled congeners: 4-monoBDE (#3), 4,4′-diBDE (#15), 2,4,4′-triBDE (#28), 2,2′,4,4′-tetraBDE (#47), 2,2′,4,4′,5-pentaBDE (#99), 2,2′,4,4′,5,5′-hexaBDE (#153), 2,2′,4,4′,5,6′-hexaBDE (#154), and 2,2′,3,4,4′,5′,6-heptaBDE (#183). The congeners of PBDEs (tetra-hepta) were monitored by gas chromatography/mass spectrometry (GC/MS) in this study. The mixture of $^{13}C_{12}$-labeled PBDE was used as a cleanup spike, and $^{13}C_{12}$-labeled 2,2′,3,4,4′,6-hexaBDE (#139) was used as a syringe spike. The organic solvents (*n*-hexane, dichloromethane, and toluene) used for extraction and cleanup were dioxin analysis grade (Kanto Chemicals, Japan). Dimethyl sulfoxide (DMSO) used for cleanup of PBDEs was of spectrochemical analysis grade (Wako Pure Chemicals Ind, Co.,Ltd., Tokyo, Japan). Silica gel (Wako Pure Chemicals Ind, Co., Ltd.) was heated for 3 h at 130 °C. Florisil (Kanto Chemicals) was heated for 3 h at 130 °C and deactivated with 1% water. Active carbon was purchased from Nacalai Tesque (Kyoto, Japan), refluxed with toluene for 1 h three times, and dried *in vacuo*; then, 500 mg of the active carbon was mixed with 500 g of anhydrous sodium sulfate (Wako Pure Chemicals Ind, Co.,Ltd.).

Table 1. Selected Ion Monitoring (SIM) Ions Used in the PBDD/DFs GC/MS Method

	ions (*m/z*)	
compound	quantification	confirmation
tetraBDD	499.6904	501.6883
pentaBDD	577.6009	579.5988
hexaBDD	655.5114	657.5094
tetraBDF	483.6955	485.6934
pentaBDF	561.6060	563.6039
hexaBDF	639.5165	641.5144
$^{13}C_{12}$-tetraBDD	511.7306	
$^{13}C_{12}$-pentaBDD	589.6412	
$^{13}C_{12}$-hexaBDD	663.5295	
$^{13}C_{12}$-tetraBDF	495.7357	
$^{13}C_{12}$-pentaBDF	573.6462	

Table 2. SIM Ions Used in the PBDEs GC/MS Method

	ions (*m/z*)	
compound	quantification	confirmation
tetraBDE	485.7113	483.7113
pentaBDE	565.6199	563.6218
hexaBDE	643.5303	641.5323
heptaBDE	721.4409	723.3338
$^{13}C_{12}$-tetraBDE	497.7516	
$^{13}C_{12}$-pentaBDE	575.6622	
$^{13}C_{12}$-hexaBDE	655.5708	
$^{13}C_{12}$-heptaBDE	733.4813	

GC/MS analysis was performed on an HP6890 gas chromatograph (Hewlett-Packard, CA) coupled to an Autospec Ultima (MicroMass, United Kingdom). The GC conditions of the PBDD/DFs were as follows: column, DB-5 (J&W Scientific, CA) 30 m, 0.25 mm i.d., 0.1 μm film thickness; column temperature program, 130−240 °C at 20 °C/min, 240−320 °C (held for 7.5 min) at 5 °C/min; injection temperature, 240 °C; injection volume, 1 μL. The GC conditions of PBDEs were as follows: column, HP-5MS (Agilent Technology, CA) 15 m, 0.25 mm i.d., 0.1 μm film thickness; column temperature program, 120 (held for 2 min) to 200 °C at 20 °C/min, 200−300 °C (held for 1 min) at 10 °C /min; injection temperature, 240 °C; injection volume, 1 μL. The MS conditions (PBDEs and PBDD/DFs) were as follows: electron energy, 38 eV; filament curret, 750 μA; ion source temperature, 270 °C; resolution, 10000. The monitoring ions used in the GC/MS method of PBDD/DFs are given in **Table 1**, and those of PBDEs are given in **Table 2**.

Sampling. Marine products were purchased from several markets in Fukuoka of Japan from September 2001 to February 2004. **Table 3** shows the data of samples prepared for this study. Dried sardines, purchased from market in October 2002, were crushed using a mill and used for the recovery test. Toasted laver, dried tangle, dried hijiki (*Hizikia fusiformis*), and dried wakame (*Undaria pinnatifida*) were also crushed using a mill. The edible parts of fish and shellfish were blended using a food processor. These food mixtures were kept below −20 °C until analysis.

Sample Preparation. For analysis, 100 g of fish and shellfish was used, and 50 g of dry foods (the toasted laver, dried hijiki, and dried wakame) was used. Blanks were run concurrently with the samples to assess laboratory contamination. To validate the analytical method, a test measuring precision was run using 20 g of dried sardine ($n = 5$), and the recoveries of congeners and relative standard deviation (RSD)

Table 3. Data of Investigated Marine Product Samples

marine product	place of production	size of sample	purchase date
horse mackerel	Nagasaki	400 g (28 cm)	September 2001
chicken grunt	Saganoseki (Oita)	400 g (26 cm)	September 2001
sardine	Hokkaido	760 g (9fishes)	September 2001
thread sailfin filefish	Kanesaki (Fukuoka)	180 g (4fishes, 19–21 cm)	September 2001
mackerel	Goto (Nagasaki)	550 g (31 cm)	September 2001
saury	Yokosuga (Kanagawa)	1290 g (8fishes)	September 2001
sea bream-1	Nagasaki	1044 g (35 cm)	September 2001
sea bream-2	Kitakyushu (Fukuoka)	551 g (33 cm)	July 2003
young yellowtail	Nagasaki	850 g (36 cm)	September 2001
yellowtail	Nagasaki (cultured)	160 g (slice)	February 2004
tuna	Taiwan (China)	271 g (slice)	February 2004
trout	Norway	263 g (slice)	February 2004
arakabu	Kitakyushu (Fukuoka)	260 g(4fishes, 15–17 cm)	July 2003
parrotfish	Kitakyushu (Fukuoka)	558 g (8fishes, 15–18 cm)	July 2003
Japanese sea perch	Kitakyushu (Fukuoka)	227 g (27 cm)	July 2003
squid	Nagasaki	160 g (17–20 cm)	February 2004
razor-shell	Korea	9–10 cm(49shellfishs)	September 2001
oyster-1	Itoshima (Fukuoka)	5kg (with shell)	November 2001
oyster-2	Buzen (Fukuoka)	5kg (with shell)	November 2001
dried horse mackerel	Yatsushiro (Kumamoto)	90–110 g (22–25 cm)	February 2004
broiled eel	Kagoshima	200 g (33 cm)	February 2004
boiled fish paste (sea bream)	Nagasaki	140 g (3peices)	February 2004
salted saury	Hokkaido	170 g (30 cm)	February 2004
sausage	Goto (Nagasaki)	95 g (4peices)	February 2004
dried sardines	Ehime	200 g (packed)	October 2002
toasted laver	Sea of Seto	55 g (10sheets)	February 2004
dried tangle	Sanriku	100 g (packed)	February 2004
dried hijiki	Japan	30 g (packed)	February 2004
dried wakame	Naruto	20 g (packed)	February 2004

values of the concentrations were checked. The food samples except dried foods were freeze-dried using an AD 2.0 ES-BC (Virtis, NY). Dried samples were stuffed in 99 mL cells and extracted with *n*-hexane by accelerated solvent extractor ASE300 (Dionex, CA). The cleanup spikes ($^{13}C_{12}$-labeled standard mixture) of PBDEs and PBDD/DFs were added to the samples before extraction. The procedure employed two 10 min extraction cycles with *n*-hexane using a 40% vessel flush at 100 °C and 10 Mpa (1500 psi). The extracts were treated with 20 mL of concentrated sulfuric acid three times and applied to the silica gel column. The column was prewashed with 100 mL of *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 about 5 mL of *n*-hexane. The *n*-hexane solution was loaded into a Florisil column (5 g), and the PBDEs fraction was eluted with 150 mL of *n*-hexane, while the PBDD/DFs fraction was eluted with 200 mL of 60% (v/v) dichloromethane/*n*-hexane. The PBDEs fraction was treated with DMSO/*n*-hexane partition in order to remove the matrix. The PBDD/DFs fraction was loaded into an active carbon column, after washing 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 50 μL, respectively. The syringe spikes [$^{13}C_{12}$-labeled-2,2′,3,4,4′,6-hexaBDE (#139) for PBDEs, $^{13}C_{12}$-octaCDD for PBDD/DFs] were added before the GC/MS measurement. These samples were analyzed using HRGC/HRMS.

RESULTS AND DISCUSSION

We attempted to analyze the congeners of PBDEs and PBDD/DFs simultaneously in food samples. They share a similarity in chemical structure, and it is important to trace each relative level in food. In advance, we checked the purity of standard by HRGC/HRMS and confirmed that the impurity levels were insignificant. The extraction process was performed using ASE in order to achieve an efficient and simple operation. After extraction, treatment with concentrated sulfuric acid was used for the first cleanup. It was considered that treatment with alkali was unsuitable, because it easily decomposed the PBDEs. For the next cleanup procedure, we used a silica gel column. The silver nitrate silica gel column was considered unsuitable because of its unacceptable blank level. On the next step, a Florisil column was used for separating PBDEs and PBDD/DFs. Choi et al. reported a cleanup method using Florisil and an active carbon column for the complete separation of PBDEs from PBDD/DFs (*29*). The recoveries of these congeners using a Florisil column for cleanup were acceptable, and PBDEs were only negligibly eluted in the fraction of PBDD/DFs (less than 0.1%). Furthermore, the PBDEs fraction was treated with a DMSO/*n*-hexane partition for the removal of lipids. The PBDD/DFs fraction was purified by an active carbon column. We used active carbon diluted by anhydrous sodium sulfate, because a large amount of solvent is needed to elute PBDD/DFs due to their strong adsorption to active carbon. We validated this analytical method of PBDEs and PBDD/DFs recovery by a test involving dried sardines (n = 5).

Table 4. Recoveries of PBDD/Fs in Dried Sardine (n = 5)

compound	recovery (%)	RSD (%)
2,3,7,8-tetraBDD	56.0	2.5
1,2,3,7,8-pentaBDD	55.8	5.2
1,2,3,4,7,8-/1,2,3,6,7,8-hexaBDD	51.4	7.2
1,2,3,7,8,9-hexaBDD	51.8	7.9
2,3,7,8-tetraBDF	50.0	5.0
1,2,3,7,8-pentaBDF	56.4	1.5
2,3,4,7,8-pentaBDF	56.0	3.9

The recoveries of PBDD/DFs from the dried sardines are given in **Table 4**. The average recoveries for PBDD/DFs were in the range of 50.0−56.4%, and the RSD values were 1.5−7.9%. The recoveries of these brominated dioxins exhibit quite low RSDs. The recoveries of PBDEs are given in **Table 5**. For PBDEs, the average recoveries were in the range of 57.7−78.5%, and RSD values were 5.4−15.9%. Although the recoveries of PBDD/DFs were low as compared with PBDEs, they were considered acceptable recoveries within 40−120%, mentioned in the analytical guideline of chlorinated dioxins in foods as determined by the Ministry of Health, Labor and

Table 5. Recoveries of PBDEs in Dried Sardine (n = 5)

compound	recovery (%)	RSD (%)
2,2′,4,5′-tetraBDE (#49)	57.7	12.5
2,2′,4,4′,5-pentaBDE (#99)	70.1	15.9
2,2′,4,4′,5,5′-hexaBDE (#153)	66.6	9.6
2,2′,4,4′,5,6′-hexaBDE (#154)	69.0	5.4
2,2′,3,4,4′,5′,6-heptaBDE (#183)	78.5	10.0

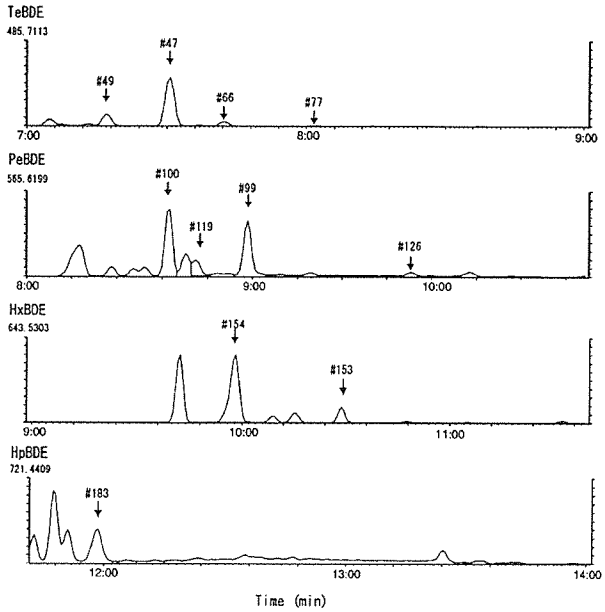

Figure 1. GC/MS SIM chromatograms of PBDEs (tetra-hepta) in dried sardine.

Table 6. Concentrations of PBDEs in Dried Sardine (n = 5)

	concentration	
compound	mean (pg/g)	RSD(%)
2,2′,4,5′-tetraBDE (#49)	55.4	4.1
2,3′,4′,6-tetraBDE (#71)	ND	
2,2′,4,4′-tetraBDE (#47)	148.8	1.7
2,3′,4,4′-tetraBDE (#66)	25.1	6.2
3,3′,4,4′-tetraBDE (#77)	1.20	9.5
2,2′,4,4′,6-pentaBDE (#100)	28.4	2.2
2,3′,4,4′,6-pentaBDE (#119)	16.2	4.3
2,2′,4,4′,5-pentaBDE (#99)	36.2	3.3
2,2′,3,4,4′-pentaBDE (#85)	ND	
3,3′,4,4′,5-pentaBDE (#126)	1.57	6.8
2,2′,4,4′,5,6′-hexaBDE (#154)	80.5	2.2
2,2′,4,4′,5,5′-hexaBDE (#153)	19.2	1.8
2,2′,3,4,4′,5′-hexaBDE (#138)	ND	
2,2′,3,4,4′,5′,6-heptaBDE (#183)	4.49	5.9
ΣPBDEs	417.1	

Welfare of Japan. The results of this determination showed that no PBDD/DFs congeners were detected in the dried sardine sample. On the other hand, 11 PBDEs congeners were detected in the same sample. **Figure 1** shows the chromatogram of PBDEs present in dried sardine, with the concentrations of PBDE congeners determined in the dried sardine given in **Table 6**. The concentration of total PBDE was 417.1 pg/g, and the major congeners detected were 2,2′,4,5′-tetraBDE (#49), 2,2′,4,4′-tetraBDE (#47), and 2,2′,4,4′,5,6′-hexaBDE (#154). 2,3′,4′,6-TetraBDE (#71), 2,2′,3,4,4′-pentaBDE (#85), and 2,2′,3,4,4′,5′-hexaBDE (#138) were not detected. The RSD values of PBDEs are satisfactory within a range of 1.7−9.5%. In regard to the lowest congener [3,3′,4,4′-tetraBDE (#77)], the RSD value was satisfactory at 9.5%. In this study, the limit of detection (SN = 3) for tetraBDEs, pentaBDEs, and hexaBDEs was 0.1 pg/g, and that for HeptaBDE was 0.2 pg/g, respectively. For PBDD/DFs, the limit of detection (SN = 3) for tetraBDD/DFs and pentaBDD/DFs was 0.01 pg/g, and that of hexaBDD/DFs was 0.05 pg/g, respectively.

In the study of chlorinated dioxins, it has been described that the food group showing the highest daily intake was fish and shellfish (*30*). Concerning PBDEs, a recent study has suggested that the daily intake of fish significantly contributes to human exposure in the same manner as chlorinated dioxins (*27*). Using this analytical method, we determined the levels of PBDD/DFs (tetra-hexa) and the PBDEs (tetra-hepta) in marine product samples, which included 17 species of raw fishes and shellfish, six kinds of processed fish, and four species of seaweed.

Table 3 shows data of investigated marine product samples. **Tables 7−9** show concentrations (pg/g whole basis) of PBDEs congeners in each sample. All of the PBDD/DFs congeners were not detected in every sample. For PBDEs, the highest concentration of total PBDE was detected in yellowtail, followed by mackerel in the raw fish. The value in yellowtail was 1161.2 pg/g on a whole basis, more than double the concentration in mackerel. In another report, a high concentration of PBDEs (1280−1720 pg/g whole basis) was detected in these fish (*27*). Yellowtail and mackerel are fish with high lipid contents. It is suggested that the high levels of PBDEs in these fishes are likely due to their high lipid contents in this case. In the processed fish, the highest concentration of total PBDE was detected in dried sardines. This value was 411.4 pg/g on a whole basis. The levels in dried fish (mackerel and sardine) appeared higher than those in other processed fishes. The haul amount of sardines is the largest in the Japanese marine products industry, and there exists a strong demand for this species as raw fish, processed food, and animal food. The dried sardine is an essential food in Japan, because it is used in traditional Japanese cooking. However, because the daily consumption of it is small (about 0.5 g), the amount of PBDEs taken in from this food does not seem to be significant. The concentrations in seaweed were low level as compared with those of fish and shellfish (1.1−10.2 pg/g whole basis). The most dominant congener was 2,2′,4,4′-tetra-BDE (#47) in all samples except grunt. This trend corresponded to the conclusion of other reports regarding the levels of 2,2′,4,4′-tetra-BDE (#47) in fish (*27*, *31*). A recent report showed that 2,2′,4,4′-tetra-BDE (#47) is a dominant congener detected in human adipose tissue (*24*). In regard to other congeners, different species expressed different patterns. It is necessary to survey various fish species and to investigate the patterns of congeners in order to obtain information regarding metabolism or bioaccumulation. Comparisons of PBDEs patterns between raw and processed horse mackerel, sardine, saury, and sea bream are presented in **Figure 2**. Interestingly, the pattern of PBDE congeners in processed fish was similar to those of raw fish in these four fish species. Although the pattern of processed fish is considered to approximately reflect the pattern of raw fish as based on the present data, more detailed data will reveal how food processing affects PBDEs congeners. The Japanese populace consumes many kinds of fish products including dried fish, salted fish, and fish sausage, and a large amount of fish is consumed in daily meals. The amount of daily consumption of fish was estimated to be 85 g in an investigation conducted by the Ministry of Health, Labor and Welfare of Japan. Supposing that 85 g of yellowtail was consumed in a day, the daily intake of total PBDEs from fish was calculated to be 98.7 ng/day and 1.97 ng/kg body

Table 7. Concentrations of PBDEs in Raw Fish and Shellfish (pg/g)[a]

compound	horse mackerel	chicken grunt	sardine	thread sailfin filefish	mackerel	saury	sea bream-1	sea bream-2	young yellowtail	yellowtail
2,2′,4,5′-tetraBDE (#49)	94.6	1.6	44.6	ND	87.6	21.4	7.9	2.6	66.8	196.1
2,3′,4′,6-tetraBDE (#71)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2′,4,4′-tetraBDE (#47)	238.8	13.1	91.4	ND	175.4	35.2	331.1	176.1	102.4	296.4
2,3′,4,4′-tetraBDE (#66)	16.1	0.8	15.9	ND	45.8	7.3	23.5	19.8	ND	61.0
3,3′,4,4′-tetraBDE (#77)	ND	0.3	2.0	ND	9.2	0.7	0.5	1.9	ND	2.6
2,2′,4,4′,6-pentaBDE (#100)	58.2	23.3	28.2	ND	62.9	6.1	74.6	38.3	52.6	260.4
2,3′,4,4′,6-pentaBDE (#119)	ND	4.8	7.3	ND	31.3	1.5	5.0	5.5	22.0	19.5
2,2′,4,4′,5-pentaBDE (#99)	0.7	3.0	14.3	ND	47.1	5.0	17.0	10.4	8.9	110.2
2,2′,3,4,4′-pentaBDE (#85)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3′,4,4′,5-pentaBDE (#126)	ND	1.0	2.5	ND	2.9	0.2	0.6	0.3	ND	2.5
2,2′,4,4′,5,6′-hexaBDE (#154)	58.8	41.4	23.1	ND	64.7	7.4	36.4	18.5	37.6	170.3
2,2′,4,4′,5,5′-hexaBDE (#153)	5.1	17.5	8.8	ND	23.4	2.0	2.4	1.7	ND	39.7
2,2′,3,4,4′,5′-hexaBDE (#138)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2′,3,4,4′,5′,6-heptaBDE (#183)	1.3	1.3	1.7	ND	3.2	0.4	0.8	0.8	0.9	2.5
∑PBDEs	473.6	108.1	239.8	ND	553.5	87.2	499.8	275.9	291.2	1161.2

[a] ND, not detected.

Table 8. Concentrations of PBDEs in Raw Fish and Shellfish (pg/g)[a]

compound	tuna	trout	arakabu	parrotfish	Japanese sea perch	squid	razor-shell	oyster-1	oyster-2
2,2′,4,5′-tetraBDE (#49)	0.9	72.8	19.4	7.1	5.4	25.5	29.7	3.0	7.1
2,3′,4′,6-tetraBDE (#71)	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2′,4,4′-tetraBDE (#47)	3.4	246.7	143.8	142.1	19.7	106.7	35.1	4.4	18.2
2,3′,4,4′-tetraBDE (#66)	0.5	20.2	7.7	3.0	0.9	12.2	ND	1.0	2.2
3,3′,4,4′-tetraBDE (#77)	0.3	0.5	0.5	1.7	0.5	0.8	ND	0.1	0.4
2,2′,4,4′,6-pentaBDE (#100)	1.9	59.7	15.2	16.9	3.8	32.1	8.6	0.5	3.4
2,3′,4,4′,6-pentaBDE (#119)	0.4	ND	2.9	3.0	1.0	6.3	ND	ND	0.4
2,2′,4,4′,5-pentaBDE (#99)	0.1	71.7	1.3	6.7	1.1	31.8	22.1	0.7	4.8
2,2′,3,4,4′-pentaBDE (#85)	ND	ND	ND	ND	ND	0.1	ND	ND	0.2
3,3′,4,4′,5-pentaBDE (#126)	ND	0.4	0.6	1.3	0.3	0.3	ND	ND	ND
2,2′,4,4′,5,6′-hexaBDE (#154)	0.9	30.6	15.0	29.6	10.0	29.5	7.2	0.4	2.6
2,2′,4,4′,5,5′-hexaBDE (#153)	0.1	14.1	3.8	12.8	2.0	9.1	4.8	0.1	0.5
2,2′,3,4,4′,5′-hexaBDE (#138)	ND	ND	ND	ND	ND	0.2	ND	ND	ND
2,2′,3,4,4′,5′,6-heptaBDE (#183)	0.1	1.3	0.2	0.3	0.2	0.9	9.7	ND	0.1
∑PBDEs	8.6	518.0	210.4	224.5	44.9	255.5	117.2	10.2	39.9

[a] ND, not detected.

Table 9. Concentrations of PBDEs in Processed Foods (pg/g)[a]

compound	dried horse mackerel	broiled eel	boiled fish paste	salted saury	sausage	dried sardines	dried tangle	toasted laver	dried hijiki	dried wakame
2,2′,4,5′-tetraBDE (#49)	57.9	40.3	0.1	13.7	0.7	56.6	0.1	1.2	0.3	1.7
2,3′,4′,6-tetraBDE (#71)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2′,4,4′-tetraBDE (#47)	242.9	180.7	0.8	21.8	5.7	146.3	0.3	3.7	1.8	4.7
2,3′,4,4′-tetraBDE (#66)	17.0	5.8	ND	3.1	0.4	23.9	ND	0.5	ND	0.7
3,3′,4,4′-tetraBDE (#77)	0.1	ND	ND	0.3	ND	1.2	ND	0.1	ND	ND
2,2′,4,4′,6-pentaBDE (#100)	40.8	33.7	0.2	3.1	1.2	27.3	0.1	0.4	0.3	0.5
2,3′,4,4′,6-pentaBDE (#119)	ND	ND	ND	ND	0.1	16.9	ND	ND	ND	ND
2,2′,4,4′,5-pentaBDE (#99)	10.6	4.6	0.1	2.3	2.8	34.4	0.2	1.2	0.8	1.7
2,2′,3,4,4′-pentaBDE (#85)	ND	ND	ND	ND	0.1	ND	ND	0.1	ND	0.1
3,3′,4,4′,5-pentaBDE (#126)	0.4	1.3	ND	0.1	ND	1.9	ND	ND	ND	ND
2,2′,4,4′,5,6′-hexaBDE (#154)	21.8	27.9	0.1	3.2	0.8	80.3	0.1	0.3	0.2	0.4
2,2′,4,4′,5,5′-hexaBDE (#153)	3.3	6.9	0.1	0.9	0.6	18.6	0.1	0.3	0.2	0.2
2,2′,3,4,4′,5′-hexaBDE (#138)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2′,3,4,4′,5′,6-heptaBDE (#183)	0.3	4.3	ND	0.2	0.2	4.0	0.2	0.5	0.2	0.2
∑PBDEs	395.1	305.5	1.4	48.7	12.6	411.4	1.1	8.3	3.8	10.2

[a] ND, not detected.

weight/day in the case of 50 kg body weight. Recently, a lowest observed adverse effect level (LOAEL) value suggested as reasonable for compounds or mixtures belonging to the PBDE group was 1 mg/kg/day (*32*), while the provisionally calculated value 1.97 ng/kg is much less than this LOAEL value. On the basis of these results, the contamination level in fish is not considered a serious problem. However, because the toxicity of PBDEs is still unclear, it is important to continue to perform studies regarding its toxicity, its levels in the environment and in food samples, and in regard to human exposure. Concerning

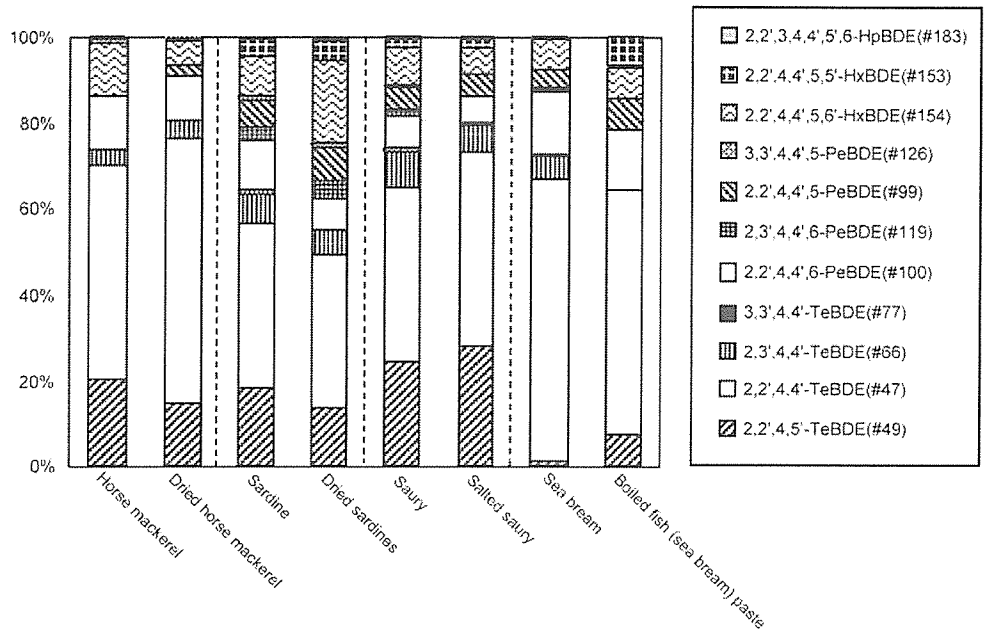

Figure 2. Comparison of PBDEs patterns between raw fish and processed fish in horse mackerel, sardine, saury, and sea bream.

PBDD/DFs, any congeners were not detected in fish samples in this study, but it is also necessary to monitor simultaneously as related compound suspected strong toxicities.

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Screening for dioxins in retail fish using a combination of a PCB ELISA and an aryl hydrocarbon receptor immunoassay (Ah-immunoassay)

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Introduction

Our study of the overall human diet in Japan showed that fish and shellfish are the main sources of PCDD/Fs and dioxin-like PCBs (dioxins)[1]. To assess the risk posed by retail fish, it is therefore important to develop screening methods for dioxins. A reporter-gene assay, such as the CALUX assay, could be a useful methodology for this application, but has the drawback of involving cell culture, which requires skilled personnel and elaborate equipment, and their introduction is also likely to require that the assays used are licensed. An ELISA-based screening tool, in the form of a commercially available kit, would be a simpler and very attractive alternative. In this study, we evaluated the effectiveness of two commercially available kits, a PCB ELISA (PCB-EIA) kit and an Ah-immunoassay (Ah-I) kit, for screening for dioxins in fish. We tested the PCB-EIA, a competitive immunoassay specific for PCB 118, as a screening method for mono-*ortho* PCBs and the Ah-I, an ELISA-based aryl hydrocarbon receptor (AhR) binding assay, as a screening method for non-*ortho* PCBs and PCDD/Fs.

Materials and Methods

Sample preparation for PCB-EIA and Ah-I: The procedure for preparing the fish samples is shown schematically in Figure 1. Samples of 20 g of retail fish were homogenized and incubated in aqueous KOH for 16 h at room temperature. The alkaline hydrolysates were extracted three times by shaking with *n*-hexane. These extracts were treated several times with concentrated sulfuric acid, and loaded onto a multi-layer silica gel column. The eluate obtained with *n*-hexane was loaded onto an alumina column. After washing with *n*-hexane, the first fraction, containing mono-*ortho* PCBs, was eluted with 2% dichloromethane/*n*-hexane, and the second fraction, containing non-*ortho* PCBs and PCDD/Fs, was eluted with 60% dichloromethane/*n*-hexane. The first fraction was dried by evaporation; the residue was re-dissolved in 100 µl DMSO and used in the PCB-EIA.The second fraction was further purified with a sulfuric acid-silica gel column. The eluate obtained with *n*-hexane was dried by evaporation, and the residue was re-dissolved in 20 µl DMSO and used in the Ah-I.

PCB-EIA: This kit was used according to the manufacturer's instructions (EnBioTec Laboratories, Japan)[2]. Briefly, PCBs in samples competed with a competitor-horseradish peroxidase (HRP) conjugate for binding to an anti-PCB 118 monoclonal antibody, coated onto microtiter plate wells. The bound competitor-HRP was detected with the enzyme substrate, 3,3',5,5'-tetramethylbenzidine. The assay used a standard curve with varying concentrations of 3,3',4'-trichloro-4-methoxybiphenyl, which is a surrogate standard for PCB 118, and had a detection limit for PCB 118 of 10 ng/ml (125 pg/well), corresponding to 50 pg/g in the test samples.

Ah-I: This kit was used according to the manufacturer's instructions (KUBOTA Co., Japan and Paracelsian Inc., USA)[3]. Briefly, samples were mixed with a reagent containing dioxin receptor element (DRE) DNA oligomers, AhR nuclear translocator protein (ARNT) and cytosol components containing Ah receptors. The mixtures were added to microtiter wells coated with DRE binding protein. The presence of dioxins promotes the formation of the AhR-ARNT complexes, which then bind DRE and so bind to the wells. Binding was detected with an anti-ARNT antibody and a second antibody conjugated to alkaline phosphatase. The assay used a standard curve with varying concentrations of 2,3,7,8-TCDD for which the detection limit was 5.0 pg/ml (1.0 pg/well). Measurements for samples containing

dioxin-like compounds were converted into Ah-I-based, 2,3,7,8-TCDD equivalents (DEQs), and were corrected by subtraction of the blank concentration for the sample preparation procedure. The minimum concentration measurable in samples was 1.0 pg/g.

HRGC/HRMS: Dioxins were extracted, prepared and analyzed as described previously[4].

Results and Discussion

Two-fold serial dilutions of the prepared extracts of the fish samples were tested in the PCB-EIA and Ah-I, to check for any interference by contaminants from the natural environment. Two or three different fish extracts were diluted with DMSO and assayed. In the PCB-EIA, the concentrations measured were within 83.5 – 107.9% of those expected from the starting concentrations (Figure 2a), suggesting that the matrix did not significantly interfere with this assay, using samples prepared in this way. In contrast, in the Ah-I, dioxin concentrations in some samples, in particular sea bass and yellowtail, appeared to increase with dilution (Figure 2b). This suggested interference, in some cases, with the Ah-I by the sample matrix or AhR antagonists in the samples. Therefore, serial dilutions of the prepared samples were measured in the Ah-I, and the maximum concentration obtained was taken as the concentration of non-*ortho* PCBs and PCDD/Fs fraction.

Dioxin concentrations were measured in twenty samples of retail fish by PCB-EIA and Ah-I, and compared to TEQ concentrations obtained by HRGC/HRMS. Both the concentrations of mono-*ortho* PCBs fraction obtained in the EIA (Figure 3a) and the concentrations of non-*ortho* PCBs and PCDD/Fs fraction obtained in Ah-I (Figure 3b) showed good correlations with TEQ concentrations measured by HRGC/HRMS ($r > 0.98$ and $r = 0.97$, respectively). These results showed that using a combination of the PCB-EIA and the Ah-I would offer a practical method for estimating the TEQ levels of dioxins in retail fish.

Further, the concentrations by the PCB-EIA and the concentrations of PCB 118 by HRGC/HRMS showed a good correlation (Figure 4), with the slope of the linear regression equation being roughly 1. This suggested that a positive reading in the EIA was mainly attributable to PCB 118 in the samples. Results obtained for concentrations in the Ah-I were compared to the expected results, calculated by multiplying the concentrations of 4 non-*ortho* PCBs and 17 PCDD/Fs determined by HRGC/HRMS and their relative potency values in the Ah-I[5]. As shown in Figure 5, a good correlation was observed between obtained and expected values, with the slope of the linear regression equation being roughly 1. This showed that a positive reading in the Ah-I was largely attributable to the target compounds in the samples, and that the difference in the toxic equivalent measurements in the Ah-I and HRGC/HRMS was mainly due to the differences between the relative potency values in the Ah-I and the WHO-TEFs in HRGC/HRMS.

Overall our results indicate that using the PCB-EIA and Ah-I in combination is a useful approach to measuring TEQs of dioxins in retail fish.

Acknowledgements

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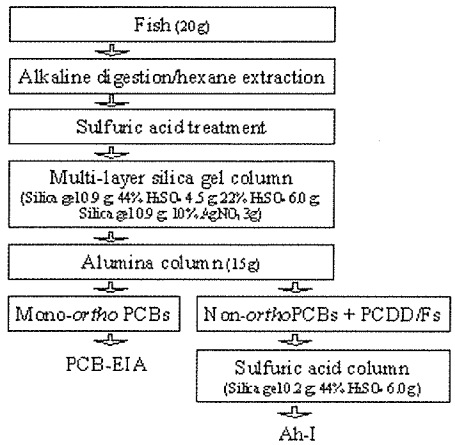

Figure 1. Sample preparation for retail fish

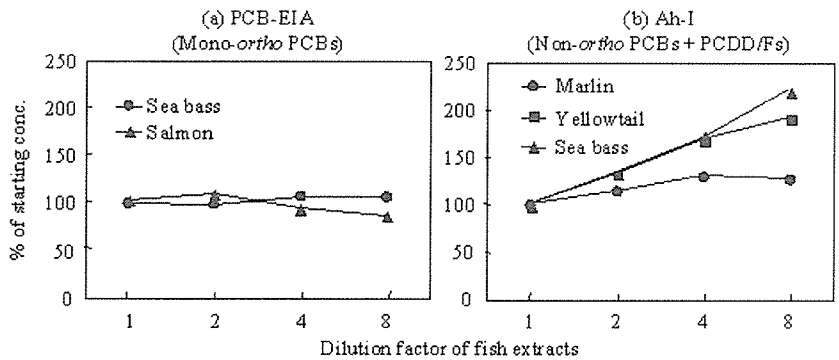

Figure 2. Effect of dilution factor on the determination of dioxins in fish

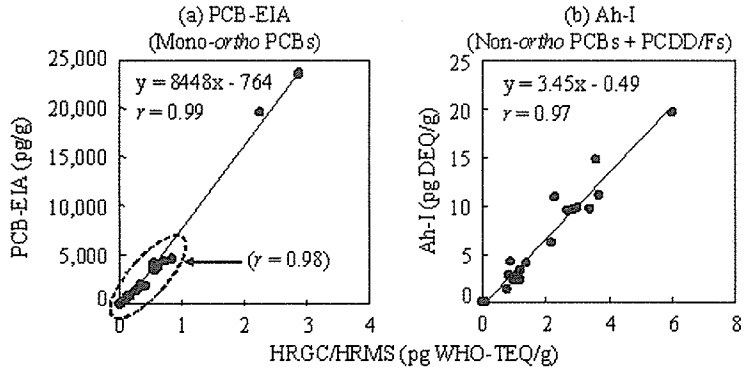

Figure 3. Comparison of EIA and Ah-I with HRGC/HRMS measurements of 20 fish samples

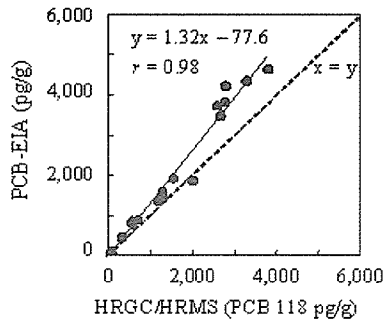

Figure 4. Comparison of concentrations measured in EIA with PCB 118 concentrations measured by HRGC/HRMS in fish samples
Two highly contaminated samples (> 5,000 pg/g of PCB 118) were excluded from the regression calculation.

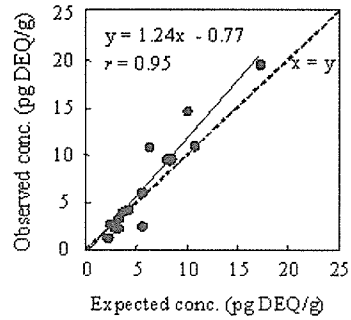

Figure 5. Comparison of observed and expected concentrations in fish, in the Ah-I
Two samples with undetectable levels were excluded from the regression calculation.

Determination of Brominated Flame Retardants in Fish and Market Basket Food Samples of Japan

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Introduction

Brominated flame retardants (BFRs) have been used all over the world and detected in effluents such as those from sewage treatment plants and textile plants[1]. In most cases, such effluents drain into rivers or estuaries. BFRs are suspected as sources of poly-brominated dioxins. Consequently, social concern is increasing regarding the pollution of the environment and marine products by BFRs. Polybrominated diphenyl ethers (PBDE), a well-known group of BFRs, are lipophic and easily bio-accumulated in organisms through the food web; however, the toxicities have not yet been fully clarified. Tetra-brominated bisphenol A (TBBPA) is another representative group of BFRs whose demand by Japan industries in 2001 has increased to nearly 32,000 tons[2], which is more than ten times the demand for DecaBDE (2,200 tons), the only PBDE used in Japan. Since TBBPA has a more polar structure than PBDEs and has been reported to be readily metabolized, there has been a limited number of studies on the pollution in food such as marine products, compared with studies on the pollution caused by PBDEs. In this paper, we report the pollution levels of these BFRs in marine products collected at food market stores in Japan. Additionally, we discuss the estimated daily intakes of the BFRs by analyzing food group samples prepared following the Market Basket Method.

Materials and Methods

Marine products: Eighteen fish of 15 species.

Market basket food samples: Thirteen mixed food samples were prepared in 2002, following the method of the Market Basket Study, alternatively termed theTotal Diet Study [3]. One hundred and sixty-six food items were chosen from 85 categories of foods that the Japanese populace commonly consumes, and the respective amounts of food items composing each of 13 food groups were decided by referring to the data of the latest national and prefectural surveys.

Preparation of samples: A homogenized sample (5~10 g) was spiked with ^{13}C-labeled TBBPA as a clean-up standard and then packed in a stainless steel tube and extracted with methanol. The extraction conditions are listed in Table 1. The methanol extract (ca. 30~50 mL) was defatted by liquid-liquid partition with 20 mL of hexane. Then, 120 mL of 5% sodium chloride solution was added to the methanol layer fraction and re-extracted twice with 30~50 mL of dichloromethane. The extract was concentrated to dryness and then 1 mL of 1N potassium hydroxide and 0.2 mL of diethyl sulfate(Cica-Reagent) were added to it and the mixture was kept at 25~30 °C for 30 min. After the mixture was treated with 4 mL of 1N potassium hydroxide at 70°C for 1 hour, 3 mL of water was added to it and the solution was re-extracted with hexane. The hexane extract was cleaned by chromatography, the first, a florisil mini-column using an elution solvent of 8 mL of 2% diethyl ether/hexane, and when necessary, the second, a sulfuric-acid-impregnated silica-gel mini-column using an elution solvent of 15 mL of dichloromethane. The final eluate was concentrated, re-dissolved in 25 mL of nonane with 5 ng of chrysene-d_{12} as a syringe spike and subjected to measurement by GC/MS. The analytical conditions are listed in Table 2.

Analysis of PBDEs was described in our paper, presented at the Dioxin 2004 in Berlin[4].

Results and Discussion

The levels of TBBPA and ΣPBDEs in 16 mixed-food samples prepared based on the nutritional classification are

shown in Table 3. In this study, the LODs were 0.1 ng/g w.w. for TBBPA and 0.0001 ng/g w.w. for each PBDE congener. TBBPA was only detected at 0.46 ng/g w.w. in one of the two X samples, constituted from fish, shellfish and processed fish products. On the other hand, PBDEs were detected ranging from 0.00012 to 1.7 ng/g w.w. in all of the mixed-food samples except VI. The total consumption of TBBPA was estimated to be 18.8 ng/day at ND=0 and 98.4 ng/day at ND=1/2LOD. The 5-fold differences in the values between the two conditions suggested dependence upon the high-level LOD, in comparison with the non-significant difference in the values of ΣPBDEs between 114 ng/day at ND=0 and 115 ng/day at ND=1/2LOD.

The levels of TBBPA and ΣPBDEs in marine products are shown in Table 4. TBBPA ranged from ND (<0. 1 ng/g) ~3.0 ng/g (saury), while ΣPBDEs ranged from ND (<0.0001 ng/g)~1.2 ng/g (yellowtail) in the raw fish. Although ΣPBDEs appeared to be correlated with the fat contents, there is no correlation for TBBPA. Considering the likelihood of it being easily metabolized by organisms to the possible metabolites such as methoxy- or glucuronized-TBBPA, the exposure to TBBPA may not be considered to continue long, even if there is temporarily highly pollution with TBBPA by sewage or sludge from plants. The exposure to ΣPBDEs is thought to occur when eating food highly contaminated by food web accumulation as same as PCBs. In this study, the medians of TBBPA and ΣPBDEs in raw fish were 0.05 ng/g and 0.22 ng/g, respectively, on the condition that ND is assumed as 1/2 LOD. Regarding all the statistical values of average, median and frequency, the latest pollution by BFRs is considered more considerable by ΣPBDEs than by TBBPA, though the demand for PBDE s (only DecaBDE is now used in Japan) was far below that of TBBPA. For the toxicities in vivo, PBDEs have been reported to pose various toxicities, such as liver toxicity, disruption of thyroid hormones, developmental neuro-toxicity and carcinogenicity on animal tests[5]. In contrast, TBBPA have shown no adverse effects in vivo[6]. However, unexpected nephro-toxicity has been recently found in newborn rats treated with TBBPA [7]. In addition, the detection of TBBPA was reported in human serum by highly sensitive analysis using LC/MS[8]. Therefore, the monitoring of those BFRs in food should be continued to prevent the further pollution that may cause and/or increase risk for the health of humans and wildlife.

Acknowledgements

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Table 1 The extraction conditions

Apparatus: ASE-300 (Dionex)
Extraction Temp.: 50°C, Extraction Pressure: 1500psi
Cell Capacity: 33mL, Exraction Time: 10 min
Extraction Cycle: 3, Flash Capacity: 90%, Purge Time: 120 sec

Table 2 The GC/MS conditions

GC/MS: HP6890/5973MSD
Column: DB-5(J&W) 0.25mmi.d.x30m, film thickness: 0.25mm
Injection Temp: 280°C
Column Temp: 120°C(1min)~20°C/min~300°C

Table 3 Average composition of total diet of average person in Japan

Group	Foods in group	Av. Weight g/day	TBBPA (ng/g,wet weight)	ΣPBDEs (ng/g,wet weight)
I	Rice and rice products	409	<0.1	0.00026
II	Grains, seeds and potatoes	192.8	<0.1	0.0019
III	Sugar and confectionaries	32.6	<0.1	0.0053
IV	Oils	15.2	<0.1	0.12
V	Legume and legume products	73.2	<0.1	0.0041
VI	Fruits	113.9	<0.1	ND
VII	Carrots and green leafy vegetables	86.9	<0.1	0.00056
VIII	White leafy vegetables, mushrooms, and seaweeds	184.6	<0.1	0.00016
IX	Seasonings and beverages	172.2	<0.1	0.00012
Xa	Fish and fish products	82.3	0.46	0.83
Xb		81.8	<0.1	1.7
XIa	Meat and eggs	110.5	<0.1	0.1
XIb		105.3	<0.1	0.07
XIIa	Milk and milk products	122.5	<0.1	0.0062
XIIb		122.5	<0.1	0.011
XIII	Other processed foods	38.1	<0.1	0.0016
XIV	Water	—	—	—
Total estimated comsumption(ng/day) at ND=0			18.8*	114*
Total estimated comsumption(ng/day) at ND=1/2LOD			98.4	115

* caliculated using the average TBBPA(or ΣPBDEs) values of Xa and Xb
LODs of 14 PBDE congeners are all below 0.0001ng/g.