2　テトラブロモビスフェノールA（TBBPA）の分析

2-1　分析方法の検討

TBBPAの分析方法については、環境庁の平成11年度化学物質分析法開発調査報告書（その1）に示されている分析法（新潟県保健環境科学研究所）に準拠した。そして、多種類の魚介類試料を分析するために、試料の抽出と誘導体化後の精製方法について予め検討を行った。試料の抽出過程は、自動化するために高速溶媒抽出（ASE）を用いた抽出法を検討した。メタノールを抽出溶媒として用いて，振とう抽出(回収率80.3%)と同等の回収率(89.5%)が得られた。高速溶媒抽出における条件を表6に示す。

TBBPA誘導体化後の精製法として，フロリジルカラム，硫酸シリカゲルカラム，ヘキサン/DMSO分配の3種類の方法を検討した。表11に各精製法の条件を示す。ヘキサン/DMSO分配による精製は回収率が低かった（図5)。また，フロリジルカラム，硫酸シリカゲルカラムのみの精製では，マトリックスの多い魚介類で妨害成分の除去が不十分であったため，両方のカラム精製を行うことにした。図6に分析操作（検体採取、抽出、精製過程）のフローを示す。アジを用いて添加回収試験（n=3）を行った結果、回収率は74.6±2.9%であり、良好であった。図7に誘導体化後のTBBPA標準溶液のクロマトグラム、図8に実試料のクロマトグラムを示す。

表11　各精製法における条件

精製法	条件
1) フロリジルカラム	カラム内径：6mm　　充填量：0.6g 溶出液：2%ジエチルエーテルヘキサン　8ml
2) 硫酸シリカゲルカラム	カラム内径：6mm　　充填量：0.5g 溶出液：ジクロロメタン　15ml
3) ヘキサン/DMSO分配	ヘキサン/DMSO分配(1：2)を3回行い、 DMSO層に2%食塩水を加えヘキサンで3回抽出

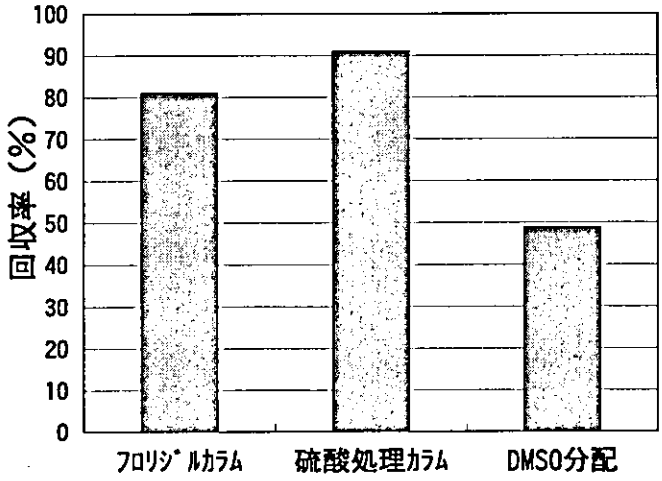

図5　各精製法におけるTBBPAの回収率（%）

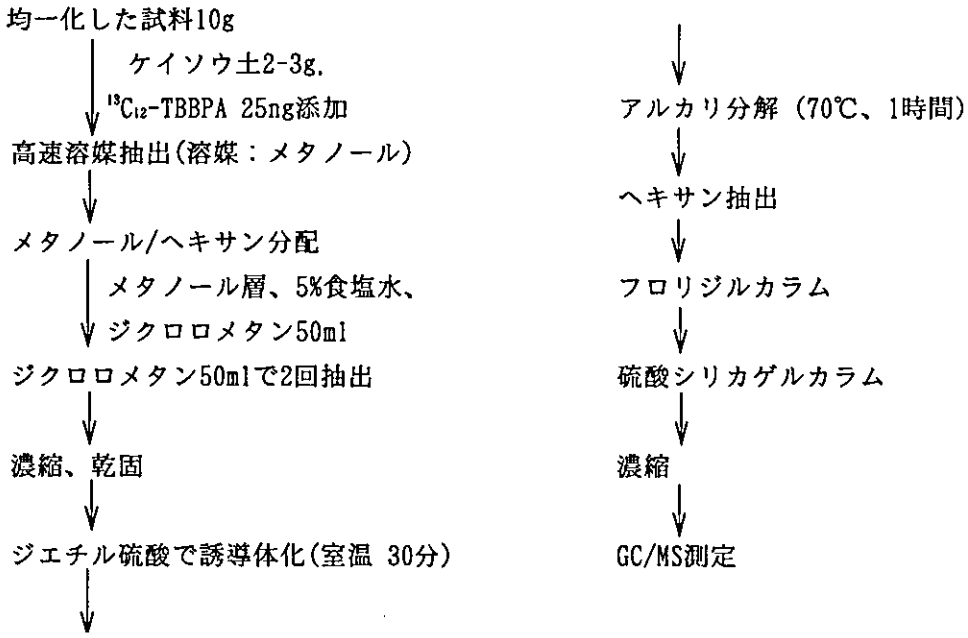

図6　魚介類試料におけるTBBPAの分析方法

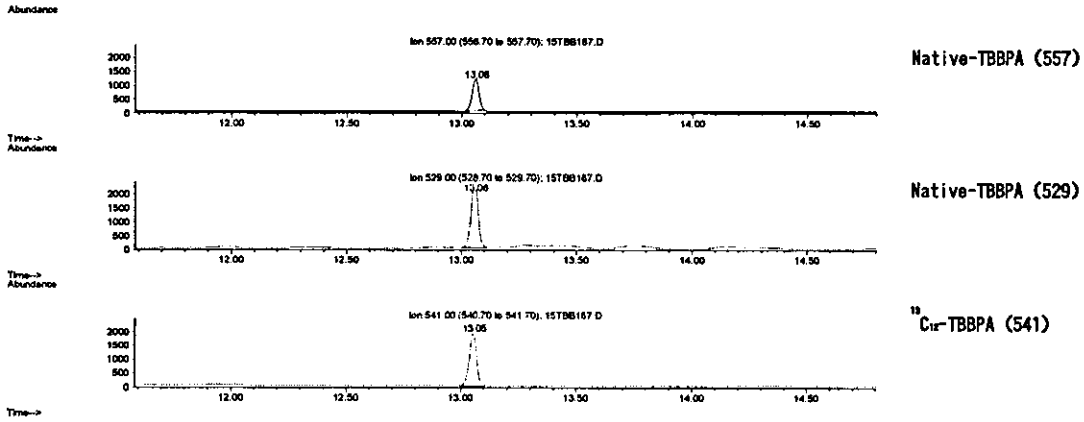

図7　TBBPA 標準溶液(0.5ppm)における SIM クロマトグラム

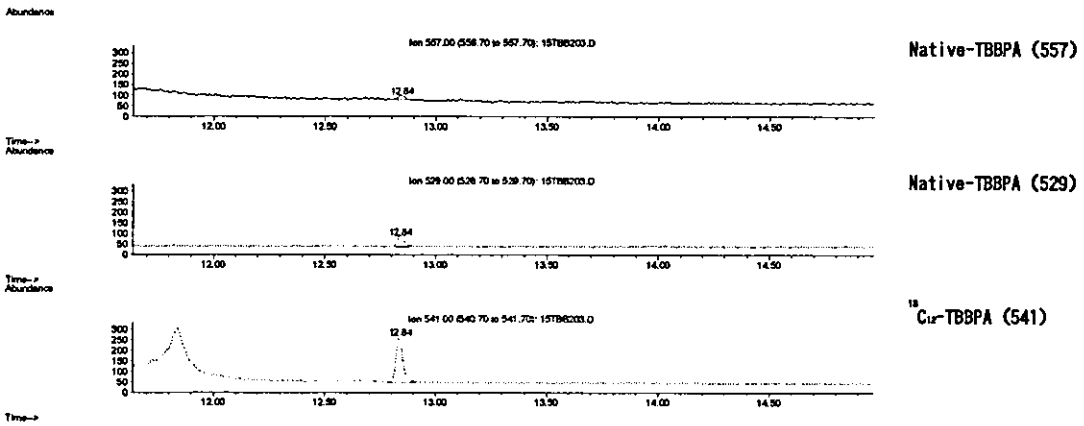

図8　試料（サンマ）における TBBPA の SIM クロマトグラム

2-2トータルダイエット試料の分析

トータルダイエット試料第1群から13群までの分析結果を表12に示す。$^{13}C_{12}$-ラベル体の回収率は50.5-95.8%の範囲で、第7群(有色野菜)以外は70%以上の良好な回収率であった。第10群（魚介類）の試料BからTBBPAが検出された（濃度0.46ng/g)。その他の食品群はすべてND（<0.1ng/g）であった。TBBPAの摂取量はダイオキシン類やPCBと同様に魚介類からの寄与が高いことが示唆された。また、分析結果より、TBBPAの一日当摂取量はND=0で計算した場合は18.8ng/day、ND=LOD/2で計算した場合は110.2ng/dayと算出された。今回の分析では第10群以外のすべての食品群でNDという結果であったため、ND=0とした場合と、ND=LOD/2とした場合での一日当摂取量に5倍以上の差が見られた。より正確に一日当摂取量と推定するためには、より高感度な分析を行う必要があると考えられた。

2-3　魚介類試料の分析

魚介類の個別試料（計27件）を分析した結果を表13（生鮮魚)、表14（加工食品)、表15（海藻類）に示す。　$^{13}C_{12}$-ラベル体の回収率は53.7-97.8%の範囲で、ブリ、開きアジ、煮干し、わかめ、のり以外は70%以上の良好な回収率であった。生鮮魚介類のサンマ、アジ、イサキ、ヤズ、サバ、カワハギ、イワシ、タイの8検体からTBBPAが検出された。最も濃度が高かったのはサンマの2.98ng/gであった。今回分析を行った加工食品と海藻類からは検出されなかった。試料の分析における検出下限値は0.1ng/g(SN=10)であった。

図9は生鮮魚介類におけるTBBPAおよびΣPBDE濃度、魚可食部中の脂肪含量を示している。昨年度測定したΣPBDE濃度が高かったブリ、マスからはTBBPAは検出されなかった。逆に、カワハギのようにPBDEはNDであったがTBBPAが検出された試料もあった。図10はTBBPA濃度とΣPBDE濃度の相関を示している。今回の結果からはTBBPA濃度とΣPBDE濃度の相関は見られなかった。今後さらに、様々な食品における臭素系難燃剤の汚染状況を明らかにするために、サンプルの種類や検体数を増やして調査を継続していきたいと考えている。

E. 健康危険情報

特になし

F. 研究発表

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・Y. Ashizuka, R. Nakagawa, T. Hori, K. Tobiishi, T. Iida: Levels of Polybrominated Diphenyl-Ethers and Polybrominated Dioxins in Fish, Total Diet Study Food Groups and Japanese Meals. Organohalogen Compounds, 66, 2524-2529, 2004.

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5. 平成15年度厚生労働科学研究費補助金研究報告書　「ダイオキシンの汚染実態把握及び低減化に関する研究」

表12　トータルダイエット試料の分析結果（試料中濃度）

	TBBPA濃度 (ng/g)	回収率 (%)
第１群　(米類)	ND	72.2
第２群　(米以外の穀類)	ND	92.2
第３群　(砂糖・菓子類)	ND	70.8
第４群　(油脂類)	ND	80.8
第５群　(豆類)	ND	86.5
第６群　(果実類)	ND	89.7
第７群　(緑黄色野菜)	ND	50.5
第８群　(その他の野菜)	ND	80.1
第９群　(調味嗜好飲料)	ND	92.7
第１０群　(魚介類) A	ND	79.3
第１０群　(魚介類) B	0.46	86.4
第１１群　(肉・卵類) A	ND	79.9
第１１群　(肉・卵類) B	ND	85.5
第１２群　(乳類) A	ND	87.0
第１２群　(乳類) B	ND	91.2
第１３群　(その他の食品)	ND	95.8

表13　生鮮魚介類の分析結果

	TBBPA濃度 (ng/g)	回収率 (%)
サンマ	2.98	81.4
アジ	1.76	74.6
イサキ	1.61	81.7
ヤズ	1.44	96.0
サバ	0.81	74.8
カワハギ	0.67	98.5
イワシ	0.19	97.8
タイ1	0.14	70.7
タイ2	ND	86.4
ブリ	ND	61.5
キハダマグロ	ND	89.5
マス	ND	90.7
スズキ	ND	78.0
ヤリイカ	ND	97.6
アゲマキ	ND	83.5
カキ1	ND	72.2
カキ2	ND	91.8

表14　魚介類加工食品の分析結果

	TBBPA濃度 (ng/g)	回収率 (%)
うなぎの蒲焼	ND	85.9
開きアジ	ND	61.9
鯛のすぼまき	ND	85.2
魚肉ソーセージ	ND	91.5
煮干し	ND	62.4

表15　海藻類の分析結果

	TBBPA濃度 (ng/g)	回収率 (%)
ひじき	ND	70.7
昆布	ND	98.8
わかめ	ND	61.5
のり	ND	53.8

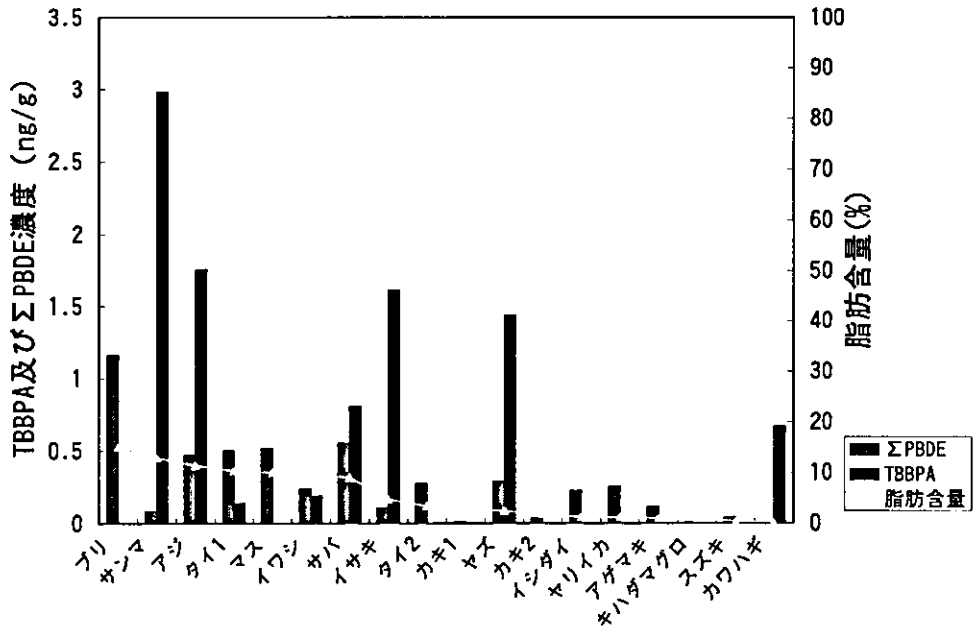

図9　生鮮魚におけるTBBPA及びΣPBDE濃度

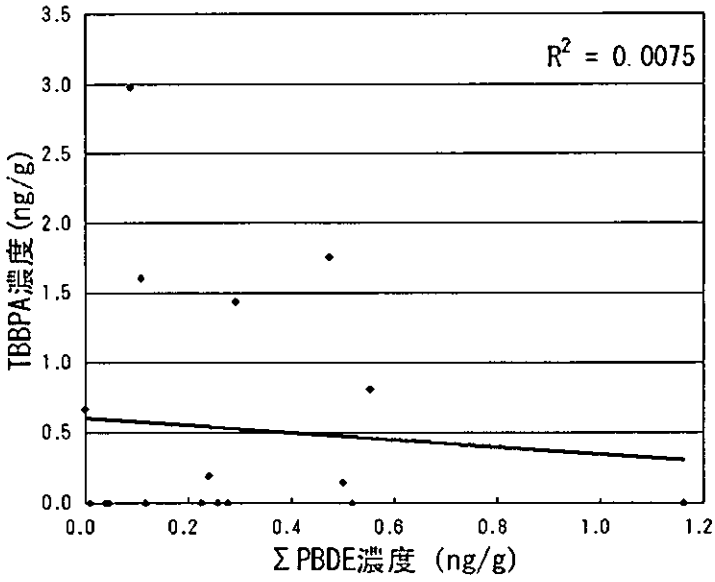

図10　生鮮魚におけるTBBPA濃度とΣPBDE濃度の相関

研究成果の刊行に関する一覧表

及び

研究成果の刊行物・別刷

研究成果の刊行に関する一覧表

雑誌

	発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
1	Hori, T., Tobiishi, K., Ashizuka, Y., Nakagawa, R., Iida, T., Tsutsumi, T., Sasaki, K.	Comparison of accelerated solvent extraction and standard shaking extraction for determination of dioxins in foods	Organohalogen Compounds	66	537-541	2004
2	Ashizuka, Y., Nakagawa, R., Hori, T., Tobiishi, K., Iida, T.	Determination of polybrominated diphenyl ethers(PBDEs) and polybrominated dibenzo-p - dioxins, dibenzofurans (PBDD/DFs) in marine products.	J. Agri. Food Chem.	43	3807-3813	2005
3	Ashizuka, Y., Nakagawa, R., Hori, T., Tobiishi, K., Iida, T.	Levels of poly-brominated diphenyl-ethers and poly-brominated dioxins in fish, total diet study food groups and Japanese meals	Organohalogen Compounds	66	2524-2529	2004

Comparison of Accelerated Solvent Extraction and Standard Shaking Extraction for Determination of Dioxins in Foods

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Introduction

We previously developed a highly sensitive method for determining dioxin content in food using a solvent cut large volume (SCLV) injection system coupled to a cyanopropyl phase capillary column[1]. The SCLV injection system coupled to a 40m-length Rtx-2330 column showed sufficient separation of 2,3,7,8-chlorine substituted isomers, and had at least five-times higher sensitivity than the conventional injection technique [2]. In the current method, a large volume of sample (generally 100g) must be treated collectively in order to attain the desirable limit of detection (LODs) at low ppt levels, namely 0.01pg/g for tetra-CDD and -CDF. The present method allowed the reduction of sample volume from 100g to 20g when such usual LODs are demanded. The SCLV injection technique is expected to improve the efficiency of laboratory performance, especially when it is coupled to an automated extraction method, such as accelerated solvent extraction (ASE).

In order to examine the applicability of ASE for the determination of dioxins in food samples, it is important to verify its extraction efficacy against that of the conventional technique. In the present study we examine the applicability of an ASE for the determination of dioxins in food samples, and the method's performance was compared with that of standard conventional shaking extraction (separatory funnel extraction) regarding recovery rates and quantitative determination. It is considered that homogeneous tissue, such as dried seaweed powder or dried milk powder, is suitable for the method's quantitative validation.

Methods and Materials

For the examination of recovery rate, extracts were prepared from homogenates of various fresh vegetables purchased at a market in Japan. The recovery rates for 17 kinds of ^{13}C-labeled 2,3,7,8-substituted PCDD/Fs and ^{13}C-labeled 12 kinds of dioxin-like PCBs were evaluated. For the comparison of quantitative determination, about 1 kg of domestic dried seaweed (*'Nori'*) was purchased and ground in a mill, giving a homogeneous powder.

The analytical procedures used in this study are summarized in Table 1. Automated extraction was performed using an ASE-300 (Dionex, USA) under the conditions of 1500psi, 150°C. Shaking extraction was twice carried out using a 1L separatory funnel for one hour each time. Four individual experiments and four simultaneous blank tests were performed for each extraction method. Dioxins were analysed using a model 6890 gas chromatograph (Agilent Technologies, USA) coupled to a model Autospec-Ultima mass spectrometer (Micromass, UK). We employed an Rtx-2330 (0.18mm x 40m) capillary column (Restek, USA) on an SCLV injection system (SGE, Australia) in order to determine tetra- and pentaCDD/Fs, and hexaCDFs. The details of the operating conditions for the SCLV injection system are described in another paper[2]. The LOD for each congener was determined according to the provisional guidelines for analysis of dioxins in foods issued by the Ministry of Health and Welfare of Japan in 1999 ('Guideline'): An absolute quantity corresponding to S/N = 3 is evaluated on HRGC/HRMS chromatograms using verification standards.

Table 1. Analytical procedures for determination of dioxins in food.

		Method 1	Method 2
Extraction		Shaking extraction* Sample size: 20g Time: 60min x 2 (120min) Solvent: acetone/n-hexane (1:1, v/v), 600mL (300mL x 2)	Accelerated solvent extraction (ASE) Sample size: 20g Time: 25min Solvent: acetone/n-hexane (1:1, v/v) 200mL
Cleanup		Sulfuric acid treatment □« Multi-layer silica gel column □« Active carbon-dispersed silica gel column	
HRGC/HRMS analysis	PCDD/DFs and non-ortho PCBs	SCLV injection Injection volume: 4 μL / 20μL Pre-column:BPX-5 (0.25mm x 5m) Analytical columns: a) Rtx-2330 (0.18mm x 40m) b) BPX-5 (0.15mm x 30m)	
	Mono-ortho PCBs	Splitless injection Injection volume: 1μL/20 μL Analytical column: HT-8 (0.22mm x 50m)	

* Method recommended for plant food samples in 'Guideline'.

Results and Discussion

As shown in Table 2, our analysis of 20 g of various plant food items according to Method 2, including the ASE and SCLV injection technique, showed recovery rates for ^{13}C-labeled 29 kinds of isomers ranging from 40.4 to 117%, within the range recommended by the Guideline (40-120%). Data regarding the quantification of principal isomers in dried seaweed are shown in Table 3. Generally, it was found that concentrations from ASE were higher than those from shaking extraction. The greatest difference between the methods was observed regarding OCDD. The ratios of estimated concentrations from ASE to those from shaking extraction ranged from 1.1 for 2,3,7,8-

TCDD, PCB#77 and PCB#123 to 3.2 for OCDD. In contrast, the average concentration of PCB#118 on ASE, that was nearly the same as that of OCDD, showed only a slight difference against shaking extraction. The averaged recovery rates for ^{13}C-labeled OCDD were 85% for ASE, which was similar to that for shaking extraction (89%). On the other hand, the results of method blank showed that the contribution of contamination was negligible on the quantification data, and chromatograms of seaweed extract also showed little interference near the retention time of OCDD. The above results suggested that ASE exhibited a superior extraction efficacy, while the extractions were incomplete on shaking extraction. However, ASE's significant predominance against shaking extraction was not observed in another examination using fresh vegetable samples (data not shown). It could be said that higher chlorinated PCDD/F isomer in the seaweed was more strongly adsorbed on the plant's structure than in the other plant foods. Actually, the solid residue after the shaking extraction process was enclosed in an ASE vessel and then re-extracted, with the result that peaks representing OCDD and other dioxin-isomers were observed on their chromatograms (Fig. 1).

Table 2. Recoveries of added 29 kinds of ^{13}C-labeled compounds on various plant food samples using ASE.

No.	Sample	Range (%)
1	*Komatsuna*	42 - 81
2	*Komatsuna*	47 - 82
3	*Komatsuna*	46 - 77
4	*Komatsuna*	43 - 94
5	*Shungiku*	44 - 87
6	*Shungiku*	44 - 72
7	*Shungiku*	48 - 91
8	Celery	42 - 93
9	Celery	43 - 94
10	Celery	44 - 94
11	Seaweed (dry)	42 - 85
12	Seaweed (dry)	55 - 88
13	Pear	45 - 90
14	Pear	51 - 84
15	Japanese parsley	50 - 93
16	*Shimeji*	52 - 90
17	Broccoli	45 - 120
18	Lotus root	40 - 93
19	Tomato	48 - 98
20	Bamboo shoot	42 - 70

Table 3. Concentrations (pg/g) of dioxins in seaweed. Comparison of ASE versus Shaking extraction.

Congeners	ASE (n=4)		Shaking extraction (n=4)		a / b
	Mean[a]	Range	Mean[b]	Range	
2,3,7,8-TCDD	0.016	0.010 - 0.021	0.015	0.013 - 0.017	1.1
1,2,3,7,8-PeCDD	0.014	0.011 - 0.017	0.011	(0.009) - 0.013	1.3
1,2,3,7,8,9-HxCDD	0.028	0.026 - 0.031	0.013	0.013 - 0.015	2.1
1,2,3,4,6,7,8-HpCDD	0.441	0.398 - 0.474	0.185	0.183 - 0.189	2.4
OCDD	3.200	3.053 - 3.470	1.008	0.946 - 1.105	3.2
2,3,7,8-TCDF	0.033	0.029 - 0.038	0.023	0.020 - 0.026	1.4
1,2,3,7,8-PeCDF	0.027	0.023 - 0.029	0.019	0.017 - 0.023	1.4
2,3,4,7,8-PeCDF	0.017	0.016 - 0.018	0.011	(0.009) - 0.012	1.6
1,2,3,4,7,8-HxCDF	0.026	0.023 - 0.033	0.014	(0.011) - 0.015	1.9
1,2,3,4,6,7,8-HpCDF	0.075	0.075 - 0.076	0.037	0.033 - 0.041	2.1
OCDF	0.051	0.047 - 0.057	0.023	0.021 - 0.026	2.2
3,3',4,4'-TCB(#77)	1.003	0.987 - 1.036	0.881	0.871 - 0.896	1.1
3,4,4',5-TCB(#81)	0.157	0.147 - 0.166	0.128	0.121 - 0.138	1.2
2,3,3',4,4'-PeCB(#105)	1.795	1.741 - 1.873	1.552	1.461 - 1.611	1.2
2,3,4,4',5-PeCB(#114)	0.377	0.360 - 0.425	0.305	0.282 - 0.323	1.2
2,3',4,4',5-PeCB(#118)	4.352	4.222 - 4.550	3.755	3.637 - 3.910	1.2
2',3,4,4',5-PeCB(#123)	0.153	0.139 - 0.166	0.134	(0.100) - 0.179	1.1
2,3,3',4,4',5-HxCB(#156)	0.299	0.256 - 0.352	0.252	0.232 - 0.291	1.2
2,3',4,4',5,5'-HxCB(#167)	0.108	0.089 - 0.126	0.084	(0.057) - 0.100	1.3

Trace data are shown in parentheses and counted in the mean value.

In conclusion, ASE could extract dioxins at high efficiency using a low-volume solvent and could provide a high level of performance for various plant matrices, especially regarding those from which dioxins are difficult to extract using conventional shaking extraction. The applicability of combined SCLV injection and ASE methodology is continuously verified for use regarding animal products.

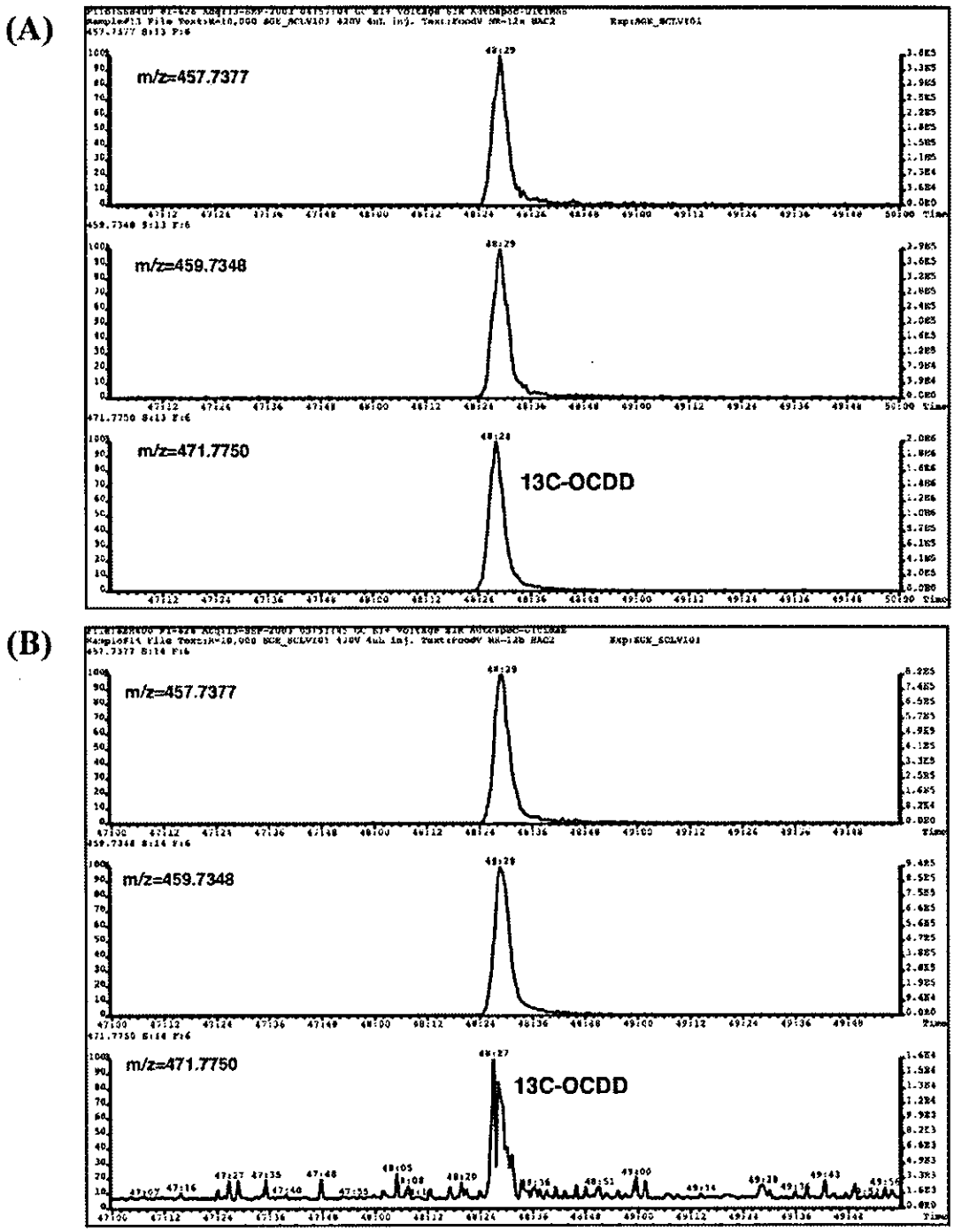

Fig. 1 HRGC/HRMS chromatograms of OCDD (A) obtained from seaweed sample using shaking extraction (B) obtained from the solid residue of shaking extraction process subsequently extracted by ASE.

Acknowledgement

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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

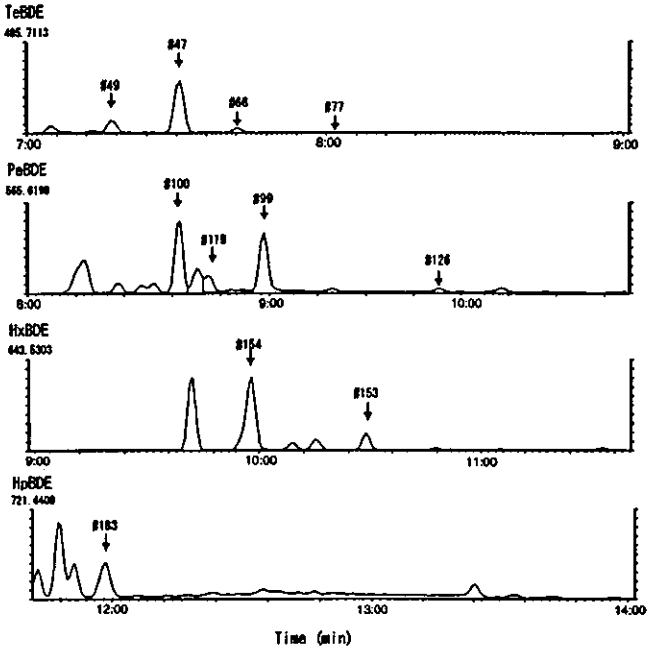

Figure 1. GC/MS SIM chromatograms of PBDEs (tetra-hepta) in dried sardine.

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
ΣPBDEs	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
ΣPBDEs	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
ΣPBDEs	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

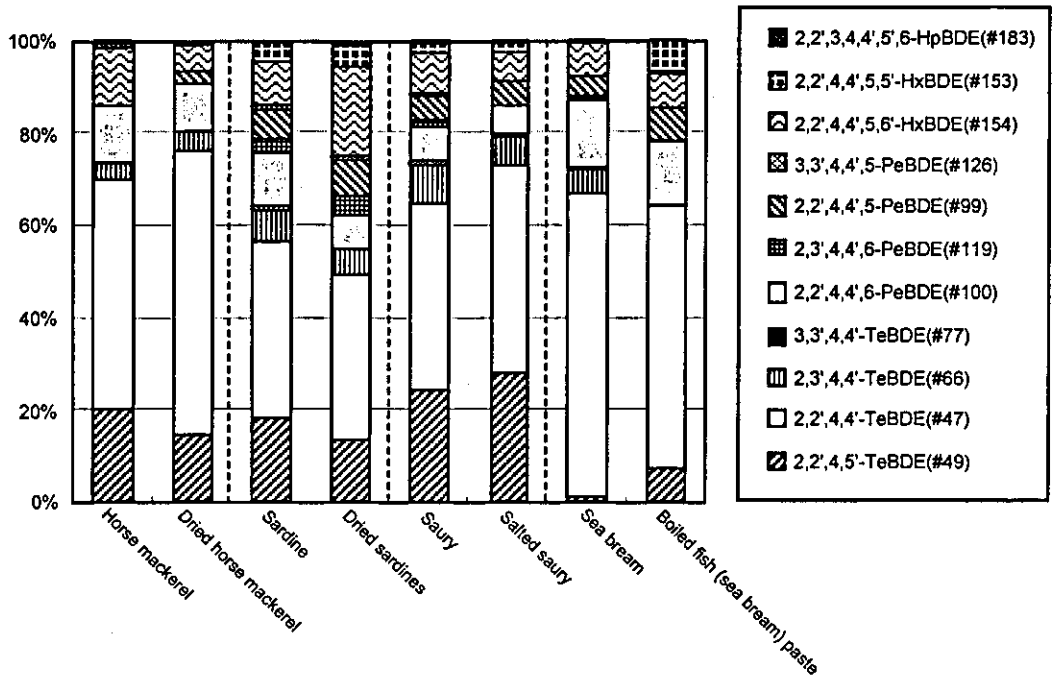

Figure 2. Comparison of PBDEs patterns between raw fish and processed fish in horse mackerel, sardine, saury, and sea bream.

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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