

## **Polychlorinated Biphenyls in Fish Species from Guanting Reservoir, Beijing, People's Republic of China**

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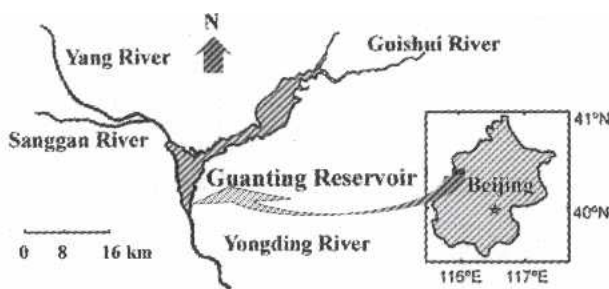
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Commercial products of polychlorinated biphenyls (PCBs) are widely used from the 1930s to the 1970s as fluids in transformers and capacitors, hydraulic fluids, lubricating oils, and as additives in pesticides, inks and paints due to their high chemical stability (Kennish, 1997). PCBs are worldwide ubiquitous anthropogenic environmental pollutants, which are extensively present in various environmental medium such as air, soil, water, sediment and biota (Fowler, 1990; Tanabe et al., 1994). The amount of PCBs in the global environment has been estimated to be about  $3.70 \times 10^8$  kg and further  $7.8 \times 10^8$  kg were estimated to be still available for utilization or deposited in different ways (Vit, 1992). Accidental leakage and improper disposal of discarded electronic equipments remain potential sources of PCB contamination in China. PCB congeners differ widely in their toxicological and physico-chemical properties according to the numbers and positions of the chlorine atoms in the molecules.

Human exposure to PCBs primarily occurs via low level food contamination (Batters hill, 1994). Fish, meat and dairy products are the most important dietary sources of PCBs for humans (Duarte-Davidson and Jones, 1994; ATSDR, 2000). Adverse health effects including skin disorders, neurochemical behavioral changes, immune dysfunction, reproductive and developmental changes, and cancer after accidental exposure have been observed (Carpenter, 1998). Even at low dietary exposure levels, endocrine disruption and relatively subtle adverse effects on neurobehavioral development have also been observed in humans (Brouwer et al., 1995). Due to the complexity of the PCBs, seven indicator congeners were often used to identify the composition of PCBs. These seven indicator congeners were PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, and PCB180, which were dominantly present in most commercial PCB products and in environmental samples (Capel et al., 1985; Frignani et al., 2001).

Fish are at the top of the food web of the aquatic ecosystem. Hence fish could be used as bioindicator for water quality and environmental pollution monitoring because they concentrate persistent organic pollutants (POPs) in their tissues directly from water, but also through their diet, thus enabling the assessment of transfer of POPs through the trophic web (Mormede et al., 2003; Fisk et al. 2001; Boon et al., 2002). Data on the presence and distribution of POPs in fish and

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**Figure 1.** Guanting Reservoir and its location in Beijing

especially edible fish species are therefore important not only for ecological perspective, but also human health. Humans are exposed inadvertently to POPs through numerous sources, of which the consumption of contaminated fish is one of the most important pathways (Fürst, 1993).

The work is a part of a continuous research program on the pollution of Guanting Reservoir and the Yongding River in Beijing. HCHs and DDTs in six kinds of small fish including feral carp, wheat head gudgeon, color gudgeon, Chinese bitterling, silver xenocypris and hemiculter leucisculus were investigated (Sun et al. 2005), but no information is available on the levels of PCBs in different fish species which were readily caught and consumed by the local inhabitants from the reservoir. The objectives of the present study were to investigate the levels and composition of PCB congeners, including the seven indicator PCBs in different fish species collected from Guanting Reservoir, to compare them with reported data from other inland water bodies and to assess their possible sources and to health-based standards for fish consumption.

## **MATERIALS AND METHODS**

Three composite stock standard solutions of PCBs (PCB Congener Mix #1~#3, which totally composed of 102 PCB congeners contained in Aroclors 1242, 1254 and 1260) were obtained from AccuStandard (New Haven, USA). The desired concentration solutions of PCBs were obtained by diluting the standard solutions with iso-octane. Tetrachloro-*m*-xylene (TCmX) (Chem Service Inc.) was used as surrogate standard. Anhydrous sodium sulfate was purified in a furnace at 600°C for 5 hr. Silica gel (100-200 mesh) was activated in drying oven at 130°C for 16 hr and then deactivated with 3% water (w/w) after it was cooled down to room temperature in a desiccator. Superclean ENVI-Carb cartridges (3mL, 250mg) (washed with 20 mL of acetone, toluene and hexane respectively before use) were obtained from Supelco (Bellefonte, PA, USA). All solvents used in the study were from Merck (Darmstadt, Germany).

10 fresh fish species including Pond smelt (*Hypomesus olidus*), karut croaker (*Wak carutta*), topmouth culter (*Erythroculter ilishaeformis*), crucian (*Carassius auratus*), Yellow catfish (*Pelteobagrus fulvidraco*), Yellow Xenocypris (*Xenocypris davidi*)

**Table 1.** Average length (cm), weight (g) and lipid content (%) and feeding habits of sampled fish species

Common name	Length	Weight	Lipid	Main food
Pond smelt	11.5	8.4	12.39	Insect larvae, amphipod and isopod,
Karut croaker	11	11.32	1.75	Omnivorous
Topmouth culter	22.0	64.63	5.58	Insect, amphipod, isopod and small fish.
Crucian	17.0	100.58	2.13	Omnivorous
Yellow catfish	23.5	111.9	4.99	Zooplankton, insect, mollusk and small fish.
Yellow Xenocypris	7.1	4.9	4.76	Algae, plant chippings,
Large icefish	19	12.42	2.20	Zooplankton, small fish and shrimp.
Stone moroko	8.1	4.4	11.59	Zooplankton, insect, aquatic plant and algae.
Chinese false gudgeon	9.5	9.5	6.78	Insect and larvae, plant chippings, diatom
Chinese bitterling	7.0	5.1	3.19	Algae and advanced plant chippings.

*Bleeker*), Large icefish (*Protoslanx hyalocranius*), Stone moroko (*Pseudorasbora pava*), Chinese false gudgeon (*Abbottina rivularis*), and Chinese bitterling (*Rhodeus sinensis* Gunther), which were expressed as GY, BTY, JZL, JY, HSY, HTY, DYY, MSY, BHY and HLP in short, respectively, were collected from local fishermen living in the Guanting prefecture in October 2003. The fishes were wrapped in pre-cleaned aluminum foil and stored at -20°C refrigerator. The information on the length, weight and habitat and feeding preferences for these fish species is presented in Table 1.

After thawing, the fish was well homogenized in a tissue homogenizer. Approximately 10 g (wet weight) of homogenized sample was transferred to a mortar, 30 g of anhydrous sodium sulfate was added, and then ground until the mixture was 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 tetrachloro-*m*-xylene solution at a concentration of 50 µg/L was added before extraction as a surrogate standard. The lipid content of each sample was determined gravimetrically. The remaining extract was dried with anhydrous sodium sulfate and then concentrated in a rotary vacuum evaporator to about 5 mL. Lipid and sulfur removal and clean-up were achieved by using sulfuric acid silica column and mercury, respectively. Organochlorine pesticides were removed using deactivated silica gel (3%) chromatography. Co-planar PCBs including Non-ortho PCBs (PCB-77, -81, -126, and -169) and mono-PCBs (PCB-105, -114, -118, -123, -156, -157, -167, and -189) and other PCBs were finally separated into two fractions for each sample using ENVI-Carb cartridge. The fractions were concentrated by a gentle nitrogen stream to 200 µL for

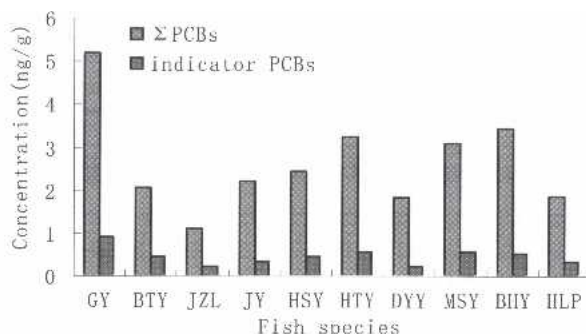


The instrumental analyses were performed by GC6890- $\mu$ ECD (Agilent 6890 series II equipped with a  $^{63}\text{Ni}$  electron capture detector). A DB-5 fused silica capillary column (30 m  $\times$  0.25 mm id, and 0.25  $\mu\text{m}$  film thickness) was used. The injection mode was splitless and 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 280°C, respectively. The GC oven temperature program was carried out as follows: initial temperature 60°C held for 1 min, increased to 150°C at 15 °C /min, then to 230°C at 6 °C /min held for 6 min, finally to 280°C and held for 10 min. Peak identification of PCB congeners were made by comparison of retention time with corresponding standards, and PCB congeners with higher concentrations in selected samples were confirmed by GC/MS using an Agilent 6890 GC equipped with a model 5973 mass selective detector (MSD) in selected ion mode. Two ions from the molecular cluster were monitored for each analyte. The data were processed by means of a G2070AA GC Chemstation and an external standard method was used for quantitative calculation.

Blank and recovery experiments were run for fish samples including the whole analytical procedure. The target compounds were not detected in the blank run for the entire procedure. For recovery experiments, PCBs in iso-octane was added to the pure corn oil. The method detection limits (MDLs) of PCB congeners 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 of the method ranged from 0.001-0.008 ng/g for PCB congeners on wet weight base. For every set of 6 samples, a procedural blank and spiked sample consisting of all reagents was run to check for interference and cross-contamination. The spiked recoveries in matrix were in the range of 64.39-108.45% for PCB congeners, and RSDs (n=3) were 7.51-16.29%. In order to monitor the recovery efficiencies of the extraction and cleanup, tetrachloro-*m*-xylene (TCmX) was used as surrogates and the recoveries of surrogate standards spiked in blank, matrix and samples tested were between 75-110 %. These results indicated that the analytical protocols used in this study are effective for determination of PCB 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. All samples were analyzed in duplicate and results reported in the study were means of duplicate analyses, both for qualitative and quantitative analysis. All results were not corrected for recoveries.

## RESULTS AND DISCUSSION

The concentration (shown in Figure 2) of total PCBs and 7 indicator PCBs ranged from 1.09 to 5.19 ng/g and 0.23 to 0.91 ng/g, with averages of 2.65 ng/g and 0.47 ng/g on a wet weight basis, respectively. The total PCB and indicator PCB concentrations were found in the order: GY > BHY, HTY, MSY > HSY, JY, BTY > HLP, DYY > JZL. It is obvious that GY contained the highest PCB concentration, while the lowest ones were found in JZL and DYY which were only about a fifth of the former.



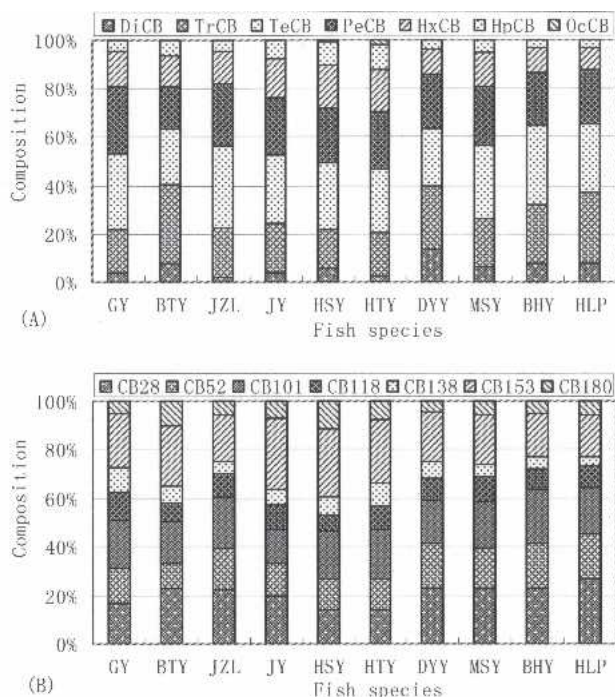
**Figure 2.** Levels of total PCBs in fish samples from Guanting Reservoir

PCBs are very persistent in the environment and easily bioaccumulate in fish. Many factors influence the uptake and bioaccumulation of PCBs in fish. Bottom feeders easily accumulate more PCBs. Older and larger fish would be expected to have higher concentrations of PCBs. Fast-growing species may have lower PCB concentrations. Fish with more lipid should accumulate higher PCB concentrations than lean fish. The different feeding behavior reflected distribution pattern of PCB congeners in fish, but it is difficult to discriminate the difference among them in this study due to their small sizes and ages. Differences in PCBs concentration in different small fish species inhabited in Guanting Reservoir are mainly associated to different lipid content as well as differences in their feeding habits and probably metabolic differences in PCBs biotransformation..

The lipid contents in each species (Table 1) were significantly different and in the descending order: GY> MSY> BHY > HLP > JZL > HSY > HTY > DYY > JY > BTY. It is obvious that fish with high lipid content accumulated more of PCBs than lean fish. But good linear relation between PCBs and lipid content was not found ( $r^2=0.50$ ) at the 0.05 level. A significantly positive correlation ( $r=0.97$ ) was found between total PCBs and indicator PCBs in fish species at the 0.01 level. Therefore, the total PCB concentration in fish samples can be estimated based on indicator PCB content. Here, the linear relation of PCB concentration with indicator PCB was as follows:

$$\text{PCBs} = 5.5534 \times \text{indicators} + 0.0432 \quad (r^2=0.9412)$$

PCB congeners were detected at low concentrations in all fish samples. A total of 86 PCB congeners were quantified. 35 PCB congeners including PCB 28, 52, 44, 87, 101, 118, 138, 149, 153 and 180 were detected with 100% detection frequencies in all fish samples. Figure 3(A) shows the composition profiles of PCB homologues in 10 fish species. The PCB profiles were similar on the whole for all the fish samples. The dominant PCB homologues in fish samples were tri-, tetra- and pentachlorobiphenyls. The major congeners detected were the lower-chlorinated congeners including 8/5, 18, 28/31, 44, 52, 56, 66, 70/76, 84, 101, 110 and 153, accounting for 44.79-59.61% with a mean of 50.70%. The lower-chlorinated



**Figure 3.** Percent contribution of PCB homologues (A) and indicator PCBs (B) in fish samples from Guanting Reservoir

congeners (di- to penta-CBs) accounted for 70.7 to 87.7% with a mean of 80.4% of the total PCBs in fish samples in the study. The occurrence of lower-chlorinated congeners and the homologue pattern indicate that atmospheric deposition was probably a major source of PCBs in Guanting Reservoir. The presence of tetrachlorobiphenyls (44 and 52), pentachlorobiphenyls (87, 101 and 118) and hexachlorophenyls (138, 149 and 153) suggest a contribution from the commercial mixtures Aroclor 1242 and 1254, which have been widely used in electrical equipment in several countries (Montone, et al., 2001).

Figure 3 (B) shows the distribution profiles of the seven indicator PCB congeners in 10 fish species. Seven indicator PCB congeners were found in all fish species, which accounted for 13.46-21.93%, with a mean of 17.89% of total PCBs analyzed in fish samples. While in Aroclor 1242, 1254 and 1260, the relative contents of indicator PCBs were 11.90, 30.41 and 31.19 %, respectively. It is obvious that the average relative content of 7 indicator congeners in fish samples was higher than that of Aroclor 1242, but far lower than that of Aroclor 1254 and 1260. The congener 153 showed the highest concentration among indicator congeners and followed in the descending order by the 28, 101, 52, 138 and 180, which also indicate atmospheric deposition was main source of PCBs.

The total concentrations of 12 coplanar PCBs were in the range of 0.03 to 0.28 ng/g



ww, which accounted for 1.66 % - 4.05 % of the total PCBs. The total mono-*ortho* PCBs in different fish samples were higher than the total non-*ortho* PCBs. The predominant coplanar PCB congener was PCB-118, and made up 30.65%-71.21% of the total coplanar PCBs. The predominant non-*ortho* PCB congener was PCB-77, and made up 55.84%-84.83% of the total non-*ortho* PCBs. In contrast, the levels of PCB-126, PCB-169, PCB-167 and PCB-169, were below their MDLs in most samples. The toxicity equivalents (TEQs) were calculated based on the Humans/mammals toxicity equivalence factors (TEFs) reported by the World Health Organization (WHO) (Van den Berg et al., 1998). The total PCB WHO-TEQs values were 0.003-0.044 pg TEQ/g ww. The contribution of mono-*ortho* PCBs to total TEQs were between 79.5% and 97.7%.

The bioconcentration of PCBs in aquatic organisms correlates with the degree of chlorination of biphenyl, the stereochemistry, and lipophilicity (Fox et al., 1994); the congeners with low degree of chlorination are more easily metabolized and eliminated than highly chlorinated ones (McFarland and Clarke, 1989). The occurrence of PCB153, 180, 118, 138, 101, 52 were due to their highly persistent and hydrophobic properties, PCB 153 is considered the most persistent congener and therefore presented higher residue levels in various fish species. While the lower-chlorinated such as PCB 8/5, 18, 28/31, 44, 56, 66, 70/76, 84, and 110 showed higher concentrations compared to other congeners due to their relatively larger individual contributions to total PCB input.

Organochlorine pollutants pose a potential threat especially to human health and aquatic biota. PCBs were included in the dozen "dirty" POPs targeted by the Stockholm Convention. The maximum permissible levels of toxic organic pollutants for protection of human health and aquatic biota have been established in many countries. PCB levels in fish taken from Guanting Reservoir are very low when compared with the guideline value of 500 ng/g proposed by the National Academy of Sciences (USA) and with the 2000 ng/g of the Food and Drug Administration for total PCBs in fish muscle. It can be inferred from the present study that atmospheric deposition was probably a major source of PCB contamination in Guanting Reservoir and the levels of PCBs in fish are lower and they do not pose a risk to human health on the basis of existing related quality guidelines.

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