Accumulation features of organochlorine pesticides and heavy metals in fish from high mountain lakes and Lhasa River in the Tibetan Plateau

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Received 26 May 2006; accepted 21 August 2006

Available online 2 October 2006

Abstract

Organochlorine pesticides and heavy metals were analyzed in fish from remote mountain lakes and Lhasa River in Tibetan Plateau. Concentrations of Σ HCH, Σ DDT and HCB in fish muscles were in the range of 0.13–2.6 ng/g, 0.78–23 ng/g, 0.31–3.2 ng/g based on wet weight, respectively. Heavy metals were routinely found with different levels in fish. The Cu and Zn levels were relatively high with the maximum concentrations reaching 2.0 and 6.9 μ g/g, respectively. Accumulation of OCPs and heavy metals are quite different in tissues and organs. Gill seems to accumulate more OCPs due to absorption by its larger surface per tissue. Heavy metal levels were generally high in liver, eggs and gill, and low in brain and muscle. Results from this study demonstrate that the Tibetan Plateau functions as a regional contaminant convergence zone by long-range atmospheric transport and orographic cold trapping.

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Keywords: HCH; DDT; HCB; POPs; Long-range atmospheric transport; Alpine lakes

1. Introduction

Evidences that alpine regions function as regional convergence zones for selected persistent organic pollutants (Drooge and Grimalt, 2004; Daly and Wania, 2005; Shen et al., 2005) and heavy metals (Yang et al., 2002) are increasing. Some anthropogenic contaminants are capable of undergoing long-range transport (LRT) in the atmosphere and undergo dry and/or deposition upon encountering relatively cold environments such as those at high elevations (Blais et al., 1998; Davidson et al., 2003) or high latitude (Atlas and Giam, 1981). A precipitate, often in the form of snow, is believed to be an efficient scavenger of atmospheric contaminants (Franz and Eisenreich, 1998; Carrera et al., 2001). Orographic effects often result in a high rate of precipitation in mountain regions.

Consequently, it is of concern whether the Tibetan plateau may be acting as a cold trap for either airborne semi-volatile contaminants or heavy metals because of its dramatic elevated

temperature and frequent precipitation. This phenomenon is exacerbated by highly populated and rapidly industrializing countries surrounding this region. In particular, India and China exhibit high levels of pesticide usage and own several polluting industries. It has been estimated that about 5×10^5 tonnes of DDT and more than 10^6 tonnes of technical HCH have been used in India (Li and Macdonald, 2005). From the 1950s until its ban in 1983, China produced and used 2.7×10^5 tonnes of DDT as well as 4.9×10^6 tonnes of technical HCH (Li et al., 1998). Lindane (pure γ -HCH) and DDT are still being used in lower amounts to control certain insects in tropical and sub-tropical countries including India and China (UNEP Regional Report, 2002). These contaminants will possibly impact the Tibetan ecosystems for years to come.

Alpine aquatic ecosystems offer “natural experiments” of exposure to atmospheric long-range transported contaminants. Thus, pollutants concentrated in organisms from aquatic environment can be uniquely related to atmospheric deposition in these areas. Fish inhabiting alpine lakes or rivers will be more vulnerable to pollutants because low temperatures and oligotrophic status cause low growth rate, which may result in

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Fig. 1. Map showing the study area and sampling sites.

accumulating higher concentration of pollutants (Kidd et al., 1995). To our knowledge, limited or no information is available concerning bioaccumulation of pollutants by fish inhabiting Tibetan lakes or rivers. In this study, we selected two high mountain lakes and an adjacent river as study areas. Fish samples were collected to explore OCPs and heavy metals in this region, and thus to validate whether the Tibetan Plateau functions as a regional contaminant convergence zone.

2. Materials and methods

2.1. Study area and sample collection

Two high mountain lakes (Nam Co Lake and Yamdro Lake) and Lhasa River were selected for study (Fig. 1). Nam Co is the largest lake (1920 km² in area) in Tibet with an altitude of 4718 m above sea level and is regarded as one of the remotest and least disturbed aquatic environment. Yamdro Lake is situated between Nhagartse and Gongkha with a coverage of 638 km². The two lakes are among the three famous holy lakes in Tibet. The Lhasa River originates from Nyangqentanglha Mountain on the Tibet Plateau. These areas are largely far from the production and usage of OCPs or industrial input of heavy metals.

Fish samples were collected in August 2005 and they were carefully identified according to the catalog provided by Wu and Wu (1992). Fish species belong to the same family (Cyprinidae) and subfamily (Schizothoracinae),

which are unique in the Tibetan Plateau. The geographical position and related information of the samples are listed in Table 1. All the samples were immediately transported to the laboratory and frozen at −20 °C until use. All the equipments used for sample collection, transportation, and preparation, were free from contamination.

2.2. Materials

The OCPs standards of α -HCH, β -HCH, γ -HCH, δ -HCH, p,p' -DDT, o,p' -DDT, p,p' -DDE, p,p' -DDD and hexachlorobenzene (HCB) were purchased from the National Research Center for Certified Reference Materials of China. The original storage solution was 100 mg/l in *n*-hexane and further diluted to obtain the desired concentrations. The internal standard 2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl (PCB-209) was purchased from Accustandard, Inc. USA. Florisil (60–100 mesh, Shanghai Chemical Reagent Factory, China) was activated in the oven at 150 °C for 8 h. The other reagents including *n*-hexane, dichloromethane and anhydrous sodium sulfate, were of analytical-grade (Beijing Chemical Reagent Factory, China). Dichloromethane and *n*-hexane were redistilled before use and a solvent check showed that there was no interference with the target compounds for acceptance. Sodium sulfate was activated in a muffle furnace at 600 °C for 8 h.

For metal analysis, nitric acid and hydrogen peroxide were guarantee reagents (GR) and purchased from Merck and Beijing Chemical Reagents Company, respectively. Element In (115) in 1% v/v nitric acid was used as calibration internal standard. All glass vessels were soaked in 1:1 nitric acid for at least 12 h then rinsed with de-ionized water for several times. The PTFE containers were boiled with

Table 1
Description of geographical location and biological data of the fish samples

Lake/River	Latitude and longitude	Altitude ^a (m)	No. of fish	Fish species	Weight (g)	Length (cm)	Moisture content (%)	Lipid content in muscle (%)
Nam Co Lake	30°53.643'N 90°52.095'E	4718	4	<i>Gymnocypris namensis</i>	245±7	29±1	79.90	1.0512
Yamdro Lake	29°85.475'N 90°28.351'E	4441	4	<i>Gymnocypris waddellii</i>	369±36	32±2	79.12	0.8496
Lhasa River	29°39.543'N 91°05.444'E	3629	3	<i>Ptychobarbus dipogon</i>	203±3	26±0	81.03	1.0001
			2	<i>Schizopygopsis stoliczkae</i>	305±12	31±5	83.44	1.9164
			3	<i>Schizopygopsis younhusbandi</i>	158±25	24±1	80.22	1.3546
			2	<i>Schizopygopsis microphalus</i>	309±9	30±3	78.00	1.2548
			2	<i>Oxygymnocypris stewartii</i>	230±18	29±4	79.65	2.2324

^a Meters above sea level.

Table 2

The recoveries, method detection limits (MDLs), and the relative standard derivation (RSD) of the used analytical procedure

Pesticides	Recovery (%)	MDLs (ng/g)	RSD (%) ($n^a=5$)
α -HCH	98	0.019	7
β -HCH	90	0.046	11
γ -HCH	95	0.022	6
δ -HCH	88	0.025	13
HCB	96	0.038	4
p,p' -DDE	103	0.029	12
p,p' -DDD	96	0.034	12
o,p' -DDT	92	0.051	10
p,p' -DDT	90	0.055	13

^a Five times repeated measurements.

Table 3

Determination of heavy metal contents ($\mu\text{g/g}$, dry wt) in mussel tissue of certified reference material (GBW 08571)

	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Pb
Certified	10.2±0.92	0.94±0.03	1.03±0.07	7.7±0.5	138±5.5	6.1±0.61	3.65±0.09	4.5±0.32	1.96±0.06
Found	9.0±0.24	0.85±0.03	1.01±0.08	7.2±0.6	112±7.8	5.5±0.55	2.95±0.03	3.6±0.24	1.84±0.04
Recovery (%)	88.2	90.4	98.1	93.5	81.2	90.2	80.8	80.0	93.9

50% v/v nitric acid and the PET bottles were immersed in 5% v/v nitric acid for 24 h followed by rinsing with de-ionized water.

2.3. Sample pretreatment and instrumental analysis

The analytical procedure of OCPs was similar to the method in our previous work (Yang et al., 2004). Briefly, a suitable amount of well homogenized tissue was ground with activated sodium sulfate until a fine powder was obtained. The samples were extracted with 100 ml of hexane–dichloromethane (1:1 v/v) using sonication method for 40 min, then stood overnight (about 10 h) and was sonicated for 20 min again. Lipid content was determined gravimetrically using 20% of the extract. The other extract was subsequently concentrated on a rotary evaporator to about 1–2 ml in a water bath at about 35 °C and then purified on a glass column packed as follows: glass wool, 6 g of activated Florisil and 2 g of sodium sulfate. The packed column was pre-rinsed with 70 ml of hexane–dichloromethane (1:1 v/v). The elution was subsequently carried out using 50 ml of hexane containing 25% (v/v) dichloromethane. The effluent was concentrated to 2–3 ml and then was reduced finally to a volume of 0.5 ml under a gentle stream of pure nitrogen for GC analysis.

The OCPs in the extracts were analyzed by an Agilent 6890A gas chromatography (GC) equipped with a ^{63}Ni electron capture detector (micro-ECD) (USA). The separation was achieved with a HP-5 fused silica capillary column (30 m length $\times$ 0.25 mm i.d.) coated with 5% dimethylsiloxane (film thickness 0.25 μm). The whole system was controlled by a Hewlett Packard workstation. The oven

Table 4

Concentrations of the selected OCPs in fish muscles from high mountain lakes and Lhasa River in the Tibetan Plateau (ng/g, wet wt)

Location	Species	HCB	α -HCH	β -HCH	γ -HCH	δ -HCH	Σ HCH	p,p' -DDE	p,p' -DDD	o,p' -DDT	p,p' -DDT	Σ DDT
Nam Co Lake	<i>Gymnocypris namensis</i>	3.2	1.63	0.66	0.28	<0.025	2.6	14	2.2	5.7	1.0	23
Yamdro Lake	<i>Gymnocypris waddellii</i>	0.85	0.43	0.96	0.17	0.031	1.6	2.5	0.26	<0.051	<0.055	2.8
Lhasa River	<i>Ptychobarbus dipogon</i>	0.29	0.18	0.056	0.050	0.027	0.32	1.7	0.23	0.42	0.14	2.5
	<i>Schizopygopsis stoliczkae</i>	0.58	0.068	0.031	0.027	0.026	0.13	0.63	0.047	0.090	<0.055	0.78
	<i>Schizopygopsis younhusbandi</i>	0.80	0.46	0.18	0.11	<0.025	0.75	2.4	0.39	0.31	0.20	3.3
	<i>Schizopygopsis microphalus</i>	0.31	0.14	0.11	0.040	<0.025	0.29	3.8	0.17	0.11	<0.055	4.1
	<i>Oxygymnocypris stewartii</i>	0.91	0.45	0.17	0.11	<0.025	0.74	5.5	0.65	0.82	0.38	7.4

Fig. 2. Compositions of HCHs (a) and DDTs (b) in fish muscles from the Tibetan Plateau. (N1: *Gymnocypris namensis*; Y1: *Gymnocypris waddellii*; L1: *Ptychobarbus dipogon*; L2: *Schizopygopsis stoliczkae*; L3: *Schizopygopsis younhusbandi*; L4: *Schizopygopsis microphalus*; L5: *Oxygymnocypris stewartii*).

temperature program conditions were 80 °C held for 2 min, ramped at 10 °C/min to 140 °C, and then at 4 °C/min to 280 °C, and held for 10 min. The temperatures of injector and detector were 250 °C and 330 °C, respectively. About 1 μl of extract was injected in the splitless mode. Highly pure nitrogen was used as the carrier gas with a flow rate of 1.0 ml/min and the make-up gas was controlled at 59 ml/min. The main peaks were confirmed in selected samples by a Finnigan GC–MS–MS (PolarisQ, USA) using a 50-m DB-5 MS column with He carrier gas.

The selected organochlorine compounds were identified by retention time comparison by reference to the corresponding standard. Quantification was performed by the internal standard method using peak area of the compounds. The correlation coefficients (r) of calibration curves of OCPs were all higher than 0.99. For each set of six samples, a procedural blank and a spiked sample with standards were run to check for the interference and cross-contamination. The IDLs of the instrument were in the range of 14 pg for α -HCH to 25 pg for α -HCH and the IQL were basically equal to the IDL. The method detection limits (MDLs) were described as 3:1 signal versus noise value (S/N). Table 2 illustrated the recoveries, MDLs, and the relative standard derivation (RSD) of the method. The MDLs ranged from 0.019 ng/g for α -HCH to 0.055 ng/g for p,p' -DDT. The spiked recoveries of OCPs using 1 ng of composite standards were in the range of 88–103% and the RSD values with five times repeatedly determined was less than 13%.

For heavy metals analysis, approximately 0.2 g (wet wt) of soft tissues was weighed in a PTFE digestion container. Each sample was added with 2 ml of

Table 5
Concentrations of heavy metals in fish muscles from high mountain lakes and Lhasa River in the Tibetan Plateau ($\mu\text{g/g}$, wet wt)

Location	Species	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Pb
Nam Co Lake	<i>Gymnocypris namensis</i>	0.61	0.041	0.094	2.0	6.9	0.24	0.36	0.025	0.047
Yamdro Lake	<i>Gymnocypris waddellii</i>	0.24	0.040	0.12	1.1	4.4	0.27	1.0	0.024	0.079
Lhasa River	<i>Ptychobarbus dipogon</i>	0.19	0.041	0.11	0.33	3.3	0.067	0.23	0.024	0.024
	<i>Schizopygopsis stoliczkae</i>	0.11	0.022	0.11	0.83	2.5	0.087	0.18	0.013	0.026
	<i>Schizopygopsis younhusbandi</i>	0.46	0.048	0.14	0.88	5.3	0.12	0.27	0.027	0.065
	<i>Schizopygopsis microphalus</i>	0.49	0.057	0.19	0.79	4.1	0.14	0.26	0.029	0.055
	<i>Oxygymnocypris stewartii</i>	0.20	0.046	0.22	0.41	3.0	0.080	0.31	0.023	0.027

concentrated nitric acid and left to predigest overnight at 40 °C. After cooling, 2 ml of 30% hydrogen peroxide was added. Thereafter the container was covered and placed in a high-pressure stainless steel bomb then put in an oven. The oven temperature was increased to 160 °C and kept for 8 h. After cooling, the solution was diluted with Milli-Q water and transferred into PET bottle to 25 g.

X series inductively coupled plasma mass spectrometer (ICP–MS) (Thermo Electron Co. Ltd, USA) was used for the determination of metals. All analyses were repeated thrice by internal calibration method. The accuracy of the method was validated by the determination of mussel (GBW 08571) certified reference materials, and the results are reported in Table 3. The found values of all elements were in good agreement with the certified values, with the recoveries ranging from 80.8% to 98.1%, suggesting that the proposed method was feasible in the determination of heavy metals in biota samples.

3. Results and discussion

3.1. Concentrations and patterns of OCPs

Organochlorine concentrations in fish muscles are summarized in Table 4. DDT is the predominant pollutant. Concentrations of ΣHCH , ΣDDT and HCB were in the range of 0.13–2.6 ng/g (mean 0.91 ng/g), 0.78–23 ng/g (mean 6.3 ng/g), 0.31–3.2 ng/g (mean 1.0 ng/g) based on wet weight, respectively. The highest OCP concentrations were found at higher altitude, e.g. the remotest sites (Nam Co). This result might be explained by “cold condensation” theory, some semi-volatile organic compounds with high vapor pressure condense at cold latitude or altitude (Grimalt et al., 2001). The OCP concentrations in fish muscles in this study are comparable with those in fish from European high mountain lakes (Vives et al., 2004, 2005). Pearson’s correlation of the concentrations of ΣHCH , ΣDDT and HCB are significantly correlated with each other (HCB and ΣHCH : $r=0.901$, $p<0.01$; HCB and ΣDDT : $r=0.939$, $p<0.01$; ΣHCH and ΣDDT : $r=0.778$, $p<0.05$), which may suggest a similar origin.

Composition differences of HCH isomers or DDT congeners in the environment could indicate contamination source (Iwata et al., 1995). The relative percentage of HCHs is shown in Fig. 2a. The compositions

Table 6
Correlation matrixes of the concentrations of heavy metals in fish muscle

	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Pb
Mn	1								
Co	0.588	1							
Ni	−0.050	0.594	1						
Cu	0.690	−0.091	−0.513	1					
Zn	0.891	0.323	−0.361	0.854*	1				
As	0.476	0.079	−0.363	0.779*	0.694	1			
Se	−0.067	0.010	−0.158	0.249	0.203	0.781*	1		
Cd	0.696	0.946	0.318	0.105	0.542	0.281	0.149	1	
Pb	0.458	0.383	−0.085	0.399	0.546	0.751	0.711	0.521	1

Bold: Correlation is significant at the 0.01 level (two-tailed).

*Correlation is significant at the 0.05 level (two-tailed).

of HCHs changed with different sampling sites and different species. Technical HCH usually contains 55–80% of α -HCH, 5–14% of β -HCH, 8–15% of γ -HCH and 2–16% of δ -HCH, respectively (Willett et al., 1998). The physicochemical properties of these isomers are different. β -HCH has the lowest water solubility and vapor pressure, which is the most stable and relatively resistant to microbial degradation. Also it should be noted that α - and γ -isomer can be photolytically converted to β -HCH in the environment (Walker, 1999). In our samples, the average concentrations of α -, β -, γ -, and δ -isomers were observed to contribute 27–64% (mean 52%), 18–40% (mean 30%), 11–18% (mean 14%) and 0–17% (mean 4%), respectively. A predominance of the α -isomer in environmental samples reflects pollution of HCH technical mixtures. India and China are probably the major contributors of OCP pollution in Tibetan Plateau because of their immediate vicinity and because of their extensive application of technical HCH in the past. The transport and fate of pollutants to the Tibetan Plateau are expected to be influenced by two distinct monsoon systems that operate in the region: the Southwest or summer monsoon and the Northeast or winter monsoon (Pant and Rupa Kumar, 1997). The contribution of β -HCH was elevated about 3 times compared with the original composition, which might be attributed to different properties of the isomers. Migration and transformation can account for the elevated β -isomer component.

Technical DDT generally contains 75% p,p' -DDT, 15% o,p' -DDT, 5% p,p' -DDE, and <5% for others. DDT can be biodegraded to more persistent DDE under aerobic conditions and to DDD under anaerobic conditions. The relative percentage of DDTs is shown in Fig. 2b. The congener of p,p' -DDE was found to be a major component ranging from 62% to 93% with a mean of 77%. Comparing the ratio of p,p' -DDT to p,p' -DDE in the fish muscle from our study (mean 0.05) with the fish muscle from other regions such as the European high mountain regions (mean 0.39) (Vives et al.,

Fig. 3. Body distributions of OCPs in *Gymnocypris waddellii* from Yamdro Lake.

Fig. 4. Body distributions of heavy metals in *Gymnocypris waddellii* from Yamdro Lake.

2004) comprehended a low ratio in the Tibetan Plateau. However, the ratio of *o,p'*-DDT to *p,p'*-DDT, ranging from 0.9 to 5.7 (mean 3.3), was surprisingly higher compared with the original composition of technical DDT (*o,p'*-DDT/*p,p'*-DDT=0.2). According to a recent study by Qiu et al. (2005), Dicofol type DDT pollution can elevate the ratio of *o,p'*-DDT to *p,p'*-DDT and of *p,p'*-DDE to *p,p'*-DDT. Therefore judging the DDT source by the ratios between congeners should be cautious because of the current production and usage of Dicofol in Asian countries such as China and India (Plassche et al., 2000).

3.2. Heavy metal concentrations

Several studies confirmed that heavy metals can reach the remote alpine regions by long-range transport through the atmosphere (Zechmeister, 1995; Schilling and Lehman, 2002; Yang et al., 2002). Anthropogenic heavy metals can affect the biogeochemical processes and the ecosystem's health for a long time.

The contents of the selected heavy metals in fish muscles are summarized in Table 5. It is apparent that all samples contaminated with different levels of heavy metals. The Cu and Zn levels were extraordinarily high with the maximum concentrations of 2.0 and 6.9 $\mu\text{g/g}$, respectively, in *Gymnocypris namensis* from Nam Co Lake. Mn, Ni and Se that were less than Cu and Zn, still higher than other metals such as Cd, Pb, Co among which As, Cd, Pb should be taken into particular account due to their harmful effects. For instance, Pb and its salts were capable of damaging the nerve system, hematosis and kidney in the body of fish or human being (Chuang et al., 2004). As a whole, all metal concentrations were below the maximum permissible levels (MPLs) according to the codes of WHO (1982).

Results of correlation analyses (bivariate correlations with Pearson's correlation coefficients) among these heavy metal concentrations are listed in Table 6 ($P < 0.05$ or $P < 0.01$). There were some correlations among concentrations of metals found in the samples (e.g., Mn–Zn, Co–Cd, Zn–Cu, As–Cu, As–Se). This result can provide some information on the metal origin or a certain bioaccumulation mechanism by an organism.

3.3. Body distribution of OCPs and heavy metals

In order to investigate the distribution status of the pollutants in the organism's tissues, *Gymnocypris waddellii* from the Yamdro Lake were dissected into six parts, i.e., muscle, gill, brain, intestine, liver and egg, and concentrations of OCPs and heavy metals of each part were

measured. The fish studied were about 10 years old according to their body weight and length (Chen et al., 2002) and therefore monitored data reflected a long-term environmental exposure. The results demonstrate that different tissues of fish have quite different concentrations of OCPs (Fig. 3). Prominently, the sum of DDTs was found much higher than that of HCB and sum of HCHs in the tissues or organs. However, the three kinds of OCPs show the similar concentration distribution in the tissues or organs. Gill, liver and brain seem to accumulate relatively higher concentrations of OCPs than other tissues. Particularly, much higher levels of OCPs were found in the gills, which can be explained by its larger surface areas per gram of tissue since gill can continuously transfer the organic pollutants from both water and suspended particles onto its surface.

Compared with the OCPs, the distribution of heavy metals was different (Fig. 4). In general, concentrations were high in liver, eggs and gill, and low in brain and muscle. The highest Cu concentration (59 $\mu\text{g/g}$ wet wt) in liver was about sixty-fold as high as those in the muscle of the fish. Interestingly, some specific distribution was also observed for the selected metals. Greater concentrations of Mn, Co, Ni and Cd were found in gill. However, levels of Zn, As, Se and Pb in the eggs were relatively high, which means that the elimination of these heavy metals through egg laying is significant.

4. Conclusion

Accumulation of the OCPs and heavy metals in fish from alpine aquatic ecosystems in Tibetan Plateau was studied. Results from this study validate that the Tibetan Plateau acts as a regional contaminant convergence zone. Because of the limited samples, this research do not allow for a complete understanding of pollution status and the factors regulating the transport of persistent pollutants such as OCPs and heavy metals in a remote environment, hence a comprehensive study is still needed.

Acknowledgements

This research was supported by the National Basic Research Program of China (2003CB415001). We express our gratitude to Prof. Shichang Kang, Zonghu Huang and other members in Nam Co Station for their kind help in sample collection. We also greatly appreciate suggestions from anonymous referees for the improvement of our paper.

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