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Distribution of endocrine-disrupting PCBs in hard roes of loaches and their potential ecological effects in Weishanhu Lake, China

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Abstract: Preliminary study is carried out on the endocrine-disrupting organic chemicals of polychlorinated biphenyls(PCBs: PCB-28, 60, 104, 153, 190) in hard roes of loaches(*Misgurnus anguillicaudatas*) in Weishanhu Lake, China. Results showed that the hard roes of loaches collected from the areas near the influx(such as Pengkou T-PCB = 0.243 μg/g) or efflux(such as Hanzhuang T-PCB = 0.221 μg/g) are characterized by higher PCB contents, about 4 times that from the central lake(T-PCB = 0.066 μg/g), suggesting great difference between their pollutions. The PCB distributions indicated that PCB congeners with more chlorine are more easily accumulated in roes of loaches. The microscopic characteristics reveal that the PCB contents at present cannot lead the roes of loaches to be abnormal. However, the roes with higher PCBs in Pengkou and Hanzhuang are obviously bigger than those with lower PCBs(T-PCBs≤0.176 μg/g) in other localities. It is suggested that PCBs have a stronger estrogenic activity on the roes of loaches, and the phenomenon is likely premonitory for the abnormal development of the hard roes.

Keywords: polychlorinated biphenyls(PCBs); environmental hormone; hard roes; loaches; Weishanhu Lake

Introduction

In recent years increasing international concern has been raised about pollutants that are able to disrupt reproductive function by interacting with the endocrine system(Markus, 2002). As oroganochlorine contaminants, polychlorinated biphenyls (PCBs) are typical endocrine-disrupting organic chemicals that have been studied frequently in aquatic systems due to their bioaccumulative and toxic nature(Heather, 2001). Higher contents of PCBs can result in many procreant anomalies, such as incretion maladjustment, reproductive failure, the weaken of procreation function, the descend of genital immunity abortion, the cancer of the reproductive organs, and so on (Tilson, 1997; Bernard, 1999; Gould, 1999; He, 2001; Kumar, 2001; Wuttke, 2001). In China, many achievements on PCBs have also been reached, but most are limited to the biological activity and toxicity in laboratory(Du, 2000; Fang, 2001).

Weishanhu Lake lies in the southwest of Shandong Province, which is the largest lake of Nansihu Lakes, with an area of about 530 km^2 and belonging to Huaihe drainage system. In the lake, there are abundant aquatic resources such as plankton, benthon, fish and waterfowl. At the same time, it is the only waterway of East-Line Project of South-to-North Water Transfer Planning. Therefore, the water quality of the Weishanhu Lake directly determines the environmental state of water supply in Weishanhu, its adjacent region, the most North China. The present paper studies the variation and distribution of polychlorinated biphenyls (PCBs: PCB-28, 60, 104, 153, 190) in hard roes of loaches in Weishanhu Lake, and discusses their potential effects on the environmental ecology.

1 Materials and methods

1.1 Sample collection

Six sampling locations in the Weishanhu Lake were chosen based on there more or less influences from rivulets(Fig.1). No.1 is near the influx of Taoyuanhe River; No.2, near the efflux of Hanzhuang waterlocks; No.3, at the central Weishanhu Lake, by Weishandao Island; No.4, neighboring the influx of a rivulet crossover Gaolou Village; No.5, Pengkou influx waterlocks; and No.6, near the waterlocks between Weishanhu Lake and Zhaoyanghu Lake. On August 18th to 24th, 2001, a mud-picker was used to collect the surficial mud samples on the lake bottom, and female fresh-loaches with the similar size(L = 9.2 ± 0.5 cm; m = 19.6 ± 1.0 g) and the same species (*Misgurnus anguillicaudatas*) were picked out. Loach samples were put into aluminum foil bag and kept at the lower temperature in refrigerator (The judgment criteria of male and female loaches: The loaches which reach sexual maturity have bigger bodies and a black spot on the tail fin with the length being longer than width of tail handle. Female loaches have expanded abdomen and small wide tail fin with obtuse-round end. Male loaches have smaller abdomen and big long tail fin with raising sharp end; additionally, there are fresh bumps on back fin and sides).

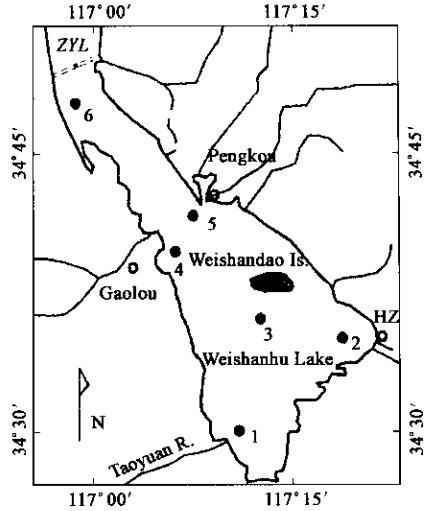

Fig.1 The Weishanhu Lake drainage system and sampling localities
● sampling sites; ▲ waterlocks between Weishanhu and Zhaoyanghu lakes; ZYL. Zhaoyanghu Lake; HZ. Hanzhuang

1.2 Sample processing and extraction

Dissect female loaches, take their hard roes into clean glass

utensils, and divide them into two portions with clean scalpel. Place the more portions into the thermostatic container to keep for 48 h at the temperature of 25℃, bake them for 36 h at 40℃ before they were ground into powder less than 6.67 hm^2 in an agate mortar. Weigh 2 g roe sample to be extracted by extractor and add 5 ml dense H_2SO_4 into extracted solution in order to remove impurity substance (Nie, 2001). Further purification of the solution was carried out on the compound column according to the methods of Gong *et al*. (Gong, 2001) and Fasola *et al*. (Fasola, 1998).

Take the other portions of roe samples on the microslides and make them into biological slice in order to observe the characteristics of the hard roes of loaches in different locations.

1.3 Chromatographic analysis

A Hewlett-Packard model 6980 plus(HP 6980 plus) gas chromatograph with ^{63}Ni electron capture detector, containing a HP-5 quartz capillary column with the length of 30 m and 0.32 mm i.d. as well as 0.25 μm film thickness, was used for this analysis. High-pure helium was used as carrier with the constant speed of 2.0 ml/min and 36.0 cm/s. The pressure before HP-5 column was 70 kPa. After 6 min, the system was incrementally heated from 80 to 160℃ over 8 min, and then at a rate of 8℃/min to 240℃ and held for 5 min. The injector port and detector were maintained at 240℃ and 300℃, respectively. Coeluting congeners were assumed to be in equal proportions. More details on the analytical procedures can be found in Pagano *et al*. (Pagano, 1999).

1.4 Quality assurance

As a measure of analytical precision and accuracy, quality assurance methods were employed by extracting laboratory matrix blank spiked with PCBs and standard reference material and performing replicate analyses. Method blanks averaged < 5% of the sample concentration. Congener method detection limits, defined as the mean of method blanks added to two times their standard deviation, ranged between 0.2—1.1 pg/g, with most between 0.6—0.8 pg/g. It was so small that no blank correction was applied. Analytical precision on replicate samples varied between 4.3%—12.1%. PCBs-28, 60, 104, 153 and 190 from Chemservice of American were selected for the standard reference materials.

2 Results and discussion

Residues of PCBs in hard roes of loaches in Weishanhu Lake are listed in Table 1. No statistical differences in the samples are significant among the sampling time, the sizes of loach samples and their species, therefore all samples are assumed to be able to reveal their environmental characteristics.

2.1 Detected proportions of PCBs in hard roes of loaches

From Table 1, five kinds of PCBs were detected from hard roes of loaches in the six sampling locations. The highest detected proportions are in No.5 (Pengkou) and No.2 (Hanzhuang). For examples, the detected proportions(DP) of 5 PCBs in No.5 all reach 100%, and those in No.2 also reach 100% except PCB-190 with DP of 90%. The lowest DP is in No.3, at the central area of the lake and near Weishandao Island, which is only 20% for PCB-60 to 50% for PCB-153. The detected proportions in No.1, No.4 and No.6 are 60%—100%, 60%—90% and 70%—100%, respectively; of which the DP of PCB-153 are the highest of 90%—100%, but the lowest detected proportions are different in the three sampling sites: No.1 is PCB-60 with DP of 60%; No.4 is both PCB-28 and PCB-190 with DP of 60%; and No.6 is PCB-190 with DP of 70%. The detected proportions indicate that the pollutions of PCBs to the hard roes of loaches differ from in sampling locations of the whole Weishanhu Lake.

Table 1 Residue of PCBs in hard roes of loaches in Weishanhu Lake (μg/g, dry weight)

PCBs	Parameters	No.1	No.2	No.3	No.4	No.5	No.6	Mean value
PCB-28	Average	0.015	0.031	0.007	0.013	0.038	0.019	0.021
	S	0.007	0.017	0.004	0.007	0.023	0.010	
	Max	0.019	0.058	0.010	0.022	0.071	0.033	
	Min	0.005	0.019	0.003	0.004	0.018	0.006	
	DP, %	70	100	40	70	100	90	
PCB-60	Average	0.002	0.004	0.001	0.002	0.005	0.003	0.003
	S	0.001	0.004	0.000	0.001	0.004	0.002	
	Max	0.002	0.007	0.001	0.002	0.007	0.004	
	Min	0.001	0.001	0.001	0.001	0.002	0.001	
	DP, %	60	100	20	60	100	80	
PCB-104	Average	0.012	0.017	0.006	0.011	0.019	0.014	0.013
	S	0.008	0.014	0.004	0.008	0.017	0.010	
	Max	0.025	0.033	0.011	0.023	0.035	0.028	
	Min	0.007	0.008	0.002	0.006	0.010	0.007	
	DP, %	70	100	30	70	100	80	
PCB-153	Average	0.062	0.102	0.033	0.059	0.110	0.088	0.076
	S	0.047	0.063	0.021	0.041	0.069	0.069	
	Max	0.103	0.199	0.054	0.098	0.203	0.143	
	Min	0.045	0.021	0.018	0.031	0.029	0.051	
	DP, %	100	100	50	90	100	100	
PCB-190	Average	0.039	0.067	0.019	0.037	0.071	0.052	0.048
	S	0.021	0.039	0.011	0.019	0.044	0.028	
	Max	0.066	0.101	0.029	0.055	0.111	0.077	
	Min	0.020	0.020	0.013	0.018	0.021	0.029	
	DP, %	70	90	30	60	100	70	
T-PCB		0.130	0.221	0.066	0.122	0.243	0.176	(0.160)

Notes: T-PCB is the total concentration of PCB-28, 60, 104, 153 and 190; S is the standard deviation; Max and Min are the maximum and minimum contents determined by GC; DP, detected proportion. Parallel tests were carried out 12 times for No.2 and No.5, and 10 times for the other samples

2.2 PCBs distribution in hard roes of loaches

Distribution proportions and patterns of PCBs in hard roes of loaches see Table 2 and Fig.2. Although their concentrations are different, their PCB patterns of all samples from different areas are almost the same(Fig. 2). The highest content is PCB-153 which accounts for 45.27%—50.00% of T-PCB(T-PCB is the total concentration of PCB-28, 60, 104, 153 and 190); the second is PCB-190, accounting for 28.79%—30.33%; next PCB-28(10.61%—15.64%) and PCB-104(7.09%—9.03%); the lowest is PCB-60(1.52%—2.06%) which is only less than 3% of T-PCB. Moreover, the proportions of the same PCB in hard roes of different loaches are characterized by slightly variation, generally less than 5%. It is suggested that the distribution of PCBs in roes of loaches follow a principle. Only two PCB congeners with more chlorine (PCB-153, 190 are 6- and 7-Cl biphenyls respectively) accounting for 74.49%—79.55% of T-PCB are much higher than the other three PCB congeners with less chlorine (PCB-28, 60, 104 are 3-, 4- and 5-Cl biphenyls respectively) accounting for only 20.45%—25.51%. It is presented that PCB congeners with more chlorine are more easily

accumulated in organisms. It possibly relates to the higher fat-solubility and difficult organism metabolism for PCB cogeners with more chlorine. This result is consistent with the researches on aquatic bird's eggs from Gong *et al*.(Gong, 2001) and Focardi *et al*. (Focardi, 1988).

Table 2 PCB percentages in hard roes of loaches(%)

PCBs	No.1	No.2	No.3	No.4	No.5	No.6
28	11.54	14.03	10.61	10.66	15.64	10.80
60	1.54	1.81	1.52	1.64	2.06	1.70
104	9.23	7.69	9.09	9.02	7.82	7.95
153	47.69	46.15	50.00	48.36	45.27	50.00
190	30.00	30.32	28.79	30.33	29.22	29.55

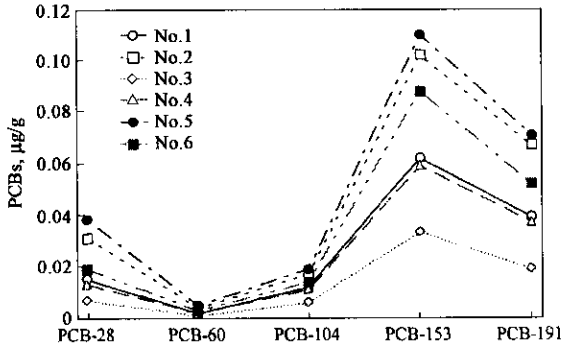

Fig.2 PCB patterns of hard roes of loaches in Weishanhu Lake

2.3 Spatial distribution of PCBs of loach's roes in Weishanhu Lake

Table 1 and Fig.2 show that PCBs of hard roes of loaches in No.5 (Pengkou) and No.2(Hanzhuang) are the highest, followed by No.6 (near the waterlocks between Weishanhu Lake and Zhaoyanghu Lake), No.1(near the influx of Taoyuanhe Stream) and No.4(neighboring the influx of a rivulet crossover Gaolou Village); those in No.3(at the central Weishanhu Lake, by Weishandao Island) are the lowest. Nevertheless, the variation characteristics of 5 kinds of PCBs in different locations are essentially similar with each other: except the contents of PCB-60 in No.6 and No.4 are the same, the ranks of 5 kinds of PCBs in 6 sampling locations are wholly consistent without crossover or overlapping. Therefore the trend of any congener of PCBs or total content of 5 PCBs(T-PCB) may indicate the variation characteristics of whole PCBs. As an example, T-PCB was selected to reveal the pollution situation of aquatic system in Weishanhu Lake.

T-PCBs of hard roes of loaches in different locations of Weishanhu Lake are also listed in Table 1. It is found that the variation potential of T-PCBs is surely consistent with that of any PCB congener. T-PCBs reveal that the residues of PCBs in No.5(Pengkou influx waterlocks) and No.2(Hanzhuang efflux waterlocks) are the largest with 0.243 and 0.221 μg/g of T-PCBs, suggesting the most serious PCB contamination in aquatic system. Second is in No.6 whose T-PCB is 0.176 μg/g. T-PCBs in No.1 and No.4 are 0.130 and 0.122 μg/g respectively, showing similarly environmental situation. No.3, however, is featured by the lowest T-PCB (0.066 μg/g), only about a half of those in No.1 and No.4, one-third of No.6, and one-fourth of No.5 and No.2. It implies that the central of the lake is characterized by the least PCB pollution.

Distinctive PCB contents in hard roes of loaches indicate that the different regions of Weishanhu Lake are characterized by different degrees of PCB pollution. The reason that resulted in this phenomenon, as we believe, is the sampling geographical positions and their contaminated states. The investigations on the pollution of Weishanhu Lake and their influx areas suggested that, although almost all factories in this district have reached the national standard in waster discharge since 1990, the lake still suffered from various contaminations such as living sewage, decomposition of remains of aquatic organisms, agriculturally chemical fertilizer and pesticides, industrial waster water (mainly from the industries of paper-making, chemicals, food, printing and dyeing, electroplating and currying etc.), waste oil discharged by shipping, and atmospheric subsiding; of which the influx and efflux of rivulets have usually suffered from the most serious pollution. In the present study, No.5(Pengkou) lies in the influx of several rivulets, and No.2(Hanzhuang) is near to the waterlocks of Hanzhuang Canal which is the main drain-off gate of Weishanhu Lake. It can be judged that higher PCBs of hard roes of loaches in the two locations were derived from their serious environmental states. No.1 and No.4 also lie in the influxes of rivers, but both have only one stream, which can explain their weaker contaminations and their lower PCBs(about a half of those in No.5 and No.2). Due to the central of the lake far from either influx or efflux of river, No. 3 with the lowest PCBs is coincident with the least contamination. These characteristics mentioned above show that the PCB contents of hard roes of loaches are well correspond to the contamination state of influx or efflux of its surroundings.

Additionally, there is few influx in the lower reach near the waterlocks between Weishanhu and Zhaoyanghu lakes, but the PCB contents in the hard roes of loaches from No.6 is considerably high and ranks next to those from No.5 and No.2. Because the waterlocks is the only one through which the water flows into the lower Weishanhu Lake from upper Zhaoyanghu Lake, it can be deduced that the higher PCBs probably correlate with the accumulation of pollutants in the upper Zhaoyanghu Lake.

As a whole, the PCBs of the roes of loaches are heterogeneously distributed in Weishanhu Lake. A great PCB differences up to 2—4 times of hard roes of loaches in different locations suggest that it is difficult to reach equilibrium for PCB distribution in an aquatic system.

2.4 Microscopic characteristics of hard roes of loaches

The hard roes of loaches in different locations of Weishanhu Lake were observed by bio-microscope and were found that although there were 2—4 times differences in PCB contents, roe tissues have not obviously abnormal development. Roes from all sampling sites are full, transparent and uniformly distributed. But there are some differences in size: roes from Nos.1, 3, 4 and 6 are similar in size with diameters of about 0.010 (0.007—0.012) mm; while those from No.2 and No.5 in which their roes have the highest PCB contents are obviously bigger, with diameters of about 0.010—0.015 mm and averaged 0.012 mm. Because these samples were featured by the same species (*Misgurnus anguillicaudatas*), similar sizes and approximate sampling time, the difference should directly reflect the toxicity from different contents of PCBs.

On the basis of the microscopic features of hard roes of loaches and their PCB residues, combining with the corresponding relationship between the roe enlargement and higher PCB contents, we suggest that lower PCBs(T-PCBs$\leqslant$0.176 μg/g) have not obvious poisoning effect on female reproductive cells; when PCB contents reach a certain level (for example, T-PCBs $\geqslant$ 0.221 μg/g in the study), their hard roes will gradually enlarge. It implies PCBs having some estrogenic activity to roes

of loaches. From the culture experiments of loaches, it has been found that the hard roes of loaches obviously enlarged and their numbers reduced when loaches were cultured in lower contents of DDVP and Pb solutions; when DDVP and Pb increased, poisoned roes developed abnormally following as concave, convex, joint each other and even without nucleolus(Kuang, 2003; 2004). In the present study, therefore, the roes with highest PCBs evidently larger than others are most likely premonitory for their abnormal development. Despite the PCBs pollutions can not lead to the abnormal development of hard roes of loaches at present, if the environmental contamination become further worse, it may result in the toxicosis of germ cells in organisms such as loaches, and even result in the destroy of regionally environmental ecology.

3 Conclusions

PCB distributions showed that PCB congeners with more chlorine are more easily accumulated in hard roes of loaches. It possibly relates to the higher fat-solubility and difficult organism metabolism of PCB congeners with more chlorine.

PCBs in hard roes of loaches are largely different in various locations of Weishanhu Lake. The highest T-PCBs in No.5 (Pengkou influx waterlocks) and No.2 (near the efflux of Hanzhuang waterlocks) are 0.243 and 0.221 μg/g respectively, indicating the most serious PCB pollutions. The central of the lake(No.3) is characterized by the lowest T-PCB (0.066 μg/g) suggesting the least contaminations. The PCB contents of hard roes of loaches are well correspond to the environmental state of influx or efflux of its surroundings.

Microscopic research reveals that at present the PCB pollutions can not lead to the abnormal development of hard roes of loaches, but the obvious enlargement of roes with the highest PCBs in No.5 and No.2 indicated that higher PCB contents have a stronger estrogenic activities. This phenomenon is probably premonitory for the abnormal development of the hard roes.

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