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Polychlorinated Dibenzo-p-dioxins in Blue Mussel from Marine Coastal Water in Japan

Hideaki Miyata,[1] Koji Takayama,[1] Junji Ogaki,[1] Takashi Kashimoto,[1] and Shigehiko Fukushima[2]

[1]Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Osaka 573-01, Japan and [2]Department of Food Hygiene, Osaka Prefectural Institute of Public Health, Higashinari-ku, Osaka 537, Japan

Polychlorinated dibenzo-p-dioxins (PCDDs) are tricyclic aromatic compounds containing 75 specific isomers. Some of the isomers with chlorine substitutions at 2, 3, 7 and 8 positions in their molecules have been reported to be extraordinary toxic (Kociba and Cabey 1985).

PCDDs have been revealed to generate as a by-product in the production process of chlorinated herbicides (Woolson et al. 1972) and in the combustion process of domestic and industrial wastes (Olie et al. 1977, Wakimoto et al. 1985, Miyata et al. in press a). In Japan, the latter case is considered to be very important in environmental pollution because most of the wastes is combusted in municipal incinerators due to her small national land.

In this paper, the pollution degree of PCDDs in marine costal water in our country was examined by using blue mussel as a biological indicator because it provided an effective trapping mechanism for many environmental pollutants (Risebrouth et al. 1976, Kashimoto et al. 1986).

MATERIALS AND METHODS

All solvents and reagents used were pesticide grade or reagent grade from Wako Pure Chemical Industrial, Ltd. (Osaka, Japan) or from Kanto Chemical Co. Inc. (Tokyo, Japan). Silica gel (Kieselgel 60, 70-230 mesh and 30-70 mesh) and alumina (neutral, activation I) was purchased from Merck Co. (Darmstadt, West Germany). Before use, the silica gel was washed with methanol, dried and activated overnight at 130°C. Florisil (60-100 mesh) was also activated overnight at 130°C. 22 % and 44 % of sulfuric acid on silica gel 1.9 % pottasium hydroxide on silica gel (70-230 mesh) and 10% silver nitrate on silica gel (70-230 mesh) were prepared with modified methods described previously (Lamparski and Nestrick, 1980).

Blue mussel (Mitilus edulis) was collected from costal areas at a central part (Osaka) and at a northern part (Hokkaido) of Japan in

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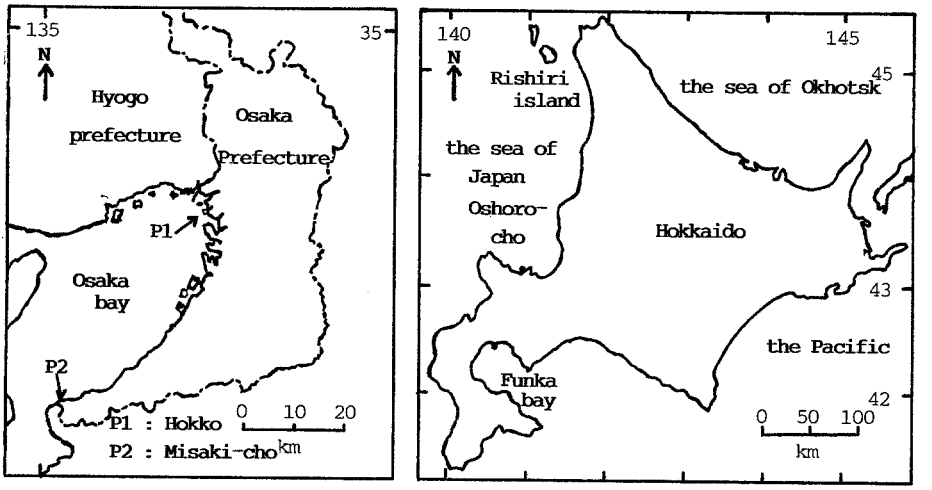

Figure 1. Sampling locations for blue mussel.

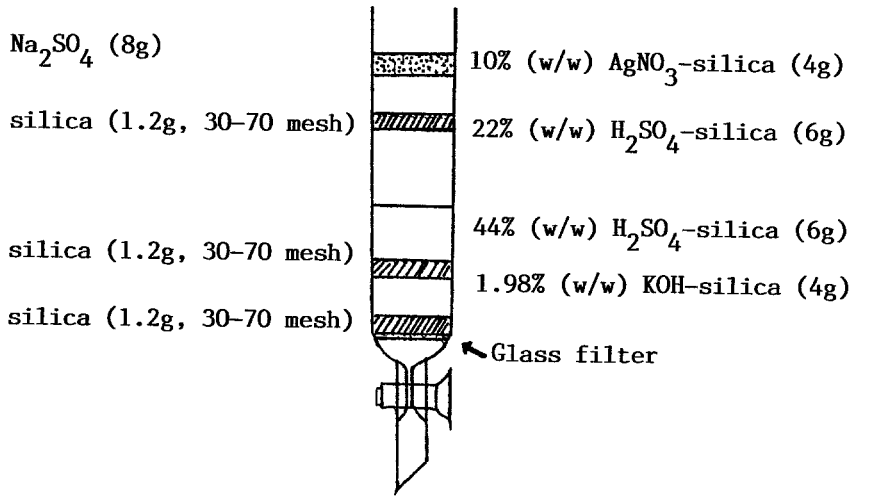

Figure 2. Multi-layer culumn.

the period of December 1984 to June 1986 (Figure 1). In Osaka, two locations (Hokko and Misaki-cho) with a fairly different degree of marine pollution with many kinds of substance (Kashimoto et al. 1985, Kuwabara et al. 1986) were designed for periodical sampling.

After addition of 2 ng of ^{13}C-2,3,7,8-tetrachlorinated dibenzo-p-dioxin (^{13}C-2,3,7,8-tetraCDD) and ^{13}C-octachlorinated dibenzo-p-dioxin (^{13}C-octaCDD) as internal standards, a shellfish content including digestive tract was cleaned-up according to our previous method (Miyata et al. in press b). The method was essentially an alkaline saponification in 350 ml of 0.5N KOH ethanol solution followed by extraction with n-hexane (twice , 150 ml) and precleaned-up on a multi-layer column (Figure 2). The n-hexane eluate from

the column was concentrated and chromatographed into two fractions on a Florisil column (3 g, 1 cm i.d.). The first fraction was analysed for polychlorinated biphenyls (PCBs) on a OV-1 column by a ECD-GC machine. The second fraction containing PCDDs was further purified on an alumina column (2 g, 1 cm i.d.). The final fraction was evaporated to dryness, dissolved with 20 μl of n-decane and then analysed for PCDDs on a SE-54 (25 m×0.31 mm, 0.17 μm film thickness) and a Supelco 2331 (30 m×0.25 mm, 0.20 μm) capillary columns in an electron impact-single ion monitoring mode by using a Hewlett・Packard 7910A gas chromatograph-JEOL DX-300 mass spectrometer with JMA 3500 computer system. The quantification followed the procedure reported previously (Miyata et al. in press a).

RESULTS AND DISCUSSION

PCDDs were detected in all samples of blue mussel from Osaka and Hokkaido. The average concentrations were 250 ppt in Hokko (Osaka), 190 ppt in Misaki-cho (Osaka), 30 ppt in Funka bay (Hokkaido) and 7.6 ppt in Rishiri island (Hokkaido). The degree of the pollution was roughly proportioned to that of the PCBs, one of representative general environmental pollutants (Table 1). Thus tendencies were also observed in our previous data (Kashimoto et al. 1985, Kuwabara et al. 1986) which reported on other pollutants (alkanes, alkylbenzenes, HCHs, dieldrine, chlordanes, dibenzothiophenes etc.). The pollution degrees of these compounds were arranged in order of Hokko > Misaki-cho > Maizuru (Kyoto) > Funka bay > Oshoro-cho (Hokkaido) > Rishiri island, and was closely related to the population density and the industrial activity. Compared to the concentration of total PCDDs in blue mussel from Rishiri island (=1.0), the relative levels of Hokko, Misaki-cho and Funka bay were 33, 25 and 3.9, respectively (Table 2). As a noteworthy thing, shellfish from Hokko was polluted 6 times heavily with PCBs than did one from Misaki-cho, whereas, in case of PCDDs, the former had

Table 1 Average concentrations of PCDDs and PCBs in blue mussel from various locations

location	PCDDs (ppt)						PCBs (ppb)
	4Cl	5Cl	6Cl	7Cl	8Cl	total	
Hokko*	140	20	23	26	42	250	65
Misaki-cho**	68	14	14	33	61	190	11
Funka bay***	8.8	4.3	4.2	6.0	6.5	30.0	1.20
Rishisi island***	2.4	1.9	3.3	n.d.	n.d.	7.6	0.56

* : Average value of 13 samples collected from December 1984 to June 1986
** : Average value of 11 samples collected from December 1984 to June 1986
*** : Value of a sample collected in May 1985
n.d.: not detected

Table 2 Relative concentration of PCDDs and PCBs in blue mussel from various locations to Rishiri island (=1.0)

location	PCDDs			PCBs
	tetraCDDs	others*	total	
Hokko	58	21	33	120
Msaki-cho	28	23	25	20
Funka bay	3.7	4.1	3.9	2.1
Rishiri island	1.0	1.0	1.0	1.0

* : PCDDs except tetraCDDs

Table 3 Congener ratios(%) of PCDDs in fly ash and blue mussel

sample	PCDDs (ppt)				
	4Cl	5Cl	6Cl	7Cl	8Cl
Fly ash*	6.5	13	20	31	24
Blue mussel					
Hokko	55	8.0	9.8	10.4	17
Misaki-Cho	38	9.9	9.2	17	26
Funka bay	30	14	14	20	22
Rishiri island	32	25	43	-	-

* : A mixture of 32 fly ash samples from 4 urban municipal incinerators

a similar level (only 1.3 times heavily) to that in the latter (Table 1). From a distance (ca. 50 km) between the two locations, this indicates that a wide coastal area of Osaka bay is polluted with almost same levels of PCDDs. A profile of PCDD congener ratio was observed to be different among sampling locations (Table 3). PCDDs in blue mussel from Osaka regions were composed of relatively larger amounts of tetraCDDs in comparison with those from Hokkaido. Especially, PCDDs from Hokko were mainly composed of tetraCDDs with an average congener ratio of 55±13%. In Misaki-cho, the ratio decreased to 38±20 % and contrary those of heptaCDDs and octaCDD increased.

The levels of the total PCDDs from Hokko and Misaki-cho were relatively constant and changed in parallel during sampling periods except September in 1985 and June in 1986. In September, 1985, they notably increased to levels of 410 ppt in Hokko and 340 ppt in Misaki-cho (Figure 3). As shown in this figure, the concentration gains in both regions were mainly attributed to increases of tetraCDDs which were confirmed to mainly contain 1,3,6,8- and 1,3,7,9-tetrachlorinated isomers on a Supelco capillary column. Taking the sampling period and the components into consideration, the cause is surmised to be derived from PCDDs present as a byproduct in a chlorinated diphenyl ether herbicide, 1,3,5-

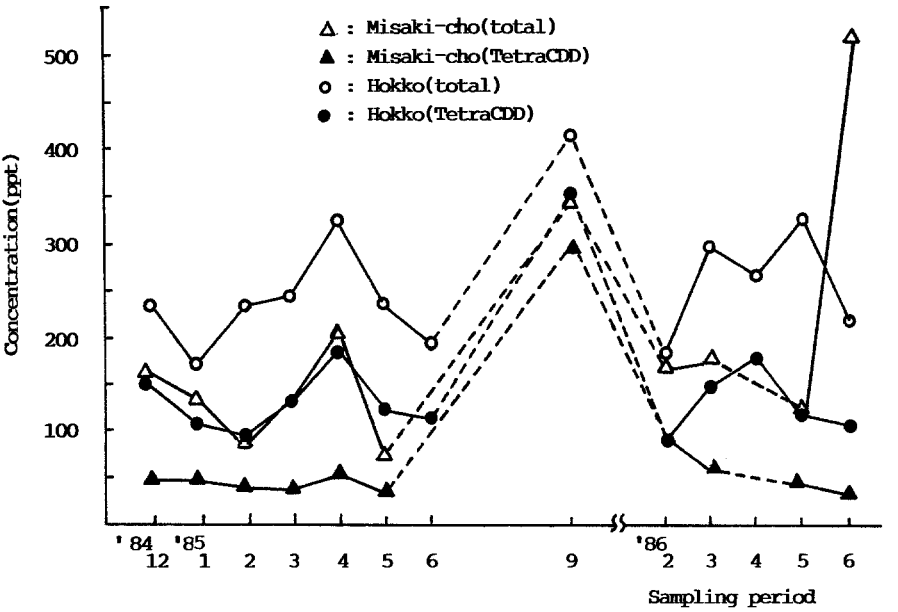

Figure 3. Time course of PCDD levels in blue mussel from Hokko and Misaki-cho

trichloro-2-(4-nitrophenoxy) benzene (CNP), which is mainly sprayed during the summer season. The main reason comes from facts that PCDDs in the herbicide used largely in Japan are mainly composed of tetraCDDs, especially 1,3,6,8-tetra- and 1,3,7,9-tetrachlorinated isomers (Yamagishi et al. 1981) and that in the season of the herbicide application, these isomers accumulated at a level of 0.2 ppb in freshwater fish (Z. platypus) and at a level of tens ppb in freshwater shellfish (Corbicula japonica prime) (Suzuki et al. 1985).

In Misaki-cho in June 1986, the level of total PCDDs was distinguishably lifted to 510 ppt, but the tetraCDDs level did not increase (Figure 3). The cause was mainly responsible for the lifted levels of heptaCDDs (130 ppt) and octaCDD (360 ppt). The elucidation is undergoing in our laboratory.

As described in detail in our previous report (Miyata et al. in press b), a distribution of specific isomers in each PCDD congener in blue mussel from Hokko or Misaki-cho was similar to that in fly ash from municipal incinerators within the distances of ca. 20 km from Hokko. However, the congener ratios of in the blue mussel was fairly different from that in the fly ash, that is, lower chlorinated congeners were predominant components in the former, whereas higher chlorinated ones in the latter(Table 2). From reports that the source of PCDDs in lake sediments in Europe and North America were the atmospheric transport of PCDDs formed by the combustion of domestic and chemical wastes (Czuczwa et al. 1985), sediments in Osaka bay are speculated to be contaminated with

higher chlorinated dioxins such as hexa- to octaCDDs from municipal fly ashes. Therefore, a part of the discrepancy in the congener distribution between the biota and the original might be attributed to a difference in water solubilities of the PCDD isomers, because Friesen et al. (1985) confirmed that water solubilities of the series of PCDDs were in a decreasing trend with increased molecular weight as the isomers contain a successively greater number of chlorine atoms. As already mentioned above, CNP herbicide was one of main pollution sources. The commercial preparation contained tetra- and pentaCDDs as major contaminants and higher chlorinated ones as minors (with levels of less than 2% of those of the formers) (Yamagishi et al. 1981). This emphasizes an appropriateness that higher chlorinated PCDDs present in blue mussel might be derived from the fly ash as the source.

Statistical calculations were performed to examined any possible correlations between the PCDDs and PCBs in blue mussel from Hokko (Table 4). Whereas the cross-correlation coefficient between tetraCDDs and pentaCDDs was 0.568, it was a negative figure between tetraCDDs and any of hexa-, hepta- and octaCDD congeners, this result again accentuates that the different sources may present for the lower and higher chlorinated CDDs. PCBs had very low possitive and negative correlations to all PCDD congeners. This also suggests that the source of PCBs may be different from that of PCDDs. Further studies on the sources of PCDDs in marine biotas are needed.

Table 4 Correlation table for PCDDs and PCBs in blue mussel from Hokko

		PCDDs					PCBs
		4Cl	5Cl	6Cl	7Cl	8Cl	
	4Cl	1.000					
	5Cl	0.568	1.000				
PCDDs	6Cl	-0.285	-0.157	1.000			
	7Cl	-0.365	-0.021	0.244	1.000		
	8Cl	-0.319	0.024	-0.017	0.925	1.000	
PCBs		0.174	0.268	0.014	-0.628	-0.549	1.000

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