ORGANIC MICRO-POLLUTANTS IN FISHES AND SHELLFISH FROM LAKE BAIYANGDIAN

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Introduction

Organic micro-pollutants (OMPs), such as hexachlorocyclohexanes (HCHs), dichlorodiphenyltrichloroethanes (DDTs), hexachlorobenzene (HCB), polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs) are ubiquitous pollutants in the aquatic environment, and have been of great concern owing to their persistence, chronic toxicity and bioaccumulation (UNEP, 2011). HCHs and DDTs were the most widely used pesticides in China between 1950s and 1980s. The amounts of HCHs and DDTs produced in China were 4.9 and 0.4 million tons, respectively, accounting for 33% and 20% of the total worldwide productions (Zhang *et al*., 2002). HCB has never been used directly as a pesticide in China, but has been used to produce pentachlorophenol (PCP) and pentachlorophenol-Na (Na-PCP), reagent and fireworks (Wei *et al*., 2007). China began to produce HCB in 1958; cumulative production of HCB after 1988 was 79,278 tons, of which 78,323 tons were used to produce Na-PCP and PCP, accounting for 98.8% of the total production. The rest was mainly used to produce fireworks. Production of HCB was reduced year by year after 2000 and completely stopped in 2004. As a part of China's National Implementation Plan (NIP) for Stockholm Convention, the government prohibited the production and use of HCB in 2009 (Wang *et al*., 2010). Many PBDE-containing plastics and polyurethane foams were once widely used in electrical or electronic products in China. The estimated annual global demand of deca-BDE (i.e., PBDE209) in 2003 as flame retardants (and for other purposes) is 56,400 metric tons (BSEF, 2006). During the early 1970s PBBs were once used as flame retardant additives in plastics, textiles, and other materials. The use of hexabromobiphenyl was, however, banned in the United States in the 1974, shortly after an accident that resulted in widespread contamination of farm products in Michigan. It is likely that the hexabromobiphenyl congener is still produced in some developing countries or in countries with economies in transition. It was reported that approximately 10 000 tons of PCBs were produced from 1965 to the early 1980s in China, accounting for about 1% of the global production (Zheng *et al*., 2010).

Lake Baiyangdian is one of the biggest lakes in the North China, with an area of 366 km^2 that located in the range of 115º45′–116º07′E and 38º43′–39º02′N. It is consisted of 143 small and shallow lakes linked by thousands of ditches. However, during the last decades, with the rapid economic development and population growth in the basin, the lake receives the considerable increasing load of OMPs. As an important base of aquaculture in North China, Lake Baiyangdian provides aquatic products for nearly seven million people. In Baiyangdian Basin, fishes and shellfish are recognized as the most important source of animal protein. Although fish products account for only about 10% of diet, it is one of the main sources of chemical contaminants which may be detrimental to human health (Alcock *et al*., 1998). The objectives of the present research are: (1) to investigate the residue levels of OMPs in the freshwater fishes and shellfish from Lake Baiyangdian; (2) to estimate the risk levels of OMPs to human health through the consumptions of freshwater and shellfish from the Lake.

Materials and methods

In October 2009, Seven species of commonly consumed freshwater fishes and shellfish, including Silver fish (*Hypophthalmichthysmolitrix*, n = 6), Crucian carp (*Carassius auratus*, n = 14), Yellow catfish (*Pelteobagrus fulvidraco*, n = 3), Grass carp (*Ctenopharyngodon idellus*, n = 3), Oriental sheatfish (*Silurus spp*, n = 3), Topmouth culter (*Culter alburnus Basilewsky*, n = 3), and Swan mussel (*Anodonta*, n = 3) were collected from Lake Baiyangdian. In order to eliminate individual diversity, the muscle tissues from 3 to 5 individuals of the same fish species were mixed as one sample. All samples were transported to the analytical laboratory as soon as possible and continued to be stored in the dark at -20°C until analysis.

The following standards were obtained from the Cambridge Isotope Laboratory (USA): 4 HCH congeners (α-HCH, β-HCH, γ-HCH, δ-HCH); 3 DDT congeners (p,p'-DDT, p,p'-DDD, p,p'-DDE), HCB, 22 PBB congeners; 27 PBDE congeners; 27 PCB congeners; 7 $^{13}C_{12}$-labeled PBDEs (PBDE15, 28, 47, 99, 153, 154, and 183); and surrogate standards pentachloro-nitrobenzene (PCNB), 2,4,5,6-tetrachloro-m-xylene (TMX), and PCB209. All solvents used (hexane, acetone, and methylene chloride) were of pesticide grade (Fisher Scientific). Silica gel (100-200 mesh) was also purchased from Merck. Sodium sulfate (granular, anhydrous, Beijing Chemical Reagent Co.) was pre-cleaned with methylene chloride and purified by heating at 450°C for 8 h in a shallow metallic enamel tray. Glassware was soaked, cleaned with chromic solution, rinsed thoroughly with distilled water and acetone, and finally heated in a baking oven (Heraeus, Germany) at temperatures programmed from 40°C to 420°C at a rate of 15°C min^{-1} for 16 h.

The fishes and shellfish samples were first homogenated individually, all freeze-dried. The dried samples were directly ground into powder in a mortar and then sieved to pass a 40 mesh for subsequent analyses. About 2-5 g of these residues from each sample was extracted by an ASE 300 instrument (Dionex, CA, USA). The extract from each sample was then concentrated to about 1 mL by rotary evaporation. The concentrated extracts were further cleaned individually by a multilayer silica gel column. The first fraction eluted with n-hexane (100 mL) was used to concentrate the PCBs, DDTs, and HCB congeners, with the second fraction (eluted with 10% methylene chloride in 80 mL n-hexane) intended for collection of the HCHs, PBB and PBDE congeners. The eluants were concentrated separately to about 1 mL, again by rotary evaporation. The solvent of each sample was concentrated by gentle nitrogen stream at 25°C and redissolved in 200 µL n-Nonane.

Chemical analysis was performed using a Varian CP3800/300 triple-quadrupole system in combination with a capillary VF-5-MS (5% phenyl/95% methyl silicone, 30 m, 0.25 mm i.d., 0.1 µm film thickness, Varian, USA). MS/MS conditions (precursor ions, product ions and collision energies) were optimized in the multiple reaction monitoring (MRM) modes for each of the DDT, HCB HCH, and PCB congeners; samples (of 1 µL) were injected in the splitless mode with a solvent delay set at 6 min; the mass spectrometer was operated in the electron capture negative ionization (ECNI) mode for analysis of PBBs and PBDEs with a capillary VF-5-MS (5% phenyl/95% methyl silicone, 15 m, 0.25 mm i.d., 0.1 µm film thickness, Varian, USA); and PBBs and PBDEs were quantified by monitoring ions $_{79}Br$ and $_{81}Br$.

To estimate the risk levels of OMPs to human health through the consumptions of fishes and shellfish from the Lake. The Actual total dietary intakes (ADI) of OMPs for these residents were estimated according to the following formula:

Actual total dietary intake (ADI) = $C_{fish} \times 42$ g/60 kg,

Where C_{fish} (µg kg^{-1} ww) is the level of each selected OMPs in fish and shellfish muscle; and 60 kg is the average body weight (BW) assumed for adult Chinese. The average daily dietary intake quantity for each adult Chinese resident was 42 g of fish (http://www.stats.gov.cn /tjsj/ndsj/2008/indexch.htm).

Results and discussion

OMPs in fishes and shellfish

γ-HCH (1.59 ng g^{-1} ww), p,p'-DDE (4.54 ng g^{-1} ww), and HCB (3.83 ng g^{-1} ww) were the most predominant OCPs in fishes and shellfish. Lindane usually contains more than 90% of γ-HCH (Li, 1999). γ-HCH is considered more readily metabolized and difficult to be detected (Willett et al., 1998). Yet High levels of γ-HCH (1.59 ng g^{-1} ww) were detected in the fish muscle in the present study. This inconsistency suggested suggested that there might be new inputs of this isomer. In fact, even though γ-HCH has been banned for use in the Chinese agriculture since 1983, small quantities of HCHs are still being used for pesticide application (Li *et al.*, 1998). It should be pointed out that, despite a ban, Lindane may still have been allowed for special applications; use of old stock may have been permissible or illegal use may have occurred. Therefore, the high contents of γ-HCH found in the fishes and shellfish could have come from old and new uses in agriculture.

Technical DDT usually contains 75% of p,p'-DDT, 15% of o,p'-DDT, 5% of p,p'-DDE, and less than 5% of other metabolites (Kim *et al.*, 2004). p,p'-DDE, which is the most persistent metabolite of p,p'-DDT (Connell *et al.*, 1999), was detected at the predominant OCPs levels in fishes and shellfish in the present study. The high proportion of p,p'-DDE found in the samples analysed compared to DDT is consistent with a long period since widespread of this compound was banned in China. This finding strongly suggested that the metabolite must have come from uses of DDT in the past since neither this metabolite nor the parent compound DDT has been used in China since the early 1980s.

In China, the characteristics of HCB production and usage were different from other countries. Large amount of HCB was produced in China from 1958 to 2003, although it was banned in most of countries before the 1980s. Most of HCB was used as an intermediate for producing PCP and PCP-Na (Wang *et al*., 2010). There was no direct use of HCB in agriculture in China. HCB was reported to be a trace contaminant in several pesticides containing chlorine, the following pesticides contained HCB as a contaminant: atrazine, simazine, picloram, pentachloronitrobenzene (PCNB), chlorothalonil, dimethyl tetrachloroterephthalate (DCPA), lindane, technical HCH, PCP and PCP-Na (USEPA, 1998). Pesticide application may be an important source of HCB in the Baiyangdian basin because of widespread use of pesticides and the lack of strict regulations on HCB impurity in pesticides. HCB was also reported as a product of incomplete combustion where chlorine is present in many studies. These combustion processes includes all kinds of waste incineration (hazardous waste, municipal waste, medical waste, and sewage sludge), cement production, iron ore sintering, coal combustion, and biomass burning may also be potential sources of HCB in the Baiyangdian basin. Lipophilic HCB is prone to be bioaccumulated by aquatic organism. Above-mentioned two facts may support the observation that HCB were the most predominant OCPs in fishes and shellfishes.

PBB30 (G.M. = 6.43 pg g^{-1} ww, n = 35) and 80 (G.M. = 19.05 pg g^{-1} ww, n = 35) were the most abundant PBB congeners; which accounted for over 40.6% of the total PBBs in fishes and shellfish samples, PBB209 was not detected in all these samples. Päpke et al. (2010) investigated fish from Europe for 5 PBBs (PBB30, 52, 101, 153, and 209) and found PBB52 and P101 to be the predominant congeners (mean values 6.1 and 3.0 pg g^{-1} ww, respectively. Gieron et al. (2010) found that 93-313 pg g^{-1} ww for PBB101 and 134-443 pg g^{-1} ww for PBB52 were high levels in cod livers from Poland. Luross et al. (2002) observed that PBB153 was the predominant congener (189-2,083 pg g^{-1} ww), followed by PBB101, 52 and 49 in lake trout from the Great Lakes in the USA. These findings suggest that PBB congeners contribution profiles in the fishes and shellfish from Lake Baiyangdian are different from the other from other countries, the low-brominated PBBs found in the fishes and shellfish could have originated from sources other than those products contained in the high-brominated compounds. Another possibility is that high-brominated PBBs may metabolize to their lower-brominated congeners through biological activity in fish body.

PBDE3, 28, and 47 were the most predominant PBDE congeners; PBDE209 were not detected in all fishes and shellfish samples from Lake Baiyangdian. Many published papers showed that BDE47 was the most predominant compound in most aquatic biota samples, including fish (Stapleton *et al*., 2004; Vives *et al*., 2004), snails and swan mussels (Gandhi *et al*.,2006). The dominance of BDE-47 in aquatic species is probably produced by debromination of higher brominated congeners (Stapleton *et al*., 2004). Gandhi et al. (2006) showed that biotransformation pathway was the debromination of BDE-100 to BDE-47 and the debromination of BDE-153 to BDE-99 and then to BDE-47. Vives et al. (2004) observed that nearly all fish samples exhibited rather uniform PBDE distributions. This uniformity is rather significant since it includes lakes from Greenland, the Alps, the Tatra, the Rila, the Pyrenees, and Norway. These facts may explain that PBDE3, 28 and 47 as the most predominant congeners in fishes and shellfish from Lake Baiyangdian are different from the other from other researchs.

Regarding the composition of PCB congeners, PCB8, 18, 28, 52, 66, 101, and 118 were the most abundant PCB congeners in fishes and shellfish from Lake Baiyangdian. From 1965 to 1974, a trichlorobiphenyl (9000 ton) and pentachlorobiphenyl mixture (1000 ton) were the two main PCB commercial products made in China. The trichlorobiphenyl mixture contained 42% chlorine, which was similar to Aroclor1242, and is widely used in transformer and capacitor oils, PCB8, 18, 28, 52, 66, 101, and 118 were the major PCB congeners in trichlorobiphenyl mixtures, accounting for 7.05%, 8.53%, 6.86%, 3.53%, 3.39%, 0.69%, and 0.66% of the total weight, respectively. The trichlorobiphenyl mixtures contained in transformer or capacitor oils are therefore considered as possible sources of the most predominant PCB congeners observed in fishes and shellfish from the Lake Baiyangdian.

OMPs in different Fish species

Yellow catfish, Topmouth culter and Oriental sheatfish were the carnivorous fish; Grass carp belongs to typically herbivorous fish and often feed on phytoplankton, zooplankton, and bacteria; and crucian carp and sliver carp belong to the detritivorous fish .The concentration of DDTs (G.M. = 54.70- 94.12 ng g^{-1}), PBBs (G.M. = 0.15 ng g^{-1} ww), PBDEs (G.M. = 4.35-8.65 ng g^{-1} ww), and PCBs (G.M. = 10.81-11.40 ng g^{-1} ww) in Yellow catfish and Topmouth culter are the first two; the concentration of HCHs (G.M. = 12.10-24.58 ng g^{-1} ww) and HCBs (G.M. = 4.42 ng g^{-1} ww) in Oriental sheatfish and Yellow catfish are the first two (Table 1), which shows that levels of OMPs in the carnivorous fish were significantly higher than that of detritivorous fish (or herbivorous fish) ($P < 0.05$), the biomagnifications of OMPs in food chain is the possible cause of higher OMPs observed in the carnivorous fish.

Actual total dietary intake (ADI) of OMPs and Health implications

The ADIs of γ-HCH, DDTs, HCB, PBBs, PBDEs, and PCBs were 1.11, 5.27, 2.68, 0.04, 1.86, and 3.82 ng/kg bw/d, respectively (Table 2). By and large, the estimated ADIs for these OMPs under study were far lower than these reference doses recommended by WHO (or Health Canada), thus suggesting that residents living around Lake Baiyangdian have been exposed to low levels of OMPs through fish muscle consumption. The total daily dietary intake (ADI) of OMPs through all kinds of food consumption by the local livings should be higher than those only through fish muscle consumption. Therefore, further research is needed into ADIs for these OMPs through other food consumption for those local residents.

Acknowledgements

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Table 1. Levels of Organic Micro-pollutants (in ng g^{-1}ww) in fishes and shellfish from Lake Baiyangdian

Species	*N*	*HCHs*	*DDTs*	*HCB*	*PBBs*	*PBDEs*	*PCBs*
Silver carp (*Hypophthalmichthysmolitrix*)	6	4.58	5.30	1.11	0.05	2.22	6.96
Crucian carp (*Carassius auratus*)	14	3.05	9.09	4.42	0.07	3.50	5.62
Yellow catfish (*Pelteobagrus fulvidraco*)	3	12.10	94.12	4.42	0.15	4.35	10.81
Grass carp (*Ctenopharyngodon idellus*)	3	1.35	0.32	2.75	0.03	1.15	1.97
Oriental sheatfish (*Silurus spp*)	3	24.58	11.17	3.97	0.02	1.16	1.82
Topmouth culter (*Culter alburnus Basilewsky*)	3	4.32	54.70	3.27	0.15	8.65	11.40
Swan mussel (*Anodonta*)	3	1.30	2.92	2.82	0.04	1.32	3.58
Fish and Shellfish	35	3.43	7.53	3.83	0.06	2.66	5.46

Table 2. Actual total dietary intake (ADI) of Organic Micro-pollutants (in ng/kg bw/d) for residents living around Baiyangdian basin, China

Pollutants	*γ-HCH*	*DDTs*	*HCB*	*PBBs*	*PBDEs*	*PCBs*
Provisional Tolerable Daily Intake	5000[a]	10000[a]	170[a]	150000[b]	1000000[c]	1000[d]
Estimated ADIs	1.11	5.27	2.68	0.04	1.86	3.82

[a]: WHO, 2010; [b]: WHO, 1994; [c]: Darnerud *et al*., 2001; [d]: Health Canada, 1996