ACCUMULATED AMOUNTS OF PERSISTENT ORGANIC POLLUTANTS IN *CARASSIUS GIBELIO LANGSDORFII* (CRUCIAN CARP) IN JAPAN

Iwamura T[1], Jinya D[1], Kadokami K[1] , Fukumoto M[2] ,Nunokawa T[2], Koezuka T[2]
[1]Graduate School of Environmental Engineering, The University of Kitakyushu, [2]Kitakyushu City Institute of Environmental Sciences, Shin-ike 1-2-1, Tobata, Kitakyushu, Fukuoka 804-0082

Abstract
Our investigations were carried out into the accumulated amounts of 10 groups of Organochlorine Pesticides covered by the Stockholm Convention on Persistent Organic Pollutants (POPs) together with HCHs in freshwater fish and bottom sediments in Japan. Samples of the fish (Crusian carp, *Carassius gibelio langsdorfii)* and bottom sediment were taken from 14 river and lake locations in Japan during autumn periods between 2003 and 2005. Furthermore, differences between the sexes in the reproductive stage for *Carassius gibelio langsdorfii* as well as transfer to the eggs were investigated using samples from Murasaki River in Kitakyushu City. Almost all target compounds were found in both fish and the bottom sediment samples. During the spawning season, concentrations in muscle tissue were found to be higher in male fish than in female fish, and this was thought to be due to transfer from the parent body to the eggs. The transfer ratio from the female body to the eggs was calculated to be 19.2%–34.1% assuming the concentration in muscle tissue was equal to the average body concentration. Furthermore, a comparison of bioaccumulation from the growth environments for *Carassius gibelio langsdorfii* at each location was carried out using the Biota-Sediment Accumulation Factor (BSAF).

Introduction
Persistent organic pollutants (POPs) are toxic, persistent, bioaccumulative, and mobile over long distances. As such, they are substances that can accumulate in ecosystems located far from their emission sources. Following the Stockholm Convention on Persistent Organic Pollutants coming into effect on May 17, 2004, efforts directed at these substances have come to be carried out on a global basis. Substances prescribed in the Stockholm Convention include polychlorinated biphenyls, DDTs, and Chlordanes, which are already regulated in some countries and which to date have been widely monitored.[1–4] Kajiwara et al. elucidated the amounts of dioxins, one of the POPs, accumulated in *Carassius gibelio langsdorfii* sampled from rivers and lakes in Japan.[5] Using the same samples that Kajiwara et al. used in their report investigating dioxins, here we report the results of accumulation amount investigations for *Carassius gibelio langsdorfii* and bottom sediments for 11 substance groups comprising all substance groups prescribed in the Stockholm Convention (excluding dioxins, PCBs, and toxaphene) together with HCHs.

Materials and Methods
Sample collection
A total of 14 sites were selected for investigation (Figure 1; Table 1) and included sites in large cities, small- and medium- size cities, agricultural areas, and other areas. Sampling of bottom sediments and the target species *Carassius gibelio langsdorfii* (*Carassius auratus grandoculis* in the case of Lake Biwa) was undertaken during autumn periods in 2003–2005. At least 30 fish samples measuring 20–25 cm in length were taken from each sampling location, and mixtures of equal quantities of muscle tissue from each fish were analyzed. In addition, 10 male samples and 20 prespawning female samples were taken from the Murasaki River in Kitakyushu City in April 2003 for the purpose of investigating differences between the sexes during the spawning period and transfer to the eggs. Mixtures of equal quantities of muscle tissue from each sample and egg masses were analyzed.

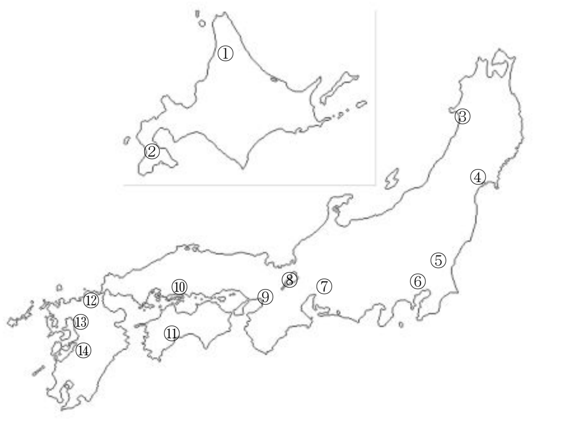

Fig.1 The locations of the 14 sampling sites in Japan (refer to Table 1)

Table 1 Sampling sites

Collecting site	Sampling area	No.	Area category	Collecting date
Tama river	Tokyo	⑥	Large city	Oct. 4, 2003
Tenpaku river	Aichi	⑦		Nov. 8 - 9, 2004
Yamato river	Osaka	⑨		Oct. 14 - 18, 2003
Masuda river	Miyagi	④	Small- and medium-size city	Nov. 8, 2004
Kurose river	Hiroshima	⑩		Oct. 20, 2005
Murasaki river	Kitakyushu, Fukuoka	⑫		April. 23 - 28, 2003 Oct. 21, 2003
Midori river	Kumamoto	⑭	Agricultural area	Nov. 1 - 16, 2004
Hachiro-gata lake	Akita	③		Oct. 11 - 29, 2003
Kasumigaura lake	Ibaraki	⑤		Oct. 4 - 22, 2005
Lake Biwa	Shiga	⑧		Oct, 21 - 22, 2003
Chikugo river	Asakura(Haki), Fukuoka	⑬		Oct. 2 - 16, 2003
Teshio river	Horonobe, Hokkido	①	Other area	Oct. 31- Nov. 2, 2004
Junsai-numa pond	Nanae, Hokkaido	②		Oct. 13 - 21, 2003
Shimanto river	Kochi	⑪		Oct. 23 - Dec. 8, 2003

Analysis of POPs

Analysis was carried out in accordance with the Ministry of Environment's POPs Monitoring Manual. Surrogate substances (^{13}C labeled compounds) were added to a 20-g sample, acetone was added, and after shaking and centrifugation, the supernatant liquid was collected and the residue Soxhlet-extracted for more than 6 hours using 300ml of dichloromethane. Sodium chloride solution (5%) was added to the resulting extract and the organic layer was collected. After dehydrating and changing the solvent to hexane, the extracts were cleaned up with Florisil column, and then bottom sediment samples were also desulfurized. Measurements were taken using high resolution GC/MS (≧10,000).

Results and Discussion

Amounts of POPs accumulated in C*arassius gibelio langsdorfii* and bottom sediments

All target substances except *trans*-heptachlor epoxide were detected in the muscle tissue of *Carassius gibelio langsdorfii*. The detected concentrations of each substance group are shown in Figures 2 and 3. Concentrations of chlordanes were the highest in large cities, small- and medium-size cities, and the Shimanto River. Concentrations of DDTs tended to be high at other locations. Hexachlorobenzene (HCB) levels in fish in the Tama River were higher than at other locations. In addition, residual mirex, which has never been manufactured and used in Japan, was detected in all fish samples, indicating that it is residual over a wide area in freshwater areas in Japan.

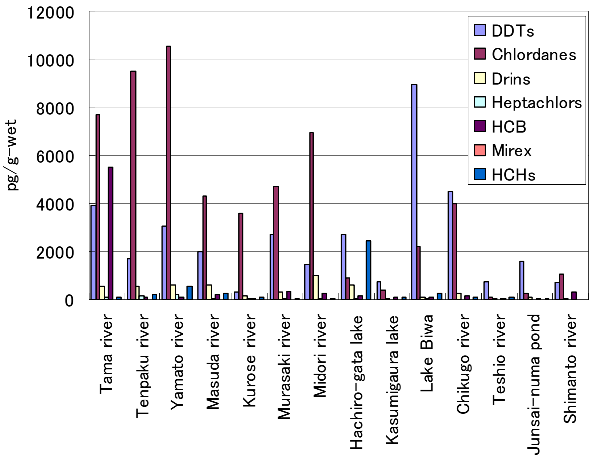

Fig.2 Concentrations of POPs in muscle tissue

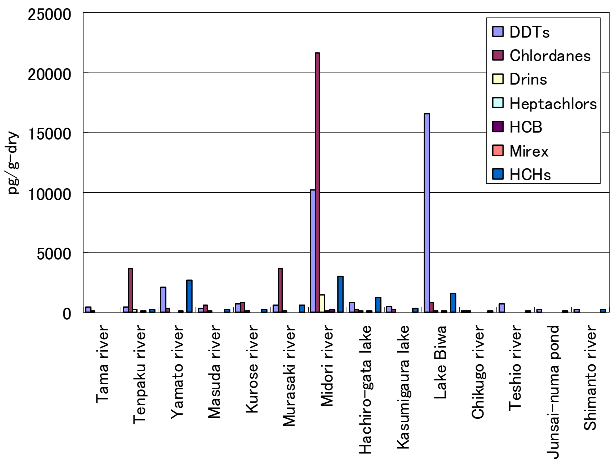

Fig.3 Concentrations of POPs in bottom sediments

With regard to concentrations of chlordanes, the proportions of *cis*-chlordane and *trans*-nonachlor were high in all fish and bottom sediment samples from all locations (Figures 4 and 5). The compositions of the DDTs in the fish samples were very different from those in the bottom sediments (Figures 6 and 7), and differences in accumulation conditions were observed.

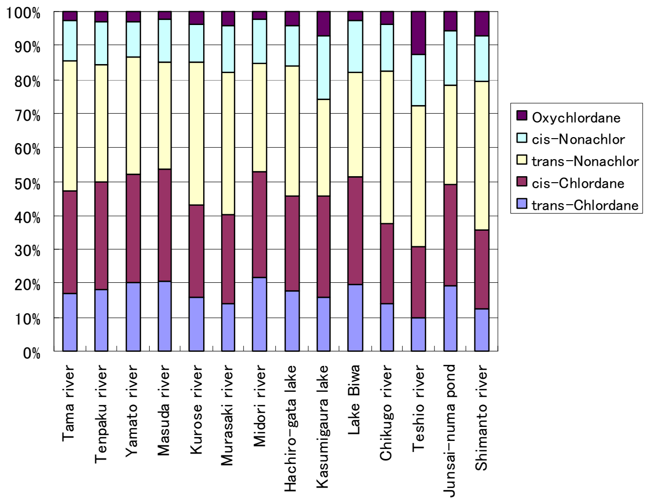

Fig.4 Compositions of chlordanes in muscle tissue

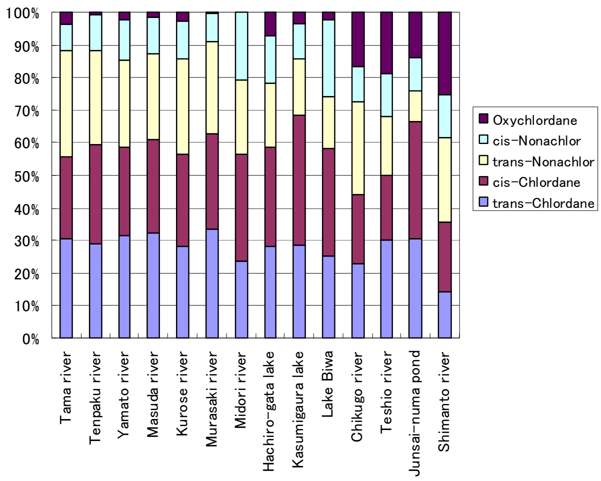

Fig.5 Compositions of chlordanes in bottom sediments

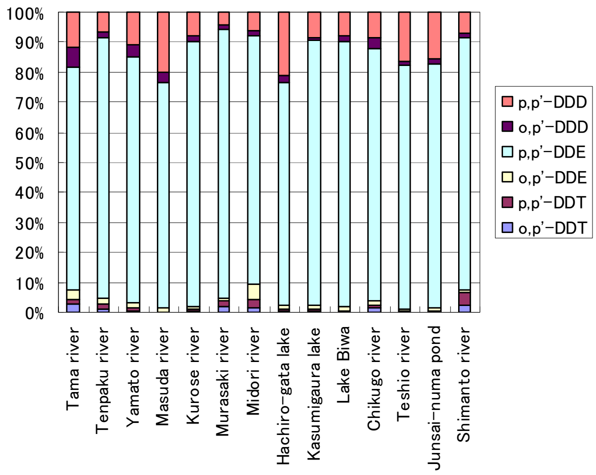

Fig.6 Compositions of DDTs in muscle tissue

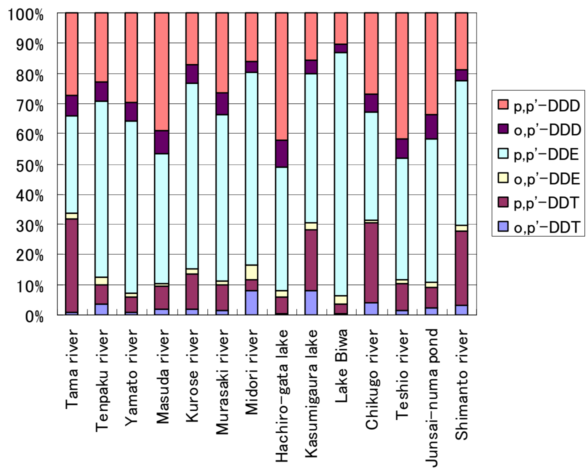

Fig.7 Compositions of DDTs in bottom sediments

Differences between sexes for concentrations of POPs, and transfer to eggs

The concentrations of POPs for male and female *Carassius gibelio langsdorfii* and eggs during the spawning season are shown in Table 2. For almost all target substances, muscle tissue concentrations were higher for the male fish than for the female fish. This tendency was also seen in the dioxins study[5] and is thought to be due to the fact that POPs are transferred from the female's body to the eggs. Assuming the concentration in muscle tissue is equal to the average body concentration, the transfer ratio from the female body to the eggs during the spawning season was calculated to be 19.2%–34.1%.

Table2 Concentration of POPs in muscle and eggs (spawning season)

(pg/g-wet)

Compound	male	female	eggs	Transfer to eggs (%)
DDTs	6,797	3,213	8,322	19.6～24.4
Chlordanes	7,840	6,170	14,240	19.2～21.8
Drins	534	255	1,045	28.1～34.1
Heptachlors	90	48	154	23.8～26.6
HCB	220	150	500	27.1
Mirex	7	4	11	23.5
HCHs	446	236	612	19.5～29.3
Average weight (g)	213	244	27.2	—
Lipid (%)	1.44	1.13	4.87	—

Bioaccumulation in *Carassius gibelio langsdorfii*

Using the results of fish and bottom sediment measurements, the bioaccumulation (Biota-Sediment Accumulation Factor [BSAF]) was calculated using the following equation.

$$\mathrm{BSAF}=\frac{\text{Concentration per unit of fat in fish}}{\text{Concentration per unit of organic matter in bottom sediment}}$$

The BSAF values for chlordanes, which were detected in all fish and bottom sediment samples, are shown in Figure 8. Differences were observed in BSAF values depending on the location. High BSAF values were observed for oxychlordane in some locations. Oxychlordane is a metabolite of chlordane and it is thought that metabolism in the bodies of organisms is larger factor than accumulation from bottom sediments. In addition, BSAF values for HCB, which was also detected in all samples, are shown in Figure 9. The BSAF value is high for the Tama River, in which the HCB concentration in fish was also high.

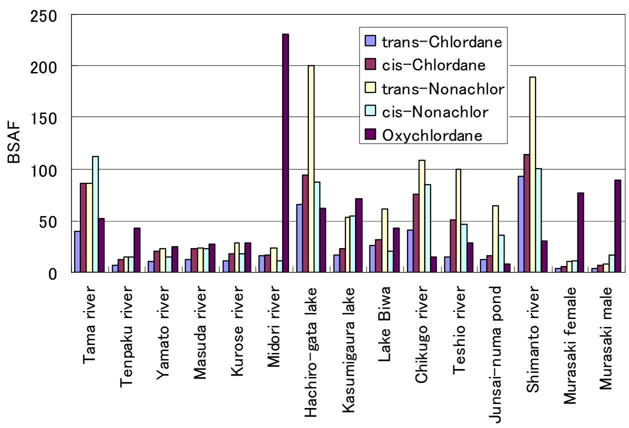

Fig.8 BSAF of chlordanes

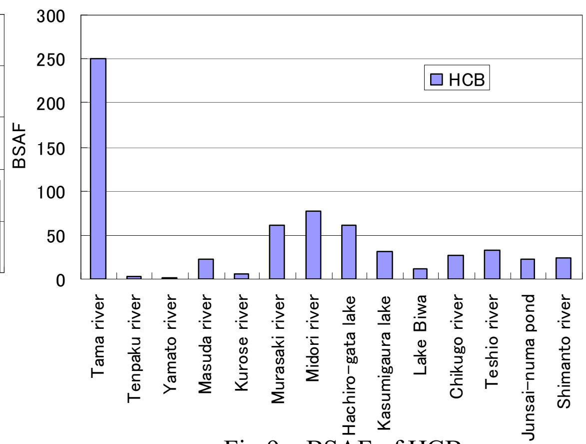

Fig.9 BSAF of HCB

Acknowledgements

This research was carried out as part of the Japanese Ministry of Environment's "Korea-Japan Co-operative Joint Research on Endocrine Disrupting Chemicals." We received advice from universities and local government regarding selection of study sites. We take the opportunity here to extend our sincere thanks.

References

1. Tsuda T. *Journal of Environmental Chemistry* 2000;10:263
2. Tsuda T. *Journal of Environmental Chemistry* 2002;12:1
3. Sapozhnikova Y, Bawardi O, Schlenk D. *Chemosphere* 2004; 55:797
4. Yang Y, Liu M, Xu S, Hou L, Ou D, Liu H, Cheng S, Hofmann T. *Chemosphere* 2006; 62:381
5. Kajiwara Y, Kashiwagi, Kadokami K. *Chemosphere* 2007; submitted.