Table 4 TBBPA and ΣPBDEs Concentrations in Various Marine Products

Raw fish and shellfish	Fat Contents (%)	TBBPA (ng/g,wet weight)	ΣPBDEs (ng/g,wet weight)
yellowtail	15	<0.1	1.2
horse mackerel	13	3.0	0.087
saury	11	1.8	0.47
sea bream A	10	0.14	0.50
salmon trout	10	<0.1	0.52
sardine	9.8	0.19	0.24
mackerel	9.1	0.81	0.55
grunt	4.8	1.6	0.11
sea bream B	3.5	<0.1	0.28
oyster-l	3.2	<0.1	0.01
amberjack	2.5	1.4	0.29
oyster-k	1.7	<0.1	0.04
striped beakperch	1.3	<0.1	0.22
cuttlefish	1.3	<0.1	0.26
razor−shell	0.98	<0.1	0.12
tuna	0.89	<0.1	0.0086
sea bass	0.54	<0.1	0.045
leatherfish	0.04	0.67	<0.0001

REMOVAL OF DIOXINS FROM RETAIL FISH BY HIGH-SPEED SOLVENT EXTRACTION

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Introduction

Studies of the Japanese diet have identified fish and shellfish as the main sources of PCDD/Fs and dioxin-like PCBs (dioxins).[1,2] Assessing the risk posed by dioxins in retail fish requires the development of rapid quantitative methods. HRGC/HRMS is the standard technique for dioxin analysis; however, the lengthy extraction process makes it time consuming. The most widely used method for extracting fish dioxins is Soxhlet extraction, although alkaline digestion followed by solvent extraction is also often used in Japan. Both conventional methods take over 16 h. Some new techniques, such as pressurised liquid extraction (or accelerated solvent extraction) have been applied to dioxins, but rarely to those in fish samples. We recently developed a high-speed method based on extraction in heated liquid solvents under near-atmospheric pressure. This technique has been used for dioxin extraction from contaminated soil and fly ash, yielding similar concentrations to Soxhlet extraction but much more rapidly.[3] Here, we report the first data from the application of this method to the extraction of dioxins from retail fish.

Materials and Methods

Samples: Retail fish samples were purchased during the years 2004 and 2005 from supermarkets in Tokyo, Japan. The muscular parts of the samples were homogenised using a food cutter and stored at –20°C until required for analysis.

High-speed solvent extraction: A model SE-100 (Dia Instruments Co., Ltd., Japan) high-speed solvent extractor was employed. Homogenised fish samples (20 g) and sodium anhydrous sulphate (80 g) were ground into powder using a mortar and pestle. The samples were then packed into 160-ml stainless-steel extraction cells. The dead volume was filled with extraction solvents and the top of the cell was sealed with a cap. $^{13}C_{12}$-labelled internal standards were used to spike samples before extraction, and also to spike extracts in order to determine the following optimal extraction conditions: 30°C and 80°C when using acetone/*n*-hexane (1:1) and toluene, respectively, as extraction solvents. The flow rate was set at 6 ml/min. A schematic diagram of the extractor is shown in Figure 1.

Alkaline digestion followed by hexane extraction: The extracts were prepared as described previously.[4] Homogenised fish samples (20 g) spiked with $^{13}C_{12}$-labelled internal standards were incubated in aqueous KOH for 16 h at room temperature. The alkaline hydrolysates were added to methanol and extracted three times by mechanically shaking with *n*-hexane.

Cleanup and HRGC/HRMS analysis: The cleanup and analysis of dioxins generally followed the methods reported previously.[4] Briefly, the extracts were treated with concentrated sulphuric acid and then purified on a silver nitrate/silica gel column. The elute obtained with *n*-hexane was loaded onto an alumina column. After washing with *n*-hexane, the first fraction (containing mono-*ortho* PCBs) was eluted with 2% dichloromethane/*n*-hexane, while the second fraction (containing non-*ortho* PCBs and PCDD/Fs) was eluted with 60% dichloromethane/*n*-hexane. The second fraction was then loaded onto an activated carbon column and eluted with toluene. Both fractions were spiked with $^{13}C_{12}$-labelled recovery standards. The quantification of dioxins was conducted using an HP6890-plus gas chromatograph coupled to a JEOL JMS-700 mass spectrometer. The determination of 2,3,7,8-chlorine-substituted PCDD/Fs was performed in DB-5MS and DB-17 columns. The determination of dioxin-like PCBs was performed in an HT-8 column. The limits of quantification were 0.01–0.2 pg/g for PCDD/Fs and non-*ortho* PCBs, and 0.5–3.0 pg/g for mono-*ortho* PCBs. The TEQ concentrations were calculated using the WHO-TEFs.

Results and Discussion

We initially determined the extraction conditions for the fish dioxins using the high-speed extractor with various extraction times and solvents. Two types of fish, sea bass and yellowtail, were treated with acetone/*n*-hexane for up to 4 h, followed by toluene for 1 h (Figure 2). The cumulative concentrations of 2,3,7,8-chlorine-substituted PCDD/Fs and dioxin-like PCBs reached a plateau after 1 h of extraction with acetone/*n*-hexane in both samples. Although the sea bass samples contained relatively high amounts of dioxin-like PCBs, the 1-h extraction period was sufficient to extract them fully. This was therefore selected as the recommended extraction condition for the practical analysis of fish dioxins.

The suitability of the high-speed solvent extraction method for analysing fish dioxins was compared with that of the conventional alkaline digestion extraction. Table 1 shows the concentrations and relative standard deviations (RSDs) for the two methods when applied to yellowtail samples. The concentration ratios of the two methods were 0.9–1.1, indicating that the concentrations of each isomer were similar for both extractions. The RSDs of the quantified isomers using the novel method were acceptable (0.0–17.4%), and were similar to those obtained using the conventional method (0.0–24.2%). The recoveries of the internal quantification standards using the new method were 72.8–109%, and were similar to those obtained using the conventional method (67.5–105%). The selected ion-mode chromatograms obtained from both extractions were visually inspected, but showed no differences in the homologous groups of dioxins present (data not shown). These results suggest that the methods tested achieved similar extraction efficiencies for dioxins to the conventional extraction method.

Finally, we used the high-speed extraction method to determine the TEQ concentrations of samples of 12 popular retail fish from Japan compared with those obtained by the conventional extraction. As shown in Figure 3, the TEQ concentrations produced by both extractions showed excellent correlations for both PCDD/Fs (r = 0.99) and dioxin-like PCBs (r = 0.99), with the slopes and y-intercepts of the linear regression equations being close to 1 and 0, respectively. This confirmed that the TEQ concentrations obtained using the present method were comparable to those obtained with the conventional extraction method.

Overall, our results indicate that high-speed solvent extraction is a useful method for extracting dioxins from retail fish. The main advantage of this method is the short extraction time (~1 h) compared with the alkaline digestion extraction method (~20 h). This method allows the rapid determination of dioxins and will therefore be a valuable tool for monitoring dioxin levels in retail fish.

Acknowledgements

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Table 1 Comparison of dioxin concentrations in yellowtail using two extractions (n=3)

Dioxins		High-speed solvent extraction (A)		Alkaline digestion-extraction (B)		Ratio
		Mean±SD, pg/g	RSD, %	Mean±SD, pg/g	RSD, %	(A/B)
PCDDs	2378-TCDD	0.13 ± 0.010	7.7	0.13 ± 0.010	7.7	1.0
	12378-PeCDD	0.27 ± 0.010	3.7	0.27 ± 0.020	7.4	1.0
	123478-HxCDD	tr [1)]	-	tr	-	-
	123678-HxCDD	0.11 ± 0.0058	5.2	0.12 ± 0.010	8.3	0.9
	123789-HxCDD	tr	-	tr	-	-
	1234678-HpCDD	0.079 ± 0.0031	3.9	0.077 ± 0.0066	8.5	1.0
	OCDD	0.15 ± 0.023	15.4	0.14 ± 0	0.0	1.1
PCDFs	2378-TCDF	2.1 ± 0.12	5.5	1.9 ± 0	0.0	1.1
	12378-PeCDF	0.28 ± 0.010	3.6	0.27 ± 0.012	4.3	1.0
	23478-PeCDF	0.91 ± 0.035	3.9	0.96 ± 0.010	1.0	0.9
	123478-HxCDF	0.052 ± 0.0091	17.4	0.050 ± 0.012	24.2	1.0
	123678-HxCDF	0.058 ± 0.0035	6.0	0.057 ± 0.0081	14.2	1.0
	123789-HxCDF	nd [2)]	-	nd	-	-
	234678-HxCDF	0.060 ± 0.0012	1.9	0.055 ± 0.0070	12.7	1.1
	1234678-HpCDF	tr	-	tr	-	-
	1234789-HpCDF	nd	-	nd	-	-
	OCDF	nd	-	nd	-	-
Non-*ortho* PCBs	33'44'-TCB	84 ± 2.1	2.5	83 ± 2.1	2.5	1.0
	344'5-TCB	4.5 ± 0.058	1.3	4.4 ± 0.20	4.5	1.0
	33'44'5-PeCB	22 ± 0.58	2.6	21 ± 0.58	2.7	1.0
	33'44'55'-HxCB	3.0 ± 0.058	1.9	3.0 ± 0.058	1.9	1.0
Mono-*ortho* PCBs	233'44'-PeCB	910 ± 25	2.8	920 ± 12	1.3	1.0
	2344'5-PeCB	62 ± 4.7	7.6	61 ± 2.6	4.3	1.0
	23'44'5-PeCB	2800 ± 0	0.0	2800 ± 58	2.1	1.0
	2'344'5-PeCB	45 ± 0.58	1.3	44 ± 2.1	4.7	1.0
	233'44'5-HxCB	290 ± 5.8	2.0	290 ± 5.8	2.0	1.0
	233'44'5'-HxCB	84 ± 1.7	2.1	84 ± 2.0	2.4	1.0
	23'44'55'-HxCB	190 ± 0	0.0	180 ± 5.8	3.2	1.1
	233'44'55'-HpCB	31 ± 1.2	3.7	29 ± 1.5	5.3	1.1

1) tr: trace (detection limits ≦tr＜quantification limits)

2) nd: not detected

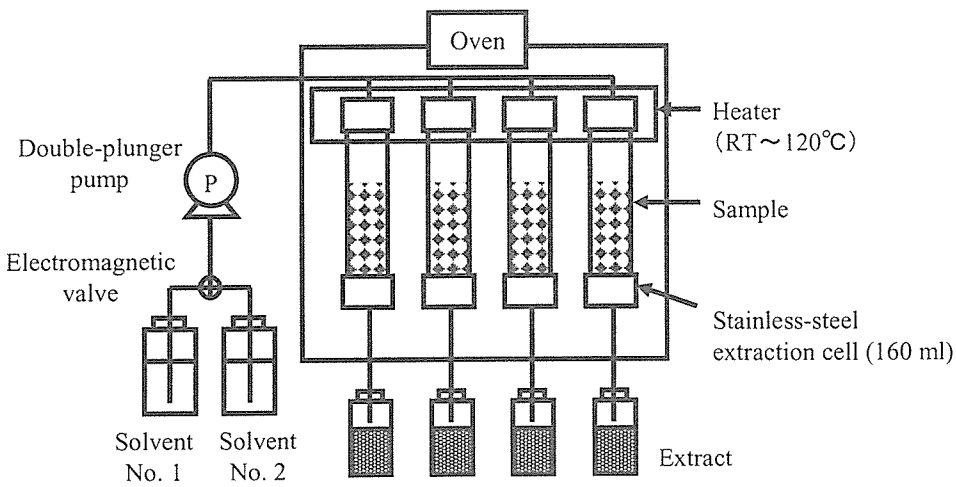

Figure 1 Schematic diagram of the extractor (SE-100)

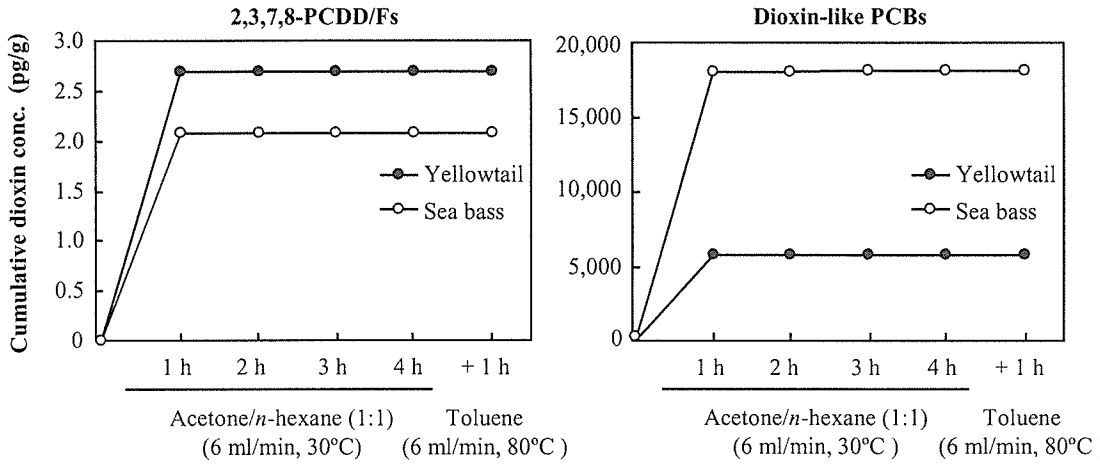

Figure 2 Dioxin concentrations in the high-speed solvent extraction under various extraction conditions. Two popular fish samples were serially extracted by the high-speed solvent extraction with acetone/*n*-hexane for up to 4 h under 30°C and then extracted with toluene for 1 h under 80°C. The hourly extracts were spiked with $^{13}C_{12}$-labelled internal standards and cleaned up for HRGC/HRMS analysis.

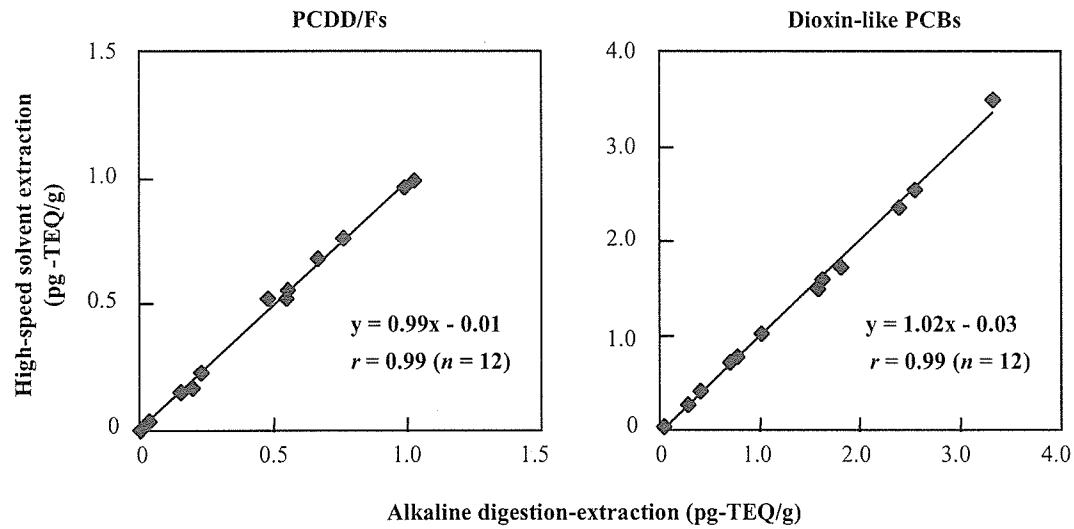

Figure 3 Comparison of TEQ concentrations of dioxins in retail fish determined by the two extraction methods. Twelve retail samples (bonito, conger eel, horse mackerel, marlin, two salmon, sardine, tuna, four yellowtail) were extracted by the two extraction methods and analyzed by HRGC/HRMS analysis.

DETERMINATION OF BROMINATED FLAME RETARDANTS AND BROMINATED DIOXINS IN FISH COLLECTED FROM THREE REGIONS OF JAPAN

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Introduction

In Japan, tetra brominated bisphenol A (TBBPA) totaling nearly 32,000 tons was consumed in 2001, which is ten times more than deca brominated diphenyl ether (DecaBDE), the only polybrominated diphenyl ether (PBDE) used in Japan (2,200 tons). Due to the worldwide usage of these brominated flame retardants (BFRs), their detection has been reported not only in environmental samples such as effluents from BFR manufacturing plants and textile plants, but also in human breast milk[1]. Therefore, the social relevance of the environmental pollution and human toxicities of BFRs is of great concern. In addition, PBDEs and TBBPA are suspected as polybrominated dioxin (PBDD/DFs) originating chemicals, because such dioxins were found in formulations of the BFRs. PBDEs and PBDD/DFs are lipophilic and likely to be bio-accumulated in organisms through the food web. Consequently the pollution in environmental samples, in particular that in food, should be cleared as quickly as possible. Additionally, it is important to investigate whether there are differences in the pollutant levels among sampling areas, to judge the pollution status of BFRs and PBDD/DFs in Japan. Here, we report the pollution levels of PBDEs, TBBPA, and PBDD/DFs in marine products purchased at food market stores in three regions of Japan from 2004 to 2005. The regions were Kyushu, a less industrialized area; Seto Inland Sea, an industrialized area; and Nagoya, a commercialized and industrialized area. Additionally, we estimate the daily intakes of PBDEs, TBBPA, and PBDD/DFs by multiplying each analyte concentration with fish weight consumed by an average Japanese adult.

Materials and Methods

Marine products: fifteen marine products were collected from each of the three regions of Nagoya, Seto Inland Sea, and Kyushu.

Analysis of PBDEs and PBDD/DFs: Forty-five marine products in total were analyzed for PBDEs and PBDD/DFs, following the procedure described in our previous report[2]. The analytical procedure consisted of freeze-drying, accelerated solvent extraction (100℃,1500 psi, n-hexane) and purification with sulfuric acid treatment, and three kinds of column chromatography of silica gel, florisil, and active carbon. Measurement was conducted by an isotope dilution method using a high resolution gas chromatograph/ high resolution mass spectrometer (HRGC/HRMS) (Table 1). Detection limits of PBDD/DFs were 0.01 pg/g, wb for tetra and penta brominated DD/DFs, 0.05 pg/g, wb for hexa brominated DD/DFs, and 0.1 pg/g, wb for hepta brominated DF. Detection limits of PBDEs were 0.1 pg/g, wb for from tetra- to octa-BDE, and 0.2 pg/g, wb for nona-BDE and 1.0 pg/g, wb of DeBDE (#209).

Analysis of TBBPA: A homogenized sample (5 g) was spiked with $^{13}C_{12}$-labeled TBBPA (0.5 ng) as a clean-up standard and then extracted with methanol. The methanol extract (ca.50 mL) was defatted by liquid-liquid partition with 10 mL of n-hexane. Then, to the methanol layer, 120 mL of 5% sodium chloride solution was added and re-extracted twice with 25 mL of dichloromethane. The extract was concentrated to dryness, and then 1mL of 1N potassium hydroxide / ethanol and 0.2 mL of diethyl sulfate were added, and the mixture was kept at 30 ℃ for 30 min. To the mixture, 4 mL of 1N potassium hydroxide / ethanol was added and kept at 70℃ for 1 hour. Then 3 mL of water was added, and it was re-extracted with n-hexane. The n-hexane extract was cleaned with florisil mini-column chromatography using an elution solvent of 8 mL of 2% diethyl ether/n-hexane The final eluate was concentrated, re-dissolved in 20 μL of nonane with 2.5 ng of

chrysene-d_{12} as a syringe spike, and subjected to measurement by HRGC/HRMS (Table 1). The detection limit of TBBPA was 0.01ng/g, wb.

Table 1 The HRGC/HRMS conditions for PBDD/DF, PBDE AND TBBPA analysis

HRGC: HP6890 (Hewlett Packard)
PBDD/DF analysis : Column:DB-5 (J&W) 0.25mmi.d. x 30m, film thickness 0.1μm **Injector temp.:260℃** **Column Temp:130℃(1min)-20℃/min-240℃-5℃/min-320℃(7.5min)**
PBDE analysis: Column: HP-5MS(Agilent) 0.25mmi.d. x15m, film thickness 0.1μm **Injector temp.:260℃** **Column Temp:120℃(2min)-20℃/min-200℃-10℃/min-300℃(7.5min)**
TBBPA analysis: Column:DB-5 (J&W) 0.25mmi.d. x 30m, film thickness 0.25μm **Injector temp.:280℃** **Column Temp:120℃(1min)-20℃/min--300℃(8min)**
HRMS: Autospec Ultima (MicroMass) **Electron energy,38eV; filament current, 750μA; ion source Temp.,270℃; resolution,10000**

Results and Discussion

The levels of PBDEs, PBDD/DFs, and TBBPA in 45 marine products from the three regions are summarized in Table 2. PBDEs were detected in all of the samples. The means and ranges of ΣPBDEs were 0.75 ng/g, wb and 0.01 to 0.70 ng/g, wb for Nagoya (N), 0.16 ng/g, wb and 0.01 to 0.53 ng/g, wb for Seto Inland Sea (S), and 0.15 ng/g, wb and 0.01 to 0.70 ng/g, wb for Kyushu (K). TBBPA was detected in a part of the samples. The detection rates were 53.3% for S and N and 86.7% for K. The means were 0.01 to 0.02 ng/g, wb for all regions. The mean of TBBPA was about one-tenth or less, compared with those of ΣPBDEs. PBDD/DFs were only detected in eight fish of the S region. They are 1,2,3,4,6,7,8-heptabrominated dibenzofuran (25.6 pg/g wb of Pike eel shown in Fig. 2, 0.42 pg/g wb of flatfish, 0.276 pg/g wb of natural sea bream A, 0.217 pg/g wb of conger eel, 0.114 pg/g wb of sole, 0.175 pg/g wb of young seerfish, 0.104 pg/g wb of sea bream B), 2,3,7,8-tetrabrominated dibenzo-p-dioxin (0.016pg/g wb of natural sea bream A shown in Fig. 3), 2,3,7,8-tetrabrominated dibenzofuran and 3-bromo-2,7,8-trichlorinated dibenzofuran (0.029 pg/g wb and 0.020 pg/g wb shown in Fig. 4 and 5) of conger eel. The mean and range of PBDD/DFs in the S region was 0.19 pg TEQ/g wb and ND to 0.256 pg TEQ/g wb. These findings would support that the S region has been polluted by PBDD/DF emissions from industry plants. Assuming that an adult person every day consumes 82.2 g of fish contaminated with the means of the three regions, that is, 0.35 ng/g of ΣPBDEs, 0.02 ng/g of TBBPA, and 0.006pgTEQ/g of ΣPBDD/DFs, the estimated daily intake of ΣPBDEs, TBBPA, and ΣPBDD/DFs from fish would result in 28.8 ng of ΣPBDEs, 1.64 ng of TBBPA, and 0.49 pg TEQ of ΣPBDD/DFs. In particular, compared with the Japanese daily intake of polychlorinated dioxins from fish (44.11 pg TEQ) investigated in 2004, the above intake of ΣPBDD/DFs is very low.

Table 2 The mean levels and ranges of PBDEs,TBBPA and PBDD/DFs in marine products from three regions of Japan

	Nagoya	Seto Inland Sea	Kyushu	Mean
ΣPBDEs (ng/g,wb)	0.75(0.01-0.70)	0.16(0.01-0.53)	0.15(0.01-0.70)	0.35
TBBPA(ng/g, wb) ND=0	0.01(ND-0.04)	0.02(ND-0.10)	0.02(ND-0.11)	0.02
ΣPBDD/DFs(pgTEQ/g, wb) ND=0	0.000	0.019(ND-0.256)	0.000	0.006

Fig. 1a and 1b show the correlations between ΣPBDEs and fat content (Fig. 1a) or fish size (Fig. 1b). Since PBDE is lipophilic, good correlation between ΣPBDEs and fat content (R^2=0.431) is reasonable. However, better correlation (R^2=0.618) was obtained between ΣPBDEs and fish size. In Table 2, one reason why ΣPBDEs in fish from the N region were higher than those in fish from other regions might have been the difference in fish size. On the other hand, TBBPA didn't correlate with fish size or fat content. Table 3

shows PBDE congener levels of cultivated or natural sea breams and conger eel. The cultivated sea breams contained relatively higher levels of ΣPBDEs than those of the natural sea breams. As a source of PBDE in cultivated fish, feed used for cultivation is suspected. For the cultivated sea breams, congeners #47 and #100 were big contributors. For the natural sea breams, the contribution of congener #47 had decreased, but that of congener #209 had increased. Particularly, for the pike eel with a high level of ΣPBDEs, contribution of #209 was also large (25%; the same as that of #47). Additionally, congeners # 206 and #207 contributed nearly 10% each. In the pike eel, 1,2,3,4,6,7,8-HpBDF was detected abundantly compared with the levels detected in the other six fish. Therefore, we speculated that congeners #209, #206, and #207 are closely related to 1,2,3,4,6,7,8-HpBDF. This is also supported by the finding of 1,2,3,4,6,7,8-HpBDF in the sea water into which effluents from DBDE(#209) manufacturing plant[3] were possibly dumped.

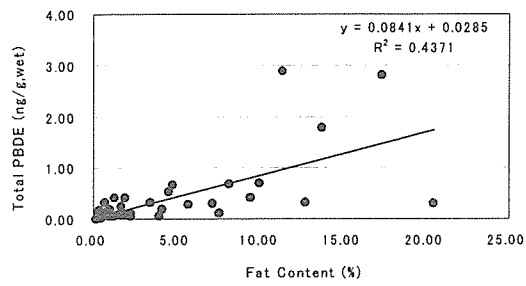

Fig.1aCorrelation between Total PBDE and Fat Content

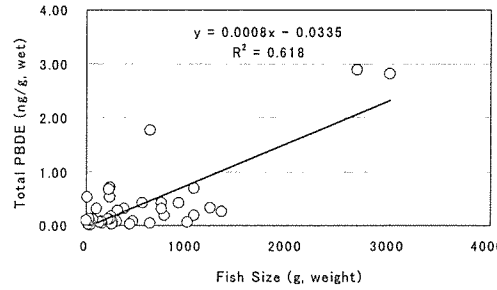

Fig.1b Correlation between Total PBDE and Fish Size

Table 3 PBDE congener levels(pg/g) in cultured and natural sea breams and a pike eel in which 1,2,3,4,6,7,8-HpBDF was detected .

	Cultivated Sea	Cultivated Sea	Natural Sea	Natural Sea bream(K)	Natural Pike eel
2,,4,4'-TriBDE(#28)	28.9	25.9	2.2	2.0	4.8
2,2',4,5'-TeBDE(#49)	52.6	4.5	0.5	1.3	20.2
2,2',4,4'-TeBDE(#47)	365.1	236.0	18.0	18.1	80.3
2,3',4,4'-TeBDE(#66)	19.2	19.8	1.7	1.8	4.5
2,2',4,4',6-PeBDE(#100)	93.0	60.4	2.2	5.0	16.6
2,3',4,4',6-PeBDE(#119)	0.0	9.6	0.3	0.8	6.2
2,2',4,4',5-PeBDE(#99)	17.8	12.2	1.9	0.8	11.2
2,2',4,4',5,6'-HxBDE(#154)	41.4	39.7	4.2	5.5	24.1
2,2',4,4',5,5'-HxBDE(#153)	4.7	1.5	1.5	0.4	8.1
2,2',3,3',4,4',5,6,6'-NoBDE(#207)	2.6	0.6	0.8	0.9	26.2
2,2',3,3',4,4',5,5',6'-NoBDE(#206)	2.2	0.0	0.7	0.7	24.5
DeBDE(#209)	51.4	5.7	13.7	10.8	79.6
Total PBDEs*	681.1	419.8	50.1	50.1	306.3

* Total PBDEs includes minor PBDE congeners not shown in the Table.

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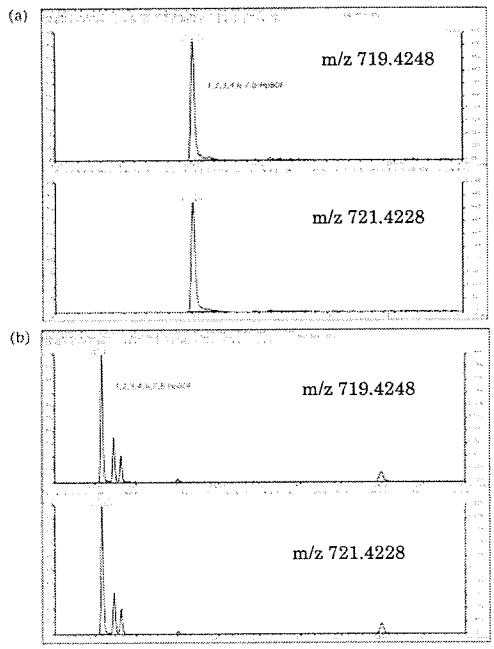

Fig. 2 MS chromatograms of1,2,3,4,6,7,8-HpBDFin natural Pike eel
(a) Column: DB-5 (b) Column: MP65HT

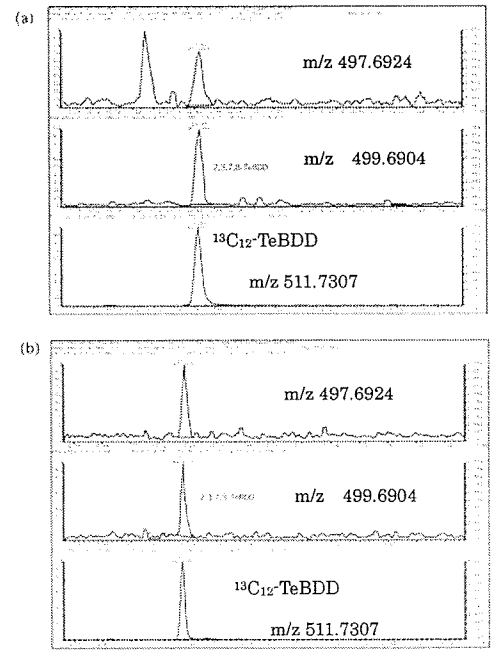

Fig. 3 MS chromatograms of1,2,3, 7,8-TeBDD in natural Sea bream
(a) Column: DB-5 (b) Column: MP65HT

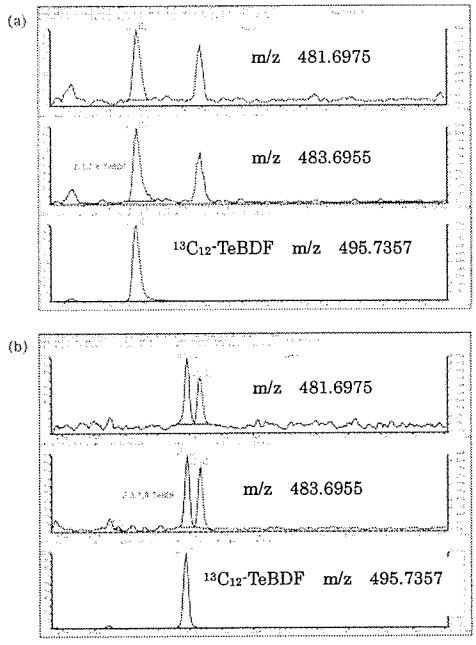

Fig. 4 MS chromatograms of1,2,3, 7,8-TeBDF in natural Conger eel
(a) Column: DB-5 (b) Column: MP65HT

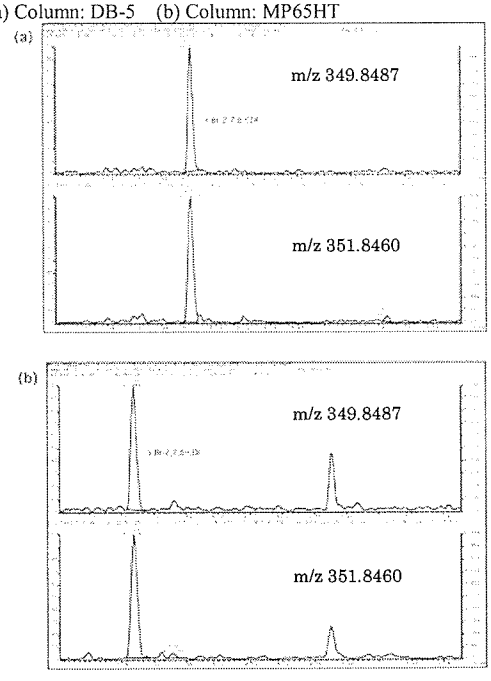

Fig.5 MS chromatograms of1 3-Br-2,7,8-CDF in natural Conger eel
(a) Column : DB-5 (b) Column: MP65HT

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Application of an ELISA for PCB 118 to the screening of dioxin-like PCBs in retail fish

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Abstract

A commercially available enzyme-linked immunosorbent assay (ELISA) kit was evaluated for the determination of toxic equivalents (TEQs) of dioxin-like polychlorinated biphenyls (PCBs) in retail fish. The ELISA was highly specific for 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118), which is generally the most abundant dioxin-like PCB isomer found in fish. The quantitative limit of the ELISA (using 3,3′,4′-trichloro-4-methoxybiphenyl as a surrogate standard for PCB 118) was 10 ng ml^{-1} (125 pg $assay^{-1}$) in the standard curve, corresponding to 50 pg PCB 118 g^{-1} in the tested sample. Good recoveries of PCB 118 (78.7–112.3%) were obtained for spiked purified fish extracts according to the ELISA. Good linearity was also obtained in dilution tests using purified fish extracts. No significant interference of the matrix was observed in the ELISA when this purification procedure was used. Recovery tests in which PCB 118 was added to fish samples also resulted in acceptable recoveries (60.2–82.3%) in the ELISA following purification. The ELISA results for fish samples correlated well with the TEQ concentrations of dioxin-like PCBs obtained by high-resolution gas chromatography/high-resolution mass spectrometry ($r = 0.92$, $n = 26$). These data indicate that the ELISA kit is suitable for screening retail fish for the TEQs of dioxin-like PCBs.

Keywords: PCBs; Dioxins; ELISA kit; Fish; Screening

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of persistent environmental contaminants that are found throughout the world. Twelve of the possible 209 PCB congeners (four non-*ortho* PCBs and eight mono-*ortho* PCBs) are generally considered to be the most toxic, showing similar levels to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). These 12 congeners are therefore termed 'dioxin-like PCBs' and have been assigned toxicity equivalent factors (TEFs) relative to 2,3,7,8-TCDD (Van den Berg et al., 1998). The populations of several countries face considerable exposure to dioxins (polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and dioxin-like PCBs) through the consumption of fish (Kiviranta et al., 2001; Tsutsumi et al., 2001). Dioxin-like PCBs often make up the majority of the toxic equivalent (TEQ) contribution in fish samples (Choi et al., 2002; Tsutsumi et al., 2003a). It is therefore important to determine the TEQ levels of dioxin-like PCBs as well as PCDD/Fs in retail fish.

Currently, high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) is generally viewed as the most reliable method for determining the TEQ levels of dioxin-like PCBs. This technique is reliable and sensitive; however, it is also time-consuming, requires expensive equipment, and the analysis must be performed

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Table 1
Cross-reactivity of the ELISA against various PCBs[a]

IUPAC no.	Cross-reactivity (%)[b]
PCB 118	100
PCB 77	17.8
PCB 66	15.2
PCB 70	14.9
PCB 31	12.9
PCB 156	7.2
PCB 5, 8, 18, 20, 28, 33, 44, 52, 81, 95, 101, 105, 110, 114, 123, 126, 138, 149, 153, 157, 167, 169, 170, 174, 180, 187, 189, 194, 196, 199, 203	<5.0

[a] Data quoted from EnBio Coplanar PCB EIA system instruction booklet.
[b] Numbers indicate the percentage of cross-reactivity to PCB 118.

by highly trained staff. Enzyme-linked immunosorbent assays (ELISAs) are a possible alternative method for detecting dioxin-like PCBs. Several ELISAs for these toxins have been reported previously (Chiu et al., 1995; Fránek et al., 2001; Ohno et al., 2003; Glass et al., 2004). However, the application of an assay to detect dioxin-like PCBs in fish samples has not yet been described. Reporter-gene assays, such as the chemical-activated luciferase gene expression (CALUX) assay, could also potentially function as alternative methods for screening fish for dioxin-like PCBs (Tsutsumi et al., 2003b; Schoeters et al., 2004). However, the CALUX assay requires specific user skills and equipment to perform cell culture. Thus, there is a definite need for an ELISA-based screening tool, in the form of a commercially available kit.

We recently developed an ELISA kit using a monoclonal antibody (MAb) that was highly specific for 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118) (Okuyama et al., 2002). The cross-reactivity of the ELISA is shown in Table 1. PCB 118 is generally the most abundant dioxin-like PCB isomer found in fish (Choi et al., 2001; Peng and Weng, 2001; Tsutsumi et al., 2003a), although it makes a relatively small contribution to the total TEQ of dioxin-like PCBs. We found that the concentrations of PCB 118 correlated well with the TEQ levels of dioxin-like PCBs in retail fish, based on the HRGC/HRMS data obtained in our national survey of dioxins in Japan. We therefore considered the PCB 118 isomer to be a good predictor of the TEQ levels of dioxin-like PCBs in fish, although the ELISA could not directly measure the TEQ levels of dioxin-like PCBs. The utility of the ELISA kit for determining the TEQ levels of dioxin-like PCBs in retail fish was assessed in the present study.

2. Materials and methods

2.1. Reagents

Dioxin-analysis grade acetone, dichloromethane and *n*-hexane were purchased from Wako Pure Chemical Co. (Osaka, Japan). Silica gel, 2% potassium hydroxide-impregnated silica gel, 10% silver nitrate-impregnated silica gel, 22% sulfuric acid-impregnated silica gel, and 44% sulfuric acid-impregnated silica gel were also obtained from Wako Pure Chemical Co. Alumina B-Super I was obtained from ICN Pharmaceuticals Inc. (Costa Mesa, CA, USA). PCB 118 was obtained from AccuStandard Inc. (New Haven, CT, USA). The ELISA kit was purchased from Amersham Biosciences Corp. (Piscataway, NJ, USA).

2.2. Fish samples

Fish samples were purchased during 2002 and 2003 from supermarkets in Tokyo, Japan. The samples (muscle tissue) were homogenized using a food cutter and stored at −20 °C until analysis.

2.3. Purification of fish tissue for the ELISA

The homogenized fish sample (20 g) was added to aqueous 2 M KOH (40 ml) and alkali digestion was performed at room temperature for 16–20 h. The alkaline hydrolysate was added to methanol (30 ml) and extracted using a mechanical stirrer (10 min) with *n*-hexane (40 ml). The extract was washed twice with 2% aqueous NaCl (40 ml), treated several times with concentrated sulfuric acid, and then passed through a multi-layer silica gel column filled from bottom to top with 0.18 g silica gel, 0.6 g of 2% potassium hydroxide-impregnated silica gel, 0.18 g silica gel, 0.9 g of 44% sulfuric acid-impregnated silica gel, 1.2 g of 22% sulfuric acid-impregnated silica gel, 0.18 g silica gel, 0.6 g of 10% silver nitrate-impregnated silica gel, and 1.2 g anhydrous sodium sulfate. The eluate obtained with *n*-hexane (50 ml) was then loaded onto an alumina column (2.5 g). After washing with *n*-hexane (10 ml), the fraction containing dioxin-like PCBs was eluted with 5% dichloromethane/*n*-hexane (45 ml). The eluate was dried using a nitrogen stream and the residue was re-dissolved into dimethyl sulfoxide (DMSO; 100 μl) before use in the ELISA.

2.4. ELISA

The ELISA kit was used according to the manufacturer's instructions (EnBioTec Laboratories, Tokyo, Japan; EnBio Coplanar PCB EIA system instruction booklet). Samples or various concentrations of 3,3′,4′-trichloro-4-methoxybiphenyl (MeO-PCB), which is a surrogate standard for PCB 118, mixed with competitor–horseradish peroxidase conjugate (1:3) were added to microtitre wells (50 μl $well^{-1}$) coated with MAb against PCB 118, and then incubated for 30 min at room temperature with gentle shaking. After washing with the solution provided, an enzyme substrate solution containing 3,3′,5,5′-tetramethylbenzidine was added to each well (50 μl $well^{-1}$) and incubated for 20 min. The enzyme reaction was stopped with 0.5 M H_2SO_4 (50 μl $well^{-1}$) and the absorbance at 450 nm was measured. All experiments were conducted in

duplicate. The standard curves were fitted using a four-parameter logistic model.

2.5. Measurement of dioxin-like PCBs in fish with HRGC/HRMS

The extraction, purification and analysis of dioxins were performed following the general procedures reported previously (Tsutsumi et al., 2003c). Briefly, the homogenized sample (50–100 g) with $^{13}C_{12}$-labelled internal standards was digested with aqueous KOH. The alkaline hydrolysate was then extracted with *n*-hexane. After treatment with concentrated sulfuric acid, the extract was purified on a silver nitrate/silica gel column followed by further purification using an alumina column. The alumina column separated the extract into mono-*ortho* and non-*ortho* PCB fractions. The latter fraction was purified further on an activated carbon column. Both the fractions were spiked with $^{13}C_{12}$-labelled recovery standards. The quantification of four non-*ortho* PCBs and eight mono-*ortho* PCBs was performed by HRGC/HRMS using an HP-6890 Plus gas chromatograph coupled to a JEOL JMS-700 MStation mass spectrometer (Tokyo, Japan). The measurement of non-*ortho* and mono-*ortho* PCBs was performed in an HT-8 fused silica capillary column (SGE, TX, USA). The TEQ was calculated using the World Health Organization (WHO) TEFs (Van den Berg et al., 1998). The limits of quantitation (LOQ) were around 0.1 pg g^{-1} for non-*ortho* PCBs and 1.0 pg g^{-1} for mono-*ortho* PCBs. Calculation of the total TEQ in a sample was carried out assuming that all isomer concentrations lower than the LOQs were equal to zero.

3. Results and discussion

3.1. PCB 118 as an indicator of the TEQ concentrations of dioxin-like PCBs in retail fish

We examined the suitability of PCB 118 concentrations as indicators of the TEQ concentrations of dioxin-like PCBs in retail fish. The PCB 118 concentrations of 178 fish samples, based on the HRGC/HRMS data produced by our national survey of dioxins in Japan, were compared with the TEQ concentrations of dioxin-like PCBs. The samples mainly consisted of fish species, such as bonito, mackerel, salmon, tuna, and yellowtail, which are popular in the Japanese market. Overall, a relatively good correlation ($r = 0.87$) was observed, as shown in Fig. 1, suggesting that PCB 118 concentrations are good indicators of dioxin-like PCBs in fish.

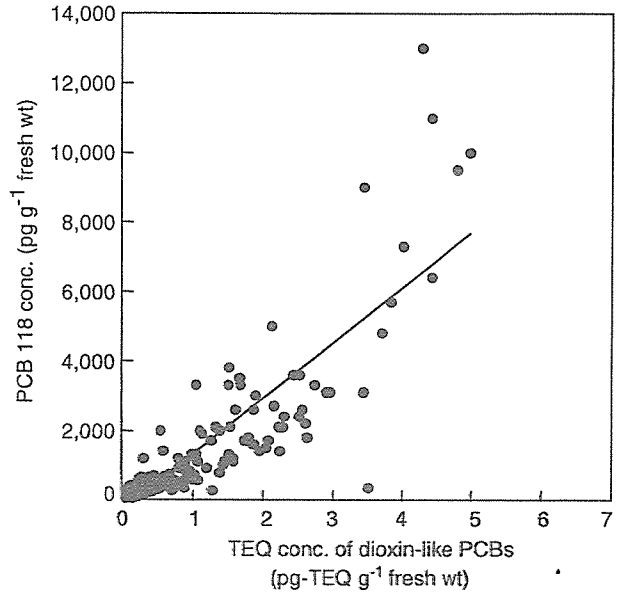

Fig. 1. Correlation between PCB 118 concentrations and TEQ concentrations of dioxin-like PCBs in retail fish. Data from our national survey of dioxins was used for this analysis. The 178 samples (mainly consisting of fish such as bonito, mackerel, salmon, tuna, and yellowtail, which are popular in the Japanese market) were analyzed by HRGC/HRMS analysis. The regression equation was $y = 1599x - 302$ and the correlation coefficient was 0.87.

3.2. ELISA standard curve

MeO-PCB was used as a surrogate standard for PCB 118 in the ELISA kit, in order to avoid using the toxic congener. The shape of the calibration curve of MeO-PCB was similar to that of PCB 118 (data not shown), although the overall reactivity of the ELISA with MeO-PCB was one-eighteenth of that of PCB 118. The standard curve derived from six different batches of kit reagents used on separate days is shown in Fig. 2. The concentration of the MeO-PCB standard was expressed as the equivalent of PCB 118. The low standard deviations (SDs) indicated that the curve was highly reproducible. The average PCB 118 half-maximal inhibitory concentration from six standard curves was 22.6 ng ml^{-1} (283 pg $well^{-1}$) and the quantitative range of the ELISA was 10–250 ng ml^{-1} (125–3125 pg $well^{-1}$). The quantitative limit corresponded to >3 SDs below of absorbance of the zero standards.

The accuracy and precision of the quantitative range of the ELISA was determined by replicate analyses of PCB 118. Known concentrations of PCB 118 (20 and 50 ng ml^{-1}) were assayed in different sets of wells on the same plate (intra-assay measurements; $n = 6$) or different plates on different days (inter-assay measurements; $n = 6$). Residual errors were small, ranging from 0.0% to 10.0% and –1.2% to 7.5% for the intra-assay and inter-assay data, respectively. The coefficients of variation (CVs) were also small, ranging from 2.4% to 3.5% and 7.2% to 9.1% for the intra-assay and inter-assay data, respectively. Therefore, the ELISA could measure PCB 118 with both accuracy and precision over the quantitative range.

3.3. Effect of fish matrix on the ELISA

The effect of the fish tissue matrix on the ELISA was measured. The purification procedure described above

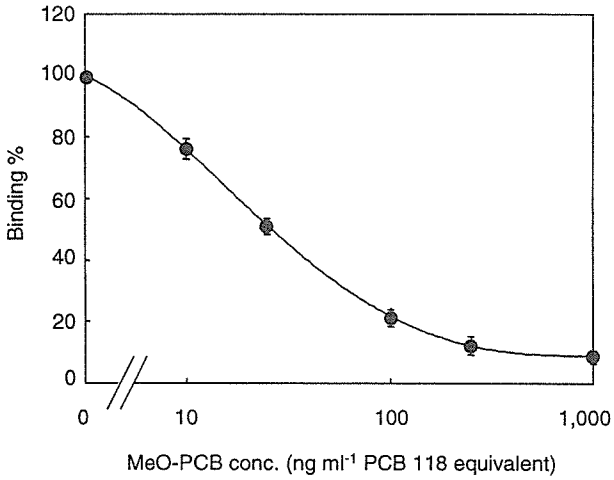

Fig. 2. Standard curve for the ELISA. Circles represent the mean binding from six separate assays performed on different days. Bars represent SDs. The y-axis shows the percentage of binding: Binding $\% = A^+/A^- \times 100$. Here, A^+ is the absorbance in the presence of standard and A^- is the absorbance in its absence.

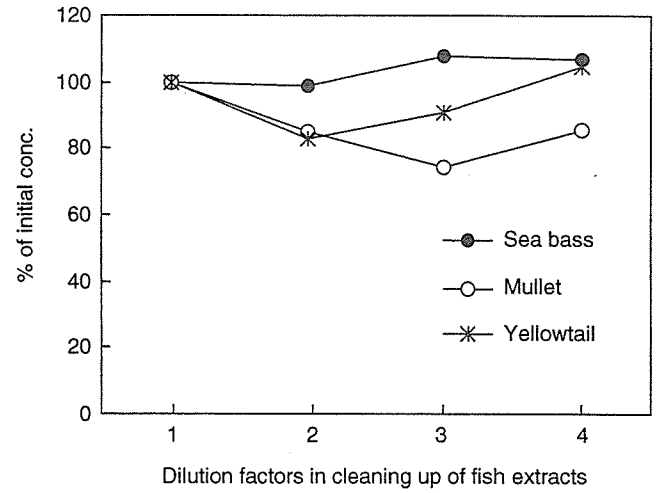

Fig. 3. Effect of dilution factor on concentrations measured in retail fish. A two-fold dilution series of purified extracts with DMSO from three varieties of fish contaminated in the natural environment was assayed by the ELISA ($n = 1$).

was employed, and the amount of PCB 118 in the purified extracts was assessed. The extracts of four varieties of fish were spiked with known amounts of PCB 118 and assayed by the ELISA. Good recoveries (78.7–112.3%) were obtained over the quantification range of the assay (Table 2).

A dilution test using the purified extracts from fish samples contaminated in the natural environment was then carried out, in order to examine further the effect of the matrix on the ELISA. Two-fold serial dilutions of three varieties of purified fish were made in DMSO and assayed by the ELISA. The levels were 74.1–107.9% of those expected based on the starting dilutions (Fig. 3). The overall results suggested that after the purification procedure, the matrix did not significantly interfere with the assay.

Table 2
Recovery of PCB 118 from spiked cleaned-up fish extracts[a]

Samples	Spiked levels (ng ml^{-1})	Observed levels (ng ml^{-1})	Recovery[b] (%)
Sea bass	0	28.2	–
	15	41.8	90.7
	30	51.8	78.7
	45	71.8	96.9
	60	82.2	90.0
Mackerel	0	22.6	–
	15	38.4	105.6
	30	56.3	112.3
	45	69.7	104.7
	60	84.1	102.5
Salmon	0	30.7	–
	15	44.1	89.2
	30	59.6	96.3
	45	75.7	99.8
	60	87.0	93.8
Tuna	0	13.7	–
	15	26.4	84.5
	30	45.7	106.7
	45	62.9	109.4
	60	77.6	106.5

[a] Cleaned-up extracts from fish samples were spiked with known quantities of PCB 118, and analyzed by the ELISA ($n = 1$).
[b] Recoveries were corrected by subtraction of the natively contaminated levels in each sample (13.7–30.7 ng ml^{-1}).

3.4. Accuracy and precision of the ELISA following the purification procedure

The accuracy of the ELISA following the purification was examined by performing a recovery test of the PCB 118 in retail fish. Fish samples were spiked with known concentrations of PCB 118, extracted, purified and assayed by the ELISA. Acceptable recoveries (60.2–82.3%) were obtained over the tested range for the two varieties of fish analyzed (Table 3). These data indicate that no significant loss of PCB 118 occurred during the purification procedure.

Table 3
Recovery of PCB 118 from spiked fish samples[a]

Samples	Spiked levels (pg g^{-1})	Observed levels (pg g^{-1})	Recovery (%)
Tuna	0	n.d.[b]	–
	150	115	76.7
	1500	1235	82.3
Yellowtail	0	194	–
	1500	1097	60.2[c]

[a] Fish samples spiked with known quantities of PCB 118 were extracted, cleaned up and analyzed by the ELISA ($n = 1$).
[b] n.d. not detected.
[c] Recovery was corrected by subtraction of the natively contaminated level (194 pg g^{-1}).

Table 4
Reproducibility of the ELISA combined with the clean-up procedure[a]

Samples	n	ELISA (pg g^{-1})		CV (%)
		Mean ± SD	Range	
Mullet	7	3836 ± 490	3330–4748	12.8
Sea bass	3	417 ± 121	340–557	29.0

[a] The two varieties of fish contaminated in the natural environment were extracted, cleaned up and assayed by the ELISA in seven separate runs for mullet, and in three separate runs for sea bass on different days.

The precision of the ELISA when used in combination with this purification procedure was tested further by repeatedly analyzing the same fish homogenized samples. The two types of fish samples were extracted, cleaned up, and assayed by the ELISA in seven separate runs for mullet, and in three separate runs for sea bass, on different days. The assay gave acceptable results for the concentrations measured: the CVs for the two varieties of fish were 12.8–29.0% (Table 4). We concluded that the ELISA would perform well when used to analyze PCB 118 levels in retail fish.

3.5. Dioxin-like PCBs measured by ELISA in retail fish samples

Fish samples usually contain many PCB isomers, in addition to PCB 118, which are recognized by the ELISA. This assay is known to have slight cross-reactivity with PCB 31, PCB 66, PCB 70, and PCB 77 (12.9–17.8% of PCB 118) (Table 1). The performance of the ELISA was investigated using 31 retail fish samples, which were analyzed by both ELISA and HRGC/HRMS. A good correlation ($r = 0.99$; $n = 26$) was observed between the ELISA values and the concentrations of PCB 118 according to the HRGC/HRMS analysis (Fig. 4(a)). At the low PCB concentrations (<5000 pg g^{-1}), a good correlation ($r = 0.94$; $n = 24$) was obtained between the two methods (Fig. 4(a), inset). The slopes of the linear regression equation were roughly 1, suggesting that positive ELISA results for the retail samples were caused mainly by their reactivity with PCB 118. However, allowing for losses during purification, the ELISA values were expected to be lower than the concentrations measured by HRGC/HRMS analysis. The values obtained in the HRGC/HRMS analysis are generally highly accurate, regardless of the recovery, because quantification is performed using isotopic dilution for the HRGC/HRMS analysis. The concentrations of PCB 77, which is classified as a dioxin-like PCB, were much lower than those of PCB 118 in the samples tested by HRGC/HRMS analysis. Therefore, the reactivity of the ELISA to PCB 77 must be negligible. The presence of high amounts of PCB 31, PCB 66, or PCB 70 might have slightly influenced the results of the ELISA, although it is not clear whether these compounds would have been present in the samples tested in the current study.

3.6. Prediction of TEQ concentrations of dioxin-like PCBs in retail fish

The values obtained in the ELISA for the 31 retail fish samples tested were plotted against the TEQ concentrations of dioxin-like PCBs according to the HRGC/HRMS

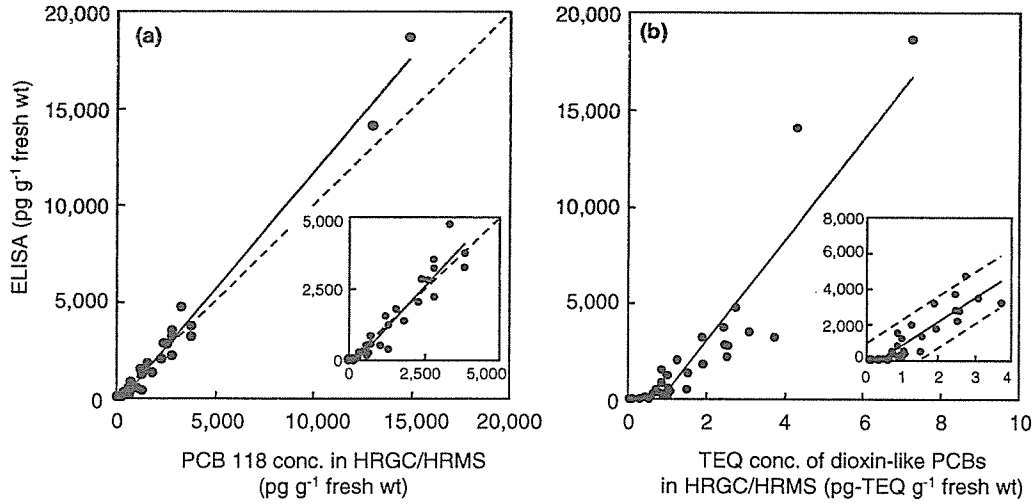

Fig. 4. Comparison of data from the ELISA and HRGC/HRMS analysis in retail fish. Thirty-one samples (two bonito, two conger, one croaker, one hairtail, one halibut, three mackerel, three marlin, five salmon, three sea bass, two tuna, and eight yellowtail) were analyzed by the ELISA and HRGC/HRMS. (a) ELISA values versus PCB 118 concentrations. The regression equation was $y = 1.21x - 391$ and the correlation coefficient was 0.99 ($n = 26$). For concentrations <5000 pg g^{-1} of PCB 118 (inset), the regression equation was $y = 1.14x - 284$ and the correlation coefficient was 0.94 ($n = 24$). Dashed lines represent $x = y$. Five samples in which PCB 118 was not detected in the ELISA were expressed as zero and excluded from the regression calculations. (b) ELISA values versus TEQ concentrations of dioxin-like PCBs. The regression equation was $y = 2631x - 2253$ and the correlation coefficient was 0.92 ($n = 26$). For concentrations < 4 pg-TEQ g^{-1} of dioxin-like PCBs (inset), the regression equation was $y = 1324x - 479$ and the correlation coefficient was 0.87 ($n = 24$). Dashed lines represent the 95% prediction interval of the regression line. Five samples in which PCB 118 was not detected in the ELISA were expressed as zero and excluded from the regression calculations.

analysis (Fig. 4(b)). A good correlation ($r = 0.92$; $n = 26$) between the ELISA and TEQ values was obtained. This indicates that, although the ELISA cannot directly measure the TEQ levels of dioxin-like PCBs, it offers a practical approach for predicting the TEQ levels of dioxin-like PCBs in retail fish. The values obtained in the ELISA were much higher than the TEQ values in HRGC/HRMS analysis, because the TEF for PCB 118 is extremely low (0.0001), and the contribution of PCB 118 to the total TEQ levels of dioxin-like PCBs was relatively small ($10.2 \pm 5.5\%$) in the samples tested.

The regression line and its 95% prediction interval at relatively low TEQ concentrations (<4 pg-TEQ g^{-1} for dioxin-like PCBs) are shown in Fig. 4(b) (inset). A good correlation ($r = 0.87$; $n = 24$) was observed between both methods. The 2.0 pg-TEQ g^{-1} in the HRGC/HRMS analysis corresponded to 670–3700 pg g^{-1} in the ELISA, based on the 95% prediction interval. The ELISA can easily detect the lower level of the predicted interval. Therefore, the ELISA will be a useful method for screening retail fish samples containing over 2.0 pg-TEQ g^{-1} with a false-negative rate < 5%. It is possible that our ELISA might be applicable only to popular fish in the Japanese market, because differences in the relative contribution of PCB 118 to the TEQ levels of dioxin-like PCBs might exist among different fish species and geographic areas. A greater number of retail samples will need to be tested in comparative studies for future assessments of the ELISA.

There are currently no internationally recognized maximum limits for dioxin-like PCBs in food. However, a draft amendment to European Commission regulations will establish action levels for dioxin-like PCBs in foodstuffs in the near future (Commission of the European Communities, SANCO/0305/2001). This directive has increased the demand for screening methods to detect these compounds. The ELISA also allows high throughput, has relatively low costs and enables small samples to be tested, compared with traditional HRGC/HRMS analysis. Thus, the ELISA would be a useful screening method for TEQ levels of dioxin-like PCBs in retail fish. Risk assessments of dioxins require TEQ concentrations of PCDD/Fs to be determined in addition to dioxin-like PCBs. The ELISA described here appears to lack cross-reactivity with PCDD/Fs (EnBio Coplanar PCB EIA system instruction booklet). Recently, several highly sensitive immunoassays for the TEQ screening of PCDD/Fs in biological samples have been reported (Sugawara et al., 2002; Okuyama et al., 2004). Together, these ELISAs will enable us to screen retail fish for the TEQ concentrations of PCDD/Fs as well as dioxin-like PCBs.

4. Conclusions

Our ELISA kit combined with the purification procedure described performed well in the analysis of PCB 118 in retail fish. When a 20-g fish sample was tested, the quantitative limit for PCB 118 was 50 pg g^{-1} per sample. A comparative study with conventional HRGC/HRMS analysis indicated that the ELISA is suitable for the TEQ screening of dioxin-like PCBs in retail fish. The ELISA will identify retail fish samples containing over 2 pg-TEQ g^{-1} of dioxin-like PCBs. This will be useful for the preliminary screening of numerous fish samples before HRGC/HRMS analysis.

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Original

Dioxin Concentrations in the Edible Parts of Japanese Common Squid and Saury

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We examined the concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dioxin-like PCBs) in muscle and gut tissues from Japanese common squid and saury. These body parts are often eaten in Japan, so it is important to measure their dioxin concentrations and evaluate the risks to consumers. The toxic equivalent (TEQ) concentrations in the squid gut samples (1.0 to 14 pg-TEQ/g fresh weight, $n=3$) were 50-fold larger than those in the muscle tissues (0.020 to 0.22 pg-TEQ/g fresh weight, $n=3$) taken from the same samples. By contrast, the TEQ concentrations in the saury gut samples (0.35 to 0.63 pg-TEQ/g fresh weight, $n=3$) were only 1.1- to 1.7-fold greater than those in the muscle tissues (0.33 to 0.37 pg-TEQ/g fresh weight, $n=3$) from the same samples. The TEQ contents in the squid gut tissues ranged from 60 to 990 pg-TEQ/squid, accounting for about 95% of the total dioxin content of the edible parts of the samples. By contrast, the TEQ contents in the saury gut tissues ranged from 4.4 to 12 pg-TEQ/saury, accounting for less than 25% of the total dioxin content of the edible parts of the samples. These tissues showed comparable PCDD/PCDF-congener and dioxin-like PCB-isomer profiles in both species. The results indicate that squid gut tissues occasionally contain high levels of dioxins, and consumption of this foodstuff could potentially significantly increase the dietary intake of dioxins.

Key words: dioxins; fishery product; gut tissue; Japanese common squid; muscle tissue; saury

Introduction

Fishery products have been identified as the main source of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs)—collectively referred to as dioxins—in the Japanese diet[1)-3)]. We previously carried out a nationwide survey of dioxin concentrations in various fishery products available on the Japanese market during the past few years[4)]. Our survey focused on dioxin concentrations in the muscle tissues, which are generally the principal edible parts of these products. However, the gut tissues of some species, such as the Japanese common squid (*Todarodes pacificus*) and saury (*Cololabis saira*), are often also consumed in Japan. Measuring the dioxin concentrations in these edible body parts is therefore essential to evaluate the risk associated with their consumption.

In general, lipid-rich tissues preferentially accumulate dioxins, which have a strongly hydrophobic nature. The dioxin concentrations in the gut tissues of fishery products are of major concern, because they include lipid-rich internal organs, such as the liver. In fact, PCDDs and PCDFs have been reported to accumulate in the liver of fish species, such as the common carp (*Cyprinus carpio*)[5)] and burbot (*Lota lota*)[6)]. Additionally, Ueno *et al.*[7)] suggested that the liver of the Japanese common squid was a suitable bioindicator for monitoring persistent organic pollutants, including PCBs[7)]. These findings have increased public anxiety in Japan regarding dioxin concentrations in the gut tissues of commercially available Japanese common squid and saury products.

Unfortunately, there are no previously published reports on the dioxin concentrations in the gut tissues of these species. Therefore, we examined the toxic equivalent (TEQ) concentrations in both the gut and muscle tissues of commercially available Japanese common squid and saury, and compared the profiles of PCDD and PCDF congeners, and dioxin-like PCB isomers, in these edible parts. Part of this work was reported previously as a preliminary communication[4)].

Materials and Methods

Reagents

Solvents (acetone, dichloromethane, diethyl ether, *n*-hexane, methanol and toluene) were obtained from Kanto Kagaku (Tokyo, Japan). Silica gel S-1 and silver nitrate–silica gel were obtained from Wako Pure Chemicals (Osaka, Japan). Alumina B-Super I was obtained from ICN Pharmaceuticals Inc. (Costa Mesa, CA, USA) and active carbon–dispersed silica gel was obtained

from Kanto Kagaku (Tokyo, Japan). All of the dioxin standards were obtained from Wellington Laboratories (Guelph, ON, Canada).

Fishery products

Japanese common squid and saury, which were caught on the Japanese coast according to the product labels, were purchased from supermarkets in Tokyo, Japan. These samples were separated into muscle (with skin) and gut tissues. The analyses of the squid tissues were carried out on individual samples ($n=3$). The analyses of the saury parts were performed using pooled samples ($n=3$), each of which consisted of three separate specimens, in order to attain the minimum sample size required for the dioxin measurements. All of the samples were homogenized using a food cutter and stored at −20℃ until the analysis.

Lipid determination

The homogenized samples (5 g) were mixed with anhydrous sodium sulfate (20 g) and extracted three times with 33.3% (v/v) diethyl ether/*n*-hexane (150 mL). The extracts were washed twice with distilled water (100 mL) and dehydrated in a funnel filled with anhydrous sodium sulfate. The eluates were evaporated and weighed in order to estimate the lipid content of the samples.

Dioxin analysis

The extraction, cleanup, and analysis of the dioxins were carried out according to a previously reported protocol[8]. Briefly, the homogenized samples (20–100 g) were spiked with $^{13}C_{12}$-labeled internal quantification standards and digested with aqueous KOH solution. The alkaline hydrolysates were added to methanol and extracted three times with *n*-hexane. The extracts were then washed twice with aqueous NaCl, treated with concentrated sulfuric acid, and purified on a silver nitrate-silica gel column. The eluates that were obtained with *n*-hexane were evaporated and loaded onto an alumina column. The column was washed with *n*-hexane, and the first fraction, which contained mono-*ortho* PCBs, was eluted with 2% (v/v) dichloromethane/*n*-hexane. The second fraction, which contained non-*ortho* PCBs and PCDDs/PCDFs, was eluted with 60% (v/v) dichloromethane/*n*-hexane. The first fraction was evaporated and spiked with a $^{13}C_{12}$-labeled recovery standard. The second fraction was further purified with an activated carbon–dispersed silica-gel column. The column was washed with 5% (v/v) dichloromethane/*n*-hexane, and the non-*ortho* PCBs and PCDDs/PCDFs were eluted with toluene. The fraction was evaporated and spiked with $^{13}C_{12}$-labeled recovery standards.

The quantification of tetra- to octa-chlorinated congeners of the PCDDs/PCDFs, four non-*ortho* PCBs and eight mono-*ortho* PCBs was performed using high-resolution gas chromatography (HRGC)/high-resolution mass spectrometry (HRMS) with an HP-6890 plus gas-chromatograph coupled to a JEOL JMS-700 MStation mass spectrometer (Tokyo, Japan). A DB-5MS column (0.32 mm i.d. ×60 m; film thickness, 0.25 μm; J&W Scientific, CA, USA) and a DB-17 column (0.25 mm i.d. ×60 m; film thickness, 0.25 μm; J&W Scientific) were used for 2,3,7,8-chlorine-substituted PCDDs/PCDFs. For analysis of the dioxin-like PCBs, we used an HT-8 column (0.25 mm i.d. ×50 m; film thickness, 0.25 μm; SGE, TX, USA). For the non-2,3,7,8-chlorine-substituted PCDDs/PCDFs, peak assignments using a DB-17 column were carried out based on the data reported by Ryan *et al.*[9]

The approximate limits of quantification were as follows: 0.01 pg/g for tetrachlorodibenzo-*p*-dioxins/tetrachlorodibenzofurans (TCDDs/TCDFs) and pentachlorodibenzo-*p*-dioxins/pentachlorodibenzofurans (PeCDDs/PeCDFs); 0.02 pg/g for hexachlorodibenzo-*p*-dioxins/hexachlorodibenzofurans (HxCDDs/HxCDFs) and heptachlorodibenzo-*p*-dioxins/heptachlorodibenzofurans (HpCDDs/HpCDFs); 0.05 pg/g for octachlorodibenzo-*p*dioxins/octachlorodibenzofuran (OCDD/OCDF); 0.1 pg/g for non-*ortho* PCBs; and 2.0 pg/g for mono-*ortho*

Table 1. TEQ concentions and contents in the muscle and gut tissues of the Japanese common squid and saury

Sample		Edible part[a]	Weight[b] (g)	Lipid (%)	pg-TEQ/g fresh wt. (g lipid wt.)			Total TEQ content pg-TEQ/sample
					PCDD/Fs	Dioxin-like PCBs	Total	
Japanese common squid	#1	Muscle	262.1	0.7	0.094	0.12	0.22 (31)	58
		Gut	70.8	14.5	5.4	8.7	14 (97)	990
	#2	Muscle	189.5	0.6	0.016	0.016	0.032 (5.4)	6.1
		Gut	77.5	26.2	0.68	0.97	1.7 (6.3)	130
	#3	Muscle	150.1	0.9	0.0072	0.012	0.020 (2.2)	3.0
		Gut	60.1	22.9	0.33	0.70	1.0 (4.5)	60
Saury	#1	Muscle	100.8	15.8	0.076	0.29	0.37 (2.3)	37
		Gut	19.8	18.8	0.13	0.50	0.63 (3.3)	12
	#2	Muscle	120.3	29.3	0.049	0.29	0.33 (1.1)	40
		Gut	12.7	33.7	0.056	0.30	0.35 (1.0)	4.4
	#3	Muscle	104.8	24.7	0.055	0.27	0.33 (1.3)	35
		Gut	13.7	30.8	0.068	0.40	0.46 (1.5)	6.3

[a] Muscle with skin.
[b] The weights of the saury muscle and gut tissues were expressed as averages of the pooled samples ($n=3$).

PCBs. The TEQ concentrations were calculated using the World Health Organization (WHO) toxic-equivalency factors (TEFs, 1998)[10]. Calculations of the total TEQ in a sample were carried out assuming that all isomer concentrations lower than the limits of quantification were equal to zero.

Results and Discussion

TEQ concentrations and dioxin contents

Table 1 summarizes the TEQ concentrations and contents of the muscle and gut tissues from three samples of Japanese common squid and saury. In the squid, the TEQ concentrations of the PCDDs/PCDFs and dioxin-like PCBs (on a fresh-weight (FW) basis) in the gut tissues were much higher than those in the muscle tissues. In fact, the total TEQ concentrations in the gut tissues were more than 50-fold greater than those in the muscles taken from the same squid samples. This was probably mainly due to the high lipid content in the squid gut tissues compared with that in the muscle tissues. In fact, when calculated on a lipid-weight (LW) basis, the total TEQ concentrations in the gut tissues were closer to the values observed in the muscles. By contrast, in the saury, there were no significant differences in the TEQ concentrations of the PCDDs/PCDFs and dioxin-like PCBs, calculated on an FW basis, between the muscle and gut tissues. The total TEQ concentrations in the saury gut tissues were only 1.1- to 1.7-fold higher than those in the muscles from the same fish samples. Again, the total TEQ concentrations in the saury gut and muscle tissues calculated on a LW basis were similar to one another. These results can probably be explained by the fact that the lipid contents in the saury gut tissues were comparable to those in the muscles. The total TEQ concentrations in the muscle tissues of both the squid and saury in the present study were within the range previously reported in our nationwide survey of dioxins in Japan[4].

The total TEQ contents in the muscle and gut tissues were also calculated (Table 1). The squid gut tissues contained relatively large amounts of dioxins compared with the muscle tissues. The TEQ contents in the gut tissues ranged from 60 to 990 pg-TEQ/squid. These organs therefore accounted for about 95% of the total dioxin content of the edible parts of the squid samples. According to the National Nutrition Survey in Japan[11], the consumption of the group of squids and octopuses is 6.6 g/person/day. If an individual consumes 6.6 g of squid gut tissues in a day, the intakes of dioxins based on the present data would range from 6.6 to 92 pg-TEQ/day. The TEQ values correspond to between 9.4 to 130% of the average dietary intake of dioxins (70.47 pg-TEQ/day) in our recent total diet study*1. Although the tested samples had a relatively wide range of TEQ contents and more analyzed samples are necessary in order to clarify in detail the TEQ intake from squid gut tissues, consumption of this food stuff could potentially significantly increase the dietary intake of dioxins.

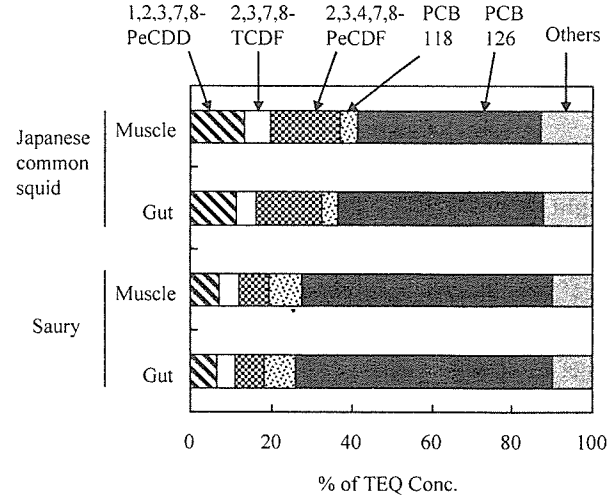

Fig. 1. TEQ profiles of the 17 PCDDs/PCDFs and the 12 dioxin-like PCBs in the muscle and gut tissues of two types of fishery product

The data are based on #1 samples.

By contrast, the saury gut tissues had much lower dioxin contents than the saury muscle tissues, probably owing to their relatively lower weight. The TEQ contents in the gut tissues ranged from 4.4 to 12 pg-TEQ/saury and accounted for 9.9 to 24.5% of the total dioxin content of the edible parts of the fish samples. These TEQ values were below 20% of the average dietary intake of dioxins. Therefore, consumption of saury gut tissues would not be expected to significantly increase the dietary intakes of dioxins.

The TEQ profiles of the dioxin isomers in the muscle and gut tissues of the most contaminated samples (designated number 1 in Table 1) are shown in Fig. 1. The isomer compositions were similar in both the muscle and gut tissues, although the precise patterns differed slightly between the two types of product. All of the samples were dominated by 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, PCB 118, and PCB 126, which accounted for about 90% of the total TEQ contents. Among these, PCB 126 was the most prevalent, with values ranging from 45.6 to 51.2% in the squid muscle and gut tissues, and from 62.2 to 64.0% in the saury muscle and gut tissues.

PCDD/PCDF-congener and dioxin-like PCB-isomer profiles

The profiles of the PCDD/PCDF congeners in the edible portions of the squid and saury samples (designated number 1 in Table 1) are shown in Fig. 2. Similar patterns were observed in the muscle and gut tissues of each sample. TCDDs and TCDFs were the dominant components of the total PCDD/PCDF contents in all of the samples. In total, these congeners accounted for 83.4 and 79.7% of the total PCDD/PCDF contents of the squid muscle and gut tissues, respective-

*1 http://www.mhlw.go.jp/topics/bukyoku/iyaku/syokuanzen/dioxin/sessyu04/index.html

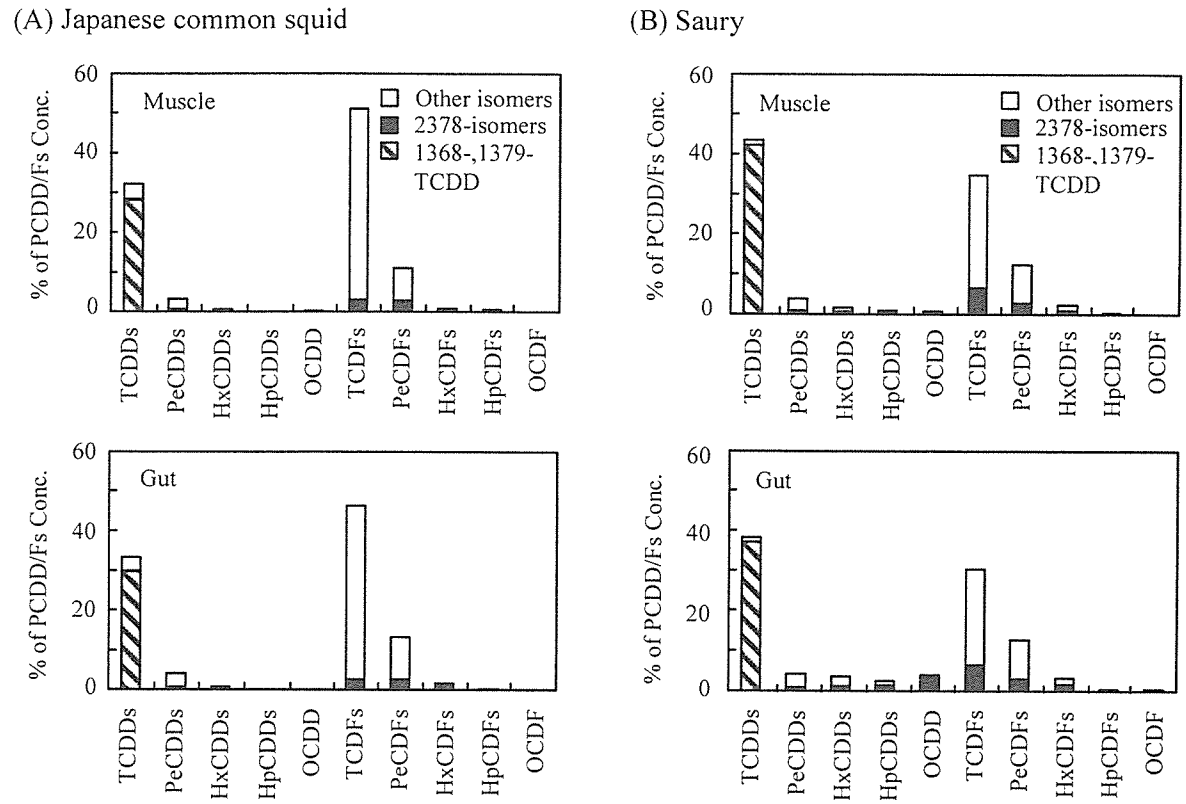

Fig. 2. Profiles of the PCDD/PCDF congeners in the muscle and gut tissues of two types of fishery product: (A) Japanese common squid (#1); (B) saury (#1)

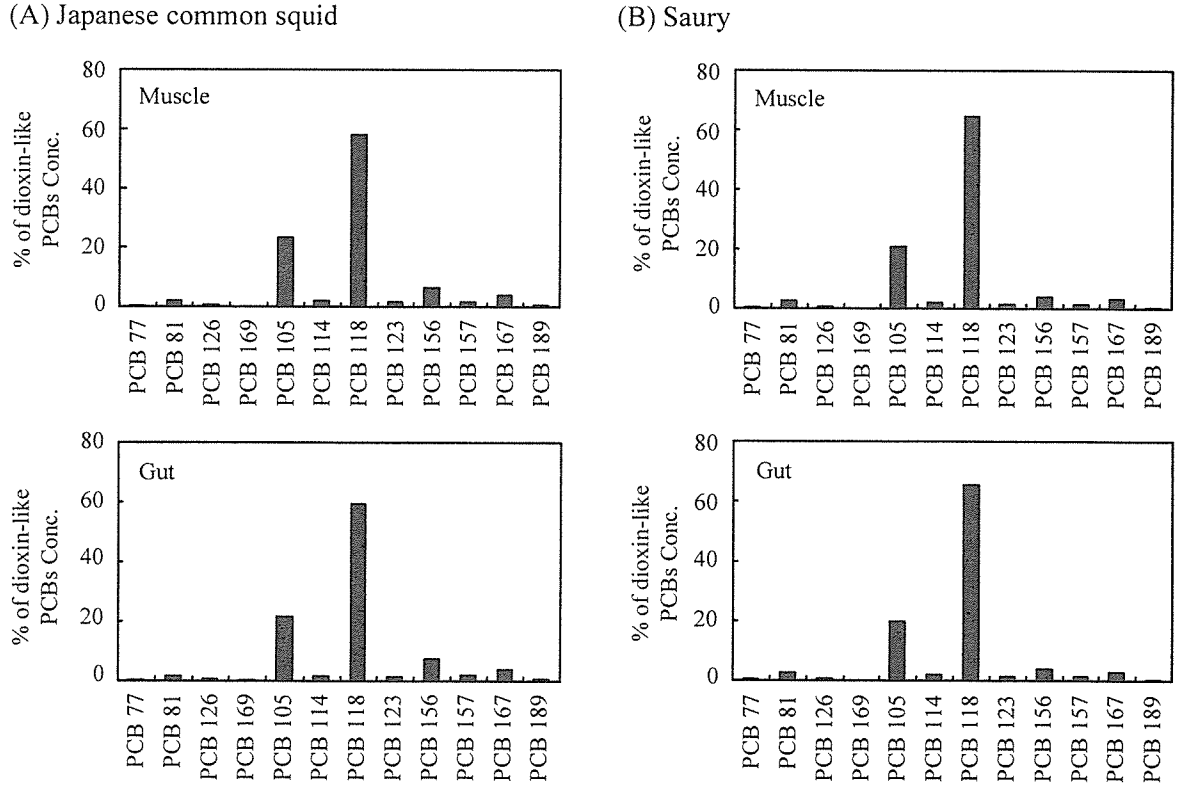

Fig. 3. Profiles of the dioxin-like PCB isomers in two types of fishery product: (A) Japanese common squid (#1); (B) saury (#1)