T4 AND T3 CONCENTRATIONS IN THE CHINESE SILVER CARP *HYPOPTHALMICTHYES MOLITRIX* EXPOSED TO ENVIRONMENTAL PERSISTENT ORGANIC COMPOUNDS

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Introduction

Previous research in our laboratories has demonstrated that exposure to TCDD (2,3,7,8-Tetrachlorinated dibenzo-p- dioxin) alters thyroid function in the early larval development of the Chinese rare minnow (*Gobiocypris rarus*)[1]. We also demonstrated depression of thyroid hormone concentrations in the liver of adult silver carp (*Hypopthalmicthyes molitrix)* from contaminated ponds in the Ya-Er Lake of the Yangtze river basin[1]. This paper reports on Thyroxine (T4) and Triiodothyronine (T3) concentrations in the serum of *H. molitrix* sampled from five ponds with a gradient of persistent organic pollutants. We also report on the bioaccumulation of dioxins in the liver of *H. molitrix.*

The field study site was the shallow and eutrophic Ya-Er Lake, in the Eastern region of Wuhan, Hubei province, sited in the median lower reaches of the Yangtze river, China[1]. From 1962 to 1987 an effluent containing a range of persistent chlorinated organic pollutants, from a large chemical factory was continuously discharged into the lake. In 1978 part of Ya-Er Lake was divided into a series of 5 oxidation ponds for the remediation of wastewater. Analysis of pond water has been shown to contain a gradient of contamination by polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F)[1] together with other toxicants. These ponds are currently stocked with *H. molitrix* and the fish from Pond 5 are harvested for human consumption.

Methods

One-year old male silver carp were sampled from ponds 1 to 5 with a gradient of contamination by polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) in the Ya-Er Lake. Fish were bled by caudal inscision, blood allowed to clot, serum separated by centrifuging at 3000 rpm for 10 minutes at 4° C and serum samples frozen at -20° C. Dissected fish livers were frozen at -20°C and all samples transported in dry ice to the GSF, Munich where samples were immediately frozen at -80°C until they were analysed. The thyroid hormones T4 and T3 in serum were determined using a specific I^{125} labelled Spectria Radioimmunoassay kit for each hormone (Orion Diagnostica, Finland) with appropriate modification of sample volumes.

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) in individual liver homogenates from a selection of the same fish samples were quantified. Thawed homogenates were ground with sea sand and anhydrous sodium sulphate and filled in a glass column. Prior to extraction they were spiked with ^{13}C-labeled standards. The clean-up procedure described previously[2] was applied to the extracts. The instrumental analysis was performed with a high resolution mass spectrometer (Finnigan MAT 95S) coupled with an HP GC 6890. Chromatographic separation was achieved by splitless injection (cold on column injector KAS4, Gerstel, Germany) of 1 µl on a Restek Rtx-2330 column (60 m, ID 0.25 mm, ft 0.1 µm). The GC oven was programmed as follows: 90°C initial hold for 1.5 min, increase at a rate of 25°C/min to 180°C followed by an increase of 2°C/min to 260°C, final hold at 260°C for 15 min. The MS was operated in SIM mode at a resolution of 10000 and the two most intense ions of the molecular ion cluster were monitored for the unlabeled and labeled isomers.

T4 and T3 from liver from another series of fish samples were extracted using barbital buffer and a methanol/chloroform separation matrix and measured using an I^{125} labelled radioimmuno-assay[3].

Results and Discussion

Fig 1 shows the T4 and T3 concentrations in serum of *H. molitrix* from the contaminated ponds. It is evident that fish from the most contaminated pond (pond 1) had significantly lower concentrations of thyroid hormones in serum. T3 concentrations were also lower in the blood of fish from pond 2 while fish in pond 5 had significantly higher concentrations of T4. The latter result should be interpreted with caution however because of the small number of samples available for analysis from pond 5.

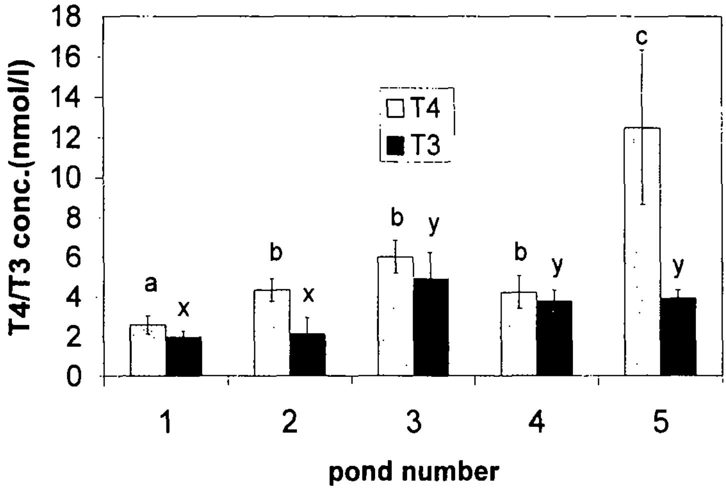

Fig. 1: T4 and T3 concentrations in serum of yearling *H. molitrix* from ponds 1 to 5 in Ya-Er Lake (mean ± SE , n=6-9 except in pond 5 where n=2). Means with the same superscript in the same series are not significantly different from each other.

It is interesting to note that the T4 and T3 concentrations in *H. molitrix* especially for samples from pond 5 corresponds to levels reported for a number of species of fish by previous authors.

Concentrations ranged from mean values of 2.6 to 12.5 n mol/l (2.0 to 9.7 ng/ml) for T4 and 2.0 to 3.9 n mol/l (1.3 to 2.5 ng/ml) for T3 from ponds 1 to 5 respectively. In addition T4 concentrations in *H. molitrix* blood were higher than T3 concentrations as is common in freshwater teleosts.

Table 1: PCDD/F concentrations in livers and T4 and T3 concentrations in serum of corresponding individual *H. molitrix* from ponds 1 to 5 with a contamination gradient (ND indicates concentration below detection limit, NA indicates result not available).

Pond	Fish ID	PCDD/F in liver TEQ(ng/kg FW)	T4 in serum nmol/l	T3 in serum nmol/l
1	11	49	0.8	1.0
	12	76	3.2	ND
	13	103	2.7	1.8
2	21	27	3.5	1.6
	22	20	2.8	3.8
	23	28	3.3	1.7
3	31	14	6.0	4.1
	32	19	4.3	11.3
	33	33	6.1	2.7
4	41	1.3	4.3	4.6
	42	2.6	3.9	4.7
	43	2.2	6.8	NA
5	51	5.0	8.7	4.4

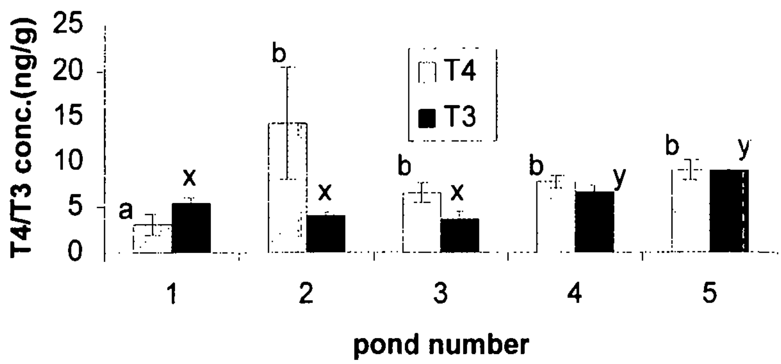

Fig. 2: T4 and T3 concentrations in the liver of yearling *H. molitrix* from ponds 1 to 5 in Ya-Er Lake (mean ± SE , n=6). Means with the same superscript in the same series are not significantly different from each other. These samples are a different series to those in Table 1.

Fig 2 shows that T4 concentrations were also significantly lower in the liver of silver carp sampled from pond 1 compared with those sampled from ponds 2 to 5. T3 concentrations in liver of fish from ponds 1, 2 and 3 were not significantly different from each other but were lower than in fish from ponds 4 and 5.

Results indicate that the high level of contamination by persistent organic pollutants of the water and sediments in Pond 1 may have resulted in depressed thyroid activity. Thyroid hormones play a significant role in the growth and metabolism of fish[4] and our results indicate that continuous exposure to dioxins could alter this role. In addition results from fish liver samples reflect the bioaccumulation of Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) corresponding to the gradient in the water of the ponds with the highest bioaccumulation in fish from pond 1 and low from pond 5. It is possible that deiodinase activity is reduced in fish from ponds 1 to 3 as a result of increasing toxicant bioaccumulation in the liver, since the liver is a major site for the deiodination of T4 to form T3. T3 is the active form of thyroid hormone and lower T3 levels could affect the growth and metabolism of fish exposed to environmental dioxins.

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