Concentration Profiles of PCDDs/DFs and Dioxin-Like PCBs in Fish from Tokyo Bay

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Abstract

For the 1953-1975 time period, pooled fish samples (goby) from the Tokyo Bay and Tateyama Bay were analyzed for polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/DFs) and dioxin-like polychlorinated biphenyls (non-*ortho*- and mono-*ortho*-substituted CBs). The results indicate that the total concentrations of PCDDs/DFs and dioxin-like PCBs in goby collected from Tokyo Bay were increased from 1953 to 1970. However, in fish samples from Tateyama Bay, the total concentrations of TEQ (ΣPCDDs/DFs-TEQ + Σdioxin-like PCBs-TEQ) were in decline. This decline tendency in 1975 might be due to differences in the sampling area between Tokyo Bay and Tateyama Bay. In fish samples from Tokyo Bay, the residue profiles of PCDD/DF congeners were found to exhibit similar temporal fluctuation from 1953 to 1970.

1. Introduction

PCDDs/DFs are not manufactured on deliberately purpose; however these toxic compounds are chemical impurities, unintentional by-products of industrial processes involving chlorine, or burning of organic matter in the presence of chlorine (Buser *et al.*, 1978; Rappe *et al.*, 1979). On the other hand, dioxin-like PCBs enter the environment from two potential sources; commerical PCB formulations or combustion processes (Brown *et al.*, 1995). These hydrophobic chemicals are highly persistent compounds with a strong affinity for sediments and a high potential for accumulating in biological tissues. Recently, many studies have reported that these hydrophobic chemicals have been found in all environmental media (Kuehl *et al.*, 1994; Kylin *et al.*, 1994; Oehme *et al.*, 1995; Brzuzy and Hites, 1996). PCDDs/DFs and dioxin-like PCBs elicit their toxic effects through a common mode of action, including the cytochrome P-450 1A1 and P-450 1A2 hemoproteins and their associated microsomal monooxygenases, which include arylhydrocarbon hydroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD) activities (Safe, 1987). It is well known that these contaminants are all capable of producing the same types of toxic responses, such as dermal lesions, body-weight loss, thymic atrophy, hepatic damage, immunotoxicity, reproductive deficits and carcinogenicity (Liem and Theelen, 1997)

Many investigations into the temporal trends of these toxic compounds have been carried out so far. The majority of historical temporal trend studies have been performed using mainly sediment cores or stored human tissues. However, information on temporal trends for wildlife is very scarce. In the case of time trend studies on residual PCDDs/DFs in wildlife, Herbert *et al.* (1994) have reported that levels of PCDDs/DFs in pooled herring gull eggs collected in the Great Lakes and the St. Lawrence River declined in most colonies between 1981 and 1984, with no clear trends after 1984.

In this study, we examine temporal changes in levels of PCDDs/DFs and dioxin-like PCBs in goby collected throughout Tokyo Bay and Tateyama Bay from 1953 to 1975. The objectives of this study were to quantify the contamination levels of PCDDs/DFs and dioxin-like PCBs in fish samples from the past and to investigate the accumulation profiles of these compounds.

2. Materials and Methods

2.1 Sample Collection

Table 1 lists the details of fish samples (goby) used for the present study. Formalin-preserved samples were obtained from Tokyo University Agricultural and Life Sciences School. All fish samples for PCDD/DF and dioxin-like PCB analysis were collected in Tokyo Bay and Tateyama Bay from 1953 to 1975. Almost all the fish samples were collected from Tokyo Bay, excluding the sample code # G75S9 from 1975. The sample collection sites are illustrated in Figure 1. Detailed information on the sampling site is unknown for the sample code # G53S2 from 1953.

2.2 Determination of PCDDs/DFs and dioxin-like PCBs(non- and mono-ortho-substituted CBs)

Approximately 50 g from the pooled fish samples was homogenized, freeze-dried and extracted using a Soxhlet apparatus for 16 h with 500 mL of dichloromethane. The extract was transferred into a separatory funnel containing 300ml of *n*-hexane and cleaned with concentrated sulfuric acid. A total of sixteen ^{13}C-labelled 2,3,7,8-chlorine-substituted PCDDs/DFs, four ^{13}C-labelled non-*ortho*- and eight ^{13}C-labelled mono-*ortho*-substituted CBs were used as internal

Table 1. Details of fish samples from 1953-1975.

Sample code	Common name	Sampling area	Sampling Year	Analyzed part	Weight (g)	Length (cm)	Fat (%)
G53S2	Goby [4]	Tokyo Bay	1953	Whole	15.6±5.1	12.3±1.1	5.6
G57S3	Goby [4]	Tokyo Bay	1957	Whole	17.6±6.7	13.1±2.1	10.8
G63S4	Goby [4]	Tokyo Bay	1963	Whole	10.6±5.5	10.6±2.8	6.9
G70S7	Goby [5]	Tokyo Bay	1970	Whole	9.5±2.5	11.2±1.1	9.0
G75S9	Goby [7]	Tateyama Bay	1975	Whole	9.2±3.6	10.1±1.3	8.5

Figures in brackets [ ] indicate the number of samples pooled.

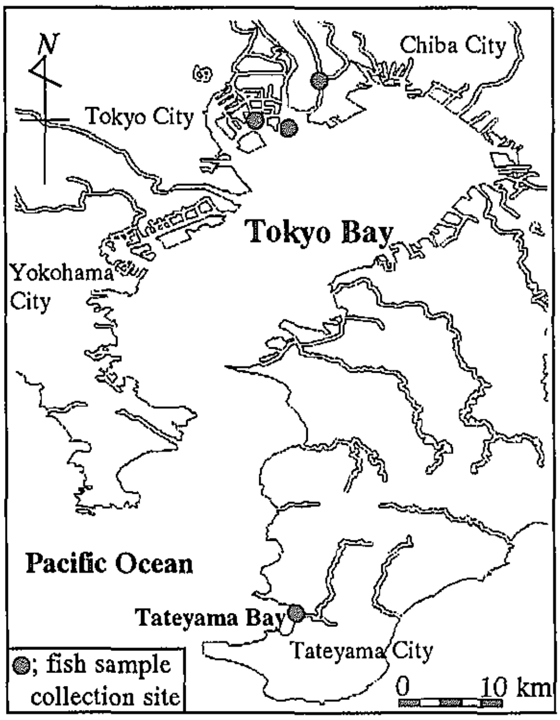

Fig. 1. Sampling locations in Tokyo Bay and Tateyama bay.

standards, spiked to the hexane extract prior to sulfuric acid treatment. Fractionation was carried out with an activated silica-gel column (Wakogel S-1, Wako Pure Chemical Co., Japan) and an alumina column (Aluminium oxide 90 Merck 1076, Germany). In the charcoal-impregnated silica-gel mixture column (Wako Pure Chemical Co., Japan) fractionation step, adsorbed PCDDs/DFs and dioxin-like PCBs were eluted into two fractions. The first fraction, eluted with 25% dichloromethane in hexane, consisted of mono-*ortho*-substituted CBs. The second fraction, eluted with toluene, comprised PCDDs/DFs and non-*ortho*-substituted CBs.

Identification and quantification of PCDD/DF homologues and non-*ortho*- and mono-*ortho*-substituted CBs was performed by high-resolution gas chromatography (Hewlett Packard 6890 Series)/high-resolution mass spectrometry (Micromass Autospec-Ultima). The HRMS was operated in electron impact and selected ion monitoring mode at a resolution R>10,000 (10% valley). The separation of PCDDs/DFs was achieved using a HP 6890 instrument equipped with DB-5 (J&W Scientific ; 0.25mm i.d.×60m length) and DB-17 (J&W Scientific ; 0.25mm i.d.×60m length) columns with splitless and solvent cut mode. Gas chromatographic separation of non-*ortho*- (IUPAC #77, #81, #126 and #169) and mono-*ortho*-substituted CBs (IUPAC #105, #114, #118, #123, #156, #157, #167 and #168) was carried out on a DB-5 capillary column. Before analysis, ^{13}C-labelled 1,2,3,4-TeCDD and 1,2,3,7,8,9-HxCDD were added for recovery estimation.

3. Result and Discussion

3.1 *Residue levels of PCDDs/DFs and dioxin-like PCBs in fish samples, 1953-1975*

The concentrations of individual congeners and the isomers of PCDDs/DFs in the fish samples collected in Tokyo Bay and Tateyama Bay from 1953 to 1975 are listed in Table 2. PCDDs/DFs were detected in all fish samples from 1953 to 1975. The concentrations on a wet weight basis of total PCDDs/DFs varied between pooled goby samples from a low concentration of 27.9 pg/g (on a wet weight basis) in 1957 to a high concentration of 109 pg/g in 1970. The total PCDD/DF concentrations in goby from 1953 to 1963 did not differ significantly. However, the total PCDDs/DFs concentrations in goby samples from the 1970s (sample codes G70S7 and G75S9) were higher than those in fish samples from the 1950s and 1960s.

Table 2. Concentrations of PCDDs/DFs (pg/g on a wet wt. basis) in fish samples.

Sample Code Isomers	WHO-TEF (humans)	G53S2	G57S3	G63S4	G70S7	G75S9
2,3,7,8-TeCDD	1	0.12	0.16	0.17	4.97	0.18
Other TeCDD		0.68	0.55	1.27	10.14	11.93
1,2,3,7,8-PeCDD	1	0.44	0.35	0.52	1.83	0.49
Other PeCDD		1.13	0.29	0.61	1.24	1.02
1,2,3,4,7,8-HxCDD	0.1	0.13	0.11	0.30	0.73	0.26
1,2,3,6,7,8-HxCDD	0.1	0.52	1.12	0.92	2.10	0.71
1,2,3,7,8,9-HxCDD	0.1	0.56	0.09	0.16	0.31	0.28
Other HxCDD		3.36	0.22	0.63	0.35	0.46
1,2,3,4,6,7,8-HpCDD	0.01	2.07	1.12	1.46	1.95	2.66
Other HpCDD		2.07	0.47	0.72	0.44	0.87
OCDD	0.0001	8.81	8.03	9.17	6.66	23.39
Σ PCDDs		19.89	12.52	15.93	30.68	42.23
2,3,7,8-TeCDF	0.1	4.68	3.40	3.75	31.2	0.51
Other TeCDF		2.32	1.04	2.59	24.31	1.19
1,2,3,7,8-PeCDF	0.05	0.44	0.44	0.53	2.09	0.12
2,3,4,7,8-PeCDF	0.5	0.76	1.40	1.27	6.02	0.34
Other PeCDF		0.91	1.07	3.07	7.05	0.71
1,2,3,4,7,8-HxCDF	0.1	0.27	1.07	0.52	1.26	0.31
1,2,3,6,7,8-HxCDF	0.1	0.10	0.15	0.30	0.82	0.15
2,3,4,6,7,8-HxCDF	0.1	0.09	0.15	0.29	0.94	0.21
1,2,3,7,8,9-HxCDF	0.1	0.01	ND	ND	0.03	0.01
Other HxCDF		0.65	2.32	2.40	3.31	0.90
1,2,3,4,6,7,8-HpCDF	0.01	0.28	1.02	0.53	0.53	0.45
1,2,3,4,7,8,9-HpCDF	0.01	0.03	0.06	0.06	0.10	0.07
Other HpCDF		0.23	1.30	0.41	0.53	0.52
OCDF	0.0001	0.50	1.95	0.35	0.32	0.41
Σ PCDFs		11.27	15.37	16.07	78.51	5.90
PCDDs/DFs WHO-TEQ		1.63	1.86	2.00	13.69	1.12
PCDDs/DFs I-TEQ		1.42	1.69	1.74	12.77	0.89

The concentrations of dioxin-like PCBs, such as non-*ortho*-and mono-*ortho*-substituted CBs in fish samples from 1953-1975, are presented in Table 3. Residues of dioxin-like PCBs in goby were confirmed from 1953 and the level gradually increased to 1970, whereas the concentration of these compounds in goby from 1975 rapidly declined. In Japan, the production of commercial PCBs by two firms (Kanegafuchi and Mitsubishi-Monsanto) began in 1954, but were banned from domestic use in 1973 (Hutzinger *et al.*, 1974; Japan Society for Chemistry, 1980). Although the domestic production of PCBs began in 1954, environmental contamination had been evidenced by the presence of dioxin-like PCBs in fish samples even before 1954, indicating the possibility of environmental contamination due to the limited use of imported PCBs by local industries prior to 1954. The presence of this residue from the early 1950s revealed that environmental contamination of PCBs started soon after these compounds were put to use in Japan.

On the other hand, we found that the residue level of dioxin-like PCBs declined in goby collected from Tateyama Bay. This seems to be due to the geographic difference rather than temporal fluctuation. Namely, the wide difference between dioxin-like PCB levels in 1970 and 1975 suggests that the contamination situations within each region were different during the 1970s.

Table 3. Concentrations of non-*ortho*- (pg/g on a wet wt. basis) and mono-*ortho*-substituted CBs (ng/g on a wet wt. basis) in fish samples.

Sample code IUPAC No.	WHO-TEF (humans)	G53S2	G57S3	G63S4	G70S7	G75S9
# 81	0.0001	0.58	6.75	54.36	409.8	4.29
# 77	0.0001	24.40	197.3	810.4	3144.4	71.45
# 126	0.1	ND	9.87	48.86	158.5	7.6
# 169	0.01	ND	0.49	2.02	6.18	0.75
Σ non-*ortho* CBs (pg/g)		24.98	214.5	915.6	3718.8	84.09
# 123	0.0001	0.10	0.19	0.73	1.84	0.19
# 118	0.0001	0.96	4.99	20.18	46.52	4.38
# 114	0.0005	0.01	0.15	0.71	1.99	0.15
# 105	0.0001	0.30	1.92	8.20	23.94	1.62
# 167	0.00001	0.01	0.07	0.33	0.71	0.08
# 156	0.0005	0.08	0.48	2.12	3.71	0.98
# 157	0.0005	0.02	0.09	0.41	0.74	0.13
# 189	0.0001	0.002	0.01	0.03	0.07	0.03
Σ mono-*ortho* CBs (ng/g)		1.48	7.91	32.73	79.52	7.55
PCBs WHO-TEQ (pg/g)		0.19	2.08	9.52	26.67	2.02
PCBs Alborg-TEQ (pg/g)		0.24	2.18	9.86	27.86	2.07

Figure 2 illustrates trends in the levels of the total toxic equivalents (Σ PCDDs/DFs-TEQ + Σ dioxin-like PCBs-TEQ) according to WHO-TEFs for humans in fish samples and the ratios of dioxin-like PCBs-TEQ versus PCDDs/DFs-TEQ from 1953 to 1975. In fish samples from Tokyo Bay, the total TEQ levels ranged from 1.72 pgTEQ/g (on a wet weight basis) in 1953 to 11.52

pgTEQ/g in 1970. The total TEQ level in Tateyama Bay goby was 3.14 pgTEQ/g in 1975.

The ratios of dioxin-like PCBs-TEQ versus PCDDs/DFs-TEQ gradually increased until 1963, after which a decline was observed until 1975. Figure 2 shows the increase of the proportion of dioxin-like PCBs-TEQ to the total TEQ concentrations, after the period of commercial PCB production in Japan. In the 1970s, however, the proportion of PCDDs/DFs-TEQ to the total TEQ concentrations also increased.

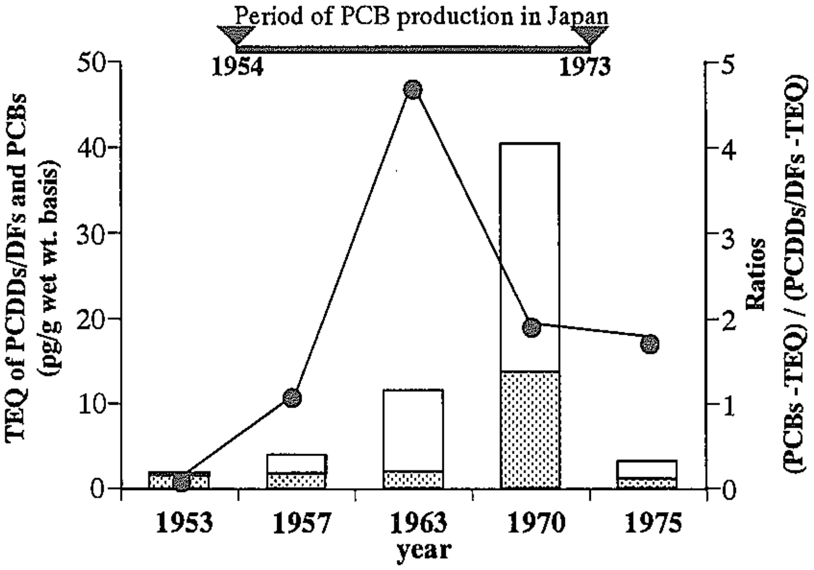

Fig. 2. The total TEQ concentrations (ΣPCDDs/DFs + Σ PCBs) and the ratios of dioxin-like PCBs -TEQ versus PCDDs/DFs-TEQ.

▨ PCDDs/DFs -TEQ ☐ PCBs -TEQ —●— Ratios of (PCBs-TEQ) / (PCDDs/DFs -TEQ)

3.2 *Congener and isomer profiles of PCDDs/DFs in goby, 1953-1975*

PCDD/DF congener profiles in fish samples collected from 1953 to 1975 are shown in Figure 3. On the whole, OCDD in goby from 1953 to 1975 was detected in the highest concentration among the analyzed PCDD/DF congeners. In fish samples collected from Tokyo Bay, although the OCDD concentration in goby from 1970 was lower than that from the 1950s or 1960s, the temporal change for PCDD/DF congener profiles in goby was not significant. In contrast, we found that the PCDD/DF congener profile of goby collected from Tateyama Bay in 1975 differed considerably from that of goby sampled from Tokyo Bay. In particular, the contribution of TeCDF to the total PCDD/DF concentrations in goby collected from Tateyama Bay was markedly lower than that in goby collected from Tokyo Bay.

In fish samples from Tateyama Bay, we found that the concentration of 1,3,6,8-TeCDD at 11% contributed most significantly to the total PCDD/DF concentrations, excluding OCDD among the detected isomers. Yamagishi *et al.*(1981) reported that the herbicide CNP (1,3,5-trichloro-2-(4-nitrophenoxy) benzene) contains 1,3,6,8-TeCDD and 1,3,7,9-TeCDD as main components of its PCDD/DF impurities. The herbicide CNP was widely used in paddy fields in Japan, in the past.

Recent studies have indicated that the sediment and soil of Japan were contaminated by PCDDs/DFs originating from impurities contained in CNP (Sakurai *et al.*, 1996; Masunaga *et al.*, 1998). Thus, we investigated the relationship between these isomer concentrations and the annual amount of CNP used in Japan (Fig. 4). The result indicates that the residues of 1,3,6,8-TeCDD, 1,3,7,9-TeCDD, 2,4,6,8-TeCDF and 1,2,3,6,8-PeCDD in goby were recorded from the 1950s and the level increased from 1970 to 1975. It is considered that the presence of these isomers in goby from the 1950s and early 1960s was due to combustion processes, whereas the increase tendency of the isomers in the 1970s was affected by CNP.

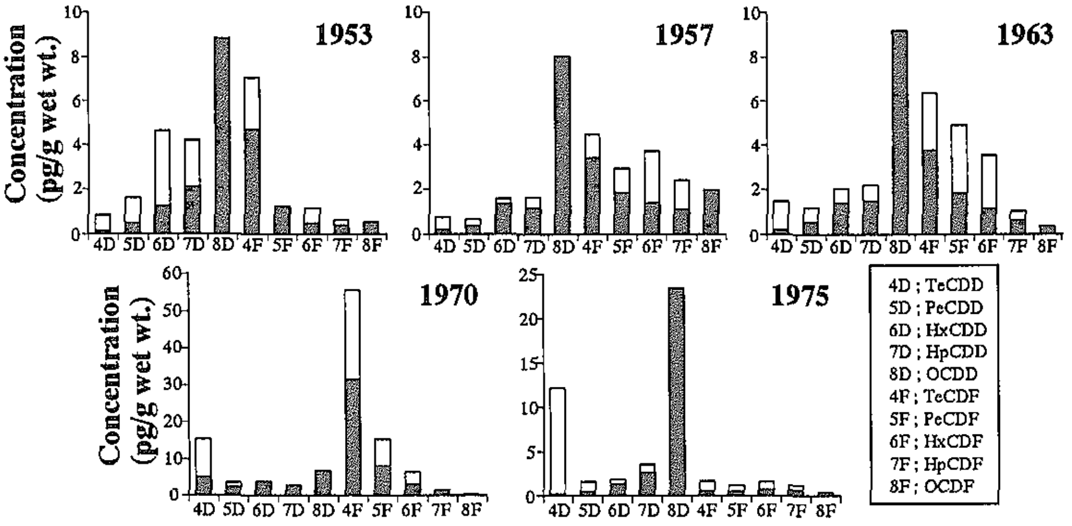

Fig. 3. Profiles of individual congeners of PCDDs/DFs in goby from 1953 to 1975.
□; Non-2,3,7,8-substituted PCDDs/DFs ▨; 2,3,7,8-substituted PCDDs/DFs

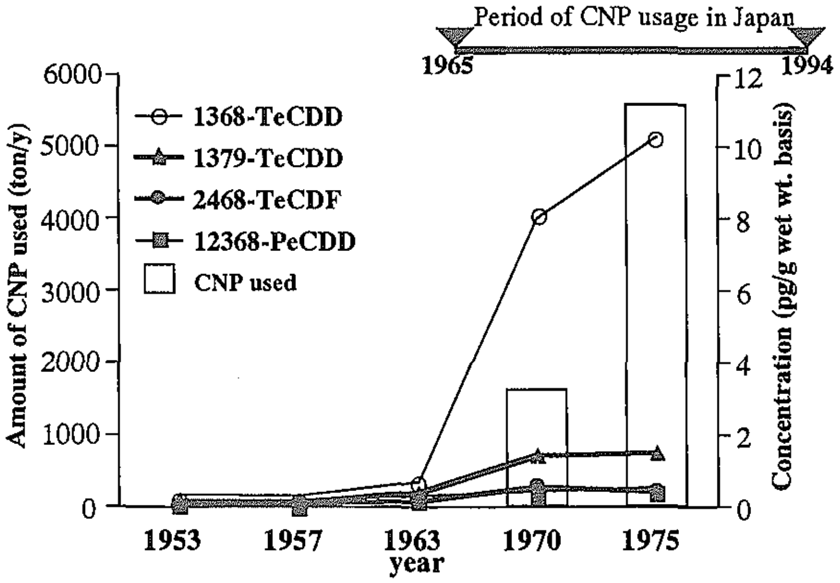

Fig. 4. Temporal trends of 1368, 1379-TeCDD, 2468-TeCDF and 12368-PeCDD concentrations from fish samples and CNP used in Japan.

4. Acknowledgment

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