Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in fish and shellfish samples from Tokyo Bay

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Abstract

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) in fish, shellfish and crab samples from Tokyo Bay were determined. Total PCDD/F concentration of the edible portion of 6 species of fish ranged between 6.5 - 106 pg/g wet weight or 0.32 - 2.07 pg I-TEQ/g wet weight, while shellfish and crab had higher values. Congener as well as isomer profile of the fish samples were quite different from that of the sediment in the region. In the case of shellfish (*Fulvia mutica*) and crab (*Charybdis japonica*), however, their isomer profile within each group of isomers were similar to that of sediment. Biota sediment accumulation factor (BSAF) of shellfish and crab decreased as number of chlorines in PCDD/Fs increased from four to eight. Little difference in BSAF was observed between 2,3,7,8-subustituted and non-2,3,7,8-substituted isomers for shellfish.

Introduction

Food is usually the major exposure route of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) to humans. Among food items, fish and shellfish are reported to constitute over 60% of the total dietary PCDD/F exposure in Japan [1)], which is significantly higher compared with other countries where they constitute less than 30% [2, 3)]. This is due to the large per capita consumption of sea food in Japan as well as to the relatively contaminated fish caught on Japanese coast. In fact, inshore fish is reported to have higher PCDD/F concentration than offshore fish [4, 5)]. Thus information on PCDD/F in inshore fish is important for estimating the exposure of these compounds in Japan. In this study, we measured PCDD/F concentration in fish, shellfish and crustacean from Tokyo Bay which are commonly consumed as food and discussed their congener and isomer profiles ("congener" in this paper refers to isomer group such as TCDDs, TDCFs, etc.).

Materials and Methods

Samples Six species of fish, one crab and one shellfish samples were collected at four sites in Tokyo Bay on May 24, June 17 and June 19, 1996 (Fig. 1). They were kept in freezer (-20°C) until analysis. The characteristics of the samples are shown in Table 1. Edible portion of the fish and crab samples and whole body excluding skin of Japanese cockle were subjected to analysis.

Analysis Homogenized sample (30g) spiked with a mixture of ^{13}C-PCDD/Fs as internal standards was digested with 300 ml of 1 N ethanolic KOH solution for 2 hours at room temperature. The saponified sample was extracted twice with 150 ml of *n*-hexane. The combined hexane extract was washed with water and dewatered with sodium sulfate. Then it was treated with concentrated sulfuric acid and cleaned by series of silica gel, alumina and activated carbon impregnated silica gel column chromatography. The final extract was concentrated and analyzed using high resolution gas chromatography / high resolution mass spectrometry (HRGC/HRMS; HP5890 series II GC and VG Autospec-Ultima). DB-5 (60m, J&W Scientific) and DB-17 (60m, J&W Scientific) were used as GC columns. Mass spectrometer was operated by EI mode at a

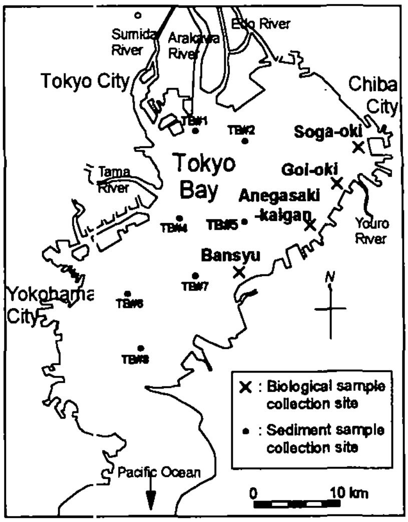

Fig. 1. Sampling Sites in Tokyo Bay

Table 1 Characteristics of fish, crab and shellfish samples

Samples Common name *Scientific name*	Sampling site *	Body length (cm)	Body weight (g)	Fat content (%)	Habitat, etc. (*Japanese Name*)
Black rockfish *Sebastes inermis*	BS	16.2	79.3	2.5	Inshore fish. Has large eyes. (*mebaru*)
Olive flounder *Paralichthys olivaceus*	AN	34.2	240	1.2	Live in sandy sea bottom. Flesh eater. (*hirame*)
Bartailed flathead *Platycephalus indicus*	GI	51.5	1089	0.5	Live in clean sandy sea bottom. Small fish & crustacean eater. (*kochi*)
Stingray *Dasyatis akajei*	SG	38.5	1886	2.4	Cartilaginous fish. Benthic shellfish & crustacean eater. (*akaei*)
Sea bass *Lateolabrax japonicus*	AN	65.5	2652	2.5	Lives inshore in summer and offshore in winter. Comes up in river. (*suzuki*)
Gray mullet *Mugil cephalus*	GI	55.0	1735	4.5	Eats Benthos in muddy sediment. Comes up in river. (*bora*)
Japanese cockle *Fulvia mutica*	SG	5.8	18.6	0.5	Bivalve. Live in shallow sea bottom. Plankton eater. (*torigai*)
Crab *Charybdis japonica*	GI	7.7	82.8	1.9	Live in shallow sea bottom. Small fish and shrimp eater. (*ishigani*)

* SG: Soga-oki, GI: Goi-oki, AN: Anegasaki-kaigan, BS: Bansyu. See Figure 1.

resolution of about 10,000.

Results and Discussion

Recovery of internal standards and blank test The recovery rates of fifteen 2,3,7,8-subustituted PCDD/F internal standards ranged from 65.3 to 96.8% for DB-5 and from 74.8 to 97.4% for DB-17 columns, respectively. As OCDD originated from activated carbon colum was detected in blank test, OCDD concentrations in samples were corrected by subtracting the blank test values.

Detected PCDD/F isomers The authors measured from tetra- to octa-chlorinated PCDD/F isomers. These 136 isomers were separated into over eighty peaks by DB-5 column, however, only 43 to 77 peaks were detected in biological samples. Japanese cockle and crab sample, which are benthic organisms, had 77 and 58 peaks, respectively, whereas fish generally had less (43 - 59) peaks.

Total PCDD/F concentration Total PCDD/F concentration ranged between 6.5 to 106 pg/g wet weight for fish sample whereas shellfish had much higher value of 1025 pg/g wet weight (Table 2). Corresponding I-TEQ values were 0.32 - 2.07 for fish and 3.56 pg/g wet weight for shellfish. These values are within or close to the range reported for inshore fish in other studies where ranges of 0.63 - 1.41 [4] and 0.1 - 2.16 [5] TEQ pg/g wet weight were obtained.

Congener profile Fig. 2 shows the congener profile of the biological samples as well as the sediment sample of Tokyo Bay. Seven sediment samples from different sites of the bay showed that their isomer as well as congener profiles were very similar to each other [6]. Hence only one sediment result is shown in the Figure. There was a substantial difference in the profile between the biological and sediment samples. OCDD was very dominant in sediment sample whereas TCDFs, PeCDFs and HxCDFs were dominant in fish samples. The shellfish and crab had high TCDD concentration showing unique congener profiles. This was due to the very high concentration of 1368-TCDD (and partly 1379-TCDD). The congener composition data were subjected to a cluster analysis. The result showed all the fish samples were relatively close to each other. The fish group, shellfish, crab and sediment, however, were quite far from each other (Fig. 3). This indicates that their bioaccumulation process and/or metabolism of each congener are quite different.

Isomer profile Cluster analysis was also applied to isomer specific data based on DB-5 separated GC peaks (Table 2). The ratios of isomer concentration divided by congener total concentration for TCDD/Fs, PeCDD/Fs and HxCDD/Fs were used in the analysis in order to neglect the congener dependent difference. The result is shown in Fig. 4. In this case, sediment and cockle (and crab to a lesser extent) were shown to have very similar isomer profile in each congener. All the fish samples were categorized into one cluster, however, the distances between each fish species were relatively far indicating that each fish have their own special isomer profiles.

Bioaccumulation The above cluster analyses indicated that fish had quite different conger/isomer profile from sediment indicating that their bioaccumulation was not directly connected with sediment. Shellfish and crab samples, however, had isomer profile within congener similar to that of sediment. This is probably because they live in benthic layer with little migration and also their PCDD/F metabolic rate is small [7]. Thus the sediment biota accumulation factor (BSAF = concentration in

Table 2. Concentration of TCDD/Fs in fish, shellfish and crab samples from Tokyo Bay

(Biological sample: pg/g wet weight. Sediment: pg/g dry sediment)

Congener	Sample / Isomer*3	Black rockfish*1	Olive flounder*1	Bartailed flathead*1	Stingray*1	Sea bass*1	Gray mullet*1	Japanese cockle*2	Crab*1	Sediment (TB#5)
TCDF	2378-TCDF	2.65	0.34	0.18	0.95	3.08	1.77	1.50	0.96	7.71
	non-2378-isomers	1.19	0.73	1.87	1.53	9.65	2.13	51.44	14.08	172
	congener total	3.84	1.07	2.05	2.48	12.73	3.90	52.94	15.04	180
PeCDF	12378-PeCDF	0.45	0.18	0.17	0.99	0.77	0.63	1.66	0.37	9.74
	23478-PeCDF	0.89	0.55	0.19	1.28	1.12	0.79	1.53	0.91	11.10
	non-2378-isomers	2.40	0.80	2.36	2.18	21.41	4.47	49.39	12.12	209
	congener total	3.74	1.53	2.72	4.45	23.30	5.89	52.58	13.40	230
HxCDF	123478-HxCDF	0.15	0.18	0.12	0.98	0.43	0.17	2.82	0.47	31.1
	123678-HxCDF	0.10	0.15	0.08	0.50	0.24	0.09	1.50	0.37	19.5
	234678-HxCDF	0.26	0.21	0.14	1.13	1.92	0.26	3.30	0.66	27.0
	123789-HxCDF	0.00	0.02	0.00	0.00	0.00	0.00	0.13	0.00	1.76
	non-2378-isomers	1.94	0.95	3.85	1.83	43.96	5.23	43.93	6.01	364
	congener total	2.45	1.51	4.19	4.44	46.55	5.75	51.68	7.51	444
HpCDF	1234678-HpCDF	0.05	0.12	0.24	0.60	0.81	0.07	13.71	0.76	344
	1234789-HpCDF	0.03	0.03	0.03	0.18	0.30	0.04	1.58	0.00	32.1
	non-2378-isomers	0.14	0.21	0.79	0.26	6.39	0.38	33.47	0.48	743
	congener total	0.22	0.36	1.06	1.04	7.50	0.49	48.76	1.24	1120
OCDF	12346789-OCDF	0.07	0.09	0.13	0.19	1.61	0.10	29.43	0.19	1050
TCDD	2378-TCDD	0.18	0.10	0.07	0.20	0.62	0.20	0.24	1.60	0.98
	non-2378-isomers	4.88	0.54	0.64	0.74	11.30	2.92	348	111	1129
	congener total	5.06	0.64	0.71	0.94	11.92	3.12	349	112	1130
PeCDD	12378-PeCDD	0.32	0.16	0.09	0.65	0.38	0.22	0.60	0.24	5.70
	non-2378-isomers	0.31	0.09	0.14	0.13	0.22	0.11	45.59	19.55	327
	congener total	0.63	0.25	0.23	0.78	0.60	0.33	46.19	19.79	333
HxCDD	123478-HxCDD	0.08	0.03	0.00	0.63	0.09	0.03	0.76	0.22	8.45
	123678-HxCDD	0.24	0.24	0.42	1.30	0.57	0.21	2.59	0.61	32.40
	123789-HxCDD	0.02	0.04	0.00	0.39	0.07	0.00	0.47	0.11	8.28
	non-2378-isomers	0.17	0.07	0.04	-0.11	0.07	0.13	12.72	2.36	221
	congener total	0.51	0.38	0.46	2.21	0.80	0.37	16.54	3.30	271
HpCDD	1234678-HpCDD	0.25	0.16	0.18	3.18	0.62	0.16	36.75	1.43	1070
	non-2337-isomers	0.07	0.09	0.06	0.12	0.09	0.06	28.28	1.36	750
	congener total	0.32	0.25	0.24	3.30	0.71	0.22	65.03	2.79	1820
OCDD*4	12346789-OCDD	0.53	0.39	0.31	2.46	0.47	0.41	313.59	2.40	11600
PCDFs	total	10.32	4.56	10.15	12.59	91.69	16.13	235.39	37.38	3030
PCDDs	total	7.05	1.91	1.94	9.69	14.51	4.46	790.38	141.19	15200
PCDD/Fs	total	17.37	6.46	12.09	22.28	106.19	20.58	1025.77	178.57	18200
I-TEQ	PCDFs	0.78	0.38	0.16	1.05	1.18	0.66	1.96	0.73	19.5
	PCDDs	0.38	0.21	0.16	0.79	0.89	0.34	1.60	1.83	31.1
	PCDD/Fs	1.16	0.59	0.32	1.84	2.07	0.99	3.56	2.56	50.6

*1: Edible portion. *2: Whole body excluding skin. *3: Individual 2378-isomers were quantified using DB-5 & DB-17 columns. Congener total values were quantified with DB-5 column. Concentration of non-2378-isomers were calculated by subtracting of 2378-isomer total from congener total concentration. *4: Corrected by blank OCDD value.

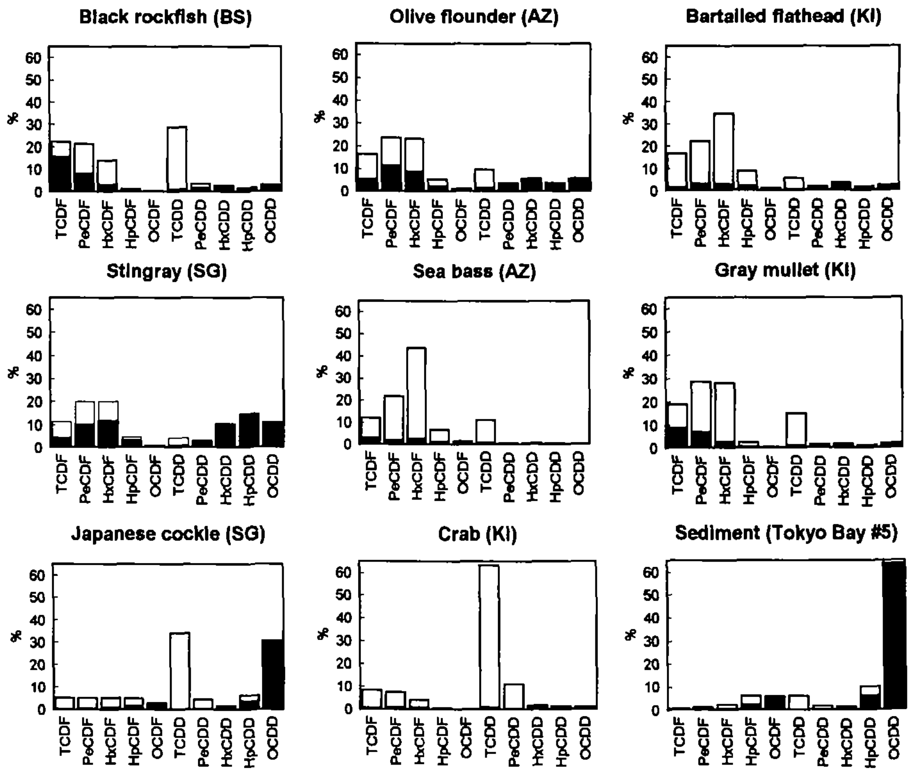

Fig. 2. Congener profile of biological sample and sediment from Tokyo Bay
Solid part of bars: 2,3,7,8-substituted isomers; Blank part: non-2,3,7,8-substituted isomers

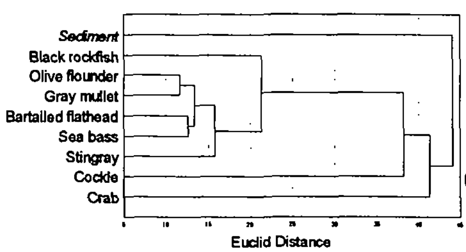

Fig. 3. Dendrogram - cluster analysis based on the congener composition

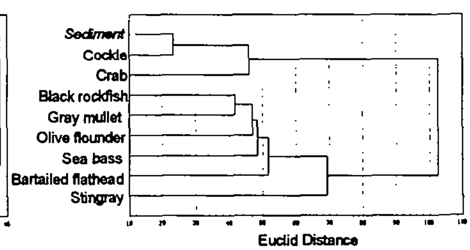

Fig. 4. Dendrogram - cluster analysis based on the isomer composition

biological samples [pg/g fat] / concentration in sediment [pg/g org-C]) was calculated for these two samples against Tokyo Bay sediment sample (TB#5 in Fig. 1) where the biological samples were collected nearby. BSAFs calculated for total concentration 2,3,7,8-subsitituted and non-2,3,7,8-subsituted isomers declined from 2.2 to 0.19 for shellfish and from 0.18 to 0.0003 (excluding 2,3,7,8-TCDD) for crab as the number of substituted chlorine increased from 4 to 8 (or as log K_{OW} increased) (Fig. 5). There was little difference between BSAFs for 2,3,7,8- and non-2,3,7,8-substituted isomers indicating that those organisms had similar bioaccumulation and metabolism potency for the two groups of isomers. We observed higher BSAF for 2,3,7,8-substituted isomers than non-2,3,7,8-substituted isomers for freshwater lake fish [8]. This may be because fish have higher metabolic rate against non-2,3,7,8-isomers than 2,3,7,8-isomers.

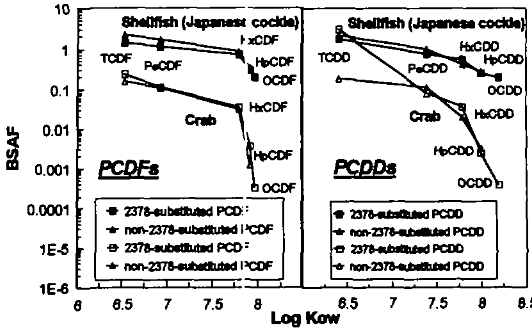

Fig. 5. Biota-sediment accumulation factors (BSAF) of PCDD/Fs in shellfish and crab
(BSAF = [pg/g fat] / [pg/g org-C])

Conclusion

PCDD/F concentrations of some fish and shellfish samples which are commonly consumed as food were determined. Total PCDD/F concentrations in the meat of 6 species of fish ranged between 6.5 - 106 pg/g wet weight (0.32 - 2.07 pg I-TEQ/g wet weight) while shellfish and crab had considerably higher values. This constitute the daily uptake of 0.53 - 3.4 pg I-TEQ/kg body weight if these fish are consumed 100g per day (common in Japan). This higher end is 1/3 of the TDI in Japan (10 pg/kg body weight/day).

Congener as well as isomer profiles of the fish samples were quite different from that of the sediment in the region indicating the unfeasibility of estimating fish contamination from sediment monitoring. In the case of shellfish and crab, however, the isomer profile within each congener were quite similar to that of sediment. Thus their BSAFs of each isomer can be regarded as uniform within the congener.

Acknowledgments

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