Bull. Environ. Contam. Toxicol. (2006) 76:766–773

DOI: 10.1007/s00128-006-0986-y

Bulletin of Environmental Contamination and Toxicology

Organochlorine Pesticides in Tissues of Catfish (*Silurus asotus*) from Guanting Reservoir, People's Republic of China

D. X. Cheng,[1] Z. Z. Yang,[1,2] X. T. Wang,[2] Y. Z. Sun,[2] X. B. Xu[2]

[1] College of Chemistry and Environmental Science, Henan Normal University, Xinxiang, Henan 453007, People's Republic of China
[2] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Post Office Box 2871, 18 Shuangqing Road, Haidian District, Beijing 100085, People's Republic of China

Received: 12 November 2005/Accepted: 8 March 2006[0]

Organochlorine pesticides (OCPs) are ubiquitous anthropogenic pollutants that can be biologically amplified to high concentration in food webs. Due to their lipophilicity, persistence and high toxicities, OCPs are readily accumulated in the tissues of non-target living organisms (Jones and Voogt 1999) where they may cause detrimental effects. It is well known that OCPs were widely used, and the amounts of HCHs and DDTs production are 4.9 and 0.4 million tons in China during 1950s to 1980s (Zhang et al. 2002). Although the use of HCHs and DDTs has been officially banned in 1983, there are still considerable levels of OCPs, such as DDTs and HCHs, in waters, sediments, soils and biologic simples (Nakata et al. 2005; Gao et al. 2005; Zhou et al. 2001; Nakata et al. 2002) which suggest that pollution sources of OCPs are present in China.

Guanting Reservoir was Beijing's second largest source of water until 1997 when its water quality failed to meet national standards. To relieve Beijing's serious problem on shortage of water, Guanting Reservoir is expected to resume drinking water source after environment management and remediation. So, in the past years, regional surveys continued to monitor OCPs residue in water and sediment (Ma and Wang 2001; Wang et al. 2003), surrounding soils (Z.Hong et al. 2004) and small-size fish (Sun et al. 2005) from Guanting Reservoir, and the further investigation is need.

Fish are on the top of food wed in the water ecosystem, they could be used as bioindicator for contaminant level of waters (Menone et al., 2000). Analysis of the tissues from fish reflects the amount of environmental exposure. In this paper, we chose catfish (*Silurus asotus*) as bioindicator of OCPs in Guanting Reservoir, not only because its flesh-eating and bottom life style but also it is a familiar edible fish species in Beijing region. The aims of this study were to investigate the levels and distribution of OCPs in selected tissues of catfish from Guanting Reservoir, and to compare them with reported data from other inland water bodies and to health-based standards for fish consumption. The information will serve as a basis for the management and remediation of the water in the future.

Correspondence to: X. B. Xu

MATERIALS AND METHODS

A composite stock standard solution of OCPs comprising α- HCH, β- HCH, γ-HCH, δ-HCH, Heptachlor, Heptachlor epoxide, α-Chlordane, γ-Chlordane, Endosulfan sulfate, Endrin ketone, Methoxychlor and p,p'-DDE, p,p'-DDD, p,p'-DDT was purchased from Chem Service. HCB and *o*,p'-DDT standard solution with a concentration of 100 mg/L were bought from National Research Center for Certified Reference Materials of China. Decachlorobiphenyl (PCB209) was from Supelco (Bellfonte, USA). The desired concentration solution of OCPs was obtained by diluting the standard solution with iso-octane. Anhydrous sodium sulfate was heated in a furnace at 600°C for 5 hr to remove impurities. Silica gel (100-200 mesh) was activated in drying oven at 130°C for 16 hr and then cooled down to room temperature in a desiccator. All solvents used were of analytical grade and redistilled in all-glass system to remove impurities prior to use.

Catfish (*Silurus asotus*) samples investigated were collected from local fishermen living in the Guanting prefecture in October 2003. There were three female fish collected, which were 44 cm, 51 cm, 54 cm in length and 0.75 kg, 0.90 kg, 0.95 kg in weight respectively. The fish were wrapped in pre-cleaned aluminum foil and stored at -20°C refrigerator.

After thawing, the fish were dissected and 10 kinds of tissues were chosen including muscle, intestine, kidney, liver, brain, skin, gill, eye, egg and mesenteric fat which were expressed as mus, int, kid, liv, bra, ski, gil, eye, egg and mf in short, respectively. The tissues were well homogenized in a tissue homogenizer, then approximately 8g (wet weight) of homogenized tissue sample (except brain, eye and kidney for the whole organ was only about 2g, 1g and 4g, respectively) was ground with sufficient anhydrous sodium sulfate until the mixture was dry and free-flowing. Finally, the resulting powder was transferred to a clean beaker, covered with aluminum foil and equilibrated for 16 hr in a desiccator for extraction. The samples were extracted with 200 mL of hexane/acetone (1:1, v/v) in hot Soxhlet extraction mode for 24 hr. 1 ml of PCB209 solution at a concentration of 60 μg/L was added before extraction as a surrogate standard. The lipid content of each sample was determined gravimetrically by evaporating an aliquot of the extract to constant weight in an oven at 105°C for 12 hr. The remaining extract was dried with anhydrous sodium sulfate and then concentrated in a rotary vacuum evaporator to about 2mL. Further clean-up was on a column packed with 15g acid silica (30% sulfuric acid, w/w), followed by a column with 10g partially deactivated silica gel (3% water, w/w) after removing sulfate by activated copper powder. The eluates were concentrated by a gentle nitrogen steam to 200 μL, and then transferred to vials for GC injection.

The instrumental analyses were performed by GC6890-μECD (Agilent 6890 series II equipped with a ^{63}Ni electron capture detector). A HP-5 fused silica capillary column (30 m × 0.25 mm id, and 0.25 μm film thickness) was used. The injection mode was splitless and the purge time was 0.75 min. Nitrogen was used as carrier gas and make-up gas. Detector and injector temperature were 300°C and

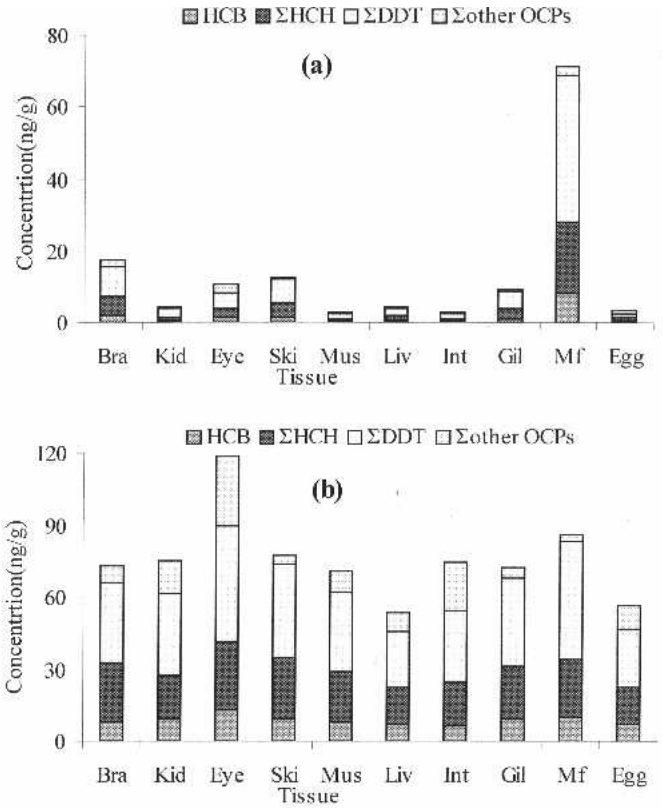

Figure 1. Mean concentrations of ΣOCPs in units of ng/g wet weight (a) and ng/g lipid weight (b) in tissues of catfish

250°C, respectively. The GC oven temperature program was carried out as follows: initial temperature 100°C held for 1 min, increased to 160°C at 10°C /min, and then to 254°C at 4°C /min, to 280°C at 10°C /min, and held for 15 min. Peak identification of these 16 OCPs was made by comparison of retention time with corresponding standards and confirmed on an Agilient 6890 GC equipped with a model 5973 mass selective detector (MSD). The quantification of the analytes was performed by comparison to external standard.

Blank and recovery experiments were run for fish tissues including the whole analytical procedure. For recovery experiments, OCP mixture in iso-octane was added to the pure corn oil. The method detection limits (MDLs) of 16 OCPs were determined as the concentrations of analytes in a blank sample that gives rise to a peak with a signal-to-noise ratio (S/N) of 3. Detection limits varied for the different OCPs and ranged from 0.001 to 0.04 ng/g. For every set of 6 samples, a procedural blank and spiked sample consisting of all reagents were run to check for interference and cross-contamination. The recoveries of OCPs spiked in matrix were in the range of 53.12-116.62%, and RSD (n=4) was 5.28-17.92%. The

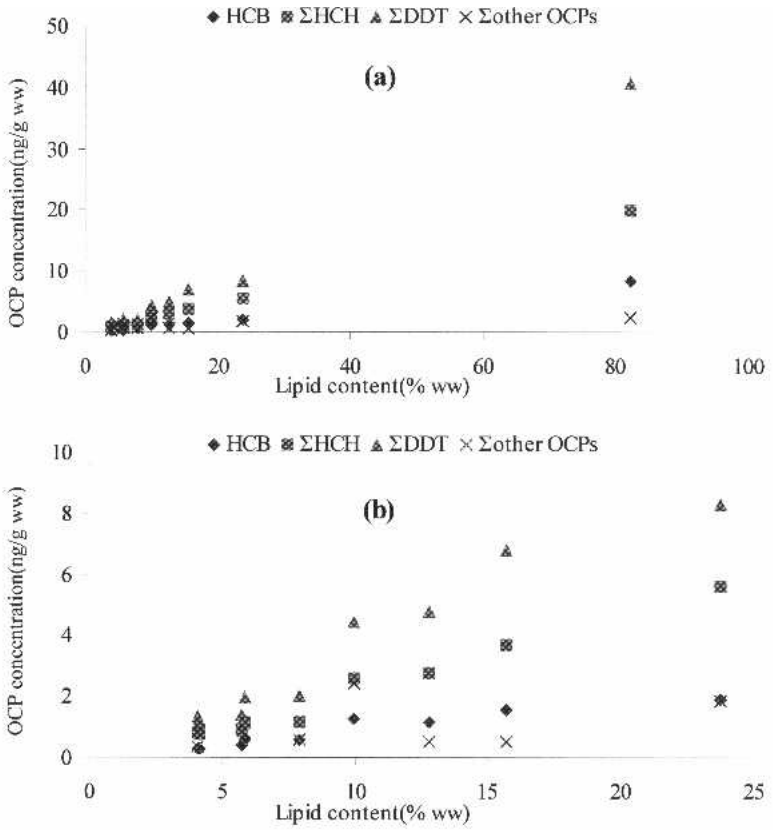

Figure 2. Linear relation between mean lipid content (% wet weight) and mean concentrations of OCPs (ng/g wet weight). Figure (b) is the magnified part of Figure (a)

recoveries of surrogate standard (PCB209) spiked in blank, matrix, samples were above 85%. These results indicated that the analytical protocols used in this study are effective for determination of 16 OCP residues in fish. All residue concentrations below method detection limits were regarded to be equal to zero in calculation of sum, means and so on. The recoveries of surrogate (PCB209) in all samples tested were between 85-110 %.

RESULTS AND DISCUSSION

The concentrations of ΣOCPs (sum of 16 organochlorine pesticides measured) in tissues of catfish are shown in Figure 1. The levels and distributions of ΣOCPs in different tissues on wet weight basis (Figure 1. (a)) showed significant differences between tissues, with significantly higher concentrations in the mesenteric fat. Pairwise comparisons showed significant differences (t-test, $p<0.05$) between wet weight ΣOCPs concentrations in brain/mesenteric fat, kidney/eye, kidney/gill, kidney/mesenteric fat, eye/muscle, eye/liver, eye/intestine

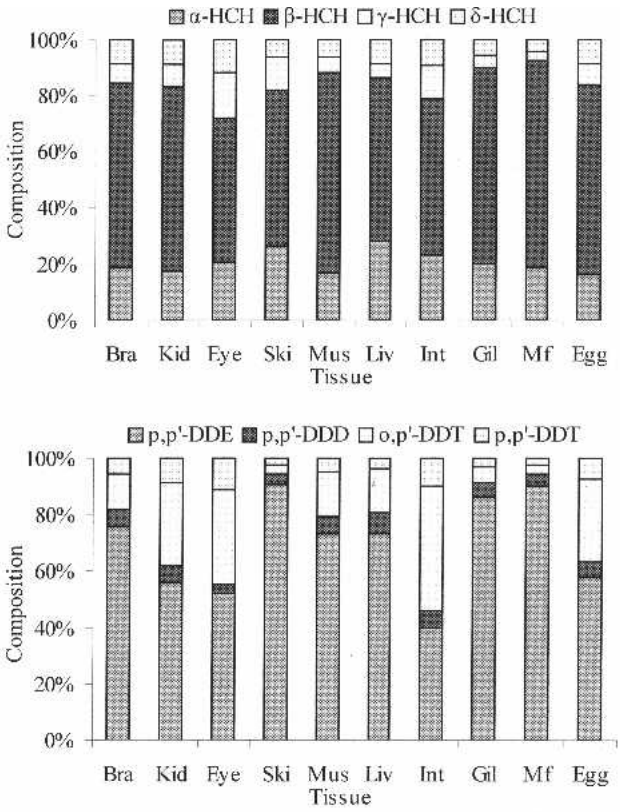

Figure 3. Percentage distribution of HCH isomers and DDT analogues in tissues of catfish

eye/mesenteric fat, eye/egg, skin/mesenteric fat, muscle/gill, muscle/mesenteric fat, liver/mesenteric fat, intestine/gill, intestine/mesenteric fat, gill/mesenteric fat, gill/egg and mesenteric fat/egg. When the data were on lipid weight basis (Figure 1. (b)), there were no significant differences in concentrations between the other tissues, except for brain/liver, liver/intestine and liver/gill (t-test, $p<0.05$), and eye had the highest concentrations of ΣOCPs.

All the 16 OCPs were detected in the 10 selected tissues, except for Methoxychlor which only found in brain, muscle and mesenteric fat range from 0 to 0.11 ng/g wet weight. The dominant part of ΣOCPs residue in tissues was ΣDDT accounted for 40.19~57.09%, with an average of 47.15%, followed by ΣHCH from 24.17% to 31.72% with an average of 27.62%. The linear relation between mean lipid content (% wet weight) and mean concentrations of OCPs (ng/g wet weight) are shown in Figure 2. High positive correlation were found in ΣDDT (R^2=0.994), ΣHCH (R^2=0.998) and HCB (R^2=0.993) to lipid content. It may be explained by their similar bioaccumulation.

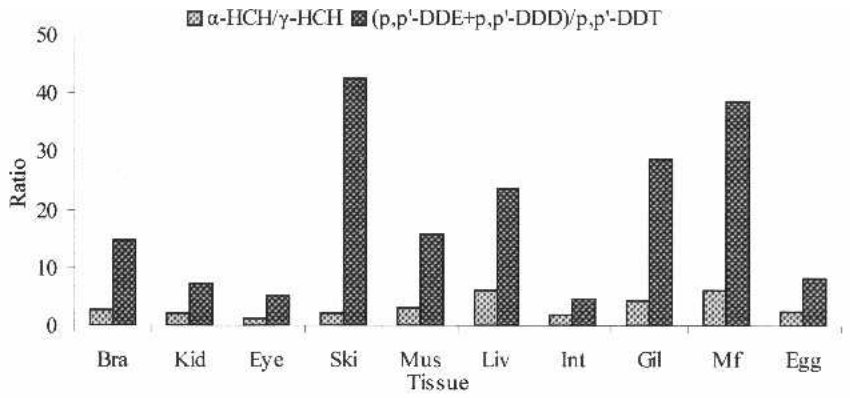

Figure 4. The ratio of α-HCH/γ-HCH and (p,p'-DDE+ p,p'-DDD)/ p,p'-DDT in tissues of catfish

Percentage compositions of HCH isomers and DDT analogues in tissues of catfish are illustrated in Figure 3. The dominant part of ΣHCH was β-HCH accounted for 51.27~74.27% with an average of 63.58%, and for ΣDDT was p,p'-DDE from 39.60% to 90.11% with an average of 69.45%. The high ratio of β-HCH/ΣHCH indicated that β-HCH is the most persistent and metabolically stable HCH isomers and that the investigated specimens had suffered an old pollution source. The ratio of α-HCH/γ-HCH and (p,p'-DDE+ p,p'-DDD)/ p,p'-DDT can be used as a rough estimation of the new input of DDT and Lindane (almost pure γ-HCH) into the environment or not. The value of α-HCH/γ-HCH would be in the range of 5.0 to 5.4 for technical HCH and nearly zero for Lindane manufactured in China (Wang et al. 2003). It can be seen from Figure 4 that the ratio of α-HCH/γ-HCH and (p,p'-DDE+ p,p'-DDD)/ p,p'-DDT in all tissues are significantly higher than 1.0 with averages of 3.19 and 18.85, respectively, which suggested mixed sources of HCH and old use of DDT.

Randall et al reported that the accumulation by fish of non-metabolized and persistent lipophilic compounds was due largely to direct exchange with the water, notably via the gills (Randall et al. 1998), they didn't give the concentrations of persistent lipophilic compounds in different tissues. In our results, on lipid weight basis, the concentrations of HCB in skin and gill had no significant differences to other tissues of catfish (t-test, $p<0.05$). As metabolized lipophilic compounds, DDT isomers have long persistence in environment, and gradually degrade to DDE and DDD by chemical and biological processes as we known. In this study, the ratio of (p,p'-DDE+ p,p'-DDD)/ p,p'-DDT in tissues of catfish was higher than in water (Wang et al., 2003), sediment (Ma et al. 2001) and surrounding soil (Z Hong et al. 2004) of Guanting Reservoir. Surprisingly, the ratios of (p,p'-DDE+ p,p'-DDD)/ p,p'-DDT in skin and gill were significantly higher than in intestine as shown in Figure 4. And there was higher ratio of (DDE+DDD)/DDT in mesenteric fat which was coincident with Silverside from a Coastal Lagoon in Argentina (Menone et al. 2000). Henriksen et al had also determined different p,p'-DDE and

p,p'-DDT lipid basis concentrations in liver, brain and subcutaneous fat of gulls (Henriksen et al. 1998). So, the different ratios of (DDE+DDD)/DDT in tissues of catfish may be due to the different composition of lipid in varied tissues.

Table 1. Comparison of ΣOCPs levels (ng/g ww) in fish samples from different fresh water bodies.

Locality	Sample	ΣOCPs	References
Guanting Reservoir, China	Various fishes	6.18-12.78	Sun et al. (2005)
Coastal Lagoon, Argentina	Silverside tissues	8.6-111.6	Menone et al. (2000)
Taihu, China	Unknow	18.1-149.5	Feng et al. (2003)
Lake Tai, China	Whole Catfish	1250 [lw]	Nakata et al. (2005)
Danube Delta, Romania	Various fishes	330-4966 [lw]	Covaci et al. (2006)
Guanting Reservoir, China	Catfish tissues	2.75-71.17	This study
Guanting Reservoir, China	Catfish tissues	53.69-118.22 [lw]	This study

lw: lipid weight basis

Compared the levels of ΣOCPs in catfish with other fish from different fresh water bodies (Table 1.), the concentrations of ΣOCPs in catfish from Guanting Reservoir were lower than those in fish from other fresh water bodies. Although the residue levels of ΣHCH and ΣDDT in catfish were far below the national food standard of China (1000 μg/kg for ΣHCH and 2000 μg/kg for ΣDDT (MOH 1994)) and OCPs' concentrations presented negligible risk to both humans and wildlife consuming the fish, further investigations on OCP pollution are needed to assess the risks of wildlife and human health in Beijing.

Acknowledgments. This study was financially supported by the Main Basic Research Development Program of China (G 1999045710) and the Fund of Natural Science of Henan province, China (NO. 0411021400)

REFERENCES

Covaci A, Gheorghe A, Hulea O, Schepens P (2006) Levels and distribution of organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in sediments and biota from the Danube Delta, Romania. Environ Pollut 140:136-149

Feng K, Yu BY, Ge DM, Wong MH, Wang XC, Cao ZH (2003) Organo-chlorine pesticide (DDT and HCH) residues in the Taihu Lake Region and its movement in soil-water system I. Field survey of DDT and HCH residues in ecosystem of the region. Chemosphere 50:683-687

Gao HJ, Jiang X, Wang F, Wang DZ, Bian YR (2005) Residual Levels and Bioaccumulation of Chlorinated Persistent Organic Pollutants (POPs) in Vegetables from Suburb of Nanjing, People's Republic of China. Bull Environ Contam Toxicol 74:673-680

Henriksen EO, Gabrielsen GW, Skaare JU (1998) Validation of the use of blood samples to assess tissue concentrations of organochlorines in glaucous gull,

Larus hyperboreus. Chemosphere 37:2627-2643
Jones KC, Voogt PD (1999) Persistent organic pollutants (POPs): State of the science. Environ Pollut 100:209-221
Ma M, Wang ZJ (2001) Contamination of PCBs and organochlorinated pesticides in the sediment samples of Guangting Reservoir and Yongding River. Environ Chem 20:238-243 (in Chinese)
Menone ML, Aizpún de Moreno JE, Moreno VJ, Lanfranchi AL, Metcalfe TL, Metcalfe CD (2000) PCBs and Organochlorines in Tissues of Silverside (*Odontesthes bonariensis*) from a Coastal Lagoon in Argentina. Arch Environ Contam Toxicol 38:202-208
MOH (1994) Hygienic standard of fresh water fish. GB 2736-94. Ministry of Health of People's Republic of China, Beijing.
Nakata H, Hirakawa Y, Kawazoe M, Nakabo T, Arizono K, Abe S, Kitano T, Shimada H, Watanabe I, Li WH, Ding XH (2005) Concentrations and compositions of organochlorine contaminants in sediments, soils, crustaceans, fishes and birds collected from Lake Tai, Hangzhou Bay and Shaanghai city region, China. Environ Pollut 133:415-429
Nakata H, Kawazoe M, Arizono K, Abe S, Kitano T, Shimada H, Li W, Ding X (2002) Organochlorine Pesticides and Polychlornated Biphenyl Residues in Foodstuffs and Human Tissues from China: Status of Contamination, Historical Trend, and Human Dietary Exposure. Arch Environ Contam Toxicol 43:473-480
Randall DJ, Connell DWK, Yang R, Wu SS (1998) Concentrations of persistent lipophilic compounds in fish are determined by exchange across the gills, not through the food chain. Chemosphere 37: 1263-1270.
Sun YZ, Wang XT, Xu XB (2005) Persistent Organochlorine Pesticide Residues in Fish from Guanting Reservoir, People's Republic of China. Bull Environ Contam Toxicol 74:537-544
Wang XT, Chu SG, Xu XB (2003) Organochlorine pesticide residues in water from Guangting Reservoir and Yongding River, China. Bull Environ Contam Toxicol 70:351-358
Z Hong, L Yonglong, W Tieyu, S Yajuan (2004) Accumulation Features of Organochlorine Pesticides Residues in Soils around Beijing Guanting Reservoir. Bull Environ Contam Toxicol 72:954-961
Zhang G, Parker A, House A, Mai B, Li X, Kang Y, Wang Z (2002) Sedimentary records of DDT and HCH in the Pearl River Delta, South China. Environ Sci Techol 36:3671-3677
Zhou JL, Maskaoui K, Qui YW, Hong HS, Wang ZD (2001) Polychlorinated biphenyl congeners and organochlorine insecticides in the water column and sediments of Daya Bay, China. Environ Pollut 113:373-384