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Toxic Equivalent (TEQ) Levels and Characteristics of PCDD/DF and CoPCB Concentrations in Marine Foods in Japan

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Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like coplanar polychlorinated biphenyls (CoPCBs), collectively referred as to dioxins, are persistent lipophilic chemicals that can induce various toxic responses, including carcinogenicity, immunotoxicity and adverse effects on reproduction and development (Feeley and Brouwer, 2000). Dietary intake is generally known as the major route of human exposure to dioxins. Consumption of fish and shellfish is also thought to contribute markedly to the Japanese population's exposure to these chemicals (MHW, 2000; MHLW, 2001). Studies on levels of PCDDs/DFs and CoPCBs in commercial fish and shellfish have therefore been conducted to assess the risk of these chemicals in Japan (MHW, 2000; MHLW, 2001; Fisheries Agency, 2003). The concentrations of dioxins, especially PCDDs/DFs, are much lower in fish and shellfish than in fly ash, soil and sediment, so the analysis of dioxins in fish and shellfish requires precision at the sub-picogram level. A good understanding of the congener profile characteristics in samples is also very useful when judging whether values generated from the analysis of dioxins are appropriate. However, certain Japanese studies to assess the risk of these chemicals from commercial marine foods, including fish, have reported only toxic equivalent (TEQ) levels, and have not described in detail the characteristics of congeners in the samples (MHW, 2000; MHLW, 2001; Fisheries Agency, 2003). Therefore, the data available on the characteristic congener profiles of PCDDs/DFs in commercial marine foods is limited in Japan. This present work focuses on dioxin levels in marine foods purchased from supermarkets and retail stores in Japan, with the aim of providing some information on dioxin congeners in marine foods that would offer a useful and effective means of analyzing fish for dioxins.

MATERIALS AND METHODS

Ten species of marine foods were purchased from supermarkets and retail stores in Osaka City, Japan, at different times during the period from 1999 to 2000 (Table 1). These marine foods occupied a market share of 36.5% of the amount (ton) of fish handled by the Osaka City wholesale market in 1999. With the exception of red sea-bream, salmon and yellow-fin tuna, the muscle, including the skin, of each species sample was homogenized and was prepared as a composite

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that contained at least three individual samples collected at same time. Red sea-bream and salmon were independently homogenized using only the muscle and skin of one fish collected at a different time. Yellow-fin tuna was also independently homogenized using only the muscle of one steak of fish collected at different time for analysis. Twenty homogenates of the marine food samples were frozen at –20 °C until analysis.

The analytical methods used have been described in a previous study (Nakatani et al. 2005). Briefly, after the addition of ^{13}C-labeled 2,3,7,8-Cl-substituted PCDDs/DFs and CoPCBs to 100 g of homogenous sample, the fat was extracted with a mixture of hexane and acetone (2:1, v/v). The fat was digested with 300 mL of 1 mol/L potassium hydroxide solution in ethanol at room temperature for 2 h, and then extracted 3 times with 100 mL of hexane after dilution with 500 mL of distilled water. The hexane extract was treated with small amounts of sulfuric acid, rinsed with distilled water, concentrated, and then passed through a multi-layer column and developed with 120 mL of hexane. The hexane eluate was concentrated and separated into a mono-*ortho*-CoPCB fraction (Fr. 1) and a PCDD/DF, non-*ortho*-CoPCB fraction (Fr. 2) using a column of aluminum oxide 90 (basic, activity I) from Merck. Fr. 1 was concentrated, treated with small amounts of fuming sulfuric acid and rinsed with distilled water. Fr. 2 was concentrated and further purified using a column of active carbon impregnated-silica gel from Wako Pure Chemical Industries Ltd. Each fraction was concentrated and then spiked with ^{13}C-labeled 1,2,3,4-TeCDD, 1,2,3,4,7-PeCDD and 2,3,4,6,7,8-HxCDF as an instrumental calibration measure. Identification and quantification of PCDDs/DFs and CoPCBs were performed with HRGC/HRMS analysis using a Hewlett Packard 6890 Series high-resolution gas chromatograph equipped with a Micromass Autospec Ultima high-resolution mass spectrometer. Chromatographic separations were achieved using a SP-2331 capillary column for Te- and PeCDDs/DFs, a HP-5MS capillary column for Hx-, Hp- and OCDDs/DFs, and a DB-5MS capillary column for CoPCBs. The limits of detection (LOD) were 0.01 pg/g for Te- and PeCDDs/DFs, 0.02 pg/g for Hx- and HpCDDs/DFs, 0.04 pg/g for OCDD/DFs, 0.09 pg/g for mono-*ortho* CoPCBs and 0.03 pg/g for non-*ortho* CoPCBs. The recoveries of the ^{13}C-labeled surrogate of the various PCDDs/DFs and CoPCBs congers were 81 ± 2% for 2,3,7,8-TeCDD, 80 ± 2% for 1,2,3,7,8-PeCDD, 78 ± 3% for 1,2,3,4,7,8-, 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD, 88 ± 9% for 1,2,3,4,6,7,8-HpCDD, 79 ± 12% for OCDD, 76 ± 2% for 2,3,7,8-TeCDF, 79 ± 3% for 1,2,3,7,8- and 2,3,4,7,8-PeCDF, 81 ± 3% for 1,2,3,4,7,8-, 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDF, 85 ± 7% for 1,2,3,4,6,7,8-HpCDF, 84 ± 4% for TeCB (PCB-77 and 81), 80 ± 3% for non-*ortho*-PeCB (PCB-126), 85 ± 4% for mono-*ortho* PeCB (105, 114, 118 and 123), 94 ± 5% for non-*ortho*-HxCB (PCB-169), 102 ± 7% for mono-*ortho* HxCB (PCB-156, 157 and 167), and 117 ± 4% for HepCB (PCB-189), respectively.

The calculations of the Spearman rank correlation coefficients were carried out using STATISTICA (Version 5.5, StatSoft Japan, Tokyo, Japan). A significance criterion of $p < 0.05$ was used for all the results.

RESULTS AND DISCUSSION

Table 1 shows the levels of PCDD/DF-TEQs, CoPCB-TEQs and total TEQs in twenty marine foods. Compared with the average values for two samples of marine foods from Japanese costal waters, mackerel, which are classified to be the top predators at trophic levels, showed the highest total TEQ levels, followed by red sea-bream, Japanese horse mackerel, sardines and Japanese common squid. Among imports, the highest total TEQ levels were for the two samples of flounder, followed by salmon. Common octopus, black tiger shrimp and yellow-fin tuna had very low total TEQ levels. The total TEQ levels resulting from some of the latest studies in Japan have ranged from 0.397 to 3.841 pg/g wet wt. in mackerel, 0.081 to 2.752 pg/g wt. in sardines, 0.266 to 3.551 pg/g wt. in Japanese horse mackerel, 0.128 to 1.916 pg/g wt. in red sea-bream, and 0.013 to 0.272 pg/g wt. in Japanese common squid (MHW, 2000; MHLW, 2001; Fisheries Agency, 2003). The total TEQ levels in marine foods in this study were within the range of data from the above-mentioned studies in Japan and showed similar concentration tendencies. Although the two samples of mackerel had somewhat high total TEQ levels compared with data from the above-mentioned studies, similar total TEQ levels have been found in some of the fish caught at Tokyo Bay, Japan (Naito et al. 2003). Total TEQ levels in flounder also ranked with those in fish from Japanese coastal waters in this study, and one flounder sample (Fl2), which was imported from Iceland, had a PCDD/DF TEQ concentration similar to that in Greenland halibut caught in the waters of Iceland or the North Sea, and analyzed in a study of PCDDs/DFs in fish on the German market (Karl et al. 2002). Very low TEQs in common octopus, black tiger shrimp, and yellow-fin tuna may be due to the low fat content in the samples, and the fact that the samples were transported from water areas far from highly industrialized countries.

Figure 1 shows the 2,3,7,8-substituted PCDD/DF congener concentrations in marine foods in this study. The congener patterns were almost the same for sample pairs in seven species, with the exceptions being common octopus, black tiger shrimp and yellow-fin tuna. The predominant congeners in fish samples, excluding yellow-fin tuna, were 1,2,3,7,8-PeCDD, 2,3,7,8-TeCDF and 2,3,4,7,8-PeCDF, and the rank of 2,3,7,8-TeCDF and 2,3,4,7,8-PeCDF concentrations were sometimes reversed between the species or samples. These results agreed with data from other studies (Naito et al. 2003; Vuorinen et al. 2004). The sum of the TEQ concentrations of the predominant congeners in the sample groups of fish and squid contributed to 76–97% of total PCDD/DF TEQs. Interestingly, 1,2,3,7,8-PeCDF concentrations in some fish samples were apparently lower than 2,3,4,7,8-PeCDF, but those in Japanese common squid were close to 2,3,4,7,8-PeCDF. One reason for this may be that the ratio of concentrations of non-2,3,7,8-substituted PeCDF to the total PeCDF congeners in squid (64 and 67%) were higher than in fish (5–42%), so non-2,3,7,8-substituted congeners were not separated from the 1,2,3,7,8-PeCDF peak when using the SP 2331 capillary column for GC analysis. Another reason is thought to be that mollusks, such as the Japanese common squid, have a higher bioaccumulation potency to 1,2,3,7,8-PeCDF compared with fish. Such explanations also hold for

Table 1. TEQ concentrations (pg/g wet weight) in marine foods purchased from supermarkets and retail stores in Osaka City, Japan.

Species	Scientific name	Sample No.[a)]	Location caught	Fat content (%)	WHO-PCDD/DF -TEQ (pg/g)	WHO-PCB -TEQ (pg/g)	Total TEQ (pg/g)
Chub mackerel	*Scomber japonicus*	CM1	Japan coast[b)]	5.6	1.8	9.3	11
		CM2	Japan coast[b)]	20.9	1.5	5.1	6.6
Flounder	NA	Fl1	The Netherland	2.0	0.96	2.0	3.0
	NA	Fl2	Iceland	20.8	2.0	3.2	5.2
Red sea-bream	*Pagrus major*	RS1	Japan coast[b)]	2.2	0.25	0.44	0.69
		RS2	Japan coast[b)]	2.5	0.47	0.89	1.4
Japanese horse mackerel	*Trachurus japonicus*	JH1	Japan coast[b)]	7.3	0.57	0.78	1.4
		JH2	Japan coast[b)]	12.7	0.26	0.38	0.64
Sardine	*Sardinops melanostictus*	Sar1	Japan coast[b)]	14.4	0.23	0.46	0.70
		Sar2	Japan coast[b)]	2.0	0.27	0.60	0.87
Salmon	NA	Sal1	Alaska	6.5	0.10	0.24	0.34
	NA	Sal2	U.S.A	6.8	0.067	0.18	0.25
Japanese common squid	*Todarodes pacificus*	JC1	Japan coast[b)]	<1	0.071	0.15	0.22
	Steenstrup	JC2	Japan coast[b)]	<1	0.074	0.12	0.19
Common octopus	*Octopus vulgaris*	CO1	Morocco	<1	0.0055	0.0093	0.015
	Cuvier	CO2	Morocco	<1	0.0027	0.011	0.014
Black tiger	*Penaeus monodon*	BT1	Indonesia	<1	0.0000080	0.0011	0.0011
	Fabricius	BT2	India	<1	0.0087	0.015	0.024
Yellow-fin tuna	*Thunnus albacares*	YT1	Indonesia	<1	0.0000050	0.0014	0.0014
		YT2	Indian Ocean	<1	ND	0.072	0.072

Toxic equivalents (TEQs) were calculated using WHO-toxic equivalency factors (TEFs). NA = not available; ND = not detected. LOD are given in the **Materials and Methods** section of the text. [a]Fl1, RS1, JH1, Sar1, JC1, CO1, BT1 and YT1: samples from June 1999; CM2, Fl2, JH2, Sal2, JC2, CO2, BT1 and YT2: samples from November 1999; CM1, RS2 and Sar2: samples from March 2000. [b]Two samples of the same species caught in different places in Japan

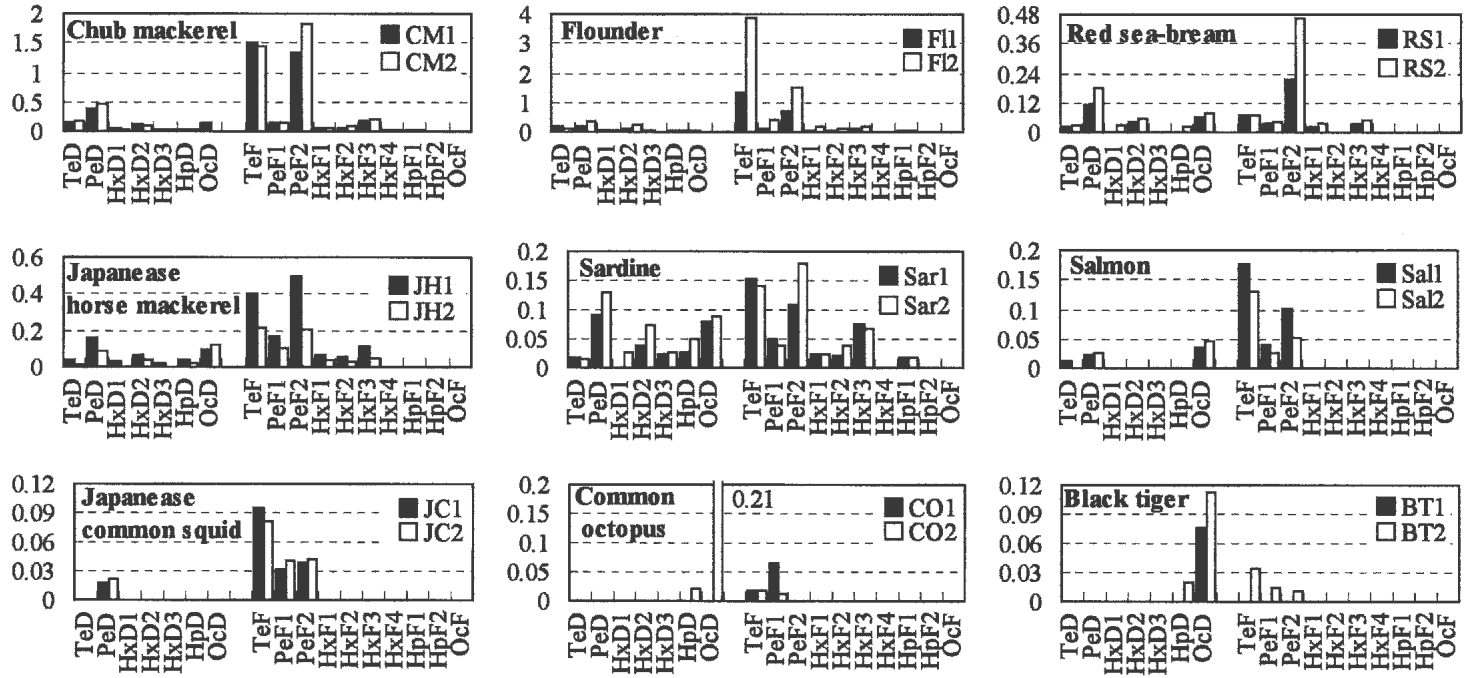

Figure 1. Concentrations of 2,3,7,8-substituted PCDDs/DFs in marine foods (pg/g wet weight). Congeners not detected are shown as 0. As for samples of yellow-fin tuna, 1,2,3,4,6,7,8,9-OCDD was detected at 0.05 pg/g in only YT1 (not shown). Abbreviations: TeD =2,3,7,8-TeCDD; PeD=1,2,3,7,8-PeCDD; HxD1=1,2,3,4,7,8-HxCDD; HxD2=1,2,3,6,7,8-HxCDD; HxD3=1,2,3,7,8,9-/1,2,3,4,6,7-HxCDD; HpD=1,2,3,4,6,7,8-HpCDD; OcD=OCDD; TeF=2,3,7,8-TeCDF; PeF1=1,2,3,4,8-/1,2,3,7,8-PeCDF; PeF2=2,3,4,7,8-PeCDF; HxF1 =1,2,3,4,6,7/1,2,3,4,7,8-HxCDF; HxF2= 1,2,3,6,7,8-HxCDF; HxF3=2,3,4,6,7,8-HxCDF; HxF4=1,2,3,7,8,9-HxCDF; HpF1=1,2,3,4,6,7,8-HpCDF; HpF2 =1,2,3,4,7,8,9-HpCDF; OcF =OCDF.

data on the common octopus. Among CoPCB congener concentrations, the highest concentration was PCB-118, followed by PCB-105, PCB-156 and PCB-167, through all marine food samples; whereas, considering the TEQ concentrations in all samples in which PCB-126 was detected, PCB-126 had the highest contribution (> 75%) to CoPCB-TEQ (data not shown).

Figure 2 shows the relative compositions of dioxin congener concentrations in fish samples from the Japanese costal waters, excluding imported fish, in consideration of the different pollution sources. Samples represented by bars along the X-axis of each congener are sequenced in the order of a high-to-low ranking of samples according to 2,3,7,8-TeCDD concentrations with the highest toxic equivalent factors (TEFs). The rank of the relative abundance of 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, a portion of HxCDDs/DFs and CoPCBs, excluding PCB-77 and -81, tended to agree with that of the 2,3,7,8-TeCDD concentrations, indicating significant Spearman rank correlation coefficients with 2,3,7,8-TeCDD concentrations (Fig. 2). In particular, the rank of relative abundance of 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, and almost all CoPCB concentrations tended to agree well with that of 2,3,7,8-TeCDD concentrations, indicating high Spearman correlation coefficients with 2,3,7,8-TeCDD concentrations. These results are tied in part to differences in bioaccumulation between dioxin congeners in fish. As is well known, the bioaccumulation of CoPCB and Te- or PeCDD/DF congeners, as indicated by the biota-sediment accumulation factor (BSAF), for example, is much higher than that of more highly chlorinated PCDDs/DFs in fish (Isosaari et al. 2004; Naito et al. 2003). It is thus thought that the magnitude of concentrations of 2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF and some CoPCB congeners that exhibit similar behavior in fish, vary together in parallel among fish samples. In this study, the associations of 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF concentrations with 2,3,7,8-TeCDD concentrations were clear in fish samples, whereas those of 2,3,7,8-TeCDF and 1,2,3,7,8-PeCDF concentrations with 2,3,7,8-TeCDD concentrations were ambiguous. However, when Spearman's correlation coefficients are also calculated using data from other Japanese studies (Kang et al. 2000; Toyoda et al. 1999), the 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF concentrations correlated more strongly with the 2,3,7,8-TeCDD concentrations than the 2,3,7,8-TeCDF and 1,2,3,7,8-PeCDF concentrations in the fish samples.

On the other hand, the rank of relative abundance of OCDD concentrations not being in agreement with that of 2,3,7,8-TeCDD is thought to derive from factors other than bioaccumulation. The uptake of OCDD/DFs in fish is hindered by steric factors, and a stronger affinity to sediment and organic matter in water (Opperhuizen et al. 1985; Loonen et al. 1994). Moreover, it is known that one characteristic source of OCDD is penta-chlorophenol (PCP) (Masunaga et al. 2001). Therefore, the magnitudes of OCDD congener concentrations in fish samples are affected mainly by factors such as the situation of equilibrium in habitat biota, the pollution source and metabolic properties of fish species; thus, the variation of concentrations of OCDD congeners may be irregular among fish samples.

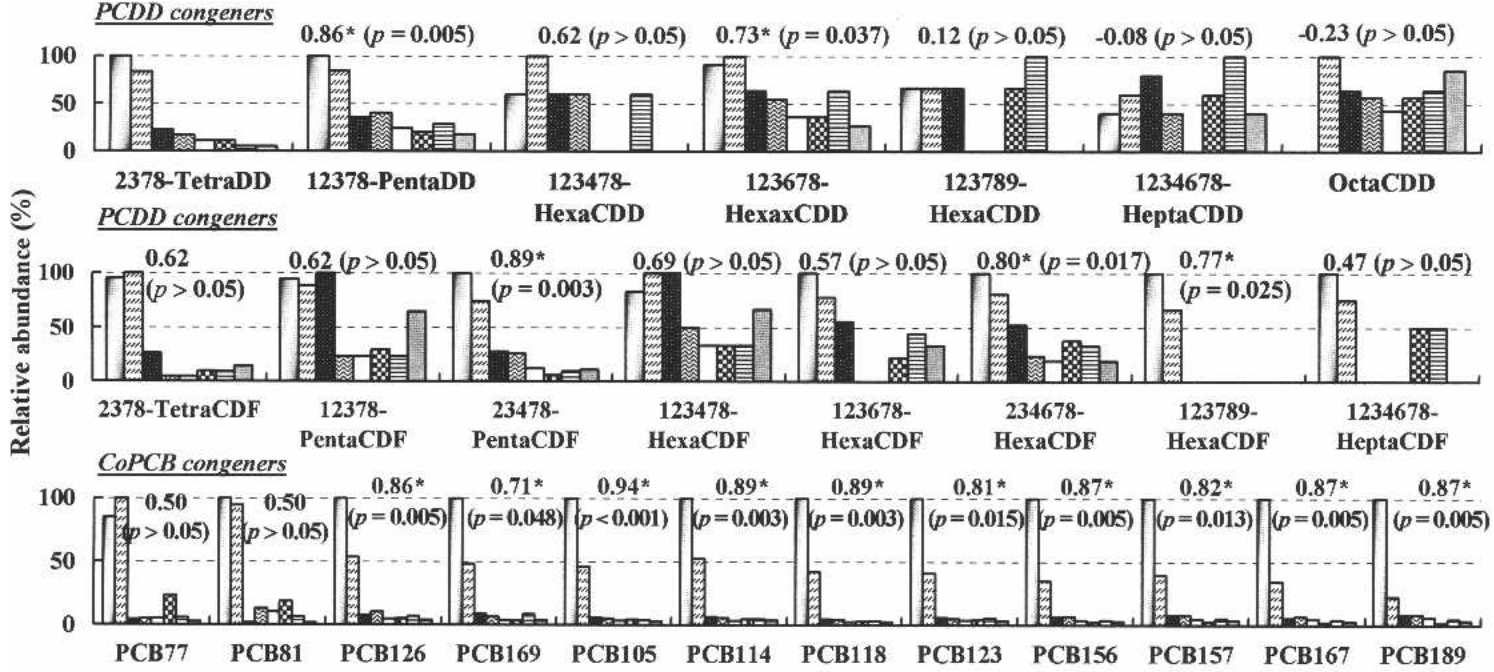

Figure 2. Relative compositions of dioxin congener concentrations in eight Japan coastal fish samples. Abundance of individual congener concentrations is normalized to the highest concentration of eight fish, which is treated as 100. Congeners not detected are shown as 0. 1,2,3,4,7,8,9-HpCDF and OCDF concentrations in all samples were below the LOD. Samples represented by bars along the X-axis of each congener (from left to right: CM1, CM2, JH1, RS2, RS1, Sar1, Sar2 and JH2) are sequenced in the order of a high-to-low ranking of samples according to 2,3,7,8-TeCDD concentrations. Values over the X-axis of each congener are Spearman correlation coefficients between concentrations of 2,3,7,8-TeCDD and other congeners in eight fish samples. Values with an asterisk indicate the significance criterion of $p < 0.05$.

REFERENCES

Feeley M, Brouwer A (2000) Health risks to infants from exposure to PCBs, PCDDs and PCDFs. Food Addit Contam 17:325–333

Fisheries Agency-Japan (2003) Results of studies on dioxins in marine foods in 1999–2002. June 2003 [in Japanese]

Isosaari P, Kiviranta H, Lie Ø, Lundebye A-K, Ritchie G, Vartiainen T (2004) Accumulation and distribution of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and polychlorinated biphenyl congeners in Atlantic salmon (*Salmo salar*). Environ Toxicol Chem 23:1672–1679

Kang Y-S, Yamamuro M, Masunaga S, Nakanishi J (2000) Isomer compositions of PCDDs/DFs and coplanar PCBs in waterfowls, fishes and shellfish from Lake Shinji. Bull Instit Environ Sci and Technol, Yokohama National University 26:11–22 [in Japanese]

Karl H, Ruoff U, Blüthgen A (2002) Levels of dioxins in fish and fishery products on the German market. Chemosphere 49:765–773

Loonen H, Parsons JR, Govers HAJ (1994) Effect of sediment on the bioaccumulation of complex mixture of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by fish. Chemosphere 28:1433–1446

Masunaga S, Takasuga T, Nakanishi J (2001) Dioxin and dioxin-like PCB impurities in some Japanese agrochemical formulations. Chemosphere 44:873–885

Ministry of Health and Welfare (MHW)-Japan (2000) The data resulting from dietary intake studies of dioxins and related PCBs in 1999. November 2000 [in Japanese]

Ministry of Health, Labour and Welfare (MHLW)-Japan [Former name: Ministry of Health and Welfare] (2001) The data resulting from dietary intake studies of dioxins and related PCBs in 2000. December 2001 [in Japanese]

Naito W, Jin J, Kang Y-S, Yamamuro M, Masunaga S, Nakanishi J (2003) Dynamics of PCDDs/DFs and coplanar-PCBs in an aquatic food chain of Tokyo Bay. Chemosphere 53:347–362

Nakatani T, Itano K, Fujita T (2005) Contamination levels of PCDDs, PCDFs and CoPCBs in fish oils used as raw materials for margarine and shortening and retailed fish. J Food Hyg Soc Japan 49:169–175

Opperhuizen A, van der Velde EW, Gobas FAPC, Liem DAK, van der Steen JMD, (1985) Relationship between bioconcentration of hydrophobic chemicals in fish and steric factors of hydrophoboic chemicals. Chemosphere 14:1871–1896

Toyoda M, Iida T, Hori T, Yanagi T, Kono Y, Uchibe H (1999) Concentrations of PCDDs, PCDFs and coplanar PCBs in Japanese retail foods. J Food Hyg Soc Japan 40:111–121 [in Japanese]

Vuorinen PJ, Parmanne R, Kiviranta H, Isosaari P, Hallikainen A, Vartiainen T (2004) Differences in PCDD/F concentrations and patterns in herring (*Clupea harengus*) from southern and northern Baltic Sea. Organohalogen Compd 66:1882–1887