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Specific Congener Profiles of Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans in Blue Mussel in Osaka Bay in Japan: Aqueous Solubilities of PCDDs and PCDFs

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We have monitored polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in the coastal waters of Japan by using blue mussel (*Mytilus edulis*) as a biological indicator (Miyata *et al.* 1987a, 1987b). The levels of both chemicals were surmised to closely relate to the number of municipal incinerators and the population densities in the cities adjoining the sampling location. The main contamination source in Osaka Bay, which is heavy polluted with PCDDs and PCDFs, was determined to be the fly ash from municipal waste incinerators based upon analytical results of blue mussel from Osaka Bay, sediments from Osaka Bay, and sediments from a river, which is located near a typical urban municipal waste incinerator(Miyata *et al.* 1988a). However, there was a remarkable difference in congener profiles of PCDDs and PCDFs between the blue mussel and the fly ash, that is, the mussel mainly contained tetraCDDs and tetraCDFs with congener ratios of 56 ± 9.7% and 62 ± 6.0%, respectively, whereas the fly ash contained the higher chlorinated PCDDs and PCDFs as major congeners. In this study, the specific congener profiles of PCDDs and PCDFs in blue mussel were investigated from the point of view of their water solubilities.

MATERIALS AND METHODS

All solvents and reagents used were described in our previous papers (Miyata *et al.* 1987a, 1987b, 1988a). Emulgen, non-ionized surface active agent composing of polyoxyethylene nonylphenylether, was provided by Kao Co., Tokyo, Japan.

Water used was prepared as follows: Laboratory deionized and distilled water was washed with n-hexane to remove lipophilic materials and then reduced to a third of the original volume by a rotory evaporator to eliminate the residual n-hexane. The water was then filtered through a Millipore membrane (0.45 μm, Ekicrodisc 13, Gelman Sciences Japan Ltd., Tokyo, Japan). An aliquot (10 mL) of the filtered water was placed in a 50-mL headspace bottle. The bottle was crimp sealed with an alumina cap with a Teflon liner and

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kept for one hour at 25°C.

A 500-µL aliquot of the headspace gas was injected into a Shimadzu GC-9A gas chromatograph (detector: FID; column: 2.1 m × 2.6 mm I.D. glass column packed with 3% OV-17 on Gas-Chrom Q, 100/120 mesh) to quantify the residual amount of n-hexane in the water. In addition, the total organic carbon in the water was determined by using a Shimadzu TOC analyser.

A PCDD and PCDF mixture was prepared from a sample of fly ash from an urban municipal waste incinerator. The fly ash (750 g) was digested with 3% hydrochloric acid and then extracted with benzene for 5 hr under refluxing according to our previous method (Miyata et al. 1988b). After evaporation of the benzene extract to dryness, the residual material was dissolved with 50 mL of n-hexane and digested in 200 mL of 0.5N KOH ethanolic solution for 2 hr at room temperature. After addition of 100 mL of water to the KOH solution, it was extracted twice with 100 mL of n-hexane each time. The hexane was washed twice with 50 mL of water each time, dried over anhydrous sodium sulfate, and concentrated to about 10 mL. The extract was successively cleaned-up on a multi-layer column containing 10% (w/w) silver nitrate on silica gel, 22% (w/w) sulfuric acid silica gel, 44% (w/w) on sulfuric acid on silica gel, and 1.9% (w/w) of potassium hydroxide on silica gel, on a Florisil column, and on an alumina column as described elsewhere (Miyata et al. 1987a). The purified fraction was analysed for PCDDs and PCDFs on a Supelco 2331 capillary column (30 m × 0.25 mm, 0.2-µm film thickness) in an electron impact-single ion monitoring mode by using a Hewlett•Packard 5890J gas chromatograph-JEOL DX-303 mass spectrometer. The quantification followed the procedure reported previously (Miyata et al. 1988b).

The experimental design for the water solubility studies consisted of placing of n-hexane solutions of a mixture preparation containing 33.8 µg of PCDDs and 28.5 µg of PCDFs into each of three 1000-mL and each of three 100-mL separatory funnels. The solvent was allowed to volatilize and disperse under a nitrogen stream at room temperature, and 500 mL of water was added to each of the 1000-mL funnels and 30 mL of 0.1% (w/w) Emulgen•water was added to each of the 100-mL funnels. Each funnel was mechanically shaken for 12 hr at 25°C. After shaking, a 450-mL aliquot of the water or 25-mL aliquot of the Emulgen•water was removed from each container. Each sample was centrifuged at 9400 x g for 30 min in a stainless steel centrifuge tube. A one-third aliquot of each supernatant was pipetted into a separatory funnel. Each solution was extracted twice with an equivalent volume of n-hexane each time. The combined hexane extract was washed twice with a half volume of water. The hexane was dried over anhydrous sodium sulfate and then reduced to a suitable volume. The 2-µL aliquot was injected into GC-MS instrument for the PCDD and PCDF analysis.

RESULTS AND DISCUSSION

Table 1. Amounts of PCDDs and PCDFs dissolved into 500 mL of water or 30 mL of 0.1% Emulgen•water

Compounds		Added(μg)	Found(μg)	
			Water	0.1% Emulgen•water
PCDDs	4Cl	0.53	0.10 (18.9)	0.18 (34.0)
	5Cl	1.45	0.19 (13.1)	0.48 (33.1)
	6Cl	12.00	1.15 (9.6)	3.60 (30.0)
	7Cl	13.50	0.95 (7.0)	3.00 (22.2)
	8Cl	6.30	0.29 (4.6)	1.17 (18.6)
	total	33.78	2.68 (7.9)	8.43 (25.0)
PCDFs	4Cl	0.45	0.08 (17.8)	0.16 (35.6)
	5Cl	1.44	0.20 (13.9)	0.51 (35.4)
	6Cl	12.50	1.60 (12.8)	4.50 (36.0)
	7Cl	12.60	1.10 (8.7)	3.30 (26.2)
	8Cl	1.51	0.08 (5.3)	0.30 (19.9)
	total	28.50	3.06 (10.7)	8.77 (30.8)

Figure in parenthesis shows a precentage of amount dissolved to amount added

It has been demonstrated that the water solubilities of chemicals are profoundly influenced by the presence of particulate matter or dissolved organic substances (Muir et al. 1985). Therefore, in order to reduce these impurities to as low a level as possible, laboratory distilled and deionized water was washed with n-hexane, filtrated through a Millipore filter, and further distilled. However, a trace amount of n-hexane used for the washing was assumed to remain in the purified water. n-Hexane in the water was determined by a headspace sampling method followed by a FID-GC analyses; the residual level was below the detection limit of 10 ppb. In addition, triplicate TOC analysis revealed that the water contained dissolved organic carbon at a level of 0.19 ± 0.01 ppm, equivalent to be only 1.9% of that used in a water solublity study by Muir et al. (1985). Taking dissolved organic compounds partition coefficients of PCDD isomers by Muir et al. (1985) into consideration, it is considered that the purified water does not contain enough amounts of organic substances to have an effect on the water solubilities of PCDD and PCDF components. To date, a water solubility has been determined only for a single component of PCDDs or PCDFs per experiment (Muir et al. 1985, Friesen et al. 1985, Webster et al. 1985, Adams and Blaine 1986, Doucette and Andren 1988). However, PCDDs and PCDFs in sediment and blue mussel from Osaka Bay were composed of their numerous congeners and isomers (Miyata et al. 1988a). Therefore, in this study, the water solubilities were investigated on a mixture of PCDDs and PCDFs purified from a sample of fly ash from a urban municipal incinerator. The PCDDs and PCDFs were composed of hexa- through octachlorinated PCDD congeners and hexa- and heptachlorinated PCDF congeners, respectively, as major constituents (Table 1).

Table 2. Solubility of PCDD and PCDF congeners from incinerator fly ash

Compounds		Solubility (ppb)		Solubility in Emulgen / Solubility in Water
		Water	Emulgen*	
PCDDs	4Cl	0.2	6.0	30
	5Cl	0.38	16	42
	6Cl	2.3	120	52
	7Cl	1.9	100	53
	8Cl	0.58	39	67
	total	5.4	280	52
PCDFs	4Cl	0.16	5.4	34
	5Cl	0.40	17	43
	6Cl	3.2	150	47
	7Cl	2.2	110	50
	8Cl	0.15	10	67
	total	6.1	290	48

*: 0.1% Emulgen•water

The total amounts of PCDDs dissolved in water and 0.1% Emulgen. Emulgen•water were 7.9 and 25.0%, respectively, of that initially added into the flask (Table 1). The total amounts of PCDFs were 10.7 and 30.8%, respectively. The water solubilities of PCDD and PCDF congeners at 25±2°C were within ranges of 0.2~2.3 ppb (ng/mL) and 0.15~3.2 ppb, respectively (Table 2). Muir et al. (1985) and Friesen et al. (1985) determined water solubilities of ^{14}C-labeled tetra- through octaCDD isomers by using a HPLC generator column method. According to their results, the solubilities decreased with an increase of the number of chlorine atoms in the molecules. However, results in this study were distinguishably different from those (Table 2), namely, the relative magnitudes were hexa- > hepta- > octa- > penta- > tetrachlorinated congener in PCDDs and hexa- > hepta- > penta- > tetra- > octachlorinated congener in PCDFs. These discrepancies indicate that the water solubilities of each congener in the PCDD and PCDF mixture preparation are readily affected by other coexistent components. Addition of Emulgen, polyoxyethylene nonylphenyl ether, as dissolved organic matter into water increased the water solubilities of all compounds, resulting in 6.0~120 ppb in PCDD congeners and 5.4~150 ppb in PCDF congeners. The water solubilities of total PCDDs and PCDFs were 52 and 48 times higher, respectively, in comparison with solubilities in pure water. The increased solubility was more pronounced with the increasing number of chlorine atoms in the molecules in both chemicals. These phenomena agree well with the data by Muir et al. (1985) which demonstrated that higher chlorinated PCDD isomers were easily adsorbed on the dissolved organic matters.

As shown in Table 3, compared to the original compounds (fly ash),

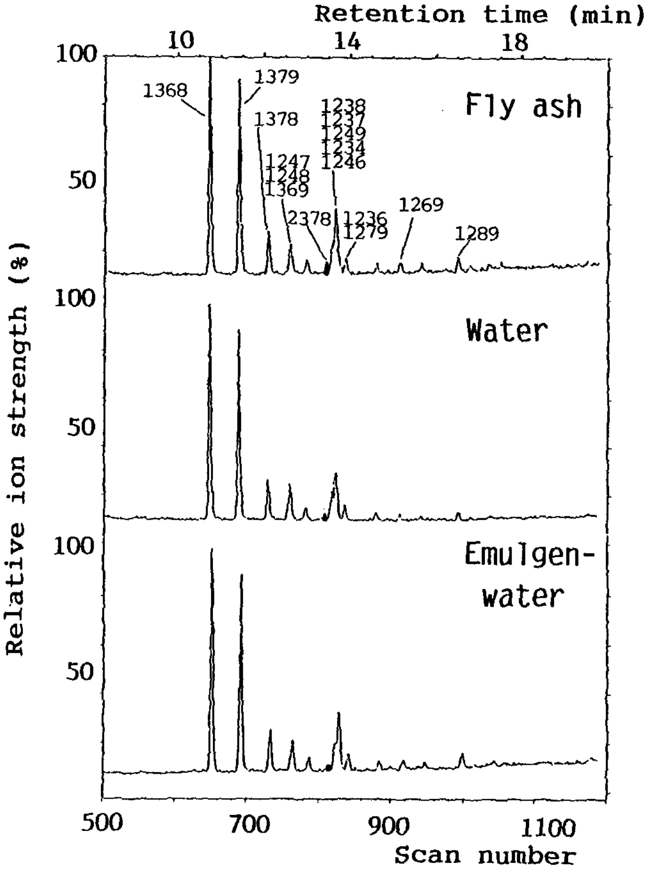

Figure 1. GC-MS SIM chromatograms at m/z 322 of tetraCDDs in various samples on a Supelco SP-2331 capillary column

PCDDs dissolved in the water included relatively larger amounts of tetra- through hexachlorinated congeners and smaller amounts of hepta- and octachlorinated ones. The relative increase was tetra- > penta- > hexa-. Similar results were also seen in case of PCDFs. On the other hand, when Emulgen was added, the congener ratios of PCDDs and PCDFs dissolved in the medium showed an increasing tendencies with an increase of the number of the substituted chlorines. This result is attributed to the higher chlorinated congeners interacting more strongly with the dissolved organic matter as described above.

As shown in Figure 1, the isomer profile of tetraCDD dissolved in the water resembles closely the profile for the fly ash extract. In addition, a similar pattern was also observed in case of 1% Emulgen in water. Similar results were also obtained from other PCDD and PCDF congeners. These indicated that each component in the compound mixture dissolves roughly in proportion to the

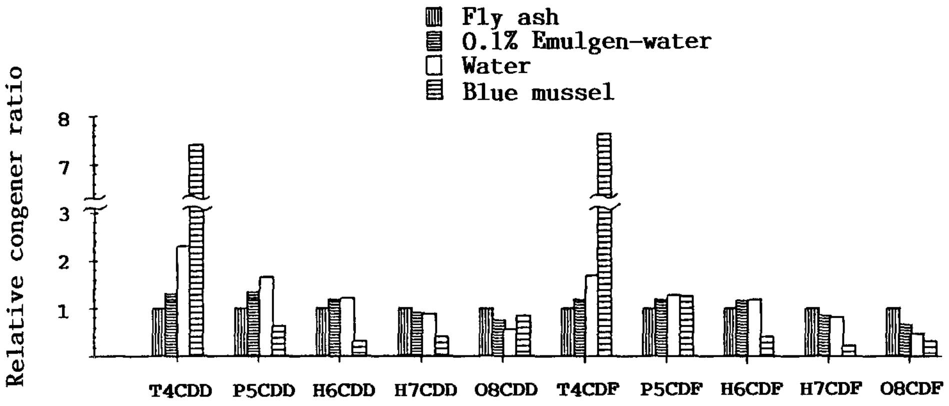

Figure 2. Relative congener ratios of PCDD and PCDF in various samples versus fly ash (=1.0)

Table 3. Congener ratio (%) of PCDDs and PCDFs

Compounds		Congener ratio (%)		
		in fly ash	in water	in Emulgen*
PCDDs	4Cl	1.6	3.7 (2.3)	2.1 (1.3)
	5Cl	4.3	7.1 (1.7)	5.8 (1.3)
	6Cl	35.5	43.0 (1.2)	42.1 (1.2)
	7Cl	40.0	35.5 (0.9)	36.0 (0.9)
	8Cl	18.7	10.7 (0.6)	13.9 (0.7)
PCDFs	4Cl	1.6	2.7 (1.7)	1.9 (1.2)
	5Cl	5.1	6.6 (1.3)	6.0 (1.2)
	6Cl	43.8	52.3 (1.2)	50.9 (1.6)
	7Cl	44.2	35.9 (0.8)	37.6 (0.9)
	8Cl	5.3	2.5 (0.5)	3.5 (0.7)

* : 0.1% Emulgen•water

Figure in parenthesis shows a ratio of each congener in water or in Emulgen•water to the corresponding one in fly ash

original amount in the medium.

Table 4 shows changes of relative component ratios of representative isomers in each PCDD and PCDF congeners. In case of PCDDs, the highly toxic 2,3,7,8-chlorine substituted isomer had less water solubilities than did other ones in each congener. This result was not true in the case of PCDFs.

Figure 2 shows relative congener ratios of PCDDs and PCDFs dissolved in the two aqueous media to the corresponding fly ash. The values of tetraCDD and tetraCDF in the water were elevated to

Table 4. Ratios of specific PCDD and PCDF isomer ratios in water or Emulgen•water to fly ash (=1.00)

PCDD isomer	Ratio		PCDF isomer	Ratio	
	Water	Emulgen*		Water	Emulgen*
1368-4Cl	1.09	1.05	2378-4Cl	1.14	0.97
1379-4Cl	1.11	1.06	3467-4Cl	1.14	0.97
2378-4Cl	0.83	0.67			
12379-5Cl	1.00	1.04	23478-5Cl	1.06	0.90
12378-5Cl	1.00	1.11	23467-5Cl	1.02	0.90
123789-6Cl	0.86	0.92	123467-6Cl	1.03	0.85
123467-6Cl	0.98	1.00	234678-6Cl	1.01	0.87
1234679-7Cl	1.05	1.05	1234678-7Cl	1.00	1.00
1234678-7Cl	0.95	0.95	1234679-7Cl	1.01	1.03

* : 0.1% Emulgen•water

2.3 and 1.7 times, respectively, of the fly ash. However, they diminished under condition that the organic substances was dissolved at a content of 1% (w/w) in water. On the other hand, samples of blue mussel collected at Hokko in Osaka Bay in 1986 contained tetraCDDs and tetraCDFs as major congeners, showing remarkably higher relative congener ratios of 7.5 and 7.6 times, respectively, of the corresponding one of the fly ash (Figure 2). Taking a fact, which actual sea water contains significant amounts of particulate matter and dissolved organic matters, it appears that the difference in the solubilities of PCDD and PCDF congeners does not have a great effect on producing the specific congener profiles of PCDDs and PCDFs in blue mussel.

Hereafter, we will investigate the specific patterns of PCDDs and PCDFs through uptake and excretion experiments of both chemicals by using blue mussel in laboratory water tanks.

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REFERENCES

Adams WJ, Blaine KM (1986) A water solubility determination of 2,3,7,8-TCDD. Chemosphere 15 : 1397-1400

Doucette WJ, Andren AW (1988) Aqueous solubility of selected biphenyl, furan and dioxin congeners. Chemosphere 17 : 243-252

Friesen KJ, Sarna LP, Webster GR (1985) Aqueous solubility of Polychlorinated dibenzo-p-dioxins determined by high pressure liquid chromatography. Chemosphere 14 : 1267-1274

Miyata H, Takayama K, Ogaki J, Kashimoto T (1987a) Monitoring of PCDDs in Osaka Bay by using blue mussel. Chemosphere 16 : 1817-1822

Miyata H, Takayama K, Ogaki J, Kashimoto T, Fukushima S (1987b) Polychlorinated dibenzo-p-dioxins in blue mussel from marine coastal water in Japan. Bull Environ Contam Toxicol 39 : 877-883

Miyata H, Takayama K, Ogaki J, Mimura M, Kashimoto T (1988a) Study on polychlorinated dibenzo-p-dioxins and dibenzofurans in rivers and estuaries in Osaka Bay in Japan. Toxicol Environ Chem 17 : 91-101

Miyata H, Takayama K, Ogaki J, Kashimoto T (1988b) Formation of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in typical urban incinerators in Japan. Toxicol Environ Chem 16 : 203-218

Muir DCG, Marshall WK, Webster GRB (1985) Bioconcentration of PCDDs by fish : Effects of molecular structure and water chemistry Chemosphere 14 : 829-833

Webster GRB, Friesen KJ, Sarna LP, Muir DCG (1985) Environmental fate modeling of chlorodioxins : Determination of physical constants. Chemosphere 14 : 609-622

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