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Effects of Short-Term Exposure to Methylmercury Chloride and Its Withdrawal on Serum Levels of Thyroid Hormones in the Catfish *Clarias batrachus*

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Natural weathering processes and large scale use of mercury (Hg) in industries, in medicine, and in agriculture (in the formulation of a large number of fungicides, insecticides and bactericides) contribute substantially to the environmental burden of Hg every year (WHO 1976; 1990). Hg which enters the aquatic ecosystems is concentrated up-through different levels of the food chain, fishes being an important and crucial link in this transfer. In fishes Hg is accumulated as the highly toxic methyl Hg (Moore and Ramamoorthy 1984; WHO 1990) and causes diverse toxic effects on renal function (Kirubagaran and Joy 1988), endocrine functions (Kirubagaran and Joy 1989; 1992). In mammals Hg has been reported to cause thyroid dysfunction (Suzuki et al. 1966; Kosta et al. 1975; Goldman and Blackburn 1979; Kawada et al. 1980; Nishida et al. 1986; Kabuto 1986; Wren et al. 1987). However, such studies on the thyroid physiology of fishes are meager (Bhattacharya et al. 1989; Kirubagaran and Joy 1989). In a previous study, we have reported that Hg compounds (mercury chloride, methylmercury chloride and emisan-6, an alkoxyalkyl Hg fungicide) inhibit radioiodine uptake and protein-bound iodine (PBI) levels, and cause histopathological changes in the thyroid of the catfish (Kirubagaran and Joy 1989). However, the profile of thyroid hormones was not investigated in that study, although the PBI measurements indicated changes on long term exposure (90 and 180 d). In toxicity studies, normally effects of acute or chronic exposure of the toxicants are usually evaluated but whether such changes are reversible or not are seldom investigated. In the present study, we have evaluated responses of the thyroid hormones (T_4 and T_3) in the catfish, C. batrachus, after exposing chronically to methyl mercury chloride and after its withdrawal.

MATERIALS AND METHODS

About 60 adult catfish of both sexes weighing 70 ± 5 g used in this study were collected from the Gangetic riverine system in and around Varanasi in December (resting phase of the reproductive cycle). They were acclimated to laboratory conditions for a fortnight before being used in the experiment and were fed minced

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goat liver daily during the course of the investigation. The fish were maintained in glass aquaria containing well-water (pH 7.3, hardness 23.2 mg L^{-1} as $CaCO_3$ and dissolved oxygen 8 mg L^{-1}) under ambient photothermal conditions (11 L: 13 D; 16°C). The fish were divided into two batches. The first batch of 30 fish was exposed to a sublethal concentration (0.125 mg L^{-1}) of methyl Hg chloride (98% W/W; Wilson Laboratory Bombay) which corresponded approximately to one-third of the LC-50 value (96 hr) reported by us (Kirubagaran and Joy 1988 for details). Water was renewed every 24 hr along with the required amount of stock solution of methyl Hg chloride. The second batch of 30 fish was maintained in well-water and served as the control. The experiment was begun in the first week of January and terminated in the second week of February.

Five fish each from the experimental and control groups were sacrificed at 1, 2 and 3 wk. At the end of the third week, the treatment was withdrawn and the remaining fish were sacrificed after 4, 5 and 6 wk along with the control fish. After sampling, blood was collected through caudal puncturing. Serum was separated by centrifuging at 2500 rpm for 10 min and stored at -20°C for a maximum of 2 mon.

Serum levels of T_3 and T_4 were assayed using the RIA kits (RIAK-4/4A and RIAK-5/5A, respectively) of Bhabha Atomic Research Centre (BARC), Bombay. Two hundred µL of 0.14 M tris (hydroxymethyl) aminomethane (pH 8.6) was taken in assay tubes alongwith 50 µL of serum samples. To this 100 µL of ^{125}I-labeled T_3 or T_4 were added. After gentle mixing, the tubes were incubated for 45 (T_3) or 30 (T_4) min at 37°C. At the end of the incubation, 1.0 mL of polyethylene glycol (PEG) was added to all the tubes except the ones used for total count. The tubes were vortexed gently and centrifuged at 1000 x g in a swingout rotor for 20 min. The supernatant was discarded and precipitate was counted in a Beckman gamma counter (DP 5500) for 1 min. Standard displacement curves were prepared using T_3(0.15-2.4 ng/mL) and T_4 (1.25-10.0 ng/mL) standards in hormone-free serum in a like manner.

Data were expressed as means ± SEM and were analyzed with Student's t-test for statistical significance between experimental and control groups.

RESULTS AND DISCUSSION

In teleosts, as in other vertebrates, the major secretory product of the thyroid is T_4 and only a small amount of T_3 is released (Leatherland 1987). Most of the T_3 in the circulation is derived from peripheral conversion of T_4. T_3 is physiologically the most active form of the thyroid hormones, the ratio of T_4 to T_3 varied from 3.07 to 3.5 in the control fish during the course of the study (Table 1). The present data are consistent with our previous observation that Hg inhibits thyroid activity (Kirubagaran and Joy 1989). Although the serum level of PBI did not vary significantly

during 45 d exposure (Kirubagaran and Joy 1989), direct hormone measurement in the present study shows that methyl HgCl inhibits thyroid hormone levels from very early period of the exposure. The

Table 1: Effects of exposure to 0.125 mgL^{-1} methylmercury chloride (methyl HgCl) on serum T_3 and T_4 levels, and T_4/T_3 ratio during 3 wk of exposure, and withdrawal in the catfish, Clarias batrachus (Means ± SEM; n=5). Significance levels (*P<0.01; **P<0.001) shown for difference between control group and group exposed to methyl HgCl.

Duration wk	T_3 ng/mL		T_4 ng/mL		T_4/T_3 ratio	
	Control	Methyl Hg	Control	Methyl Hg	Control	Methyl Hg
Exposure						
1	1.28±0.06	1.22±0.08	4.49±0.19	3.08±0.18*	3.50	2.52
2	1.37±0.04	0.92±0.07**	4.61±0.21	2.41±0.31**	3.36	2.61
3	1.41±0.06	0.55±0.05**	4.85±0.31	1.86±0.32**	3.43	3.38
Withdrawal						
4	1.58±0.05	0.57±0.04**	5.04±0.28	2.15±0.40**	3.19	3.77
5	1.75±0.12	1.02±0.08**	5.38±0.34	2.44±0.36**	3.07	2.39
6	1.77±0.09	1.64±0.16	5.68±0.37	2.53±0.29**	3.21	1.54

discrepancy may be due to the fact that the PBI measurement is not a sensitive index as compared to the RIA procedure. The significant and progressive reduction of serum T_4 levels at all durations of Hg exposure up to 3 wk indicates that it impaired directly the hormone synthesis and its release into the circulation. A similar decrease in T_4 level was reported in the teleost, Channa punctatus exposed to mercury chloride (Bhattacharya et al. 1989) who attributed the inhibition to disturbance in the activity of lysosomal protease (cleaves T_4 from thyroglobulin for release into the blood) and cytosolic iodide peroxidase (oxidizes inorganic iodide to active elemental iodine). In mammals, Goldman and Blackburn (1979) and Kawada et al. (1980) have suggested that Hg may not affect organification (binding to tyrosine residues) of iodide but interferes with the enzyme coupling reaction during the synthesis of T_3 and T_4. Nishida et al. (1986) have reported that Hg inhibits T_4 but not the T_3 level. Wren et al. (1987) did not find any differences on serum T_4 or T_3 levels of adult mink exposed to methyl Hg. In the catfish, the serum T_3 level showed a significant reduction only in the 2 and 3 wk of Hg exposure. The reduction in T_3 level may be indirect due to the significant decrease of T_4 at all durations and not due to inhibition of monodeiodination of T_4 by methyl HgCl. This is indicated by the fact that the T_4/T_3 ratio increased from 2.52 in the I wk to 3.38 (close to control value) in the 3 wk of Hg exposure, despite the progressive decrease in T_4.

In the withdrawal study, the T_3 level, which was significantly low compared with control fish at 4 and 5 wk was restored to that of the control fish in the 6 wk. This early restoration of T_3 level appears to be due to increased peripheral conversion of T_4 because the T_4/T_3 ratio decreased from 3.77 to 1.54. However, the T_4 level was not restored to the normal range and remained significantly low even after the 6 wk, despite the fact that there was a quantitative increase in the T_4 value in comparison to that of the 3 wk Hg group. This suggests that Hg did not irreversibly affect hormone synthesis and its release. The process of restoration of T_4 synthesis and release appears to be very slow perhaps due to extensive damage of several large thyroid follicles and decreased radioiodine uptake (Kirubagaran and Joy 1989). The regeneration and maturation of new follicles may take time to augment T_4 output to the normal range.

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