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Endocrine Disruption by Hexachlorobenzene in Crucian Carp (*Carassius auratus gibelio*)

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Up to now, more and more evidence have indicated that human, domestic and wildlife species have suffered adverse health effects after exposure to endocrine disrupting chemicals. The adverse health effects include increasing risk of cancers, reducing reproduction and declining population (Campbell et al. 1998). Many organochlorine compounds have been identified as endocrine disrupters, such as DDT and its metabolites, dioxins, PCBs and some pesticides. These endocrine-disrupting chemicals can disturb endocrine system in biological organism, especially during the critical stages of life cycle and finally lead to permanent toxicity effects (Campbell et al.1998, Kavlock et al. 1996).

Hexachlorobenzene (HCB) was once intensively used as a fungicide for treating agricultural seed. But in 1980s, its use was stopped in China because of the cacinogenicity in rodents and the environmental persistence. At present, there are still a number of emission sources that HCB continues to be release to the environment, including the disposal of chlor-alkali industry effluent, the production of chlorinated chemicals and municipal incineration etc. According to our previous study, HCB concentration was found in lake sediment contaminated by organochlorines at few magnitudes higher levels than PCDD/F and other chlorinated contaminants (Wu et. al. 1997). They often occur together with Hexachlorocyclohexane (HCH), a cheap broad spectrum pesticide, which was also widely applied in China. Among the four isomers of HCHs, β-HCH is the final chemical speciation of the residual HCH in environment (Xu et al. 1994). HCB is also a biological metabolite of HCH (Ahlborg et al. 1995). In view of the realistic contamination status by organochlorines in China, HCB and it together with β-HCH with graded doses exposure design was selected to investigate their endocrine disruptions in fish in the present study.

MATERIALS AND METHODS

HCB (purity> 99.5%) was purchased from BDH Chemicals Ltd., England and β-HCH (purity> 99.5%) was purchased from Labor Dr. Ehrenstorfer, D-8900

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Augsburg, Germany. Acetone, hexane and dichloromethane (pesticide residue grade) were ordered from Promochem, Germany. The concentrations of stock solutions were measured by GC prior to the experiments.

200 one-year old male and 200 one-year old female Crucian Carp (*Carassius auratus gibelio*), with ca.12-15cm length and 150g body weight, were obtained from the hatchery of the Institute of Hydrobiology (far from industry area without any contamination). After one-week acclimation in dechlorinated tap water (pH 8.0, dissolved oxygen close to saturation), the male and female fish were randomly assigned into control group, receiving 5ml acetone, and the graded dose groups, receiving 5ml graded HCB and β-HCH acetone solutions in 50 liters water under thoroughly stirring. For each sex group, the fish in replicate were respectively exposed to HCB in the doses of 0, 50, 200μg/L HCB and 200μg/L HCB plus 50μg/L β-HCH for two weeks. During the exposure, the temperature in water was controlled at 20±2°C and the light-dark rhythm was 12:12 (L:D). The test solution water was changed every other day and the fish were fed 1% of the body weight daily with commercial fish pellets.

Under the laboratory semistatic condition, the concentration levels of 17β-estrodiol (Estrogen) and 11-ketotetesestoster(Androgen) in serum of Crucian carp (*Carassius auratus gibelio)* were measured by Radioimmunassay (RIA) method (Sufi et. al., 1987) after 2 weeks respectively. Meanwhile, HCB in fish livers were collected and dry-frozen. Then they were extracted with toluene, followed by the clean up with 6% Florisil column. The determination of HCB was finally carried out on a GC-ECD (HP6890, USA) system. The chromatographic condition: HP-5 capillary column sized 30m×0.25mm i.d., 0.25μm df. was applied. The oven temperature program was 100°C to 190°C with rate of 10°C/min, then to 250°C. The sample amount injected: 1μl, splitless mode; injector temperature 250°C and ECD detection temperature 290°C. Statistic data analysis was performed by using ANOVA,followed by Bonferroni/Dunn test for the multiple comparison procedures.

RESULTS AND DISCUSSION

HCB is the major contributor for residual organochlorines and it exists together with β-HCH in the typical contaminated area in China (Xu et al. 1994, Wu et. al. 1997). Therefore, 50ug/L HCB, 200 μg/L HCB and 200 μg/L HCB plus 50 μg/L HCH were considered as representing the environmental concentrations for general and heavily contaminated Chinese areas. Fish livers have high lipid content, they were collected as the organochlorine target organ to determine the bioaccumulation of lipophilic HCB. The alterations of the sex hormones were used as the bioindicators for assessing the endocrine disruption of the

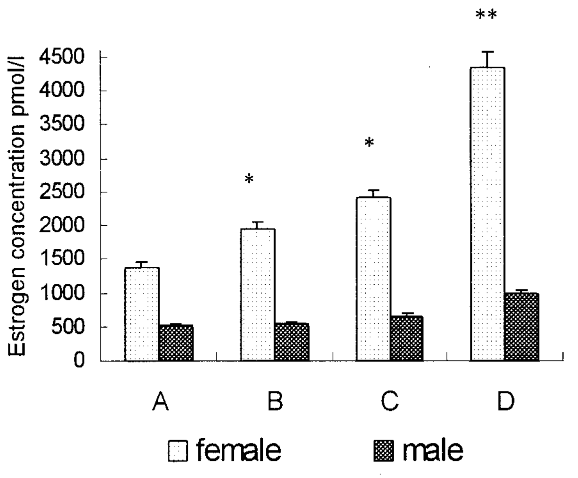

Figure 1. Estrogen (17β-estrodiol) alteration in fish serum exposed to organochlorine for two weeks
A-control, B-50ug/L HCB, C-200ug/L HCB, D-200ug/L HCB + 50ug/L β -HCH
Data (n=50) are expressed as means±S.E.M. Significant difference in female group found between means in a column (P= 0.04)

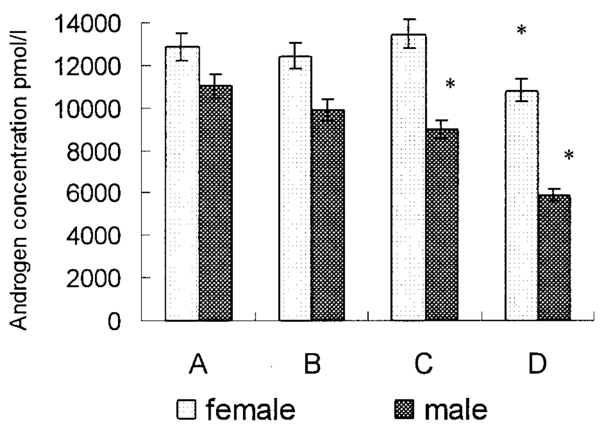

Figure 2. Androgen (11-ketotestosterone) alteration in fish serum exposed to organochlorine for two weeks
A-control, B-50ug/L HCB, C-200ug/L HCB, D-200ug/L HCB + 50ug/L β -HCH
Data (n=50) are expressed as means±S.E.M. Significant difference in male group found between means in a column (P= 0.04)

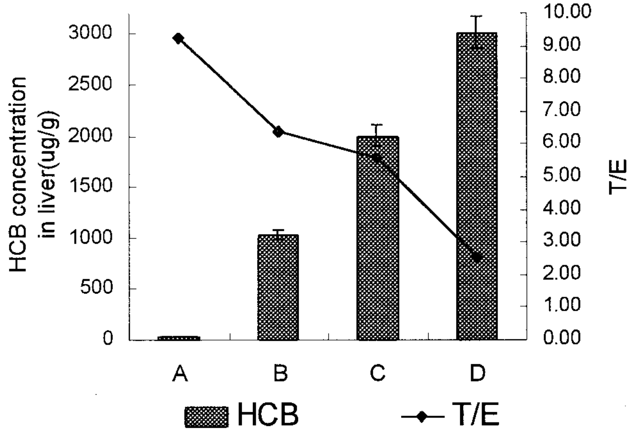

Figure 3 Relationship between HCB concentration in male fish liver and T/E
A-control, B-50ug/L HCB, C-200ug/L HCB, D-200ug/L HCB + 50ug/L β-HCH
Data (n=50) are expressed as means±S.E.M.

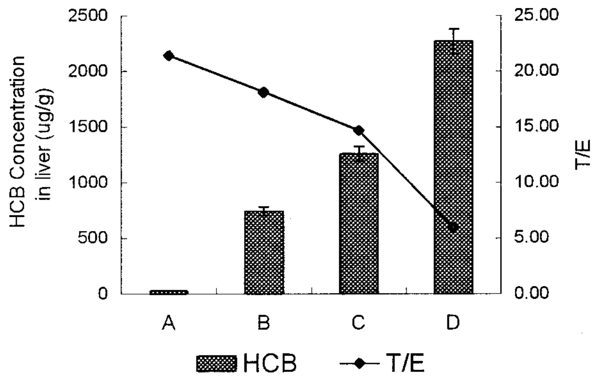

Figure 4. Relationship between HCB concentration in female fish liver and T/E
A-control, B-50ug/L HCB, C-200ug/L HCB, D-200ugL HCB + 50ug/L β-HCH
Data (n=50) are expressed as means±S.E.M.

organochlorines during their sex maturity period.

As shown in Figure 1 and 2, the effects of HCB and β-HCH on 17β-estrodiol (E) and 11-ketotestosterone (T) in serum of Crucian Carp (*Carassius auratus gibelio)* were studied. We selected 17β-estrodiol and 11-ketotestosterone as the representatives of sex hormones. That is due to. their high biological activity in teleostean reproduction. It was observed in Figure 1, both in female and male groups, 17β-estrodiol, the main estrogen levels in serum was significantly increased from A to D group, indicating the effect was dose-dependent. The increasing trend was more apparent in female groups than that in male groups. In Figure 2, there was a slightly reducing trend of 11-ketotestosterone, the main androgen levels in male groups, but not in female groups. Significant increase of estrodiol levels and simultaneous relative decrease of testosterone level indicates the alterations of sex hormonal balance due to the exposure of HCB. The androgen alterations may be related with the increase of hypothalamus-pituitary system sensitivity to steroid and depression of gonadotropin production (Moore, 1991). The mechanism of HCB effects on sex steroid metabolism and disturbed steroid regulation are unknown.

Figure 3 and Figure 4 displayed the relationship between HCB accumulated in fish liver and T/E in fish serum. Higher bioaccumulations of HCB in livers were found in female groups than males, owing to their higher lipid contents. Serum T/E values in exposure groups significantly reduced with increasing exposure concentrations of HCB to fish, especially in the coexistence of β-HCH. The reductions of T/E in serum were in direct proportion to the concentrations of HCB accumulated in liver. The presence of β-HCH was considered to have a synergistic effect, enhanced the bioavailability of HCB in Crucian Carp (*Carassius auratus gibelio)*. β-HCH itself was reported having endocrine disruptions (Ahlborg et.al. 1995). In addition, the results from our fish exposure tests demonstrated that female groups were more sensitive than males to the sex hormone alterations caused by HCB exposure. This is consistent with those short-term and subchronic exposure studies with mammals. Their results indicated that female rats were more sensitive than males to the porphyrinogenic effects (Smith et. al. 1990, D'Amour & Charbonneau, 1992) and the EROD activity inductions of expose to HCB (Smith et. al. 1990, Goerz et. al. 1994). The T/E reductions in fish serum by HCB exposure may mean the antiandrogenic and/or estrogenic effects to teleosts. Further research is required to understand the toxic effects of HCB on sex steroid metabolism and disturbed steroid regulation, resulting in hormonal imbalance in teleosts.

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