LEVELS OF PCDDS, PCDFS AND NON-ORTHO CHLORINE SUBSTITUTED COPLANAR PCBS IN FISH AND CRAB FROM CULTURE PONDS AND A COASTAL AREA NEAR INCINERATION SITES FOR METAL RECLAMATION IN WAN-LI, TAIWAN, REPUBLIC OF CHINA

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1. INTRODUCTION

We previously reported that four surface soil samples from six incineration sites of waste electric wire and/or magnetic card for metal reclamation in Wan-Li, southern Taiwan, Republic of China, were heavily polluted by PCBs, polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)[1]. Four sediment samples from nine culture ponds for freshwater fish in this Wan-Li area were also found to be remarkably exposed to PCDDs, PCDFs and non-ortho chlorine substituted coplanar PCBs (Co-PCBs), showing their levels to be similar to those of surface soil specimen from waste incineration sites for metal reclamation[2,3].

In addition, some of sediment samples from six river sides and one coastal area near the waste incineration sites were observed to have significantly high levels of PCDDs, PCDFs and Co-PCBs. The detail result will be presented at poster session of this DIOXIN '94 International Symposium[4]. These results suggest a possibility that organisms in Wan-Li area might be polluted by highly toxic PCDDs, PCDFs and Co-PCBs. Therefore, in this study, freshwater fish from culture ponds and crab from a costal area near waste combustion sites in Wan-Li were analyzed for PCDDs, PCDFs and Co-PCBs in order to survey the pollution by them.

2. EXPERIMENTAL

Sampling

Samples of freshwater fish (chanos) were obtained from culture ponds and a market in Wan-Li, and from a market in Tainan City, southern Taiwan in February, July and October, 1993. Crabs were caught at a freshwater culture pond and at a coastal area near the mouth

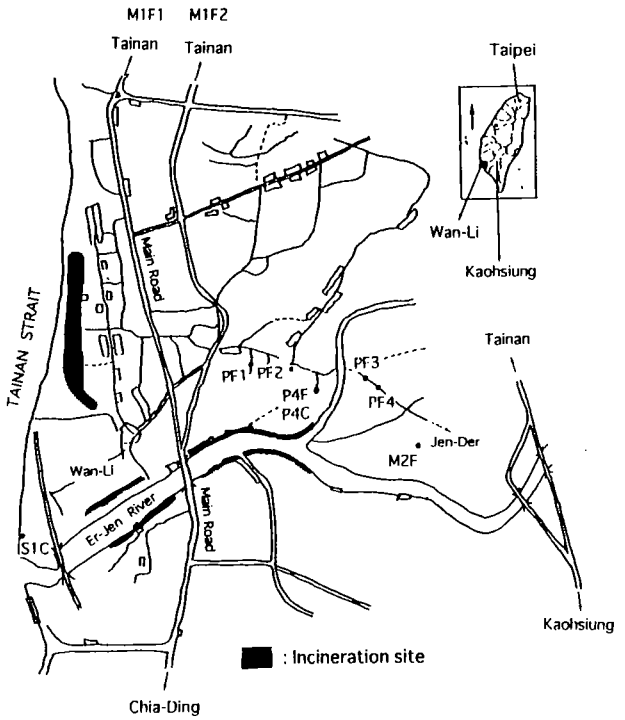

Fig. 1. Sampling sites of freshwater fish and crab

Table 1. The details on samples of fish and crab used in this study

Sample	Sample No.	Sampling time	Sampling location	No. of sample	Average length (cm)	Average weight(g)
Crab	S1C	Feb., 1993	Coastal	13	11.8	15.7
Crab	P4C	Feb., 1993	Pond	37	9.9	11.5
Fish	P4F	Feb., 1993	Pond	11	16.5	44.7
Fish	PF2	Jul., 1993	Pond	1	40.0	710
Fish	PF1	Oct., 1993	Pond	3	29.0	243
Fish	PF3	Oct., 1993	Pond	1	37.0	505
Fish	PF4	Oct., 1993	Pond	3	33.3	330
Fish	M1F1	Jul., 1993	Market[1]	1	31.0	366
Fish	M1F2	Oct., 1993	Market[1]	1	40.0	671
Fish	M2F	Oct., 1993	Market[2]	1	37.0	506

Market[1]: in Tainan City Market[2]: in Wan-Li

of Er-Jen River in Wan-Li in February, 1993. The sampling sites are shown in Fig. 1 and the details of samples were described in Table 1.

Extraction

After spiking of internal standards (five $^{13}C_{12}$-PCDDs and five $^{13}C_{12}$-PCDFs, each 250 pg; three $^{13}C_{12}$-Co-PCBs, each 1250 pg), homogenized sample (ca. 50 g) was saponified in 300 ml of 0.5N ethanolic KOH solution for 2 hrs. in room temperature. The saponified sample was extracted twice with 150 ml of n-hexane and precleaned on a multi-layer column containing Na_2SO_4-silica (12 g), 10% (w/w) $AgNO_3$-silica (8 g), silica (2.4 g), 22% (w/w) H_2SO_4-silica (12 g), 44% (w/w) H_2SO_4-silica (12 g), silica (2.4 g) and 2% (w/w) KOH-silica (8 g) with an eluent of n-hexane (360 ml). The eluate was concentrated to 5 ml and chromatographed into three fractions with successive eluents of 90 ml of n-hexane, 70 ml of 1% methylene chloride in n-hexane and 160 ml of 50% methylene chloride in n-hexane on an alumina column (10 g, Merck neutral, activate I). The third eluate containing PCDDs, PCDFs and Co-PCBs was concentrated to 3 ml. After addition of keeper solvent (n-decane, 30 μl), the concentrated eluate was left for complete evaporation of n-hexane in room temperature and then adjusted to a volume of 30 μl with n-decane.

Above finally purified extract was analyzed on J&W DB-5 (30 m x 0.32 mm, 0.20 μm)(held for 1 min at 120℃ and programmed to 180 at 20℃ /min, to 260℃ at 4℃ /min and to 310℃ at 20℃ /min) for Co-PCBs, (held for 1 min at 140℃, programmed to 220℃ at 20℃ /min and 310℃ at 8℃ /min, and held for 4 min) for hepta- and octachlorinataed PCDDs and PCDFs, and on Supelco 2331 (60 m x 0.32 mm, 0.20 μm)(programmed from 150 to 180℃ at 20℃/min and to 250℃ at 3℃/min, and held for 29 min) for tetra- through hexachlorinated PCDDs and PCDFs in an electron impact-single ion monitoring mode at a resolution of 7000 using a Hewlett Packard 5890J gas chromatograph-JEOL SX-102 mass spectrometer. The results were corrected for the recovery of $^{13}C_{12}$-labeled internal standards.

3. RESULTS AND DISCUSSION

Wastes of wire, magnetic card and electric instrument part, etc. have been incinerated at the areas around northwest part of Main road and around both river sides of the Er-Jen River in Fig. 1.

As shown in Table 1, samples of crab (P4C) and fish (P4F) were obtained from a culture pond near waste incineration site in Wan-Li in Feb., 1993. In addition, the pond sediment was also sampled at the same time.

In general, the hepatopancreas of crustacea was reported to be an excellent indicator for monitoring PCDDs and PCDFs at a local contamination area, because the organ gave remarkably higher levels of the contaminants than did the muscle, but also showed resemble congener patterns to those in water and sediment at the biota's living area[5-7]. On the other hand, a limited number of selected congeners with chlorine atoms at 2, 3, 7 and 8 positions in their molecules was found in the muscle tissue of crustacea and fish[5]. Therefore, we analyzed the hepatopancreas of crab and the meat of fish for PCDDs, PCDFs and Co-PCBs.

The hepatopancreas of crab showed 19 times higher concentrations of PCDDs (66.5 pg/g)

Table 2. Concentrations of PCDDs, PCDFs and Co-PCBs in samples of crab and freshwater fish from fish culture ponds and a coastal area near waste incineration sites for metal reclamation in Wan-Li, and in samples of freshwater fish from a market in Wan-Li and from a market in Tainan City

Compound	Crab		Culture pond Fish							Market Fish		
	S1C	P4C	P4F	PF2	PF1	PF1	PF1	PF3	PF4	M1F1	M1F2	M2F
	Hepatop.	Hepatop.	Meat	Meat	Meat	Gut	Liver	Meat	Meat	Meat	Meat	Meat
2,3,7,8-TCDD	N.D.	N.D.	1.04	N.D.	0.01	0.08	0.04	0.02	0.01	0.02	0.02	0.01
1,2,3,7,8-PeCDD	29.3	34.4	0.26	N.D.	0.05	0.37	0.07	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,3,4,7,8-HxCDD	5.13	N.D.	0.05	N.D.	N.D.	0.04	N.D.	N.D.	N.D.	0.78	N.D.	N.D.
1,2,3,6,7,8-HxCDD	N.D.	20.8	0.01	0.08	N.D.	0.12	N.D.	0.06	N.D.	N.D.	0.04	N.D.
1,2,3,7,8,9-HxCDD	N.D.	10.8	0.12	0.24	N.D.	N.D.	0.24	N.D.	0.02	N.D.	N.D.	N.D.
1,2,3,4,6,7,8-HpCDD	52.0	0.19	0.17	0.62	0.09	0.43	2.00	0.34	0.46	0.21	0.54	0.36
OCDD	609	0.28	1.85	0.85	1.66	2.78	19.3	2.22	6.15	0.29	13.2	4.30
Total PCDDs	696	66.5	3.50	1.79	1.81	3.82	21.6	2.64	6.64	1.30	13.8	4.67
TEQ of total PCDDs	16.3	20.4	1.19	0.039	0.038	0.288	0.138	0.032	0.023	0.100	0.043	0.018
2,3,7,8-TCDF	449	456	9.84	0.14	1.26	0.06	6.09	0.28	0.02	0.03	N.D.	N.D.
1,2,3,7,8-PeCDF	40.3	54.0	0.85	0.01	0.02	0.65	0.28	0.01	0.02	0.09	N.D.	0.01
2,3,4,7,8-PeCDF	157	133	3.59	N.D.	0.12	5.63	1.08	0.02	N.D.	N.D.	N.D.	N.D.
1,2,3,4,7,8-HxCDF	38.5	29.0	0.04	0.01	N.D.	N.D.	0.15	0.03	0.01	0.04	N.D.	0.01
1,2,3,6,7,8-HxCDF	N.D.	19.4	N.D.	N.D.	0.02	0.11	0.14	0.02	0.01	N.D.	N.D.	N.D.
1,2,3,7,8,9-HxCDF	N.D.	N.D.	N.D.	0.11	0.06	N.D.	0.39	N.D.	N.D.	N.D.	N.D.	0.05
2,3,4,6,7,8-HxCDF	13.7	N.D.	0.13	N.D.	0 03	0.16	0.65	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,3,4,6,7,8-HpCDF	14.7	0.30	0.05	N.D.	0.01	0.11	0.64	0.03	0.05	N.D.	0.26	0.05
1,2,3,6,7,8,9-HpCDF	0.19	N.D.	0.03	N.D.	0.01	0.11	0.64	0.04	0.12	1.42	0.26	N.D.
OCDF	3.93	0.03	0.02	0.12	0.05	0.27	1.37	0.03	N.D.	0.18	0.04	0.06
Total PCDFs	718	691	14.6	0.39	1.58	8.10	11.4	0.46	0.23	1.76	0.56	0.18
TEQ of total PCDFs	131	119	2.84	0.027	0.198	2.93	1.31	0.044	0.007	0.026	0.005	0.007
3,3',4,4'-TCB	183000	9680	10100	354	2470	4730	7735	184	52.2	55.0	12.6	8.06
3,3',4,4',5-PeCB	6840	2720	459	27.2	240	2120	1200	25.5	5.68	3.31	1.69	0.87
3,3',4,4',5,5'-HxCB	669	335	23.4	0.28	9.56	109	48.3	2.62	0.19	0.39	0.05	0.04
Total Co-PCBs	190000	12700	10600	381	2720	6960	8990	213	58.1	58.7	14.4	8.79
TEQ of total Co-PCBs	2550	386	149	6.28	49.2	265	201	4.52	1.10	0.900	0.298	0.170
Total TEQ	2700	525	153	6.35	49.4	268	202	4.60	1.13	1.03	0.346	0.195

than did the meat of fish (3.5 pg/g). The ratio (243) of PCDFs in crab (691 pg/g) versus fish (2.84 pg/g) became more greater. A similar observation was reported in the case of blue crab and striped bass from Newark Bay, New Jersey, USA[5]. However, the both organisms contained similar levels of Co-PCBs. These results indicate that the uptake and accumulation of Co-PCBs are different from those of PCDDs and PCDFs. Furthermore, in the both biotas, the levels of Co-PCBs notably higher in comparison with those of PCDDs and PCDFs (Table 2).

The level (3.5 pg/g) of PCDDs in the culture pond fish P4F was a slightly higher than those (0.04 to 1.50 pg/g)[8] of Japanese marketing fish, and was same to those (1.6 to 4.3 pg/g) [8] of Japanese coastal fish, whereas the level (14.6 pg/g) of PCDF was lifted than those (0.01 to 7.6 pg/g)[8] of marketing and coastal fishes in Japan. Regarding Co-PCBs, the fish P4F contained larger amount (10600 pg/g) than did coastal fishes (0.54 to 1500 pg/g)[8]. The pond sediment was contaminated with PCDDs of 663 pg/g dry weight, PCDFs of 1030 pg/g dry weight and Co-PCBs of 1860 pg/g dry weight. The ratios of PCDDs, PCDFs and Co-PCBs in crab and fish versus sediment were respectively 0.10 and 0.67, 6.8 and 0.0052, and 0.014 and 5.7. Thus discrepancy is attributable to a difference in physical properties such as water solubility and octanol/water partitioning coefficiency etc. among the three chemicals.

The concentrations of PCDDs, PCDFs and Co-PCBs in hepatopancreas of crab (S1C) from a coastal area were 696, 131 and 190000 pg/g fresh weight, showing the levels of PCDDs and Co-PCBs to be equivalent to 10.5 and 15 times greater of those of crab P4C from a culture pond. A sample of sediment from the coastal site, however, included 30 times lower levels (63 pg/g) of Co-PCBs than did one from the culture pond (1860 pg/g). The discrepancy might be interpreted as follows. The coastal crab burrows in sediment hole, whereas the pond crab lives in the water, and the burrow living habit may result in significant exposure through direct contact with the contaminated sediment. A similar observation was also reported in soil-borne insects, beachmice and meadowlarks[9].

In a case of culture pond fish PF1, the meat, gut and liver were analyzed for chlorinated contaminants in order to investigate their transfer to the fish body, because in general, environmental contaminants enter through the route of gut → liver → meat. As shown in Table 2, a magnitude in the exposure level was arranged in order of gut, liver and meat in PCDDs, liver, gut and meat in PCDFs, and liver, gut and meat in Co-PCBs. The ratios of liver to gut and of meat to liver were 5.7 and 0.088 in PCDDs, 1.4 and 0.14 in PCDFs, and 1.3 and 0.3 in Co-PCBs, respectively. This indicates that PCDDs can more easily transfer to liver through gut than do PCDFs and Co-PCBs ,and that PCDFs and Co-PCBs can more easily transfer to meat through liver than do PCDDs. In addition, a magnitude of the translation into meat via liver was arranged in order of Co-PCBs, PCDDs and PCDFs. Consequently, the uptake rate (meat/gut) into meat through gut was put PCDDs (0.47), Co-PCBs (0.39) and PCDFs (0.20) in the magnitude order.

The concentrations of PCDDs, PCDFs and Co-PCBs in meat samples of marketing fish from Wan-li (M2F) and Tainan City (M1F1 and M1F2) were in the ranges of 1.30 to 13.8, 0.18 to 1.76 and 8.79 to 58.7 pg/g, respectively. Samples of fish from culture ponds in Wan-Li were roughly found to contain at the same level of PCDDs (1.79 to 6.64 pg/g) to that of the marketing fish. In a case of PCDFs, a similar observation was seen in four pond samples

except P4F (14.6 pg/g). Regarding Co-PCBs, however, remarkably higher contamination (58.1 to 10600 pg/g) was confirmed in the pond samples. Especially, the levels of samples P4F and PF1 were 10600 and 2720 pg/g, respectively.
Compared to the marketing fish (0.195 to 1.03 pgTEQ/g), the pond fish showed higher total TEQ concentration (1.13 to 153 pgTEQ/g) of PCDDs, PCDFs and Co-PCBs . The levels (153 and 49.4 pgTEQ/g) of samples P4F and PF1 were respectively 15 and 4.8 times higher than the average (10.27 pgTEQ/g) of Japanese coastal fish samples[8], and 278 and 90 times higher than one (0.55 pgTEQ/g) of Japanese marketing fish samples[8]. Furthermore, it was cleared that 97.3 to 99.5% of the total TEQ was attributed to Co-PCBs.
From above results, it was revealed that fish samples were heavily polluted by Co-PCBs.
Now, we are going to examine another fish samples from culture ponds in Wan-Li, and will present the data in this symposium.

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