ACCUMULATION LEVELS OF 2,3,7,8-SUBSTITUTED DIOXINS IN THE AMPHIBIANS FROM THE BASINS OF MAJOR RIVERS IN S. KOREA

Gi Ho Jeong[2], Dong-Ho Moon[1], Chang-Han Joo[1], and Jae-Cheon You[1]

[1]Analytical Research Center, Environmental Management Co, Boondang-gu Hasanwoon-dong San 26-4, Sungnam City, Kyonggi Do, 463-430, S.Korea.
[2]Department of Chemistry, Pusan National University. Kumjeong-gu Jangjeon-dong San-30. Busan 609-735, S. Korea

Introduction

S. Korea has four major river systems such as the Han, Nakdong, Kum, and Youngsan River. Almost all wastewater has been discharged through these rivers. As a part of the grand national survey of the accumulation levels of the endocrine disrupters, we determined 2,3,7,8-substituted PCDDs and PCDFs in the muscular tissues of amphibians from 31 sampling sites along the basins of the major rivers and two well-known wetlands. Leopard frog (*Rana pipiens*) and bullfrog (*Rana catesbeiana*) were caught as the representative amphibians. We determined the TEQs of PCDDs and PCDFs contained in these frogs. This is the first systematic and widespread investigation to determining the dioxin levels in the frogs. The highest TEQ value of leopard frog, 0.636 pg-TEQ/g, is around ten times higher than that of bullfrog, 0.069 pg-TEQ/g.

Methods and Materials

Sampling fishes

We caught leopard frogs from 30 sites and bullfrogs from 26 sites located along the several rivers and two wet lands. We failed to collect them from the other sites. The locations are illustrated in another issue[1] of Organohalogen Compounds and site names are listed in Table 1. Sampling period was from August 1999 to June 2000. Only the muscle was separated and stored at below -20 °C before analysis.

Homogenization

Samples were homogenized for about 30 minutes at 10,000-18,000 rpm by using a homogenizer (SMT). The quantity of muscle sample before treatment was around 50 grams.

Lipid determination

Lipid levels were determined by the Soxhlet extraction[2] with reference to the U.S. EPA method 1613. Lipid was extracted by using dichloro methane for more than 20 hr.

Extraction

A total of 17 congeners 2,3,7,8-substituted ^{13}C-PCDD/Fs (500 pg/sample, CIL EDF 8999) were used as internal dioxin standards, and added to the samples before extraction. For the determination of PCDD/Fs, 200 mL of 2-N KOH-Ethanol solution was put into a separatory funnel containing specific amount of a sample and the internal standard. The funnel was shaken until decomposition was completed for about 2 h. The 150 mL of 2 % NaCl solution was added to the sample solution and then it was liquid-liquid extracted three times with 100 mL of n-hexane.

Purification and Analysis

The raw extract was treated with sulfuric acid and then purified over a multi layer silica gel column, an activated alumina column, and finally cleaned up with an active carbon impregnated silica gel column. The purified sample was then concentrated to give a final sample for analysis. The analyses were performed with a fused silica capillary column (SP2331, 60 m × 0.32 mm × 0.2 μm, Supelco) and a double focusing type mass spectrometry (Autospec Ultima, UK, resolution over 10,000) by applying the selected ion monitoring (SIM) method. The TEQs were obtained by multiplying the concentration and TEFs established by NATO/CCMS Working group.

Results and Discussion

The lipid level was determined only for bullfrogs and its average value was 4.2 %. The average recovery rates obtained from the internal standards ranged from 77.1 % to 96.7 %. The 2,3,7,8-TCDD substituted TEQs values are listed in Table 1 and also shown in Fig. 1. The lower limit of quantification was 0.10 pg/g for tetra- and penta-, 0.20 pg/g for hexa- and hepta-, and 0.50 pg/g for octa-chlorinated dioxins. The total TEQs ranged from 0.063 to 0.636 pg-TEQ/g (wet weight) for leopard frogs and from ND to 0.069 pg-TEQ/g for bullfrogs. The average TEQs were 0.191±0.121 and 0.003±0.014 pg-TEQ/g for leopard frogs and bullfrogs respectively. The median level of leopard frog was 0.167 pg-TEQ/g and ND for bullfrogs. Even though the body size of leopard frog is much smaller than that of bullfrog, the accumulated dioxin levels were much higher in leopard frogs. We observed dioxins from leopard frogs above detection limit at all 30 sampling sites, but only two sites from bullfrogs. In comparison with the dioxin levels in fresh water fishes[1] from the same sites, 0.46±0.94 and 0.12±0.27 pg-TEQ/g for crucians and minnows respectively, the levels in amphibians were more evenly distributed. This result also indicates that the dioxin levels in amphibians are comparable to those in fresh water fishes. In leopard frogs collected from Wisconsin U.S.A.,[3] one PCDD and two PCDFs were observed at total TEQ values of 3.221 pg-TEQ/g. This level is much higher than the highest TEQ values observed in this study. We need more data for amphibians to compare with the other species. The levels of PCDFs are much higher than that of PCDDs. The percent ratios of PCDFs observed from both amphibians are higher than 70 %, i.e. 71.4 % and 100 % for leopard frogs and bullfrogs, respectively. Among 17 congeners of 2,3,7,8-substituted dioxins, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD occupied most of the TEQs. As shown in Table 2, 2,3,4,7,8-PeCDF occupied 86.1% and 95.8% of total TEQs of 2,3,7,8-substituted PCDFs of leopard frogs and bullfrogs, respectively. The 1,2,3,7,8-PeCDD occupied 76.8% of total TEQs of 2,3,7,8-substituted PCDDs of leopard frogs. These two congeners occupy 83.4% and 95.8% of total TEQs of total 2,3,7,8-substituted dioxins of leopard frogs and bullfrogs, respectively. In fresh water fish case[1], e.g. for crucian (*Carassius auratus*), we observed very similar trend; these two congeners occupied 87.2 %, 65.3 %, and 82.7 % of total TEQs of 2,3,7,8-substituted PCDFs, PCDDs, and dioxins respectively.

Table 1. The 2,3,7,8-TCDD substituted TEQ levels obtained from leopard frog and bullfrog. pg-TEQ/g (wet weight)

Site No. and Name			Leopard frog (PCDFs+PCDDs)	Bullfrog (PCDFs+PCDDs)
Han River	1	Uiam-Dam	0.063 (0.059+0.004)	-
	2	Seom-River	0.258 (0.163+0.094)	-
	3	Bokha-Stream	0.113 (0.108+0.005)	ND
	4	Kyungan-stream	0.120 (0.120+0.000)	ND

	5	Paldang-Dam	0.636 (0.359+0.277)	ND
	6	Anyang-Stream	-	ND
Nakdong	7	Koomee	0.182 (0.115+0.067)	ND
River	8	Koryoung	0.188 (0.133+0.055)	ND
	9	Kumho-River	0.185 (0.130+0.055)	ND
	10	Nam-River	0.215 (0.156+0.059)	ND
	11	Namji	0.144 (0.094+0.051)	0.003 (0.003+0)
	12	Moolgum	0.115 (0.112+0.003)	ND
	13	Nakdong-Estuary	0.087 (0.087+0.001)	ND
Kum	14	Dachung-Dam	0.330 (0.198+0.132)	ND
River	15	Moosim-Stream	0.169 (0.114+0.156)	ND
	16	Kongjoo	0.200 (0.147+0.053)	ND
	17	Booyeo	0.215 (0.162+0.053)	ND
Youngsan	18	Damyang-Dam	0.165 (0.103+0.062)	ND
River	19	Kwangjoo-Stream	0.148 (0.145+0.003)	ND
	20	Najoo	0.154 (0.096+0.058)	ND
	21	Mooan	0.096 (0.090+0.007)	0.069 (0.069+0)
Other	22	Kosan	0.450 (0.314+0.136)	ND
Small	23	Oncheon-Stream	0.089 (0.089+0.001)	ND
Scaled	24	Myungchon	0.142 (0.139+0.003)	ND
Rivers	25	Yangyang	0.063 (0.061+0.003)	-
	26	Hadong	0.192 (0.102+0.091)	ND
	27	Samcheok	0.108 (0.094+0.013)	-
	28	Kangnung	0.106 (0.093+0.013)	-
	29	Hwangku-Tributary	0.372 (0.229+0.144)	ND
Wet lands	30	Woopo	0.216 (0.148+0.068)	ND
	31	Joonam	0.220 (0.145+0.075)	ND
		The average TEQs	0.191±0.121	0.003±0.014

Blank(-) : Sites where amphians were not caught. ND: none detected

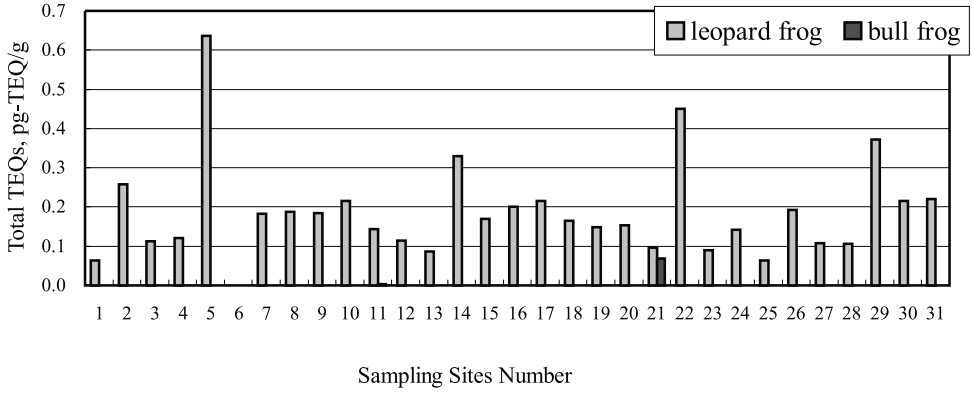

Figure 1. The 2,3,7,8-TCDD substituted TEQs obtained from the muscular tissue of leopard frog and bullfrog. pg-TEQ/g (wet weight)

Table 2. Total TEQs of three major congeners observed from the amphibians

	Leopard frog (pg-TEQ/g)	Bullfrog (pg-TEQ/g)
2,3,4,7,8-PeCDF	3.53	0.069
2,3,7,8-TCDD	0.15	0
1,2,3,7,8-PeCDD	1.26	0
2,3,7,8-substituted PCDFs total	4.10	0.072
2,3,7,8-substituted PCDDs total	1.64	0
(PCDFs + PCDDs) total	5.74	0.072

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