Bull Environ Contam Toxicol (2007) 79:45–48
DOI 10.1007/s00128-007-9101-2

Profiles of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Fish Livers and Gills from Dongting Lake, China

L. P. Fang · M. H. Zheng · B. Zhang · W. B. Liu · L. R. Gao

Received: 31 July 2006 / Accepted: 15 March 2007 / Published online: 30 June 2007

Dongting Lake is located in the northeastern part of Hunan Province in the People's Republic of China. The lake covers about 2,740 km^2, making it the second-largest freshwater lake in China. The lake is an important source of freshwater fish. However, it is also an endemic region of schistosomiasis. *Oncomelania hupensis gredler* is the only lodging host of schistosome and plays a vital role in schistosomiasis spread. To kill *Oncomelania hupensis gredler* large amounts of sodium pentachlorophenate (Na-PCP), which was found containing polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) impurities, had been sprayed for over 30 years (Bao et al., 1995). PCDD/Fs are chemically stable, persistent hydrophobic organic chemicals and are thought to be biomagnified via the food chain. Those isomers with chlorinated substitutions in the 2, 3, 7, and 8 positions are thought to pose a risk to human health due to their toxicity, carcinogenic potency, and potential effects on animal reproductive and immunological systems. Although the spraying of Na-PCP has been prohibited in the region since 1996, recent studies have indicated that the sediments, soils, and breast milk around Dongting Lake area are contaminated by PCDD/Fs (Zheng et al., 1997; Gao et al., 2005; Zheng et al., 2003).

Fish are usually used as bioindicators for the environmental medium and food webs, and can reflect the PCDD/Fs contaminant status. Moreover, humans are consumers of fish, and exposure assessments now routinely consider fish ingestion as a potential route of human exposure to chemicals in the environment. Consequently, monitoring programs establish limits for the maximum content of these toxicants in a variety of foods. An important regulation is that the European Community (EC) has set a limit of 4 pg/g wet weight of toxic equivalents ($WHO_{PCDD/F}$-TEQ) for the muscle meat of fish and fishery products (EC, 2001). As do most organic contaminants, PCDD/Fs accumulate in certain organs with the highest proportions found in the fish liver (Wu et al., 2001). This paper describes the levels and bioaccumulation profiles of PCDD/Fs in different fish species livers and gills from Dongting Lake region.

L. P. Fang · M. H. Zheng (✉) · B. Zhang · W. B. Liu · L. R. Gao
State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China
e-mail: zhengmh@rcees.ac.cn

Materials and Methods

Fish were caught in Dongting Lake in March 2004. Samples were wrapped in aluminum foil, and stored at –20°C until analyzed.

Once thawed, fish 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 U.S. EPA Method 1613. Before extraction, samples were spiked with $^{13}C_{12}$-labeled 2,3,7,8-substituted PCDD/F internal standards. Extraction was carried out by Soxhlet extraction using 250 mL n-hexane/dichloromethane (1:1) for 24 h. The extract volumes were reduced by rotary evaporation. Lipid contents were calculated with residues of the extracts. Lipids were then dissolved with n-hexane and subjected to sulfuric acid wash. The volumes of eluates were reduced by rotary evaporation, and a multistep-cleanup was performed with adsorption chromatography using a multilayer 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% $AgNO_3$ silica-gel, 1 g silica-gel) and eluted with 100 mL n-hexane, which contained PCDD/Fs. The hexane extract was further concentrated and passed through basic alumina column for purification and fraction, then eluted with 100 ml of 5% dichloromethane in n-hexane as a first fraction. The second fraction eluted with 50% of 50 mL dichloromethane in n-hexane contained PCDD/Fs. The final eluates were condensed to about 20 μL by a stream of nitrogen. The final step involved the reduction to the volume necessary for the analysis. Prior to analysis, $^{13}C_{12}$-labeled injection standards were added for calculating recovery. All $^{13}C_{12}$-labeled internal and performance standards, as well as the calibration solution, were purchased from Wellington Laboratories.

PCDD/Fs were analyzed by high-resolution gas chromatograph (HRGC) coupled with high-resolution mass spectrometry (HRMS) (an Agilent 6890 series gas chromatograph coupled with Micromass Autospec Ultima series, Waters Micromass, USA) by tracing the M^+, $(M+2)^+$, or the most intensive ions of the isotope cluster. PCDD/F congeners were analyzed by a 60 m DB5ms-column (60 m × 0.25 mm i.d. × 0.25 μm). Gas chromatography (GC) temperature program: 80°C for 2 min rising 15°C/min to 150°C, then rising 2.5°C/min to 270°C, and finally rising 150°C/min to 330°C, before holding for one minute. The injection temperature, 290°C, the transfer-line temperature, 290°C, the carrier gas was He 1.2 mL/min, and the injection volume was 1 μL in splitless mode with a 60 s splitless period. The mass spectrometry (MS) was operated at a resolution of over 10,000, under positive electron ionization (EI) conditions (38 eV), and data were obtained in the selected ion monitoring (SIM) mode. Additionally QA/QC measures included running a procedural blank, a replicate, and a certified reference material.

Results and Discussion

The recoveries for the PCDD/Fs analyzed in this study were in the range of 53.2–105%. 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. Toxicity equivalents (TEQs) for 2,3,7,8-PCDD/F congeners were calculated for each sample using the World Health Organization toxicity equivalency factors (WHOTEFs) for fish (Van de Berg et al., 1998). When the value of PCDD/Fs congener was less than LOD, the total WHO-TEQ was calculated with 1/2 LOD. The levels of PCDD/Fs congeners and WHO-TEQ from fish tissues were given in Table 1.

From Table 1, it could be seen that OCDD had the highest values in fish tissues, followed by 1,2,3,4,7,8-HpCDD. The pattern of PCDD/Fs in the fish tissues characterized by high OCDD was consistent with the pattern of sediment. Previous studies showed that the sediment and soil around Dongting Lake region were contaminated by PCDD/Fs originating from impurities contained in Na-PCP (Zheng et al., 1997). In general, it is known that Na-PCP contained highly chlorinated dioxin and furan impurities, such as OCDD, 1,2,3,4,6,7,8-HpCDD, and OCDF, as its major isomers (Hagenmaier and Brunner, 1985; Bao et al., 1995). Considering this, it could be concluded that Na-PCP used in the Dongting Lake area is the main input source of PCDD/Fs, and the fish species analyzed in this study were also influenced by Na-PCP.

The biota-sediment accumulation factor (BSAF) is a very useful parameter to investigate the bioavailability of contaminants in the aquatic environment. The BSAF is defined as the ratio of the concentrations of contaminant accumulated in 1 g of lipid within the organism to the concentration in 1 g of organic carbon from the sediment. In this study the organic carbon content of sediment was 2.5%. The BSAFs of 2,3,7,8-substituted PCDD/F congeners in fish livers and gills against values of log K_{ow} of individual congeners were shown in Fig. 1. On the whole, the BSAFs recorded in the present study ranged from 3.0×10^{-5} to 8.0 for fish tissue samples. Reported BSAFs for PCDD/Fs from the other studies ranged from 2.0×10^{-4} to 2.0×10^{-1} for fish samples and 3.0×10^{-5} to 2.2×10^{-1} for eel (Kang et al., 2002; van der Oost et al., 1996). It could be seen that the BSAF values tend to decrease with increasing log K_{ow}. Notably, when log K_{ow} equals approximately 7.5–8.5, the tendency of BSAF to decline was very apparent. The isomers with log $K_{ow} > 7.5$ include most of the higher chlorinated compounds. The molecular cross-sectional > 0.95 nm for the PCDD/Fs is less efficiently taken up by organisms (Opperhuizen et al., 1985). This declining trend appears also to be consistent with the BSAFs for aquatic biota previously reported (Burkhard et al., 2004; Sakurai et al., 2000; Lyytikäinen et al., 2003; Wu et al., 2001). The relatively low BSAF values observed for the more highly chlorinated congeners in this study are generally understood in terms of a low bioavailability of these congeners due to their low solubility in comparison to less-chlorinated congeners.

Fish tissue was enriched in the less-chlorinated homologs and depleted in the highly chlorinated homologs relative to sediment from the same site. In contrast, concentrations of more highly chlorinated congeners were less in fish tissue than in sediment. The difference between sediment and tissue concentrations increased with the number chlorinated. For example, OCDD accounted for more than 95% of total PCDD/F in sediment, but was on

Table 1 Levels of PCDD/Fs in livers and gills from different fish species in Dongting Lake (pg/g lipid wt.)

Congeners	Carp liver(1)	Carp liver(2)	Catfish liver	Pomfret liver	Carp gill	Catfish gill	Sediment*
Lipid (%)	3.32	3.22	3.55	33.5	2.97	2.17	-
2378-TCDF	21.1	7.85	12.1	7.34	<6.56	17.5	0.60
12378-PeCDF	3.01	<2.27	<2.87	<0.55	<6.26	<5.99	<0.04
23478-PeCDF	4.54	<2.36	6.47	<0.50	<6.56	<6.08	<0.04
123478-HxCDF	<5.12	<1.65	<2.62	0.36	<4.2	<3.91	1.50
123678-HxCDF	<6.19	<1.68	<2.81	<0.24	<4.07	<3.64	0.70
234678-HxCDF	<4.25	<1.63	<2.45	<0.27	<3.87	5.07	0.60
123789-HxCDF	<7.02	<2.72	<3.94	<0.48	<7.47	<6.96	0.70
1234678-HpCDF	11.8	1.68	1.68	0.78	1.35	12.4	5.40
1234789-HpCDF	1.21	<1.12	<1.27	<0.28	<3.23	<4.42	0.30
OCDF	3.62	<0.95	1.97	0.15	<4.67	<2.99	29.0
∑2378-PCDFs	56.6	16.7	35.0	9.79	24.8	52.0	38.8
2378-TCDD	<2.52	<2.16	2.81	0.42	<6.69	<6.77	<0.06
12378-PeCDD	<7.14	<3.65	4.78	<0.60	<13.7	<10.2	<0.20
123478-HxCDD	<1.72	1.12	11.5	<0.22	4.04	22.1	8.20
123678-HxCDD	2.41	<1.18	<1.18	<0.21	<3.50	7.37	1.70
123789-HxCDD	<1.48	<1.24	1.41	<0.22	<3.67	2.30	6.00
1234678-HpCDD	22.9	8.69	16.9	1.85	42.1	39.2	60.0
OCDD	141	22.7	77.6	4.06	579	155.8	2980
∑2378-PCDDs	173	36.6	115.6	6.95	639	235	3056
∑2378-PCDD/Fs	230	53.4	150.6	16.7	664	287	3095
Ratio DFs to DDs	0.20	0.28	0.28	1.21	0.08	0.19	0.013
∑WHO-TEQ	10.1	4.93	18.0	1.38	15.3	23.6	3.60**

*Data come from Gao et al. (2005), unit is pg/g dry wt

** I-TEQ

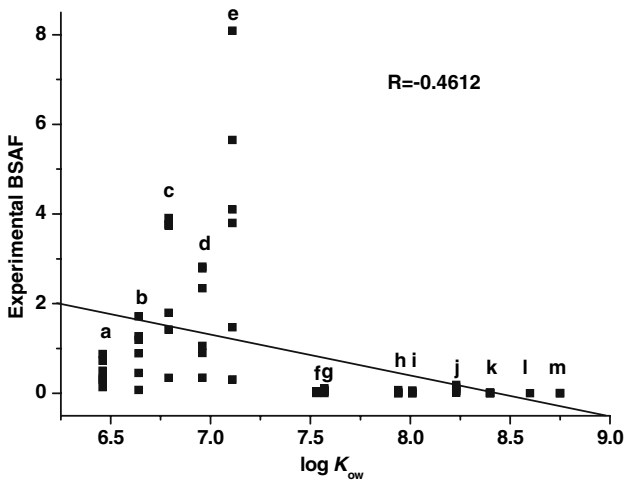

Fig. 1 Relationship between logK_{ow} and BSAF of 2,3,7,8-substituted PCDD/Fs in fish livers and gills. a, 2378-TCDF; b, 12378-PeCDD; c, 12378-PeCDF; d, 2378-TCDD; e, 23478-PeCDF; f, 123478-HxCDF; g, 123678-HxCDF; h, 123478-HxCDD; i, 1234678-HpCDF; j, 1234789-HpCDF; k, 1234678-HpCDD; l, OCDF; m, OCDD

the average of 54% in the fish tissues. It was reported that the difference in homolog profiles between tissue and sediment may indicate that fish obtain these compounds from the water column rather than from sediment (Bonn, 1998).

Concentrations of total 2,3,7,8-substituted PCDD/Fs of fish livers and gills ranged from 16.7 up to 664 pg/g lipid weight. Ratios of total PCDF congeners against total PCDD congeners were less than one, excluding that of the pomfret liver sample, with 1.21. The character of accumulation between the different fish species tissues was probably due to differences in feeding habits and metabolic capability toward PCDD/Fs for different fish species and fish internal organs. Figure 2 showed that 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 1,2,3,4,7,8-HxCDD were major contributions to the TEQs. The five congeners were responsible for about 90% of the total TEQs of PCDD/Fs. Total WHO-TEQ values for the samples analyzed were between 1.38 and 23.6 pg TEQ/g lipid basis, whereas the levels were from 0.18 to 0.64 pg

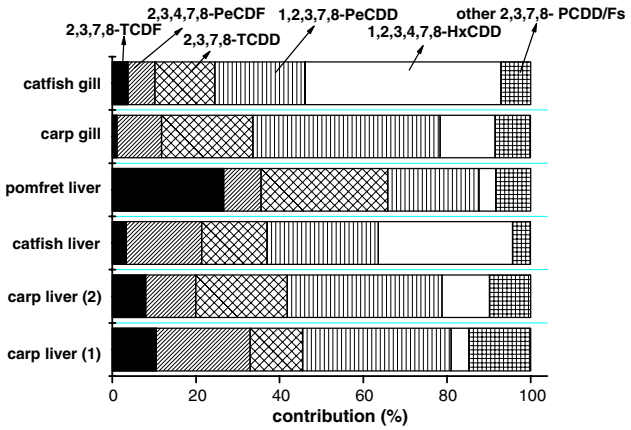

Fig. 2 Contribution of individual PCDD/F congeners to TEQs (WHO, 1998)

WHO-TEQ/g when the results were expressed wet weight. These levels meet the limit for dioxins established by the European authorities of 4 WHO-TEQ pg/g for PCDD/Fs for fish and fish products on whole weight basis (EC, 2001). In view of the measuring results, there is not much serious dioxins pollution in fish around the Dongting lake area due to the dilution effect of a large amount of water (average flood discharge peak flow: 40,200 m^3/s) and sand deposition from upper reaches (0.9251×10^8 m^3/year).

Acknowledgments This study was supported by the National Natural Science Foundation of China (20621703, 40332023) and the National Basic Research Program of China (2003CB415006).

References

Bao ZC, Wang KO, Kang JX, Zhao LW (1995) Analysis of polychlorinated deibenzo-p-dioxins and polychlorinated dibenzofurans in pentachlorophenol and sodium pentachlorophenate. Chin Environ Chem 14:317–321

Bonn BA (1998) Polychlorinated dibenzo-p-dioxin and dibenzofuran concentration profiles in sediment and fish tissues of the Willamette Basin, Oregon. Environ Sci Tech 32: 729–735

Burkhard LP, Cook PM, Lukasewycz MT (2004) Biota-sediment accumulation factors for polychlorinated biphenyls, dibenzo-p-dioxins, and dibenzofurans in southern lake Michigan Lake Trout (Salvelinus namaycush). Environ Sci Technol 38: 5297–5305

Council Regulation (EC) No. 2375/2001 of 29 November 2001 amending Commission Regulation (EC) No. 466/2001 setting maximum levels for certain contaminants in foodstuffs. Official J Eur Communities L 321, 06/12/2001: 1–5

Gao LR, Zheng MH, Zhang B, Liu WB, Zhao XR (2005) Declinging dioxins levels in the sediments from Dongting Lake in China. Organohalogen Compd 67:1085–1087

Hagenmaier H, Brunner H (1987) Isomerspecific analysis of pentachlorophenol and sodium pentachlorophenate for 2,3,7,8-substituted PCDD and PCDF at sub-ppb levels Chemosphere 16: 1759–1764

Kang YS, Yamamuro M, Masunaga S, Nakanishi J (2002) Specific biomagnification of polychlorinated dibenzo-p-dioxins and dibenzofurans in tufted ducks (*Aythya fuligula*), common cormorants (*Phalacrocorax carbo*) and their prey from Lake Shinji, Japan. Chemosphere 46: 1373–1382

Lyytikäinen M, Hirva P, Minkkinen P, Hämäläinen H, Rantalainen AL, Mikkelson P, Paasivirta J, Kukkonen JVK (2003) Bioavailability of sediment-associated PCDD/Fs and PCDEs: relative importance of contaminant and sediment characteristics and biological factors. Environ Sci Technol 37: 3926–3934

Opperhuizen A, van den Velde EW, Gobas FAPC, Liem DAK, van den Steen JMD (1985) Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. Chemosphere 14: 1871–1896

Sakurai T, Kim JG, Suzuki N, Matsuo T, Li DQ, Yao Y, Masunaga S, Nakanishi J (2000) Polychlorinated dibenzo-p-dioxins and dibenzofurans in sediment, soil, fish, shellfish and crab samples from Tokyo Bay area, Japan. Chemosphere 40: 627–640

Van den Berg M, Birnbaum L, Bosveld BTC, Brunström B, Cook P, Feeley M, Giesy J, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, Van Leeuwen FXR, Djien Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T (1998) Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for human and wildlife. Environ Health Perspec 106: 775–792

Van den Oost R, Opperhuizen A, Satumalay K, Heida H, Vermeulen NPE (1996) Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*) I. Bioaccumulation: biota-sediment ratios of PCBs, OCPs, PCDDS and PCDFs. Aquat Toxicol 35: 21–46

Wu WZ, Schramm KW, Xu Y, Kettrup A (2001) Accumulation and partition of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) in the muscle and liver of fish. Chemosphere 43: 633–641

Zheng MH, Bao ZC, Wang KO, Yang H, Xu XB (1997) Polychlorinated dibenzo-p-dioxins and dibenzofurans in lake sediments from Chinese schistosomiasis areas. Bull Environ Contam Toxicol 59:653–656

Zheng MH, Yang LC, Zhang B (2003) Polychlorinated dibenzo-p-dioxins and dibenzofurans in breast milk from Chinese schistosomiasis areas. Bull Environ Contam Toxicol 70: 189–192