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Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in different tissues of the cormorants (*Phalacrocorax carbo*) from Dongting Lake, China

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Abstract

Tissue distribution provides important information regarding the pharmacokinetic behavior of pollutants and is invaluable when analyzing the risk posed to avian species by the exposure to such kind of pollutants. In this study, concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were determined in muscle, liver, spleen, kidney, stomach, gall bladder, skin, heart, pancreas, intestine and lung tissue extracts of cormorants collected from Dongting Lake, China. Tissue distribution results showed preferential accumulation of PCDD/Fs in both liver and skin. The total concentration of PCDD/Fs ranged from 421 to 5696 pg/g lipid weight. Octachlorinated dibenzo-*p*-dioxin (OCDD) was the predominant congener in all tissues and contributed between 31% and 82% to all 17 PCDD/Fs in different tissues. The liver/muscle ratios progressively increased with the degree of chlorination of PCDDs, except for OCDD. The relative toxic potential of PCDDs and PCDFs in tissues were calculated using the World Health Organization (WHO) Toxic Equivalency Factors (TEFs) for birds. The concentrations of WHO-toxic equivalent in different tissues ranged between 14.8 and 2021 pg/g lipid weight. These results indicated PCDD/Fs may have been bio-accumulated in cormorant via food-web. Furthermore, when compared with studies reported in the literatures, the PCDD/Fs levels in the cormorant collected from Dongting Lake were still relatively high.

Key words: polychlorinated dibenzo-*p*-dioxins; dibenzofurans; cormorant; Dongting Lake; distribution; tissue

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Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzo-furans (PCDD/Fs) are persistent organic pollutants, displaying high toxicity and bioaccumulation potential. They tend to biomagnify into the higher trophic levels of the aquatic food web (US EPA, 2000). PCDD/Fs are mainly byproducts of industrial processes including smelting, chlorine bleaching of paper pulp and the manufacturing of some herbicides and pesticides. Although the formation of dioxins is local, environmental distribution is global. PCDD/Fs are found throughout the world. The highest levels of these compounds are found in some soils, sediments and food, especially dairy products, meat, fish and shellfish. Very low levels are found in plants, water and air.

Dongting Lake is located in the southern of China. It is the second-largest freshwater lake in China, with a surface area of 2691 km^2, and it plays an important role in regulating the volume of water in the Yangtze River. The parasitic disease Asian schistosomiasis has been endemic in the Dongting Lake region for centuries and it has a devastating effect on the public health of the local people (Li et al., 2000). Large amounts of the pesticide sodium pentachlorophenol (Na-PCP) have been sprayed over the lake in an attempt to control schistosomiasis. PCDD/Fs are found as impurities in commercial Na-PCP products (Bao et al., 1995). Because of the adverse effect of Na-PCP, its use has been prohibited in this region since 1996. However, PCDD/Fs are chemically inert and could remain in the environment for many years. Studies conducted during the 1990s in Dongting Lake region found high concentrations of PCDD/Fs in sediments, which raised the question of whether the bioaccumulation of PCDD/Fs could pose a potential ecological risk for the Dongting Lake aquatic food web.

The cormorant (*Phalacrocorax carbo*) is a major predator in the freshwater food chain, and it was therefore expected that they would be highly contaminated by PCDD/Fs (Williams et al., 1995; Kannan et al., 2001).

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The pharmacokinetic behavior of these pollutants can be assessed by analyzing their distribution in the tissues of wild avian species. The knowledge on tissue distribution of PCDD/Fs can help to evaluate the exposure risk of PCDD/Fs pose to the species. Reports of PCDD/Fs concentrations in cormorant tissues, such as liver, muscle, fat and kidney are sparse. In this study, cormorants were collected from Dongting Lake. Analysis of several tissues extracts from the cormorants would provide information on the partitioning of PCDD/Fs among body tissues, including the physiologically important organs, such as the liver and kidneys. This information can be used to calculate concentration quotients, which are useful in risk assessments. Toxic Equivalents (TEQs) were calculated using the World Health Organization (WHO) toxic equivalency factors (TEFs) (Van den Berg et al., 1998) for birds, and compared with thresholds for toxicity reported in the literature.

1 Experimental

1.1 Sample collection

The cormorants collected from Dongting Lake during the spring of 2004. The collection of cormorants is strictly restricted by Nature Reserve Management Office at Dongting Lake. The skin of the defeathered birds was washed with both an ordinary detergent and acetone. Then the cormorants were immediately dissected. Tissue samples weighed, transferred to glass bottles and sealed and stored frozen at –20°C prior to analysis.

1.2 Lipid determination and PCDD/Fs analysis

Samples were thawed at room temperature and then homogenized for PCDD/Fs analysis. After homogenization, the tissues were freeze-dried, ground and mixed with anhydrous sodium sulfate (Na_2SO_4). The PCDD/Fs analysis was performed using the isotope dilution technique based on US EPA Method 1613B. Before extraction, samples were spiked with a mixture containing 15 $^{13}C_{12}$ labeled 2,3,7,8-substituted PCDD/Fs internal standards (Wellington Laboratories, Canada). Extraction was carried out by Soxhlet extraction using 250 mL *n*-hexane/dichloromethane (1:1) for 24 hr. The extracts were reduced by rotary evaporation, and then dried with a stream of nitrogen. Lipid contents were calculated with residues of the extracts. The lipids were dissolved with *n*-hexane and subjected to sulfuric acid wash. The eluates were reduced by rotary evaporation, and a multi-step clean-up was performed with adsorption chromatography column. A multiplayer silica column (from top to bottom: anhydrous sodium sulfate, 1 g silica-gel, 10 g 44% silica-gel-sulfuric acid, 1 g silica-gel, 5 g 33% silica-gel-sodium hydroxide, 1 g silica-gel, 2 g 10% silver nitrate silica-gel, 1 g silica-gel) was used and eluted with 100 mL *n*-hexane. The hexane extracts were further concentrated and passed through a basic alumina column for further purification. Samples were then eluted with 100 mL 5% dichloromethane/hexane followed by 50 mL 50% dichloromethane/hexane. The 50% dichloromethane/hexane eluates were concentrated to approximately 20 μL by a stream of nitrogen. Prior to injection, a $^{13}C_{12}$-labeled injection standard (containing $^{13}C_{12}$-labeled 1,2,3,4-TCDD and 1,2,3,7,8,9-HxCDD, Wellington Laboratories) was added for calculation of the percentage recovery. PCDD/Fs were analyzed by an Agilent 6890 gas chromatograph coupled with Micromass Autospec Ultima high-resolution mass spectrometry (HRGC-HRMS) by tracing the M+, (M+2)+, or the most intensive ions of the isotope cluster. PCDD/F congeners were analyzed by a DB5 MS column (60 m × 0.25 mm i.d. × 0.25 μm). The carrier gas was helium at 1.2 mL/min. Injection volume was 1 μL in splitless mode with a 60 sec splitless period. The MS was operated over 10,000 resolution with EI (35 eV), and data were obtained in the selected ion monitoring (SIM) mode. The total tera-octa PCDD/Fs were calculated based total peaks with the precise molecular weight in the GC window and isotope ratios of the two monitored ions for each compound had to be within 15% of the theoretical chlorine values.

1.3 Quality assurance and quality control

The instrument stability and relative response factor variance were obtained from the analysis of calibration standard solutions during each sample batch. For quality control, the retention times of the analytes in a sample had to be within 2 sec of the retention times of the internal standards. Isotope ratios of the two monitored ions for each compound had to be within 15% of the theoretical chlorine values. The limit of detection (LOD) for PCDD/Fs in a given sample was defined by a signal to noise ratio greater than three times the average baseline variation. The recoveries of the PCDD/Fs in this study were in the range of 55%–110%. A laboratory method blank run was performed to demonstrate freedom from contamination. The duplicate sample was analyzed in the laboratory, along with the regular sample, as a further quality control tool, to ensure valid results. In addition, a certified reference material (CARP-2 sample) purchased from Wellington Laboratories Inc. (Canada) was analyzed, and the analysis results of PCDD/Fs were in the scope of the reference values.

2 Results and discussion

2.1 PCDD/Fs levels in different tissues of cormorant

The wet weight concentrations of 2,3,7,8-PCDD/Fs, in different tissues of cormorant were between 23.4 and 338 pg/g, as shown in Table1. The concentrations below detection limit were presented as '< LOD'. The concentrations of 2,3,7,8-PCDD/Fs were higher in skin (337.6 pg/g) when compared with those found in kidney (224 pg/g) or heart (206 pg/g). The lowest concentration was 23.4 pg/g found in the spleen. The concentrations of 2,3,7,8-PCDD/Fs in lungs and pancreas were also relatively low. The stomach (139 pg/g), liver (102 pg/g) and intestines (85.6 pg/g) all had similar concentrations of 2,3,7,8-PCDD/Fs. The concentrations of 17 PCDD/Fs were also relatively low in

Table 1 Concentration of PCDD/Fs in different tissues of the cormorant (unit: pg/g wet weight)

	Gall bladder	Skin	Heart	Pancreas	Intestines	Spleen	Lungs	Muscle	Kidney	Liver	Stomach
Lipid (%)	22	31	49	2.6	6.1	1.9	1.7	3.1	9.1	1.8	4.8
2,3,7,8-TCDF	< 0.01[b]	0.001	< 0.001[b]	0.042	< 0.03[b]	< 0.05[b]	< 0.03[b]	< 0.001[b]	< 0.02[b]	< 0.001[b]	< 0.001[b]
1,2,3,7,8-PeCDF	< 0.33[b]	0.353	< 0.03[b]	< 0.34[b]	< 0.03[b]	< 0.025[b]	< 0.11[b]	< 0.03[b]	< 0.62[b]	< 0.06[b]	< 0.04[b]
2,3,4,7,8-PeCDF	10.2	24.5	3.5	0.5	5.8	1.4	1.0	2.0	26.5	11.9	5.0
1,2,3,4,7,8-HxCDF	2.9	5.5	0.8	0.5	1.2	< 0.16[b]	< 0.03[b]	0.5	4.7	2.1	1.1
1,2,3,6,7,8-HxCDF	2.5	4.7	0.6	0.3	1.0	< 0.17[b]	0.1	0.4	3.5	1.4	0.9
2,3,4,6,7,8-HxCDF	2.0	3.7	0.6	0.3	0.9	< 0.13[b]	< 0.03[b]	0.3	9.0	1.1	0.8
1,2,3,7,8,9-HxCDF	< 0.11[b]	0.08	< 0.02[b]	< 0.23[b]	< 0.04[b]	< 0.14[b]	< 0.04[b]	0.01	< 0.37[b]	< 0.03[b]	< 0.03[b]
1,2,3,4,6,7,8-HpCDF	0.8	0.8	0.1	0.2	0.2	< 0.11[b]	0.0	0.1	1.0	0.3	0.2
1,2,3,4,7,8,9-HpCDF	0.2	0.1	< 0.03[b]	0.6	< 0.03[b]	< 0.15[b]	< 0.03[b]	< 0.03[b]	< 0.25[b]	< 0.02[b]	< 0.03[b]
OCDF	1.2	0.5	0.2	0.5	< 0.03[b]	< 0.18[b]	< 0.03[b]	< 0.04[b]	1.4	0.2	0.2
Total tetrafurans	0.1	0.1	0.1	1.8	0.1	2.0	1.5	0.2	0.2	0.3	0.1
Total pentafurans	11.0	35.9	10.0	1.3	9.8	1.4	1.8	3.8	33.7	16.3	9.0
Total hexafurans	9.7	38.5	8.6	1.2	8.6	2.5	1.3	5.0	21.0	11.7	13.0
Total heptafurans	5.9	44.4	11.8	2.4	12.1	1.3	1.0	7.6	6.4	7.8	26.0
Total PCDFs	27.9	119.3	30.6	7.2	30.7	7.1	5.6	16.5	62.6	36.2	48.2
2,3,7,8-TCDD	2.1	5.0	0.7	0.4	19.0	0.2	< 0.03[b]	0.5	1.1	0.5	0.8
1,2,3,7,8-PeCDD	8.4	21.0	2.8	1.3	< 0.05[b]	< 0.24[b]	< 0.03[b]	1.5	< 0.49[b]	< 0.07[b]	3.4
1,2,3,4,7,8-HxCDD	5.2	11.2	1.9	1.6	2.6	< 0.16[b]	0.7	1.3	18.2	4.1	2.3
1,2,3,6,7,8-HxCDD	4.3	9.0	1.2	< 0.17[b]	1.9	< 0.13[b]	< 0.05[b]	0.8	7.7	3.2	1.7
1,2,3,7,8,9-HxCDD	< 0.09[b]	2.2	0.3	< 0.17[b]	0.5	< 0.14[b]	< 0.05[b]	0.2	2.8	0.6	< 0.02[b]
1,2,3,4,6,7,8-HpCDD	4.6	6.1	1.0	0.4	1.6	0.4	0.4	0.5	12.0	3.2	1.4
OCDD	679.0	67.3	12.3	12.5	15.6	7.9	10.0	6.9	67.6	27.1	13.9
Total tetra-dioxins	4.0	54.3	7.2	1.1	7.9	0.2	0.4	9.0	3.4	4.2	24.5
Total penta-dioxins	9.1	27.1	4.3	1.6	1.8	1.7	1.6	3.2	0.5	5.4	4.6
Total hexa-dioxins	12.3	30.0	34.9	1.6	7.3	3.3	1.8	3.7	30.1	10.1	8.2
Total hepta-dioxins	25.5	39.6	117	3.9	22.4	3.2	4.5	4.8	60.2	19.5	39.7
Total PCDDs	119	218	176	20.6	55.0	16.3	18.3	27.7	162	66	90.8
Total PCDD/Fs	147	338	206	27.8	85.6	23.4	23.9	44.2	224	102	139
PCDD/Fs (l.w.)[a]	1333	1089	421	1070	1403	1230	1405	1425	2466	5696	2896
PCDD/PCDFs	2.1	1.8	5.7	2.8	1.8	2.3	3.3	1.7	2.6	1.8	1.9
WHO-TEQ	21.7	52.8	7.2	2.5	25.3	1.6	1.0	4.3	30.6	13.2	9.7
WHO-TEQ (l.w.)[a]	98.8	170	14.8	94.2	415	84.9	58.9	137	336	731	2021

[a] l.w.: lipid weight; [b] limit of detection.

muscle with 44.2 pg/g wet weight.

When the values were expressed on a lipid weight basis, the highest concentration of total PCDD/Fs was found in the liver (5696 pg/g) (Fig. 1). The levels of total PCDD/Fs in stomach tissue (2896 pg/g) were higher than those in kidney (2466 g/g). The concentrations of PCDD/Fs in gall bladder, skin, pancreas, intestines, spleen, lungs and muscle were relatively low, between 1070 and 1425 pg/g lipid weight. The lowest concentrations were found in heart tissue (421 pg/g). These results are consistent with those found in the literatures for other species (Wan et al., 2006; Kumar et al., 2002; Wu et al., 2000).

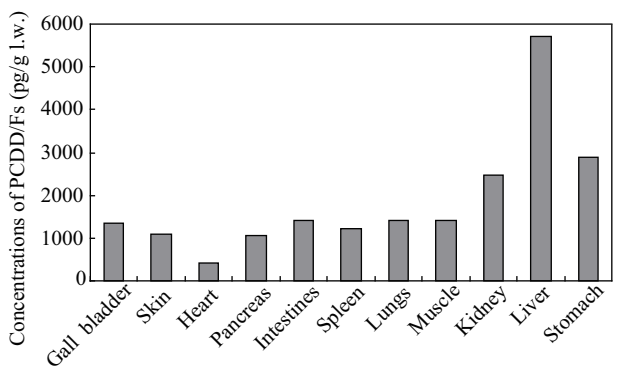

Fig. 1 Total concentrations of PCDD/Fs in different tissues in cormorant from Dongting Lake, China.

2.2 PCDD/Fs patterns in different tissues of cormorant

The concentrations of PCDDs were much higher than those of PCDFs in both the sediments and the water of Dongting Lake (Gao et al., 2005). The ratio of the concentration of PCDDs to that of PCDFs varied among the tissues analyzed in this study. The ratios were in the range of 1.7–5.7. The highest ratio of PCDDs/PCDFs was found in heart tissue (5.7), whereas those of the skin, intestines, muscle, liver and stomach were found to be of approximately the same value (1.7–1.8). The ratios were lower compared with previous studies (Wu et al., 2000). It has previously been reported that the concentrations of PCDDs were lower than PCDFs in all tissues but the brain tissue of the piscivorous birds in Ya-er Lake, which had been exposed to the pollutants hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), and pentachlorophenol (PCP) (Wu et al., 2000).

Historically, the concentrations of PCDD/Fs in the sediments of the Dongting Lake region were high; however, the levels of PCDD/Fs declined dramatically because of the dilution effect of several serious floods that have occurred since 1996 (Zheng et al., 1997; Gao et al., 2005). Octachlorinated dibenzo-*p*-dioxins (OCDD), 1,2,3,4,6,7,8-heptachlorinated dibenzo-*p*-dioxins (1,2,3,4,6,7,8-HpCDD) and octachlorinated dibenzofurans (OCDF) were major congeners of PCDD/Fs

in sediments. The contribution of OCDD to all 17 PCDD/Fs ranged from 31% to 82% in the various tissues. OCDD made a much greater contribution to the overall concentration of PCDD/Fs in the sediments when compared with that found in the tissues. The lowest proportion of OCDD was found in the heart and the highest proportion in the pancreas. This phenomenon is consistent with that reported by Wan et al. (2006). OCDD has also been found to be the predominate congener in spleen, brain, muscle and liver tissues extracted from Wild Herring Gulls from Bohai Bay, North China (Wan et al., 2006).

2.3 Liver/muscle concentration ratios

Kubota et al. (2004) used liver/muscle concentration ratios to describe hepatic sequestration. It was reported that the liver/muscle ratios progressively increased with the degree of chlorination of PCDDs. Molecular size and limited solubility may reduce the uptake of highly chlorinated congeners, including 1,2,3,4,6,7,8-HpCDD/F and OCDD, from the gastrointestinal tract. In this study, the average liver/muscle concentration ratios of PCDD/Fs, on a lipid weight basis for individual congeners, were used. As shown in Fig. 2, the liver/muscle ratio for PCDD/Fs were all higher than 1, indicating that all the PCDD/Fs were primarily deposited in the liver. This is constant with previous research that showed the concentration of PCDD/Fs was greater in muscle tissue (18–2700 pg/g wet weight) extracted from bald eagles from the Upper Peninsula of Michigan (Kumar et al., 2002). OCDD had the highest liver/muscle ratios, reaching a maximum value of 30 in the cormorant, as reported by Kubota et al. (2004). Interestingly, the liver/muscle ratios progressively increased with the degree of chlorination of PCDDs, except for OCDD, in this study. The liver/muscle concentration ratios for 1,2,3,4,6,7,8-HpCDD (10.5) were higher than other congeners. OCDD had the liver/muscle ratio 6.7. The liver/muscle concentration ratios in Herring gulls (Kubota et al., 2004) increased significantly with an increase in logK_{ow}, however, the correlation was not statistically significant in this study. The differences may be due to differences between the species when considering their sequestration ability. The liver/muscle ratios of PCDF congeners showed no increase with the degree of chlorination, and 2,3,4,7,8-PeCDF showed the highest concentration ratio in this study, which agrees with the result of Kubota et al. (2004).

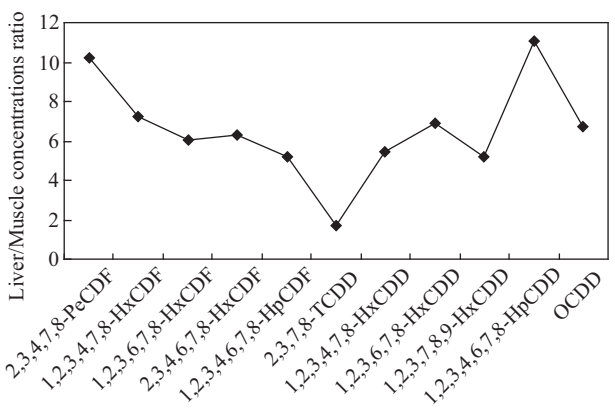

Fig. 2 Liver/muscle concentration ratios in different tissues in cormorant from Dongting Lake, China.

2.4 TEQs in different tissues of cormorant

The relative toxic potential of PCDDs and PCDFs, in tissues, was calculated using the WHO Toxic Equivalency Factors (TEFs) for birds reported by Van den Berg et al. (1998). The wet weight concentrations of TEQ ranged from 1.0 to 52.8 pg/g. This was consistent with previous studies, where the wet weight concentrations of TEQ, found in the gill and liver of fish collected in Dongting Lake, ranged between 0.16 and 0.52 pg/g (Fang et al., 2007). These results indicate that cormorant bio-accumulate PCDD/Fs through the food-web. The concentrations of TEQs in skin were 52.8 pg/g, followed by kidney (30.6 pg/g), intestines (25.3 pg/g), gall bladder (21.7 pg/g), liver (13.2 pg/g), stomach (9.7 pg/g), heart (7.2 pg/g), muscle (4.3 pg/g), pancreas (2.5 pg/g), spleen (1.6 pg/g) and lungs (1.0 pg/g) based on a wet weight basis. When the values were expressed on a lipid weight basis, the concentrations of TEQ ranged between 14.8 and 2021 pg/g (Fig. 3). The concentration of TEQ in liver tissue was relatively higher than those in other tissues. The TEQ concentrations in this study were higher than that in the tissue of wild herring gulls (1.9–190.8 pg/g lipid weight) from Bohai Bay (Wan et al., 2006). The concentrations of TEQ in the tissue of piscivorous birds, collected from Ya-er Lake, were between 4.6 and 552 pg/g lipid weight (Wu et al., 2000). The TEQ concentrations in this study were in the same magnitude as those from birds of Ya-er Lake. So the PCDD/Fs levels in the cormorant collected from Dongting Lake were still relatively high. It is interesting to note that the portion of 2,3,7,8-TCDD was relatively high in the intestinal tissue. It has not previously been reported that the concentrations of 2,3,7,8-TCDD in intestines were higher those in other tissues. Hence, the bio-accumulation of 2,3,7,8-TCDD requires further study.

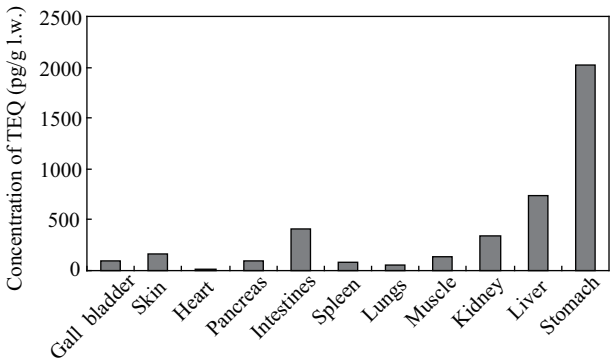

Fig. 3 Concentrations of TEQs in different tissues in cormorant from Dongting Lake, China.

3 Conclusions

In this study, levels and patterns of PCDD/Fs were determined in different tissues of cormorants collected from Dongting Lake, China. The total concentrations of

seventeen 2,3,7,8-PCDD/Fs ranged from 23.4 to 338 pg/g wet weight. The concentrations of PCDDs were found to be higher than those of PCDFs. Tissue distribution results showed preferential accumulation of PCDD/Fs in both liver and skin tissues. The liver/muscle ratios progressively increased with the degree of chlorination of PCDDs, except for OCDD and no increase with the degree of chlorination PCDFs. The concentrations of WHO TEQ in different tissues ranged between 1.0 and 52.8 pg/g lipid weight. These results indicate that the cormorant is in the high trophic level in the food-web. The PCDD/Fs levels in the cormorant collected from Dongting Lake were still relatively high compared with results reported in the previous publications.

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