JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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

YUKI ASHIZUKA, REIKO NAKAGAWA,* KAZUHIRO TOBIISHI, TSUGUHIDE HORI, AND TAKAO IIDA

Fukuoka Institute of Health and Environmental Sciences, 39, Mukaizano, Dazaifu-shi, Fukuoka 818-0135, Japan

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 ∑PBDEs 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-

* To whom correspondence should be addressed. Tel: +81 92 921 9946. Fax: +81 92 928 1203. E-mail: nakagawa@fihes.pref.fukuoka.jp.

10.1021/jf0485786 CCC: $30.25
Published on Web 04/14/2005

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

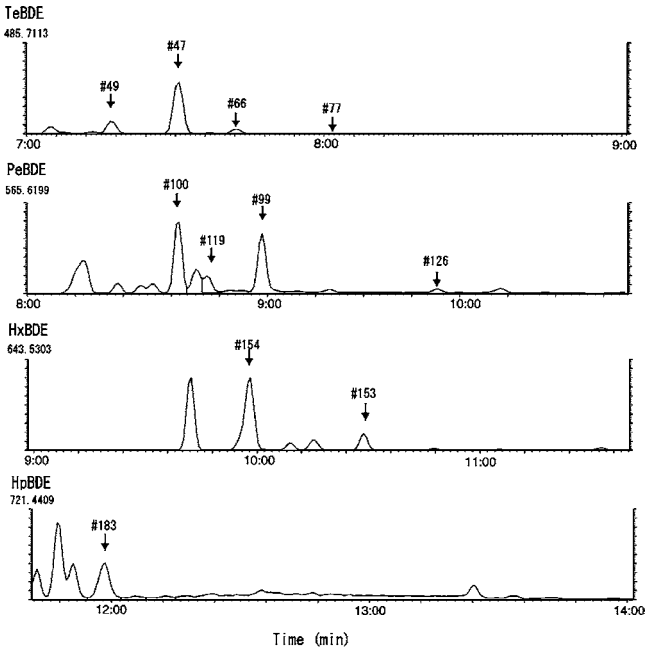

Figure 1. GC/MS SIM chromatograms of PBDEs (tetra-hepta) in dried sardine.

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
∑PBDEs	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

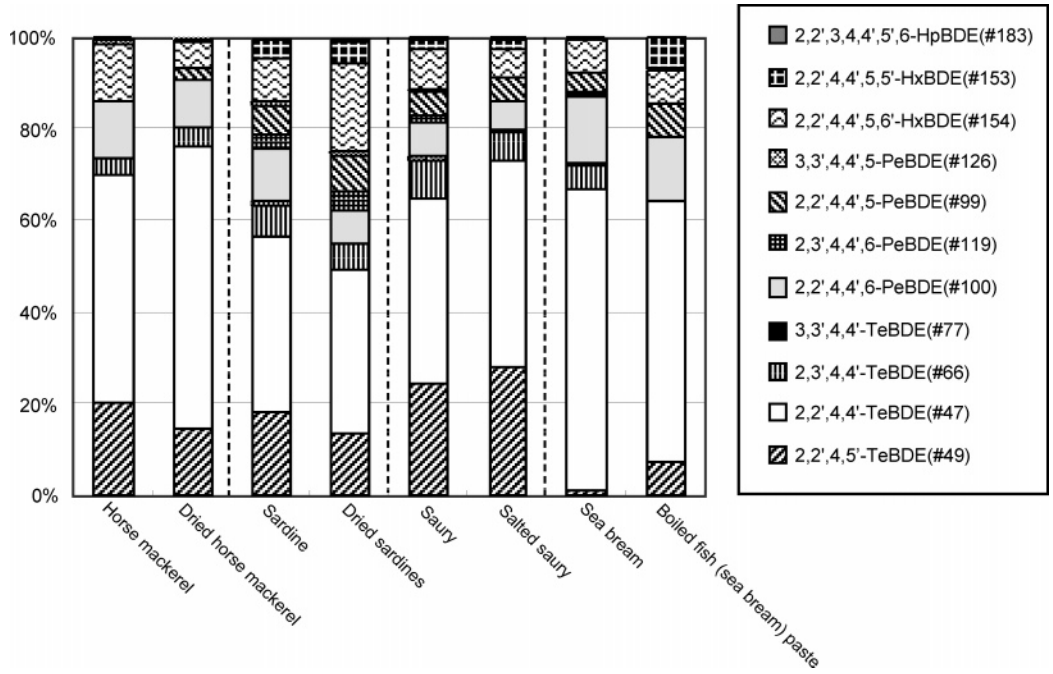

Figure 2. Comparison of PBDEs patterns between raw fish and processed fish in horse mackerel, sardine, saury, and sea bream.

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.

LITERATURE CITED

(1) Ohta, S. Environmental influence by organic brominated compounds. *Waste Manage. Res.* **2001**, *12*, 363−375.

(2) Chen, G.; Konstantinov, A. D.; Chittim, B. G.; Joyce, E. M.; Bols, N. C.; Bunce, N. J. Synthesis of polybrominated diphenyl ethers and their capacity to induce CYP1A by the Ah receptor mediated pathway. *Environ. Sci. Technol*. **2001**, *35*, 3749−3756.

(3) Meerts, I. A. T. M.; Luijks, E. A. C.; Marsh, G.; Jakobsson, E.; Bergman, A.; Brouwer, A. Polybrominated diphenyl ethers (PBDEs) as Ah-receptor agonists and antagonists. *Organohalogen Compd.* **1998**, *37*, 147−150.

(4) McDonald, T. A. A perspective on the potential health risks of PBDEs. *Chemosphere* **2002**, *46*, 745−755.

(5) Meerts, I. A. T. M.; Letcher, R. J.; Hoving, S.; Marsh, G.; Bergman, Å.; Lemmen, J. G.; Burg, B.; Brouwer, A. *In vitro* estrogency of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. *Environ. Health Perspect.* **2001**, *109*, 399−407.

(6) Viberg, H.; Fredriksson, A.; Eriksson, P. Neonatal exposure to polybrominated diphenyl ether (PBDE153) disrupts spontaneous behavior, impairs learning and memory, and decreases hippocampal cholinergic rereceptors in adult mice. *Toxicol. Appl. Pharmacol*. **2003**, *192*, 95−106.

(7) Eriksson, P.; Fischer, C.; Fredriksson, A. Co-exposure to a polybrominated diphenyl ether (PBDE99) and an ortho-substituted PCB (PCB52) enhances developmental neurotoxic effects. *Organohalogen Compd.* **2003**, *61*, 81−83.

(8) Lichtensteiger, W.; Ceccatelli, R.; Faass, O.; Ma, R.; Schlumpf, M. Effect on polybrominated diphenyl ether and PCB on the development of the brain-gonadal axis and gene expression in rat. *Organohalogen Compd*. **2003**, *61*, 84−87.

(9) Buser, H. R. Polybrominated dibenzofurans and dibenzo-*p*-dioxins: Thermal reaction products of polybrominated diphenyl ether flame retardants. *Environ. Sci. Technol*. **1986**, *20*, 404−408.

(10) Wichmann, H.; Dettmer, F. T.; Bahadir, M. Thermal formation of PBDD/F from tetrabromobisphenol A- a pomparison of polymer linked TBBP A with its additive incorporation in thermoplastics. *Chemosphere* **2002**, *47*, 349−355.

(11) WHO. Polybrpminated dibenzo-*p*-dioxins and dibenzofurans. *Environ. Health Criteria* **1998**, *205*.

(12) Lacorte, S.; Guillamon, M.; Martines, E.; Viana, P.; Barcelo, D. Occurrence and specific congener profile of 40 polybrominated diphenyl ethers in river and sediments from Portugal. *Environ. Sci. Technol*. **2003**, *37*, 892−898.

(13) Watanabe, I. Polybrominated biphenyl ethers in marine fish, shellfish and river and marine sediments in Japan. *Chemosphere* **1987**, *16*, 2389−2396.

(14) Choi, J. W.; Fujimaki, S.; Kitamura, K.; Hashimoto, S.; Ito, H.; Sakurai, T.; Suzuki, N.; Nagasaka, H.; Tanabe, K.; Sakai, S.; Morita, M. Historical trends of PBDD/Fs, PBDEs, PCDD/Fs and dioxin-like PCBs in sediment cores from Tokyo bay. *Organohalogen Compd.* **2003**, *61*, 119−122.

(15) Lee, R. G. M.; Thomas, G. O.; Jones, K. C. PBDEs in the atmosphere of three locations in western Europe. *Environ. Sci. Technol*. **2004**, *38*, 699−706.

(16) Hassanin, A.; Breivik, K.; Meijer, S. N.; Steinnes, E.; Thomas, G. O.; Jones, K. C. PBDEs in European background soils: Levels and factors controlling their distribution. *Environ. Sci. Technol*. **2004**, *38*, 738−745.

(17) Hale, R. C.; La Guardia, M. J.; Havey, E. P.; Mainor, T. M.; Duff, W. H.; Gaylor, M. O. Polybrominated diphenyl ether flame retardants in Virginia freshwater fishes (USA). *Environ. Sci. Technol*. **2001**, *35*, 4585−4591.

(18) Manchester-Neesvig, J. B.; Valters, K.; Sonzogni, W. C. Comparison of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in lake Michigan salmonids. *Environ. Sci. Technol*. **2001**, *35*, 1072−1077.

(19) Luross, J. M.; Alaee, M.; Sergeant, D. B.; Cannon, C. M.; Whittle, D. M.; Solomon, K. R.; Muir, D. C. G. Spatial distribution of polybrominated diphenyl ethers and polybrominated biphenyls in lake trout from the Laurentian Great Lakes. *Chemosphere* **2002**, *46*, 665−572.

(20) WHO. Polybrominated diphenyl ethers. *Environ. Health Criteria* **1994**, *162*.

(21) Wolkers, H.; Bavel, B. V.; Derocher, A. E.; Wiig, O.; Kovacs, K. M.; Lydersen, C.; Lindstrom, G. Congener-specific accumulation and food chain transfer of polybrominated diphenyl ethers in two arctic food chains. *Environ. Sci. Technol*. **2004**, *38*, 1667−1674.

(22) Choi, J. W.; Fujimaki, S.; Kitamura, K.; Hashimoto, S.; Ito, H.; Suzuki, N.; Sakai, S.; Morita, M. Polybrominated dibenzo-*p*-dioxins, dibenzofurans, and diphenyl ethers in Japanese human adipose tissue. *Environ. Sci. Technol*. **2003**, *37*, 817−821.

(23) Hirai, T.; Fujimine, Y.; Watanabe, S.; Hata, J.; Watanabe, S. Concentration of polybrominated diphenyl ethers (PBDEs) in human sample in Japanese. *Organohalogen Compd*. **2003**, *61*, 151−154.

(24) Akutsu, K.; Kitagawa, M.; Nakagawa, H.; Makino, T.; Iwazaki, K.; Oda, H.; Hori, S. Time-trend (1973−2000) of polybrominated diphenyl ethers in Japanese mother's milk. *Chemosphere* **2003**, *53*, 645−654.

(25) Ohta, S.; Ishizuka, D.; Nishimura, H.; Nakao, T.; Aozasa, O.; Shimidzu, Y.; Ochiai, F.; Kida, T.; Nishi, M.; Miyata, H. Comparison of polybrominated diphenyl ethers in fish, vegetables, and meats and levels in human milk of nursing woman in Japan. *Chemosphere* **2002**, *46*, 689−696.

(26) Watanabe, K.; Takemori, H.; Abe, M.; Iseki, N.; Masunaga, S.; Ohi, E.; Takasuga, T.; Morita, M. Polybrominated -dibenzo-*p*-dioxins (PBDDs), -dibenzofurans (PBDFs), -biphenyls (PBBs), and-diphenyl ethers (PBDEs) in common cormorant (*Pharacrocorax Carbo*) from Japan. *Organohalogen Compd*. **2003**, *61*, 159−162.

(27) Utkina, N. K.; Denisenko, V. A.; Scholokova, O. V.; Virovaya, M. V.; Gerasimenko, A. V.; Popov, D. Y.; Krasokhin, V. B.; Popov, A. M. Spongiadioxins A and B, two new polybrominated dibenzo-*p*-dioxins from an Australian marine sponge *Dysidea dendyi*. *J. Nat. Prod.* **2001**, *64*, 151−153.

(28) Utkina, N. K.; Denisenko, V. A.; Virovaya, M. V.; Scholokova, O. V.; Prokof'eva, N. G. Two new minor polybrominated dibenzo-*p*-dioxins from the marine sponge *Dysidea dendyi*. *J. Nat. Prod.* **2002**, *65*, 1213−1215.

(29) Choi, J. W.; Hashimoto, S.; Suzuki, N.; Onodera, J.; Ito, H.; Morita, M. Cleanup method for PBDD, PBDF and PBDE by active carbon column- and its application to sediments. *Organohalogen Compd*. **2001**, *52*, 53−57.

(30) Toyoda, M.; Uchibe, H.; Yanagi, T.; Kono, Y.; Hori, T.; Iida, T. Decreased daily intake of PCDDs, PCDFs and Co-PCBs from foods in Japan from 1977 to 1998. *J. Food Hyg. Soc. Jpn*. **1999**, *40*, 494−499.

(31) Akutsu, K.; Obana, H.; Okihashi, M.; Kitagawa, M.; Nakazawa, H.; Matsuki, Y.; Makino, T.; Oda, H.; Hori, S. GC/MS analysis of polybrominated diphenyl ethers in fish collected from the Inland Sea of Seto, Japan. *Chemosphere* **2001**, *44*, 1325−1333.

(32) Darnerud, P. O.; Eriksen, G. S.; Jóhannesson, T.; Larsen, P. B.; Viluksela, M. Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. *Environ. Health Perspect*. **2001**, *109* (Suppl. 1), 49−68.

Received for review August 27, 2004. Revised manuscript received March 18, 2005. Accepted March 20, 2005. This study was supported by a Grant from the Ministry of Health, Labor and Welfare of Japan.

JF0485786