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Alterations in Retinoids, Tocopherol, and Microsomal Enzyme Activities in the Liver of Silver Carp (*Hypophthalmichthys molitrix*) from Ya-Er Lake, China

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Retinoids (Vitamin A) and tocopherol (Vitamin E) are two kinds of important nutriments and exogenous hormones, which support a variety of normal physiological processes. α-Tocopherol is the most active form of Vitamin E, whereas retinal (R) and retinyl palmitate (RP) is the most active and the main storage forms of Vitamin A, respectively. Fish liver is the major storage organ for retinoids and tocopherol and the important target for the detoxication of many pollutants. There is evidence that some persistent organic pollutants (POPs) such as PCDD/F, PAHs, PCBs in waters may contribute to skin disease, edema and neoplasia in fish, which resemble those of Vitamin A deficiency (Palace et al. 1994; Besselink, et al. 1997). Moreover, the studies in mammals and birds indicate that PCB, PAH and PCDD/F can induce the biotransformation enzyme activities through Ah receptor pathway (Besselink et al. 1997). Many investigations focus on the increasing synthesis of P4501A1 using EROD induction as a biomarker. Also the activities of some phase II enzymes such as certain forms of UDP-glucuronyltransferase (UDPGT), and mammalian acylcoA: retinol acyl transferase (ARAT) have been suggested to be induced in an AhR related manner (Besselink et al. 1997). Ah-mediated induction of these enzymes can lead to retinoid depletion by ARAT inhibition or UDPGT induction and reduction store of tocopherol in tissue by oxidative stress increase.

Ya-Er Lake, China had been continuously polluted by HCH-isomers and other chlorine aromatic hydrocarbons from 1962-1987 through direct discharge of the industry effluent. High levels of HCB, HCHs, PCB, PAH, PCDD/F and heavy metals have been detected in the sediment and they are considered as the principal contaminats in Ya-Er Lake area (Schramm, et al. 1999). The purpose of the study is to investigate the effects of these POPs on biotransformation enzymes and the storage of retinoids and tocopherol in the liver of silver carp, the predominant fish species in Ya-Er Lake.

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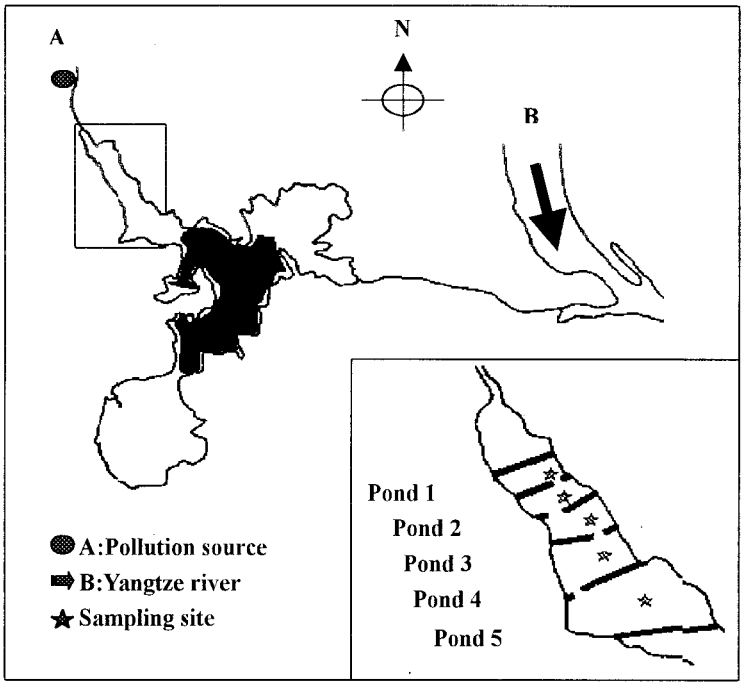

Figure 1. The map of Ya-Er Lake showing the sampling sites. A, pollution source; B, Yangtze River. The shaded area stands for the original lake, which is dry land now.

MATERIALS AND METHODS

Samples were taken in October 1999. The sampling locations for the study are indicated in Fig1. The five ponds were chosen for the study based on their historical episodes of exposure to waste discharge from the plant. The five-oxidation ponds were built for self-purification of the effluent in 1979. Silver carp about one-year old, weighing from 500 to 700g were captured from five ponds on the same day. Livers were carefully dissected free from gall bladder and quickly frozen in liquid nitrogen for further processing.

Individual pieces of liver were homogenized in 4 volumes of buffer (0.1M phosphate, 0.15M KCl, pH7.4) in a glass homogenizer. The homogenate was centrifuged at 4 ^{0}C at 1000g for 20 min. An aliquot of supernatant was withdrawn for EROD measurement. The remaining supernatant was further ultracentrifugation at 105, 000g for 60 min. The resulting pelllet was suspended in a buffer containing 20% glycerol (V/V) corresponding to 1g liver per ml. Microsomal preparations were quickly frozen in liquid nitrogen and stored at

-80^0C for UDPGT assays. All procedures were performed at 0-4 ^{0}C. EROD activity was determined by fluorometric methods as described previously (Li et al, 1997). UDPT activity was measured spectrophotometrically as described by Sturm et al. (1999) with p-nitrophenol (p-NP, Sigma) as aglycone and uridine 5-diphospho-glucuronic acid (UDPGA, Sigma, ammonium salt) as the glucuronyl donor. Protein was determined according to the method of Lowrry et al. (1951) with bovine albumin as a standard.

Retinoids and tocopherol (Sigma) were extracted from hepatic homogenates and analyzed by HPLC according to a modification of the method previously described (Palace & Brown, 1993). The fortified recoveries determined by addition of known amounts of tocopherol, retinol and retinyl palmitate in homogenate were 92.9%, 97.2%, 92.7% respectively. The extraction efficiencies of tocopherol acetate varied in 79.6±4.2%. All extractions were conducted without direct daylight. Retinoids and tocopherol were separated and quantified by isocratic reversed-phase HPLC method. The HPLC system included a Hewlett-Packard 1100 series quaternary pump and autosampler, diode array detection, and an HP-Chem Station employing a C_{18} reversed-phase analytical column (25.0×4.0mmID, 5um). The mobile phase was methanol at a rate of flow of 1.2 ml/min. Retinoids and tocopherol were detected at the wavelength of 325nm and 292nm, respectively.

After data were tested for normality and homogeneity of variance using the Chisquare test, data analysis was performed by using one-way ANOVA, followed by a Duncan's test for the multiple comparison procedures. Data that did not pass the Chi-square test were analyzed by Kruskal-Wallis test for differences between Ponds. The correlations between hepatic EROD and other biochemical parameters were determined using the software package SPSS/PC^{TM}, version 10.0 (SPSS Inc., Chicago, IL). The data are expressed as the means±SD.

RESULTS AND DISCUSSION

Fig. 2 showed clearly that hepatic EROD and UDPGT activities decreased in silver carp from Pond 1 to Pond 5, which coincided with the flowing direction of the effluent. Multiple comparisons indicated that both means of EROD and UDPGT activities in silver carps from heavy polluted Pond 1 were the significantly the highest among fish from five ponds ($P<0.05$, Duncan's). Previous studies have demonstrated that the concentrations of HCHs, CBs, PAHs, PCBs, PCDD/F and heavy metals decreased from Pond 1 to Pond 5 through particles adsorption and suspension interaction in water (Schramm, et al. 1999).

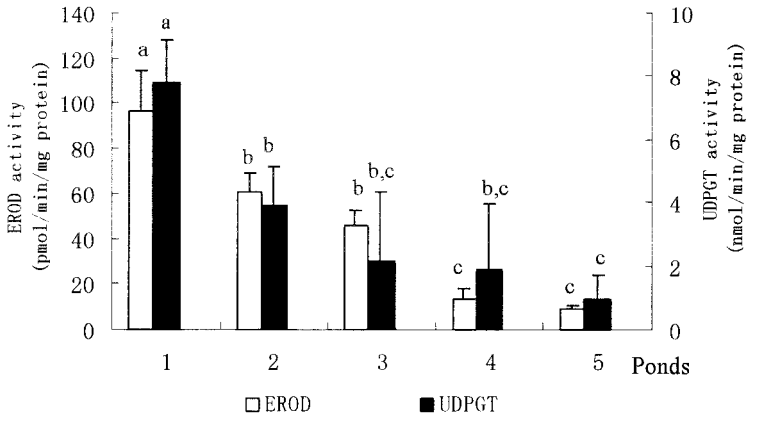

Figure 2. Hepatic EROD and UDPGT activities in one-year old silver carps from different ponds of Ya-Er lake (n=4).The bars are means±SD, and the means with different small letter indicates significance at 0.05 level (one-way analysis of variance followed by Duncan's test) .

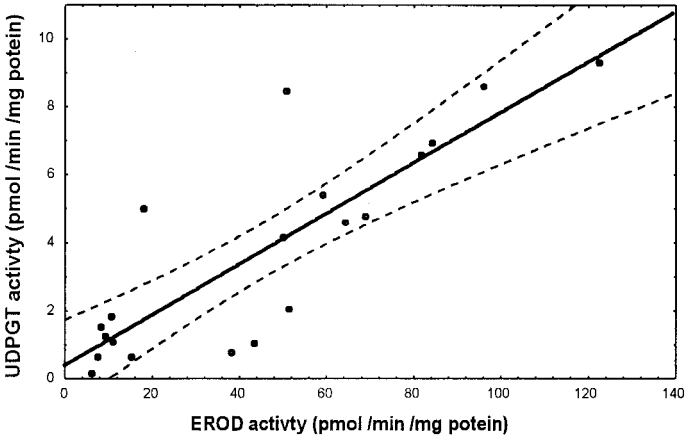

Figure 3. Positive relationship between hepatic UDPGT and EROD activity in one-year-old silver carps from the different ponds. of Ya-Er Lake (y=0.38+0.074*EROD; r=0.84, $p<0.05$).The area between the dotted lines represents the 95% confidence interval.

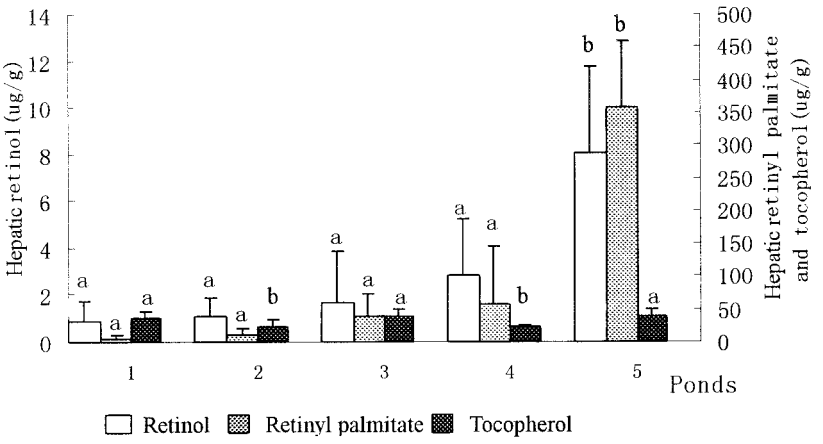

Figure 4. Hepatic retinol, retinyl palmitate and tocopherol levels in one-year old silver carp from different ponds of Ya-Er Lake (n=4).The bars are means±SD, and the means with different letter indicates significance at 0.05 level (one-way analysis of variance followed by Duncan's test) .

Throughout the five oxidative ponds, there is a distinct downward gradient level of pollutants in sediments (Schramm, et al. 1999). Biotransformation enzymes play a key role in contaminant metabolism. Their presence and activity determine potential biological effects of contaminant exposure. The cytochrome P450 (CYP) enzyme system is considered as the most important in the phase I biotransformation for endogenous and exogenous compounds (Wolkers et al. 1998). Studies have indicated that EROD can be induced by Ah receptor-active chemicals such as PAH, PCDD/F, PCB to assess their exposure. Increased catalytic activity of CYP1A-dependent EROD is commonly used as a diagnostic tool. UDP glucuronosyl transferase, known as to be under regulation of the Ah receptor (Besselink et al. 1997), is involved in the detoxification of exogenous and endogenous compounds by catalyzing glucuronidation. A significantly positive relationship between EROD and UDPGT activities was found in silver carps for individual fish from all ponds: UDPGT=0.38+0.074*EROD; r=0.84, $p<0.05$ (Fig.3). The results of the present study suggest EROD and UDPGT activities in silver carp liver are responsive to Ah receptor-active chemicals in Ya-Er Lake. AhR regulates the transcription both for phase I and phase II enzymes, which is in agreement with the studies in mammals and birds.

Several retinoid compounds were present in liver tissue, some unknown vitamin A metabolites also eluted on the HPLC column before retinyl palmitate, which have

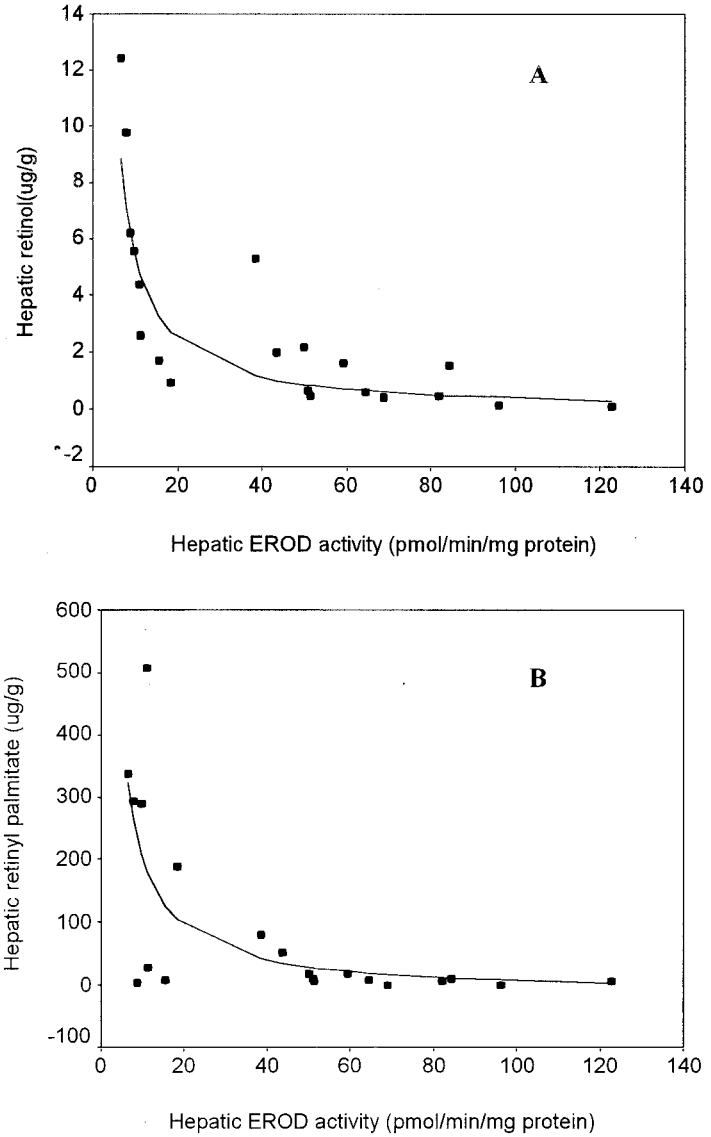

Figure 5. Negative correlation between hepatic EROD and retinoids concentrations in one-year-old silver carps from the different ponds of Ya-Er Lake. Calculations were carried out using SPSS software package, version 10.0 according to the following equation: $R= 72.17*(EROD^{-1.13})$, F-statistic= 37.79, $r^2=$ 0.68 (A); Rp= -14.77 + 2154.52 / EROD, F-statistic= 16.73, $r^2=$ 0.48 (B).

not been identified but could represent retinoid esters with a shorter fatty acid tail, or other unsaturated fatty acids. This finding is consistent with the semi-field

study of flounder (*platichthys flesus)* (Besselink, et al. 1997) and the field study of brown bullhead (*Ameiurus nebulosus*) (Lisa, et al. 1999). As it is presented in Fig.4, Hepatic retinoid concentrations were decreased in silver carp from Pond 5 to Pond 1 with the increase of POPs levels. But there was no statistically significant difference in the concentrations of retinol and retinyl palmitate observed in silver carps from Pond 1 to Pond 4. Mean value of hepatic retinoids in silver carp from Pond 5 was found the significantly highest compared to the fish from the other 4 ponds ($P<0.05$). It was found significant negative non-linear association between hepatic retinoid and EROD activities in the carps from each pond: $R=72.17*(EROD^{-1.13})$, F-statistic=37.79, $r^2=0.68$; RP= -14.77 + 2154.52/ EROD, F-statistic=16.73, $r^2=0.48$ (Fig.5). It is suggested the modulation of Vitamin A homeostasis by xenobiotics may involve in oxidation and glucuronidation reactions of retinoid metabolism in silver carp. EROD and UDPGT enzymes also play an important role in the balance of retinoids. The contaminants inducing activities of phase I and phase II enzymes may disrupt vitamin A homeostasis. The results indicate that POPs bound to Ah receptor induce biotransformtion enzyme activities, increase metabolism of retinoids in liver, finally leading to hepatic retinoids reduction in silver carps of Ya-Er Lake. Retiniods in fish liver may be used as an effective biomarker in assessing the long-term toxic effects of organochlorine pollution on wild fish.

Silver carp from the different ponds were exposed to the mixture of heavy metal and organochlorine, which might act either alone or together in a synergistic or antagonsitic manner. The interactive effects of the mixture may complicate of oxidative stress in liver of silver carp. As shown in Fig. 4, the hepatic concentration of tocopherol in silver carps was not significantly decreased from Pond 1 to Pond 5. Organochlorine via Ah-mediated induced biotransformation enzyme can increase oxidative stress, leading to reduction of hepatic storage of tocopherol (Palace, et al. 1993). Heavy metals may also act as oxidants, but they could be part of some enzymes such as superoxide dismutase, catalase and caeruloplasmin, which are needed for elimination of radicals (Reijo, et al.1999). Therefore, the presence of heavy metals in water might avoid tocopherol reduction from the oxidative stress of Organochlorine. Tocopherol level in silver carp liver from Pond 1 to Pond 5 may be not only related to organochlorine exposure, but also associated with heavy metal existence. Further studies are needed to understand interactive effects between heavy metal and organochlorine exposure on silver carps from Ya-Er Lake.

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REFERENCES

Besselink, HT, Denison, MS, Hahn, ME, Vethaak, AD, Koeman, JH, Brouwer, A (1997) High induction of cytochrome P4501A activity without changes in retinoid and thyroid hormone levels in flounder (*platichthys flesus*) exposed to 2,3,7,8- tetraachlorodibenzo-*p*-dioxin. Environ Toxicol Chem 16: 816-823

Palace, VP, Brown, SB (1993) HPLC determination of tocopherol, retinol, dehydroretinol and retinyl palmitate in tissues of lake char (*salvelinus namaycush*) exposed to coplanar 3,3', 4,4', 5-pentachlorobiphenyl. Environ Toxicol Chem 13: 473-476

Lisa D AH, Chris DM (1999) Biomarks of exposure of brown bullheads (ameiurus *nebulosus*) to contaminants in the lower Great Lakes, north America. Environ Toxicol Chem 18: 740-749

Li W, Wu WZ, Xu Y, Zhang YY, Schramm KW, Kettrup A (1997) 7-Ethoxyresorufin -o-deethylase (EROD) as bioindicator of 2, 3, 7, 8 - Tetachlorinated -dibenzo-p-dioxin toxicity to exposed *gobiocypros rarus*. Acta Hydrobiol Sinica 21: (Suppl Sept) 214-220

Reijo K, AK, HH, Juha A, S-KD (1999) Vitamins A_1, A_2 and E in minks exposed to polychlorinated biphenyls (aroclor 1242) and copper, via diet based on freshwater or marine. Environ Toxicol Chem 18: 2259-2599

Schramm KW, Henkelmann B, Kettrup A, Y Xu, Wu WZ (1999) Sources, fate, bioaccumulation and sinks of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in People's Republic of China, especially in Ya-Er Lake area near Wuhan. GSF-Bericht 04/99, ISSN 0721-1695

Sturm A., Hodson, PV, Carey, JH, Hansen, P D (1999) Hepatic UDP-glucuronosyltransferase in rainbow trout (*oncorhynchus mykiss*) and preliminary assessment of response to pulp mill cooking liquor. Bull Environ Contam Toxicol 62: 608-615

Scott BB, Peter DD, Rebert EE, W, Derek CG, Fred JW (1997) Biochemical and histological reponses in rainbow trout (*oncorhynchus mykiss*) exposed to 2,3,7,8-pentachlorodibenzofuran. Environ Toxicol Chem 17: 915-921

Susan MC, Michael RM, CM, BS, Rodney DJ, Joseph ET (2000) Ttemporal trends in ethoxyresorufin-o-deethylase activity of brook trout (*salvelinus fontinalis*) fed 2,3,7,8-tetraachlorodibenzo-*p*-dioxin. Environ Toxicol Chem 19: 462-471

Wolkers J, Wikamp RF, Nijneijer SM, Burknow LC, de Groene EM, Lydersen C, Dahle S, Monshouwer M (1998) Phase I and phaseII enzyme activities in Ringed seals (*phocahispida*): Characterization of hepaticcoytochrome P450 by activity patterns inhibition studies mRNA analyses and westerm blotting. Aquat Toxicol 44:103-115.