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

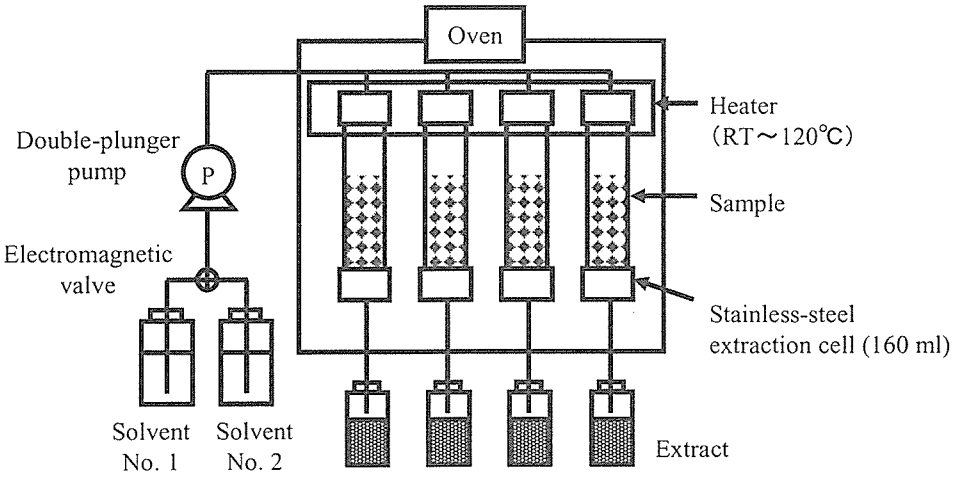

Figure 1 Schematic diagram of the extractor (SE-100)

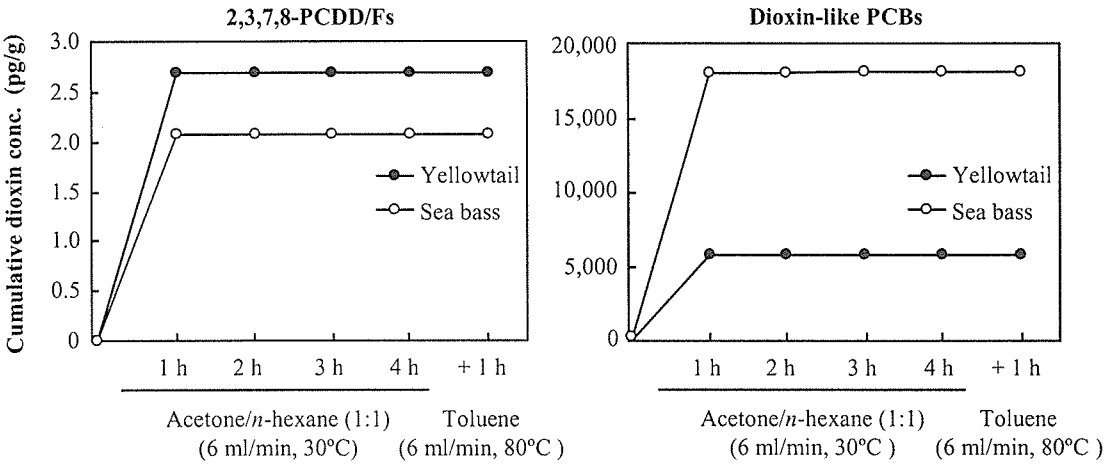

Figure 2 Dioxin concentrations in the high-speed solvent extraction under various extraction conditions. Two popular fish samples were serially extracted by the high-speed solvent extraction with acetone/*n*-hexane for up to 4 h under 30°C and then extracted with toluene for 1 h under 80°C. The hourly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

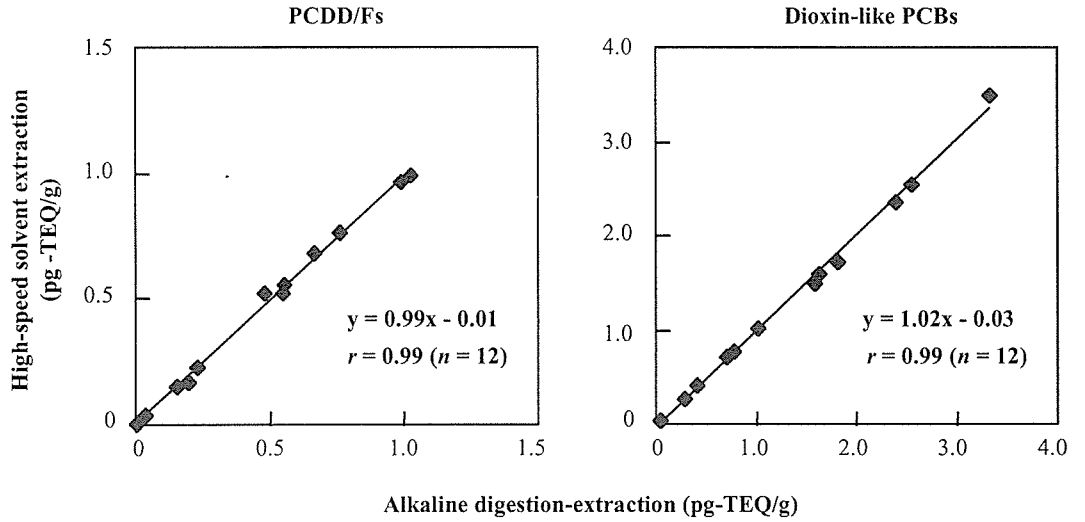

Figure 3 Comparison of TEQ concentrations of dioxins in retail fish determined by the two extraction methods. Twelve retail samples (bonito, conger eel, horse mackerel, marlin, two salmon, sardine, tuna, four yellowtail) were extracted by the two extraction methods and analyzed by HRGC/HRMS analysis.

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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.

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

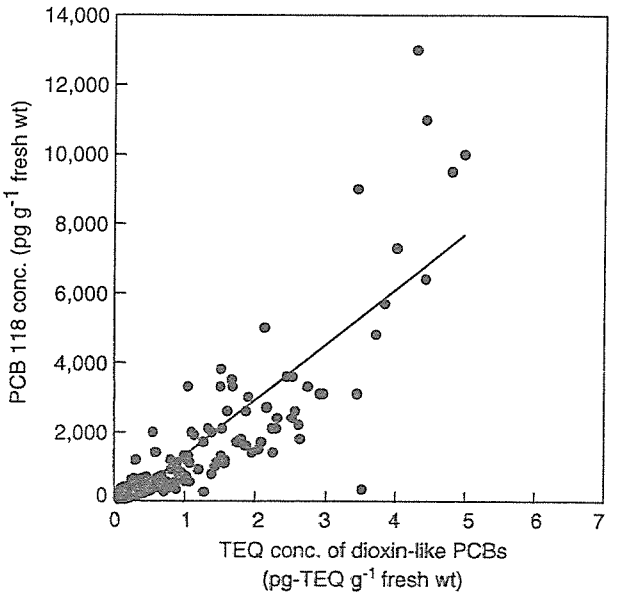

Fig. 1. Correlation between PCB 118 concentrations and TEQ concentrations of dioxin-like PCBs in retail fish. Data from our national survey of dioxins was used for this analysis. The 178 samples (mainly consisting of fish such as bonito, mackerel, salmon, tuna, and yellowtail, which are popular in the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

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

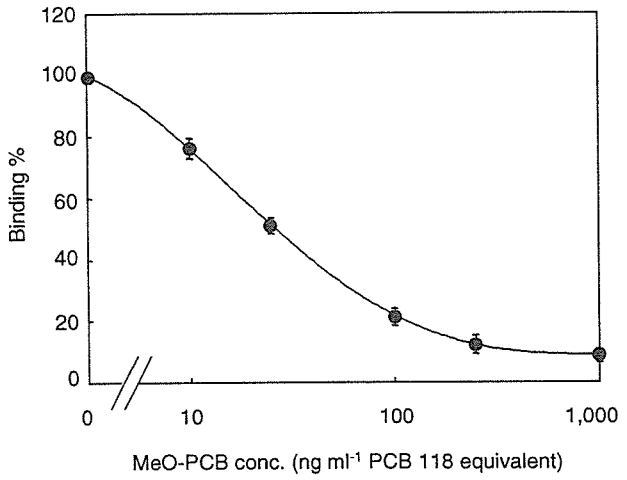

Fig. 2. Standard curve for the ELISA. Circles represent the mean binding from six separate assays performed on different days. Bars represent SDs. The y-axis shows the percentage of binding: Binding $\% = A^+/A^- \times 100$. Here, A^+ is the absorbance in the presence of standard and A^- is the absorbance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

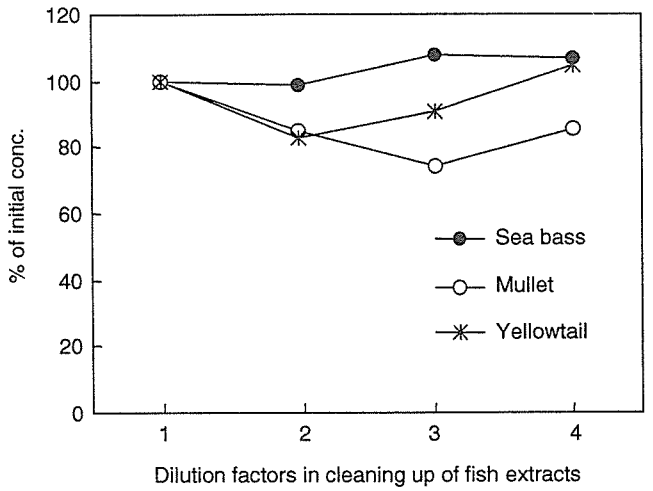

Fig. 3. Effect of dilution factor on concentrations measured in retail fish. A two-fold dilution series of purified extracts with DMSO from three varieties of fish contaminated in the natural environment was assayed by the ELISA ($n = 1$).

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

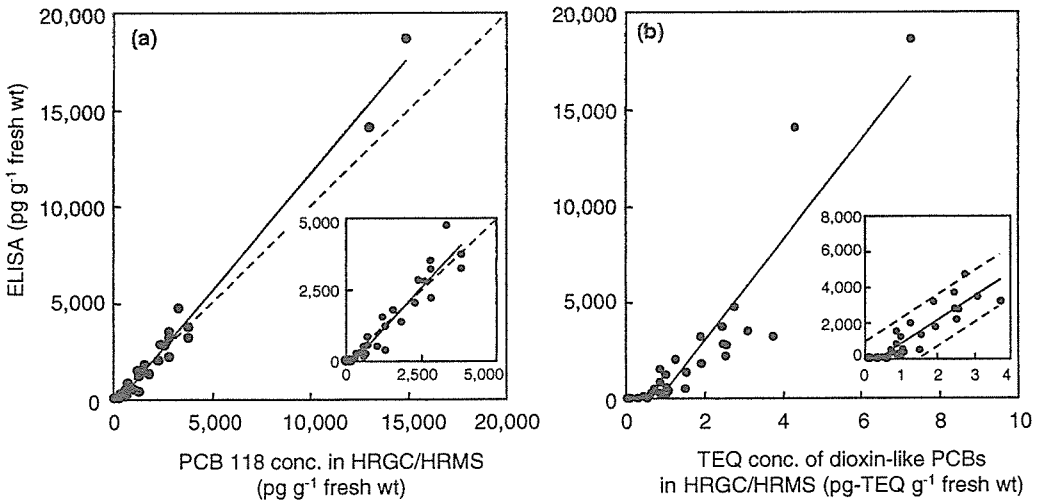

Fig. 4. Comparison of data from the ELISA and HRGC/HRMS analysis in retail fish. Thirty-one samples (two bonito, two conger, one croaker, one hairtail, one halibut, three mackerel, three marlin, five salmon, three sea bass, two tuna, and eight yellowtail) were analyzed by the ELISA and HRGC/HRMS. (a) 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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References

Chiu, Y.W., Carlson, R.E., Marcus, K.L., Karu, A.E., 1995. A monoclonal immunoassay for the coplanar polychlorinated biphenyls. Anal. Chem. 67, 3829–3839.

Choi, Y.S., Eom, J.H., Jung, J.H., Eom, S.W., Kim, M.Y., Yu, M.J., Ahn, S.G., 2001. Dioxin like PCBs levels in fish from the Han-river. Organohalogen Compd. 51, 356–359.

Choi, D., Hu, S., Jeong, J., Won, K., Song, I., 2002. Determining dioxin-like compounds in selected Korean food. Chemosphere 46, 1423–1427.

Commission of the European Communities, a draft amending Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs. SANCO/0305/2005-rev.3-minor.

EnBio Coplanar PCB EIA system instruction booklet. EnBio Tec Laboratories Co. Ltd., Time 24 Bldg. 4th floor, 2-45, Aomi, Koto-ku, Tokyo 135-8073, Japan.

Fránek, M., Deng, A., Kolár, V., Socha, J., 2001. Direct competitive immunoassays for the coplanar polychlorinated biphenyls. Anal. Chim. Acta 444, 131–142.

Glass, T.R., Ohmura, N., Saiki, H., Sawadaishi, K., Kataoka, C., Takagi, Y., Ohiwa, T., 2004. Development and characterization of new monoclonal antibodies specific for coplanar polychlorinated biphenyls. Anal. Chim. Acta 517, 161–168.

Kiviranta, H., Hallikainen, A., Ovaskainen, M.L., Kumpulainen, J., Vartiainen, T., 2001. Dietary intakes of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and polychlorinated biphenyls in Finland. Food Add. Contam. 18, 945–953.

Ohno, Y., Usuki, Y., Iida, S., Kato, I., Kitamura, K., Nagasawa, S., Yanaihara, C., 2003. Development of enzyme-linked immunosorbent assay specific for non-*ortho* coplanar-polychlorinated biphenyls with use of nobel type of labeled antigen. Organohalogen Compd. 60, 287–290.

Okuyama, A., Takenaka, H., Nishi, K., Mizukami, H., Kozaki, S., Kirihata, M., Miyatake, K., Takigami, H., Sakai, S.-I., Morita, M., 2002. Development of enzyme-linked immunosorbent assay for the pre-screening of coplanar polychlorinated biphenyls. Organohalogen Compd. 58, 333–335.

Okuyama, M., Kobayashi, N., Takeda, W., Anjo, T., Matsuki, Y., Goto, J., Kambegawa, A., Hori, S., 2004. Enzyme-linked immunosorbent assay for monitoring toxic dioxin congeners in milk based on a newly generated monoclonal anti-dioxin antibody. Anal. Chem. 76, 1948–1956.

Peng, J.H., Weng, Y.M., 2001. PCDDs, PCDFs and dioxin-like PCBs in fish from Tung Kang, Kao Ping rivers in Taiwan. Organohalogen Compd. 51, 364–367.

Schoeters, G., Goyvaerts, M.P., Ooms, D., Van Cleuvenbergen, R., 2004. The evaluation of dioxin and dioxin-like contaminants in selected food samples obtained from the Belgian market: comparison of TEQ measurements obtained through the CALUX bioassay with congener specific chemical analyses. Chemosphere 54, 1289–1297.

Sugawara, Y., Saito, K., Ogawa, M., Kobayashi, S., Shan, G., Sanborn, J.R., Hammock, B.D., Nakazawa, H., Matsuki, Y., 2002. Development of dioxin toxicity evaluation method in human milk by enzyme-linked immunosorbent assay–assay validation for human milk. Chemosphere 46, 1471–1476.

Tsutsumi, T., Yanagi, T., Nakamura, M., Kono, Y., Uchibe, H., Iida, T., Hori, T., Nakagawa, R., Tobiishi, K., Matsuda, R., Sasaki, K., Toyoda, M., 2001. Update of Daily Intake of PCDDs, PCDFs, and Dioxin-like PCBs from Food in JAPAN. Chemosphere 45, 1129–1137.

Tsutsumi, T., Amakura, Y., Yanagi, T., Nakamura, M., Kono, Y., Uchibe, H., Iida, T., Toyoda, M., Sasaki, K., Maitani, T., 2003a. Levels of PCDDs, PCDFs and dioxin-like PCBs in retail fish and shellfish in Japan. Organohalogen Compd. 62, 93–96.

Tsutsumi, T., Amakura, Y., Nakamura, M., Brown, D.J., Clark, G.C., Sasaki, K., Toyoda, M., Maitani, T., 2003b. Validation of the CALUX bioassay for the screening of PCDD/Fs and dioxin-like PCBs in retail fish. The Analyst 128, 486–492.

Tsutsumi, T., Amakura, Y., Sasaki, K., Toyoda, M., Maitani, T., 2003c. Evaluation of an aqueous KOH digestion followed by hexane extraction for analysis of PCDD/Fs and dioxin-like PCBs in retailed fish. Anal. Bioanal. Chem. 375, 792–798.

Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., Van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.

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.

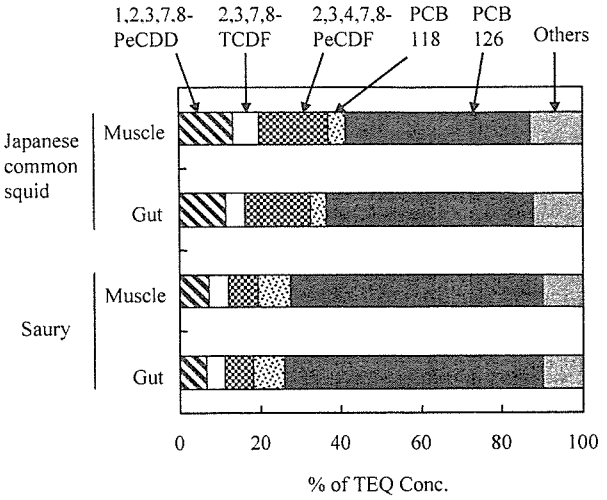

Fig. 1. TEQ profiles of the 17 PCDDs/PCDFs and the 12 dioxin-like PCBs in the muscle and gut tissues of two types of fishery product

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/syoku-anzen/dioxin/sessyu04/index.html

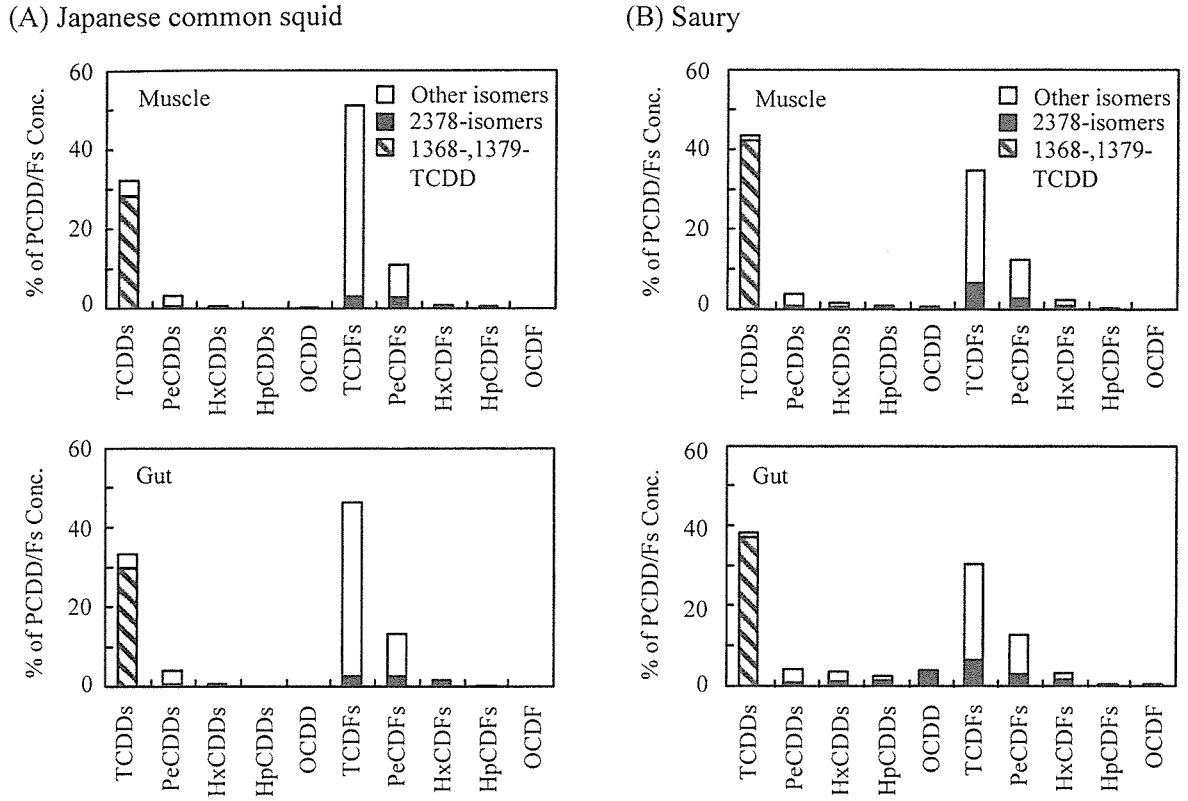

Fig. 2. Profiles of the PCDD/PCDF congeners in the muscle and gut tissues of two types of fishery product: (A) Japanese common squid (#1); (B) saury (#1)

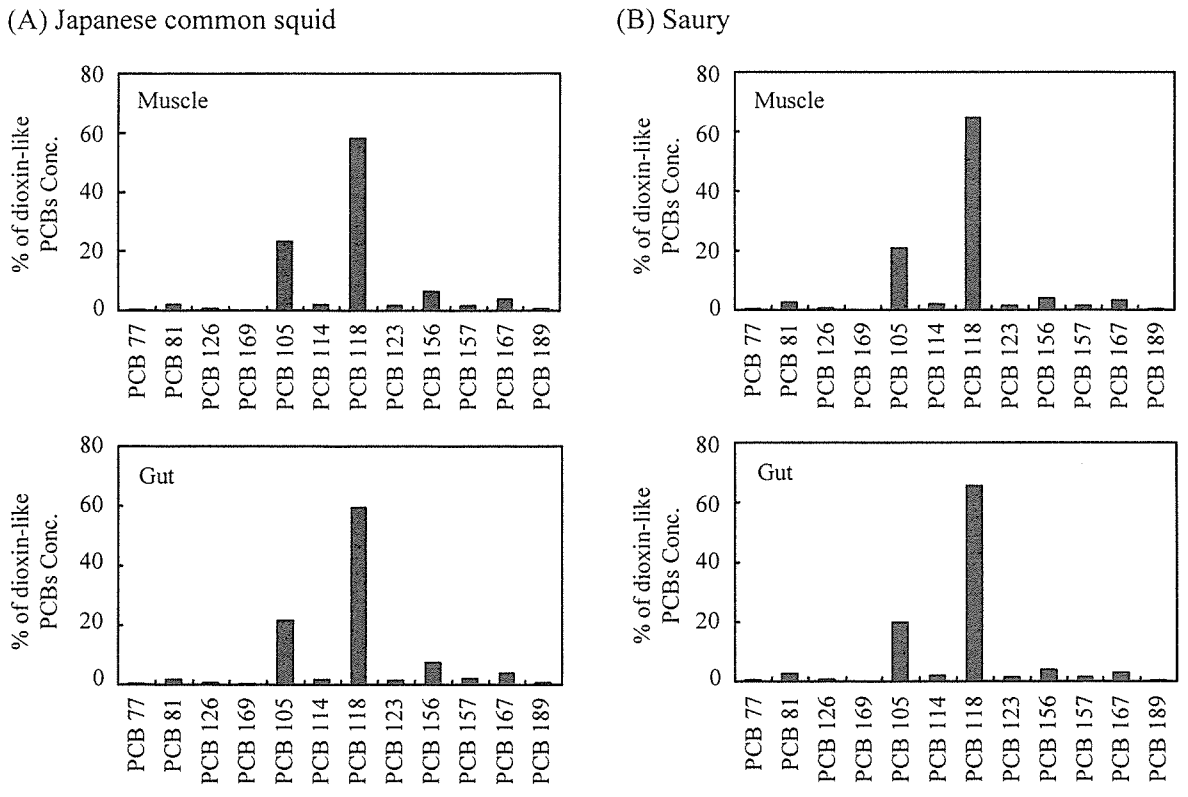

Fig. 3. Profiles of the dioxin-like PCB isomers in two types of fishery product: (A) Japanese common squid (#1); (B) saury (#1)

ly, and 78.1 and 68.7% of those in the saury muscle and gut tissues. Additionally, 1,3,6,8- and 1,3,7,9-TCDDs were dominant isomers in all of the samples. These dioxins are known to be impurities in the agrochemical chloronitrofen (CNP)[12], which was one of the principal herbicides used in paddy fields in Japan in the past. These findings suggest that CNP might have been a major source of the PCDDs/PCDFs in the samples. OCDD is also known to be present as an impurity in the agrochemical pentachlorophenol (PCP)[12], which was one of the principal herbicides used in paddy fields in Japan. However, the isomer was not dominant in the total PCDD/PCDF contents in all of the samples. 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF had relatively high contributions to TCDFs and PeCDFs in all of the samples.

The profiles of the dioxin-like PCBs isomers in the samples are shown in Fig. 3. Again, the patterns observed in the muscle and gut tissues were similar in both the squid and fish. PCB 118 was the dominant isomer, followed by PCB 105. In total, these isomers accounted for 81.3 and 81.0% of the total dioxin-like PCB contents of the squid muscle and gut tissues, respectively, and 85.0 and 85.2% of those in the saury muscle and gut tissues. These profiles were similar to those of the commercial PCB mixtures Kanechlor 400 and 500[13]. This finding suggests that these PCBs could have been the main sources of the dioxin-like PCBs in our samples. The similarity of the above-mentioned profiles between the two tissues also implies that the internal organs comprising the gut tissues are unlikely to accumulate specific dioxin isomers in Japanese common squid and saury. The profiles of PCDD/PCDF congeners and dioxin-like PCBs isomers in the other samples (designated numbers 2 and 3 in Table 1) are similar to those in Fig. 2 and 3 (data not shown).

In conclusion, our data suggest that consumption of the gut tissues of the Japanese common squid could potentially significant increase the dietary intake of dioxins, whereas saury gut tissues should have little effect. Our data should prove useful for further risk evaluations of these fishery products.

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References

1) Takayama, K., Miyata, H., Aozasa, O., Miura, M., Kashimoto, T. Dietary intake of dioxin-related compounds through food in Japan. Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Japan), **32**, 525–532 (1991).
2) Tsutsumi, T., Yanagi, T., Nakamura, M., Kono, Y., Uchibe, H., Iida, T., Hori, T., Nakagawa, R., Tobiishi, K., Matsuda, R., Sasaki, K., Toyoda, M, Update of daily intake of PCDDs, PCDFs, and dioxin-like PCBs from food in Japan. Chemosphere, **45**, 1129–1137 (2001).
3) Sasamoto, T., Ushio, F., Kikutani, N., Saitoh, Y., Yamaki, Y., Hashimoto, T., Horii, S., Nakagawa, J., Ibe, A. Estimation of 1999–2004 dietary daily intake of PCDDs, PCDFs and dioxin-like PCBs by a total diet study in metropolitan Tokyo, Japan. Chemosphere, **64**, 634–641 (2006).
4) Tsutsumi, T. Amakura, Y., Yanagi, T., Nakamura, M., Kono, Y., Uchibe, H., Iida, T., Toyoda, M., Sasaki, K., Maitani, T. Levels of PCDDs, PCDFs and dioxin-like PCBs in retail fish and shellfish in Japan. Organohalogen Compd., **62**, 93–96 (2003).
5) Wu, W., Schramm, K. W., Xu, Y., Kettrup, A. Accumulation and partition of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F) in the muscle and liver of fish. Chemosphere, **43**, 633–641 (2001).
6) Korhonen, M. Verta, M., Lehtoranta, J., Kiviranta, H., Vartiainen, H. Concentrations of polychlorinated dibenzo-*p*-dioxins and furans in fish downstream from Ky-5 manufacturing. Chemosphere, **43**, 587–593 (2001).
7) Ueno, D., Inoue, S., Ikeda, K., Tanaka, H., Yamada, H., Tanabe, S. Specific accumulation of polychlorinated biphenyls and organochlorine pesticides in Japanese common squid as a bioindicator. Environ. Pollut., **125**, 227–235 (2003).
8) Tsutsumi, T., Amakura, Y., Sasaki, K., Toyoda, M., Maitani, T. Evaluation of an aqueous KOH digestion followed by hexane extraction for analysis of PCDD/Fs and dioxin-like PCBs in retailed fish. Anal. Bioanal. Chem., **375**, 792–798 (2003).
9) Ryan, J. J., Conacher, H. B. S., Panopio, L. G., Lau, B. P. Y., Hardy, J. A., Masuda, Y. Gas chromatographic separations of all 136 tetra- to octapolychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans on nine different stationary phases. J. Chromatogr., **541**, 131–183 (1991).
10) Van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., van Leeuwen, F. X. R., Liem, A. K. D., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Warn, F., Zacharewski, T., Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect., **106**, 775–792 (1998).
11) Ministry of Health, Labor and Welfare, Japan. The National Nutrition Survey in Japan, 2002.
12) Masunaga, S., Takasuga, T., Nakanishi, J. Dioxin and dioxin-like PCB impurities in some Japanese agrochemical formulations. Chemosphere, **44**, 873–885 (2001).
13) Takasuga, T., Inoue, T., Ohi, E. All congener specific analytical method for polychlorinated biphenyls (PCBs) with various chromatographic clean-up and HRGC/HRMS. J. Environ. Chem., **5**, 647–675 (1995).