Bull. Environ. Contam. Toxicol. (2004) 73:911–918

DOI: 10.1007/s00128-004-0513-y

Bulletin of Environmental Contamination and Toxicology

Hematological Parameters of Tench (*Tinca tinca* L.) after Acute and Chronic Exposure to Lethal and Sublethal Mercury Treatments

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Received: 30 July 2003/Accepted: 8 September 2004

Heavy metals are recognized as serious pollutants of the aquatic environment. The contamination of aquatic ecosystems by metals poses a threat to aquatic organisms in particular and to whole ecosystems in general. Non-biodegradable metals such as mercury accumulate in living organisms and cause various diseases and disorders, including renal lesions in *Oncorhynchus mykiss* (Iliopoulou-Georgudaki and Kotsanis 2001); hepatic, splenic, intestinal and ovarian lesions in *Heteropneustes fossilis* (Bano and Hasan 1990) and endocrine impairment in *Etroplus maculates* (Veena et al. 1997). Mercury compounds have also been reported to react directly or catalytically with cell membrane lipids in cells of the central nervous system and promote hydrolysis and hydrolytic decomposition. Mercury is a major and common aquatic pollutant, and is present in many aquatic ecosystems (Iliopoulou-Georgudaki and Kotsanis 2001). It is released in an inorganic form mainly from power plants and waste incinerators and in water is converted by microbes into more toxic form of methyl mercury, which accumulate in fishes (Schrope 2001).

Fish constitute a valuable commodity from the standpoint of human consumption, and aquatic pollution has direct effect on fish health and survival. Animals with different foraging behaviours may have different metal burdens (Chen and Folt 2000). Tench is a primary fish species of commercial importance in some countries, such as Germany (Grosch et al. 2000). It is also a good test organism for metal contamination because of its bottom feeding behaviour. Significant concentrations of metals have been reported in bottom feeding fishes (Campbell 1994).

Erythrocytes and associated hematological indices reflect the energetic and respiratory status of animals, and are commonly used as indicators of metal pollution in the aquatic environment (Shah et al. 1995). This present study was conducted based on the hypothesis that tench is more resistant fish and can survive in the environment, wherein, the other fish are eliminated, and therefore, it may show more resistance demonstrated hereby resilience of hematological parameters to mercury toxicity.

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MATERIALS AND METHODS

Tench (*Tinca tinca* L., 1758) were collected from Mogan Lake near Ankara, Turkey with cast nets and transported to the wet laboratory of the Department of Biology, Ankara University, Ankara. Fifty liters capacity water tanks supported with air pumps were used for transportation of fish. Animals were allowed two weeks to acclimatize in laboratory conditions. Fish were observed for overall health based on behaviour and presence of gross lesions. Any fish with dubious health status was culled from the group. Transportation stress resulted in fungal disease (Saprolegniasis) in some fishes, however, such fishes were separated immediately, and healthy fishes only were used for experiments. Fish were fed commercial pellet food twice a day, maintained at 12 D/ 12 L photoperiodicity, and the water replaced twice a week with stored dechlorinated water. Physio-chemical parameters of laboratory water were measured daily with standard laboratory apparatus/meters and were as follows: dissolved oxygen 7.68±0.13 mg/L, water temperature 20.67±0.49 °C, pH 7.49±0.9, EC 0.29±0.02 mS/cm. Other water chemistry parameters were determined at the DSi, TAKK, Chemical laboratories, Ankara at the start of experiments only and were determined to be: bicarbonates 97.6 mg/L, total alkalinity 80 mg/L, chlorine 10.3 mg/L, sulphates 26.1 mg/L, calcium 29.0 mg/L, magnesium 1.2 mg/L and mercury <0.005 mg/L.

Seven mercury exposures (1.0 ppm/48 hrs; 0.1 ppm/24 hrs; 0.1 ppm/96 hrs; 0.1 ppm/3 wks; 0.25 ppm/24 hrs; 0.25 ppm/96 hrs; 0.25 ppm/3 wks) were tested using fish in 120 litre capacity glass aquaria. These concentrations were chosen based on the Lc_{50}. 96-hrs Lc_{50} of mercury (1.0 ppm) was calculated from percent mortalities of tench following Veena et al. (1997). Mercury (Merck) was obtained from Chemical laboratory of department of Chemistry, Ankara University, as a mercuric chloride ($HgCl_2$), and introduced in to the water in concentration of ppm from the stock solution, prepared following Allen (1994). Circulation of a metal in aquaria was ensured by two air pumps devoid of filters, as filters were previously observed to accumulate metals from water. Mercury levels were not determined in aquaria throughout experimental duration and therefore, are considered as approximations. However, aquaria were covered with glass lids and water was replaced to counter evaporative loss and to keep concentration of mercury constant. A group of 8 healthy fish was used for each of seven exposures, and the same number for control with each exposure (total 112, 53 male and 59 females of age group II-III, average total length 23.17±0.49 cm and average weight 199.19±6.11 g).

Blood was collected within 35-40 seconds through cardiac puncture in 2 ml disposable heparinized syringes with 21-gauge needle after stunning fish by a blow on the head. Syringes were kept at 4 °C up to complete study of blood parameters. For haematocrit (Hct) determination, a three fourth of microhaematocrit capillaries (75 mm L ×1.1 mm ID, Superior Germany) were filled with blood, sealed at one side by capillary sealer (Marion Feld, Germany) and centrifuged at 11000 rpm for 6 minutes in microhaematocrit centrifuge

(Hawksley and Sons, Co Sussex, England). Haematocrit (%) was determined by a microhaematocrit reader (Blaxhall and Daisley 1973). Hemoglobin (Hb) was determined with a hemoglobin test kit (No.124729, Roach GmbH Mannheim, Germany) using the cyanmethemoglobin method. Briefly, 0.02 ml of aliquot blood was mixed with 5 ml of test reagent (potassium hexacynoferrate 0.6 mmol/L and potassium cyanide 0.75 mmol/L), incubated at room temperature for 10-20 minutes, and absorbance was read at 546 nm using Shimadzu spectrophotometer (UV-120 IV, Shimadzu Cooperation, Japan). Absorbance values were converted to hemoglobin measurements (g/dl) based on standards included with the test kit. Total red blood cells (tRBCs) were counted using an improved Neubaur haemocytometer (Clay, Adams, NY). Blood was diluted 1: 200 with Hayem`s fluid (Mishra et al. 1977). Erythrocytes were counted in the loaded hemocytometer chamber and total numbers were reported as 10^6 cells/ mm^3, as described by Wintrobe (1967). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined as follows (Tort and Torres 1988):

MCV (μm^3)= Hct (%)/RBC ($mm^3 \times 10^6$) $\times 10$

MCH (pg)= Hb (g/100 ml)/RBC ($mm^3 \times 10^6$) $\times 10$

MCHC (g/dl)/Hct (%) $\times 100$.

Values of treated groups were compared statistically with control groups by student's t-test. Significance was established at $P \leq 0.05$ using the Microsoft Excel 2000 Programme. Significance of data was further checked with the percent change (+ increase and – decrease) in blood parameters of tench (Singh and Reddy 1990).

RESULTS AND DISCUSSION

In acute lethal treatment (1.0 ppm Hg/48 hrs), a significant decrease was observed in Hct, Hb and RBC count ($P \leq 0.05$). A 3.11, 12.06 and 15.79 % increase was observed in MCHC, MCV and MCH respectively, however, it was non-significant when compared with control ($P \geq 0.05$). In lower acute sublethal treatment (0.1 ppm Hg/24 hrs), a significant increase was observed in Hct and RBC count ($P \leq 0.05$). A non-significant increase of 0.38 % in MCV and 15.89 % in Hb level ($P \geq 0.05$), and a non-significant decrease of 9.48 and 10.09 % in MCH and MCHC respectively were also observed ($P \geq 0.05$). In second lower acute sublethal treatment (0.1 ppm Hg/96 hrs), a significant increase in MCV ($P \leq 0.05$) and a significant decrease in MCHC ($P \leq 0.05$) were observed. Haematocrit only increased 5.58 % ($P \geq 0.05$), and MCH, RBC count and Hb decreased 1.16, 12.5 and 13.69 % respectively ($P \geq 0.05$). In lower chronic sublethal treatment (0.1 ppm Hg/3 wks), a significant decrease was observed in Hct, Hb and RBC count ($P \leq 0.05$). MCHC showed 2.15 % decrease and MCV 5.84 % increase ($P \geq 0.05$). In higher acute sublethal treatment (0.25 ppm Hg/24 hrs), no significant change was observed in any blood parameter. A 3.39 and 13.42 % increase was recorded in Hct and RBC count respectively ($P \geq 0.05$), and 1.12, 3.79, 12.03 and 15.30 %

decrease was observed in Hb, MCHC, MCV and MCH respectively ($P \geq 0.05$). In second higher acute sublethal treatment (0.25 ppm Hg/96 hrs), a significant increase in Hct, Hb and RBC count ($P \leq 0.05$) and a significant decrease in MCHC ($P \leq 0.05$) were observed. MCH showed non-significant decrease of 3.06 % ($P \geq 0.05$) and MCV showed non-significant increase of 12.64 % ($P \geq 0.05$). In higher sublethal chronic treatment (0.25 ppm Hg/3 wk), a significant decrease in Hct and RBC count ($P \leq 0.05$) and significant increase in MCH ($P \leq 0.05$) were observed. Hemoglobin decreased 7.58 % ($P \geq 0.05$) and MCV and MCHC increased 12.11 and 12.45 % respectively ($P \geq 0.05$) (Table 1 & 2).

Metal-induced alterations in hematological parameters generally occur due to the osmotic changes resulting in hemodilution or hemoconcentration (Tort and Torres 1988). Anemia in flounders after cadmium exposures has been attributed to an increased plasma volume caused by disturbed water balance, and to the decreased rate of production and/or increased loss or destruction of RBCs (Larsson 1976). A decline in Hct, Hb and RBCs in *Anguilla rostrata* after chronic cadmium exposure has been attributed to: (i) impaired erythropoisis due to direct effect of metal on haematopoietic centers (kidney/spleen), (ii) accelerated erythroclasis due to altered membrane permeability and/or increased mechanical fragility, and (iii) defective Fe metabolism and/or impaired intestinal uptake of Fe due to mucosal lesions (Gill and Epple 1993). Similar physiological impairments may also occur with mercury. A decreased Hct, Hb and tRBCs in *Aphanius dispar* after mercury exposure has been attributed to the permeability of erythrocytes to mercurial compounds and the balance of the number of binding sites between intracellular and extracellular components of blood. Mercurial compounds have been reported to penetrate cell membranes and inhibit both Na+-K+-ATPase and Na+-K+-insensitive ATPase (Hilmy et al. 1980). Histopathological lesions in spleen and intestine of *Heteropneustes fossilis* after mercury exposure have been reported (Bano and Hasan 1990). Additionally, Gill and Pant (1985) described poikilocytosis, vacuolation, ruptured cytoplasm, nuclear displacement in erythrocytes of *Barbus conchonius* after mercury exposures. Hemolysis during cell handling and hemorrhaging during fish dissection were observed almost in all groups of tench in present study possibly indicating increased RBC fragility among the treated fish compared to the control.

Elevations in hematological parameters associated with metals have been reported, such as increased Hct, Hb and RBCs in *Cyprinus carpio* exposed acutely to copper (Svobodova 1982). An elevation in RBC numbers resulting in increased Hct and Hb could be a consequence of blood cell reserve release. Spleens of some teleosts have been reported to serve as a potent blood storage organ, sequestering blood cells under resting conditions and releasing them to circulation during contraction associated with stress (Yamamoto 1988). An increased RBC count due to splenic contraction- a common stress response, has been reported in *Oreochromis auraus* after acute mercury exposure. Splenic contraction may occur due to osmotic disturbances. An increase in blood parameters may be due to stimulated erythropoiesis by elevated demand for O_2 or CO_2 transport as a result

Table 1. Hematological parameters of *T. tinca* after acute and chronic exposure to lethal and sublethal mercury treatments.

Mercury (ppm)	Time	Hct (%)		Hb (g/dl)		RBCc ($mm^3 \times 10^6$)	
		Control	Treated	Control	Treated	Control	Treated
1.0	48 hrs	24.06±1.01 (20.40-28.86)	*17.48±1.44 (11.57-22.1) - 27.34	5.51±0.32 (4.1-7.0)	*3.90±0.06 (3.7-4.3) - 29.22	1.15±0.05 (0.95-1.35)	*0.73±0.04 (0.58-0.92) - 36.52
0.1	24 hrs	22.90±0.69 (20.20-26.53)	*29.53±1.39 (22.58-36.84) + 28.95	5.41±0.37 (4.2-7.2)	6.27±0.39 (4.0-7.8) + 15.89	1.10±0.07 (0.8-1.5)	*1.38±0.09 (0.92-1.75) + 25.45
0.1	96 hrs	22.05±0.84 (19.38-25.51)	23.28±0.63 (20.43-25.53) + 5.58	5.77±0.31 (4.7-7.5)	4.98±0.25 (3.9-6.1) - 13.69	1.2±0.03 (1.01-1.31)	1.05±0.06 (0.74-1.24) - 12.5
0.1	3 wks	21.74±0.86 (18.75-26.04)	*16.52±1.12 (11.57-20.83) - 24.01	5.58±0.42 (4.4-7.5)	*4.03±0.09 (3.8-4.7) - 27.77	1.35±0.09 (1.05-1.70)	*0.95±0.03 (0.75-1.08) - 29.63
0.25	24 hrs	28.24±1.04 (25.26-33.69)	29.20±1.07 (22.68-32.29) + 3.39	7.15±0.42 (5.9-9.9)	7.07±0.23 (5.9-7.8) - 1.12	1.49±0.17 (1.02-2.40)	1.69±0.12 (1.25-2.34) + 13.42
0.25	96 hrs	21.15±0.90 (18.36-24.74)	*29.73±0.57 (26.88-31.91) + 40.56	5.72±0.33 (4.7-7.6)	*6.86±0.19 (5.9-7.5) + 19.93	1.17±0.03 (0.99-1.30)	*1.55±0.17 (1.12-2.35) + 32.47
0.25	3 wks	26.89±0.68 (23.95-30.52)	*22.06±0.76 (18.94-25.26) - 17.96	6.33±0.27 (5.1-7.6)	5.85±0.32 (3.9-6.9) - 7.58	1.51±0.07 (1.15-1.90)	*1.11±0.04 (0.9-1.27) - 26.49

n=8, ± SE, ( ) range, + % increase, - % decrease, * $P \leq 0.05$

Table 2. Hematological parameters of *T. tinca* after acute and chronic exposure to lethal and sublethal mercury treatments.

Mercury (ppm)	Time	MCV (μm^3)		MCH (pg)		MCHC (g/dl)	
		Control	Treated	Control	Treated	Control	Treated
1.0	48 hrs	212.57±15.19 (164.88-292.94)	238.22±15.07 (192.83-327.87) + 12.06	47.60±1.48 (40.36-52.63)	55.12±3.12 (42.39-66.15) + 15.79	23.15±1.55 (17.96-29.59)	23.87±2.10 (17.64-32.84) + 3.11
0.1	24 hrs	214.87±15.85 (163.20-296.37)	215.70±6.65 (184.90-245.43) + 0.38	50.11±3.71 (35.59-62.60)	45.36±1.04 (39.49-49.60) - 9.48	23.49±1.14 (19.82-29.54)	21.12±0.55 (17.71-23.25) - 10.09
0.1	96 hrs	184.71±8.35 (155.04-215.13)	*225.54±10.31 (198.34-290.54) + 22.10	48.24±2.55 (37.6-59.52)	47.68±1.01 (43.54-52.70) - 1.16	26.14±0.85 (22.37-30.31)	*21.34±0.67 (18.13-24.66) - 18.36
0.1	3 wks	166.40±12.15 (114.32-210.75)	176.13±16.12 (116.86-250.0) + 5.84	41.28±1.14 (36.92-45.45)	42.64±1.86 (37.38-54.66) + 3.29	26.0±2.21 (19.20-37.5)	25.44±1.95 (19.2-32.84) - 2.15
0.25	24 hrs	201.96±15.62 (140.05-265.86)	177.66±10.27 (137.99-224.96) - 12.03	50.98±4.55 (36.08-72.54)	43.18±2.75 (32.9-58.4) - 15.30	25.27±0.97 (21.94-29.38)	24.31±0.62 (21.56-27.33) - 3.79
0.25	96 hrs	181.28±8.59 (151.73-215.54)	204.20±15.91 (135.56-267.47) + 12.64	48.91±2.65 (38.84-61.29)	47.41±4.09 (26.95-64.34) - 3.06	27.01±0.86 (23.28-31.05)	*23.08±0.51 (19.88-24.68) - 14.55
0.25	3 wks	179.89±7.42 (150.70-208.26)	201.69±11.99 (161.88-280.66) + 12.11	42.45±2.45 (33.77-52.20)	*53.45±3.83 (36.11-73.33) + 25.91	23.53±0.72 (18.64-25.24)	26.46±1.04 (19.5-30.24) + 12.45

n=8, ± SE, ( ) range, + % increase, - % decrease, * $P \leq 0.05$

of increased metabolic activity or by destruction of gill epithelium causing faulty gaseous exchange (Buckley 1976). Gill tissue in some fishes such as *Etroplus maculatus* are reported to be one of the main targets of mercury action (Veena et al. 1997). Erythropoiesis may be due to either direct action of metal on haematopoietic organs or indirect action by impairing hemoglobin synthesis. Pollutants are widely reported to enter the RBCs and either oxidize or denature hemoglobin by inhibiting glycolysis or metabolism of the hexose monophosphate shunt. As a result, increased amounts of hemoglobin are required to replace oxidized or denatured hemoglobin, which is achieved by stimulation of erythropoietic tissues (Cyriac et al. 1989). A slight increase and/or decrease in red cell indices may be due to disproportion between RBCs and Hb concentration. Some treatments of mercury did not show any significant effect, which may be due to compensation of fish to pollutants or physiological adjustments to the stressor (Dethloff et al. 2001).

It is evident from the data that both acute and chronic mercury exposures caused some physiological impairments in tench. The hematological data, as well as gross observations from sample handling and fish necropsy, suggest that these findings may have been particularly related to erythrocyte fragility and hemorrhaging. It is possible that the decrease in blood parameters may be due to hemolysis and damage to haematopoietic tissues by mercury, and the increase in parameters may be the result of feed back response of fish body to compensate erythrocyte loss. Further, greater resistance to mercury toxicity shown hematologically in tench, compared to other fish species, did not prove true, as initially hypothesized. This notion, however, would need to be demonstrated more conclusively with more detailed studies in both field and laboratory trials.

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