Journal of Environmental Sciences Vol.17, No.5, pp.722—726, 2005

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2005)05-0722-05 **CLC number**: X132 **Document code**: A

Distribution of persistent organochlorine pesticides in tissue/organ of silver carp (*Hypophthalmichthys molitrix*) from Guanting Reservoir, China

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Abstract: The concentration of organochlorine pesticides (OCPs) in tissues and organs of silver carp (*Hypophthalmichthys molitrix*) from Guanting Reservoir were investigated to evaluate the pollution potential and distribution of OCPs. A total of 16 OCPs were measured and the concentrations were in the range of 1.61—69.01 ng/g wet weight (ww) for total OCPs, 0.16—0.75 ng/g ww for HCB, 0.75—26.80 ng/g ww for ΣHCH (sum of α-, β-, γ- and δ-HCH) and 0.68—35.94 ng/g ww for ΣDDT (sum of *p*, *p'*-DDE, *p*, *p'*-DDD, *o*, *p'*-DDT and *p*, *p'*-DDT). The mean concentrations of total OCPs, HCB, ΣHCH, and ΣDDT were 18.04, 0.96, 7.14 and 9.28 ng/g ww, respectively. Among the organochlorine pesticides, β-HCH and *p*, *p'*-DDE were the most dominant compounds in tissue and organ with the average concentrations of 4.42 and 8.14 ng/g, respectively. The results obtained in this study show that the levels of 16 OCP residues found in silver carps are low and pose no threat to human health and wildlife fed upon them on the basis of existing related quality guidelines. However, recent input of lindane and DDT might still exist in the area investigated and further investigation should be carried on.

Keywords: organochlorine pesticides; silver carp; distribution; Guanting Reservoir

Introduction

Persistent organochlorine pesticides (OCPs) such as HCH and DDT are ubiquitous anthropogenic chemicals in the environment. The products of HCH, DDT have been widely used from 1950 to 1983 in China. These compounds were once used on a large scale in agricultural practices. Being lipophilic, persistent and toxic in nature, these organochlorines are readily accumulated in the tissues of non-target living organisms (Phillips, 1980; Connell, 1995) where they may cause detrimental effects, and they have been detected in a wide range of environmental media, including biota (Fowler, 1990; Tanabe, 1994).

Guanting Reservoir was once one of main water sources as agricultural, industrial and drinking water for the people living in Hebei Province and Beijing Prefecture. But it was contaminated seriously by agricultural, industrial, manufacturing discharge and municipal sewage disposal practices from the upriver estuaries in the 1980s, so it could not be used as drinking water source since then. Fish is on the top of the food web of the water ecosystem and therefore higher concentration of persistent organic pollutants (POPs) is accumulated by biomagnification in its body. Hence fish could be used as bioindicator for water quality (Mormede, 2005). Freshwater fish species represent important sources of food protein in many regions of China. Silver carp is consumed by the population inhabited in the area. This is due to the higher accessibility of theses species for the fishermen and their low costs on the local markets, so its contamination is also a matter of concern for human health.

The contamination of organochlorines (including OCPs and PCBs), polycyclic aromatic hydrocarbons (PAHs) in water, suspended particle matters and sediment in Guanting Reservoir and the Yongding River have been investigated comprehensively (Wang, 2003; 2004), HCB, 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 also investigated (Sun, 2005), but no information is available on the distribution of OCPs in tissue/organ of fish from the reservoir. The objectives of this study were to determine the levels and distribution of persistent organochlorine pesticides in different tissues and organs of silver carps collected from Guanting Reservoir and to compare them with reported data from other inland water bodies and to build health-based standards for fish consumption.

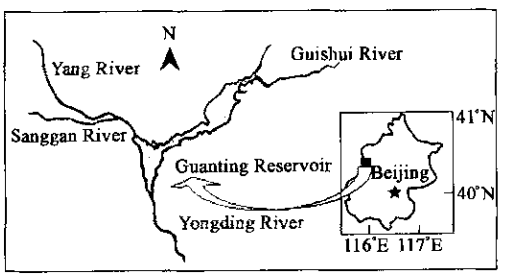

Fig.1 Guanting Reservoir and its location in Beijing

1 Materials and method

1.1 Standards and reagents

A composite stock standard solution of OCPs comprising, α-HCH, β-HCH, γ-HCH, δ-HCH, heptachlor, heptachlor epoxide, α-chlordane, γ-chlodane, endosulfan sulfate, endrin ketone, methoxychlor and *p*, *p'*-DDE, *p*, *p'*-DDD, *p*, *p'*-DDT were purchased from Chem Service Inc., USA. 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℃ for 5 h to remove impurities. Silica gel (100—200 mesh) was activated in drying oven at 130℃ for 16 h and then deactivated with 3% water (w/w) after it was 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.

1.2 Sampling

Silver carp (*Hypophthalmichthys molitrix*) samples were

Foundation item: The Main Basic Research Development Program of China (No. G1999045710) and KIP of CAS (RCEES No. 9902); * Corresponding author

collected from local fishermen living in the Guanting Reservoir in October 2002, which were almost 2 kg in weight and 50 cm in length. The fishes were wrapped in precleaned aluminum foil and stored at −20℃ refrigerator.

1.3 Extraction and clean-up

After thawing, the fishes were dissected and the tissue and organ were well homogenized in a tissue homogenizer. 11 kinds of tissue/organs were analyzed including muscle, intestine, kidney, liver, heart, brain, skin, gill, eye, scale and bladder which were expressed as mus, int, kid, liv, hea, bra, ski, gil, eye, sca and bla in short, respectively. Approximately 10 g ww of homogenized muscle sample were 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 h 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 h. 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℃ for 12 h. The remaining extract was dried with anhydrous sodium sulfate and then concentrated in a rotary vacuum evaporator to about 5 ml. Further lipid removal and clean-up were achieved by using concentrated sulfuric acid wash and silica gel (partially deactivated) chromatography sequentially. The eluates were concentrated by a gentle nitrogen steam to 200 μl, and then transferred to vials for GC injection.

1.4 Analytical procedure

The instrumental analyses were performed by GC6890-μECD (Agilent 6890 series Π equipped with a 63Ni electron capture detector). A DB-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℃ and 280℃, respectively. The GC oven temperature program was carried out as follows: initial temperature 50℃ held for 1 min, increased to 280℃ at 4℃ /min, then held for 10 min. Peak identification of HCB, HCHs (including α-, β-, γ- and δ-HCH) and DDTs (including *p*,*p*′-DDE, DDD, DDT and *o*, *p*′-DDT) was made by comparison of retention time with corresponding standards and confirmed on an Agilent 6890 GC equipped with a model 5973 mass selective detector (MSD). The quantification of the analytes was performed by comparison to external standard.

1.5 Quality control and quality assurance

Blank and recovery experiments were run for fish tissues and organs 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 ww. 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* ($n = 3$) was 5.28%—17.92%. The 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 expressed as nd and 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 90%—110%. Concentrations were expressed in ng/g on a wet weight basis. 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 expressed in ng/g ww and all results were not corrected for recoveries.

2 Results and discussion

2.1 Total OCPs

The concentration of ΣOCP (sum of 16 persistent organochlorine pesticides measured) in tissue/organ of silver carp is shown in Fig.2. The levels and distributions of ΣOCP in different tissue/organs of silver carp were different and are shown in Fig.2. The concentrations of ΣOCP in fish tissue/organ were in the range of 1.61—69.01 ng/g with a mean value of 18.04 ng/g. The maximum concentration of ΣOCP was found in brain (69.01 ng/g), followed by skin (45.06 ng/g) and eye (32.12 ng/g). The concentration of ΣOCP in muscle of silver carp was only 4.29 ng/g, which was similar to that of liver and higher than that of scale and gill.

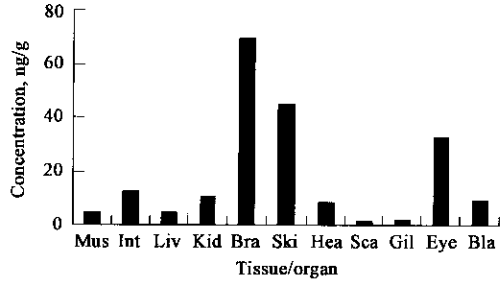

Fig.2 Concentration of ΣOCP in tissue/organ

2.2 HCB

HCB occurs in the environment as a result of wide range used as a fungicide, manufacturing, combustion (Vallack, 1998; Bailey, 2001) and by-product of the manufacture of chlorinated hydrocarbons (Villanueva, 1974). HCB was used as an industrial chemical to produce pentachlorophenol (PCP) in China, whose total production was about 20% of total PCP in the world. HCB is characterized of its toxicity, very strong environmental persistence and long range transport ability, and significant bioaccumulation in biota. The frequencies of detection of HCB were 100 % in tissue and organ of silver carps.

The concentrations of HCB in silver carp fish tissues/organs were in the range of 0.16—0.75 ng/g with a mean value of 0.96 ng/g. The maximum concentration of HCB was found in brain (3.75 ng/g), followed by eye (2.01 ng/g), next kidney (1.07 ng/g), intestine (1.02 ng/g). The concentration of HCB in muscle of silver carp was only 0.26 ng/g, which was higher only than that of scale and gill. Its low body burden may be explained from its persistence and

physical properties which favors relatively high mobility via atmosphere and deposition from its high emissions.

2.3 Chlordanes

Technical chlordane is a broad-spectrum contact pesticide and was extensively used as an agricultural pesticide for corn, as a soil pesticide for home lawns and gardens, and as a termiticide for buildings (Mattina, 1999; Jantunen, 2000; Xia, 2001; Bidleman, 2002). In China, technical chlordane is still being extensively used against termites, with an estimated amount of over 200 t/a in recent years (POPs news, 2002). The α-chlordane and γ-chlordane were measured in the study. The concentrations of chlordanes (sum of α-chlordane and γ-chlordane) in tissue/organ of silver carp were in the range of nd—0.85 ng/g with a mean of 0.21 ng/g. Among chlordane isomers, γ-chlordane was a dominant component with a mean concentration of 0.17 ng/g, which was 3.76 times higher than that of γ-chlordane.

2.4 Heptachlor and heptachlor epoxide

The cumulative production of heptachlor was 17 tons between 1967—1969 in China. After then only a small amount of heptachlor was produced and the production was completely phased out in 1978. The main source of heptachlor was from production and use of chlordane. Heptachlor accounts for 5% in technical chlordane. The frequencies of detection of heptachlor and its degradation product—heptachlor epoxide were 100% and 45%, respectively. Their concentrations in tissue and organ were in the range of 0.01—0.04 ng/g and nd—0.72 ng/g with means of 0.02 ng/g and 0.14 ng/g, respectively.

2.5 HCH isomers

The concentration of ΣHCH (sum of α-, β-, γ- and δ-HCH) in tissue/organ of silver carp is shown in Fig.3.

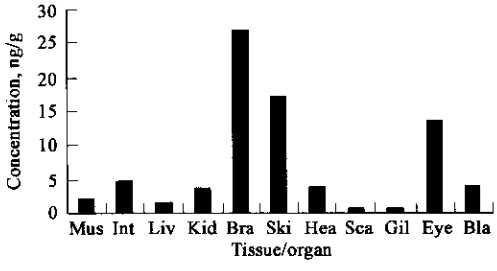

Fig.3 Concentration of ΣHCH in tissue/organ

The concentrations of ΣHCH in tissues/organs were in the range of 0.75—26.80 ng/g with a mean value of 7.14 ng/g for silver carp, which accounted for 35.01%—46.75% with an average of 40.58%. It indicated that HCH was still one of the predominant pollutants among persistent organochlorine pesticides. The maximum concentration of ΣHCH was found in brain (26.80 ng/g), followed by skin (17.01 ng/g) and eye (13.73 ng/g), next intestine (4.59 ng/g), bladder (3.94 ng/g) and kidney (3.55 ng/g) for silver carp.

As to the individual HCHs, the frequencies of detection of α-, β-, γ- and δ-HCH in all tissues and organs in silver fish were 100%, indicating widespread contamination in water environment and biota by this pesticide. The concentration in most tissue/organ of silver carp decreased in the order: β-HCH > α-HCH > γ-HCH > δ-HCH. The β-HCH was the predominant HCH isomer in most tissue and organ

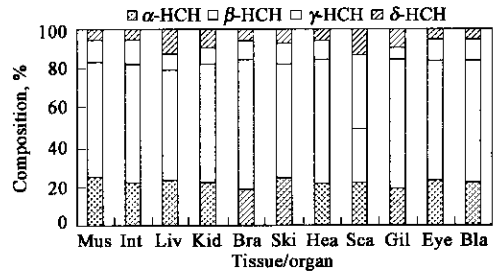

Fig.4 Composition of HCH isomers in tissue/organ

and ranged between 0.21—17.79 ng/g with a mean value of 4.42 ng/g. Environmental β-HCH originates from the residue of extensive use of technical HCH in the past and lindane, which isomerizes in the environment to α- and β-HCH. Being the most persistent HCH isomer, it easily accumulates in lipid-rich tissue/organ. In scale γ-HCH was the predominant isomer.

Percentage compositions of HCH isomers in different fish species are illustrated in Fig. 4. α-, β-, γ- and δ-HCH isomers were also detected in all tissues/organs. The main residue component of HCH isomers in fish was β-HCH, which had the highest concentration in all species and was in the range of 0.21 to 1.77 ng/g, with an average of 0.85 ng/g, followed by γ-HCH and α-HCH from 0.21 to 0.70 ng/g and 0.17 to 0.68 ng/g, with means of 0.40 and 0.36 ng/g, respectively. β-HCH is metabolically inactive and the most persistent one of HCH isomers, which can explain the reason for the highest percentage in the composition of HCH isomers in most tissues/organs.

The ratio of α-HCH to γ-HCH (α/γ) can be used to determine whether recent exposure to technical HCH and/or lindane occurred. The value of α/γ would be in the range of 5.0 to 5.4 for technical HCH and nearly zero for lindane manufactured in China (Wang, 2003). Low ratios, particularly below one, indicate recent lindane input. The ratios found in different tissues/organs of silver carp in present study were in the range of 0.58 to 3.52. The mean ratios (2.22) in silver carp is lower than that of the technical HCH mixture, suggesting that a relatively recent use of lindane (pure γ-HCH—the most toxicological active HCH isomer) maybe have occurred. Nevertheless, the ratio in scale of silver carp studied were only 0.58. The low ratio might further confirm that a fresh input of γ-HCH isomer in the water body investigated have occurred, because the OCPs in scale of fish originate mainly from adsorption from water and/or partition between water and scale, which is almost not metabolized by fish. The new input of γ-HCH might come from illegal application of lindane.

2.6 DDT and its metabolites

The concentrations of ΣDDT (sum of *p*, *p*′-DDE, *p*, *p*′-DDD, *o*, *p*′-DDT and *p*, *p*′-DDT) in tissue/organ of silver carp (Fig. 5) ranged from 0.68—35.94 ng/g with a mean value of 9.28 ng/g, which accounted for 41.06%—58.64% with an average of 47.83%. It indicated that DDT was still the predominant pollutant among persistent organochlorine pesticides. The maximum concentration of ΣDDT was found in brain (35.94 ng/g), followed by skin (26.42 ng/g) and eye (15.37 ng/g), next intestine (6.26 ng/g) and kidney (5.05 ng/g) for silver carp.

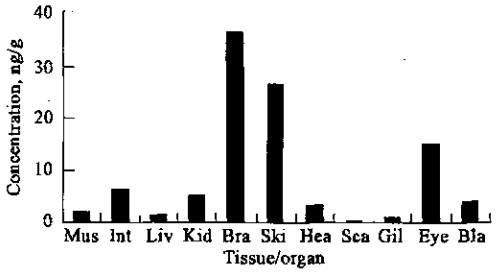

Fig.5 Concentration of ΣDDT in tissue/organ

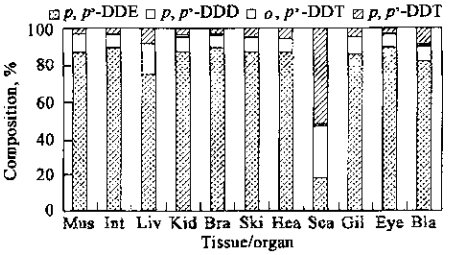

Fig. 6 Composition of DDT isomer and metabolites in tissue/organ

Percentage compositions of p,p'-DDT and its isomer and metabolites in different tissue/organ are illustrated in Fig.6. As to the individual DDTs, the concentration in most tissue/organ of silver carp decreased in the order: p,p'-DDE > p,p'-DDD > p,p'-DDT > > o,p'-DDT. As we know DDT has been extensively used in China and abroad in the past decades and is still significantly applied for vector control against malaria in the low latitudes. Because p,p'-DDE is the most persistent metabolite of p,p'-DDT, p,p'-DDE is dominant component in DDTs in different tissue/organ. The frequencies of detection of p,p'-DDE, p,p'-DDD and p,p'-DDT were 100% in all tissue/organ. The frequency of o,p'-DDT was 82%, which was not detected or lower than its LOD only in liver and gill. The residue concentration of p,p'-DDE in tissue/organ was in the range of 0.12—32.16 ng/g, with an average of 8.14 ng/g, followed by p,p'-DDD, from 0.11 to 2.48 ng/g, and with a mean of 0.70 ng/g. Concentrations of p,p'-DDT and o,p'-DDT in all tissues/organs were significantly lower than those of p,p'-DDE and p,p'-DDD.

The ratio of(p,p'-DDE + p,p'-DDD)/p,p'-DDT was also used to determine whether recent exposure to DDT occurred, with the ratio increasing over time as the DDT degraded. The ratios found in tissues/organs ranged from 0.89 to 33.29 with a mean of 21.13 for solver carp. The ratios were greatly above 1 in most tissues/organs, indicating an old use of technical DDT. Nevertheless, the ratio in scale of silver carp studied were only 0.89. The low ratio might suggest a fresh input of DDT in the water body investigated, because the OCPs in scale of fish originate mainly from adsorption from water and/or partition between water and scale, which is almost not metabolized by fish. The new input of DDT might come from illegal application of DDT and/or the use of technical dicofol mixture, which contains about 3.5%—10.8% DDT. To understand the status of OCPs contamination in silver carps, the levels of HCB, HCHs and DDTs determined in the present study were compared with other results from other water bodies in China and other countries(Table 1). It is clear that there is no evidence to suggest that Guanting Reservoir is seriously contaminated currently with HCB, HCHs, and DDTs compared to the reported data.

Table 1 Representative examples of OCPs in fish samples from different fresh water bodies(ng/g ww)

Locality	Fish	HCB	HCHs	DDTs	References
Mumbai, India	Dogfish	NA	33.73[a]	32.56[a]	Pandit, 2002
Arctic Lake	Lake trout	1.0[a]	1.0[a]	2.8[a]	Wilson, 1995
South Sinai	Bouri fish	20.3[a]	NA	18.2[a]	Nemr, 2004
Texas	Various fishes	NA	NA	76—2117	Wainwright, 2001
San Francisco	Various fishes	NA	NA	5.3—85	Davis, 2002
Baiyangdian Lake	Carp	NA	110.7[a]	124.4[a]	Dou, 1996
Taihu Lake	Various fishes	NA	3.7—132	3.7—23.5	Feng, 2003
Guanting Reservoir	Silver carps	0.96	7.14[a]	9.28	This study

Notes: [a] Average concentration; NA. not available

Organochlorine compounds pose a potential threat especially to human health and aquatic biota. DDT and HCB 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. The residue levels of HCHs and DDTs in fish were far below the national food standard of China (1000 μg/kg for HCHs and 2000 μg/kg for DDTs) (MOH, 1994). The Canadian tissue residue guidelines(CCME, 1999) and US Great lakes water quality initiative criteria(EPA, 1995) for protection of fish-eating wildlife are much lower than human health guidelines with values of 14 and 39 ng/g wet weight respectively. Even compared with these stricter quality guidelines mentioned above, the results in fish species were not exceeded, suggesting that HCHs and DDTs concentration present negligible risk to both humans and wildlife consuming these fish.

3 Conclusions

It can be inferred from the present study that the levels of 16 OCP residues found in silver carps investigated is low and pose no threat to human health and wildlife fed upon them on the basis of existing related quality guidelines. However, recent input of lindane and DDT might still exist in the area, further investigation should be carried on in order to ascertain whether recent input of lindane and DDT into the studied area occurred and where it came from if it really existed.

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(Received for review January 6, 2005. Accepted February 10, 2005)