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# Distribution of Mercury in the Soft Tissues of the Blue Tilapia *Oreochromis aureus* (Steindachner) after Acute Exposure to Mercury (II) Chloride

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Mercury has no known biological functions in the animal body and is described as an ultratrace element (Lall 1989). Consequently, there is no well defined regulatory mechanism present in the animal body and it tends to accumulate readily if available in an animal's environment. Sources of mercury include the chloroalkali industry, the manufacture of electrical equipment, paint, fungicides and dentistry (WHO 1989). The use of mercury in the gold mining industry has caused extensive pollution in the Amazon Basin (Martinelli et al. 1988). Whether fish take up organic or inorganic mercury, most of it accumulates in the tissues in the organic form (WHO 1989). Most cases of mercury poisoning arising from fish consumption are due to methylmercury because mercury entering the aquatic system rapidly becomes methylated (Jensen and Jernelov 1969). Minamata disease in humans was first reported in 1956 due to consumption of contaminated fish and shellfish from Minamata Bay (Mance 1987). Therefore it is important to monitor the mercury content of fish which are caught or farmed for human consumption. Since many commercial animal feeds contain a fish meal component, monitoring is important from the aspect of contamination of farm animals intended for human consumption. Oreochromis aureus (Steindachner) is a species of tilapia often cultured in ponds (Hepher and Pruginin 1982) and also in cages in North and Latin America. Therefore, it is a suitable model to use for studying the effects of mercury exposure on the distribution of mercury in different tissues of fish. Distribution is important, because different cultures consume different fish organs, not just the muscle portion alone. The tissues which have a high content of mercury will be most dangerous from a toxicological viewpoint (Hendricks and Bailey 1989). Removal of the tissues known to contain the highest concentrations of mercury would reduce the mercury content of fish meal. Since fish are often species-

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specific in their responses to heavy metals (Sorensen 1991), it is important to study a species which is actually farmed and cultured as a food fish.

## MATERIALS AND METHODS

O. aureus were purchased from the Primary Production Department, Freshwater Fisheries Laboratory, Singapore. Prior to experimentation the fish were maintained in 120-L glass aquaria for a minimum observation period of one month, to ensure that they were disease-free. The fish ranged from 90 to 130 mm in total length. During the experiments the following conditions applied to the water: The temperature ranged from 25.5 to 28.5 $^{o}C$; oxygen concentration did not fall below 7.7 mg $L^{-1}$; hardness ranged from 9.10 to 11.88 mg $L^{-1}$ as calcium and 0.83 to 1.00 mg $L^{-1}$ as magnesium; pH ranged from 6.1 to 6.5. All aquaria and glassware used were presoaked in a solution of 5% (v/v) nitric acid to remove any contaminating heavy metals. The fish were randomly assigned to plastic aquaria, one fish per tank, each tank containing 25 L of tap water. The number of fish was 24 for each of the 6 treatments and controls. However, tissues from only 12 fish per treatment were analyzed for mercury content. Tissues from the remaining 12 fish were used for other analyses, except for the gonads, which were required for mercury analysis. Both gonads from each fish were combined for mercury analysis. All chemicals used were purchased from Merck (Darmstadt, Germany) and were of the highest purity available. A stock solution of 1 g $L^{-1}$ mercury was made in double distilled water from mercury (II) chloride. The volumes of stock solution required to obtain the designated concentrations of 0.5 or 0.1 mg $L^{-1}$ were added to the tanks. No additions were made to the control tanks. The fish were fed ad libitum on a commercial pellet food during the experimental period, but food was witheld for the first 24 hr of each experiment and for 24 hr prior to sacrifice. Every 48 hr, 10 L of water were siphoned from each tank, enabling feces and uneaten food to be removed. Subsequently, 10 L of tap water containing the designated concentration of mercury were added to each tank, with the exception of the control tanks which received 10 L of water without mercury.

Fish were sacrificed by decapitation. Red-Tip microhematocrit capillary tubes (Monoject, USA) were used for the collection of blood from the severed dorsal aorta. Plasma was obtained by centrifuging the capillary tubes at 11800 RPM (RCF = 14000 x g) for 5 min in a Micro-hematocrit Mk.5 centrifuge (Hawksley and Sons Ltd., England). The bile was removed from the gall bladder with a 1 mL insulin syringe (Monoject, USA).

Tissues were dissected free and rinsed in 0.9% (w/v) saline, blotted dry, weighed into 100 mL conical flasks and placed in a drying oven at 60 $^{o}$C until constant weight was attained. The dried tissues were digested using the wet oxidation method described by Gergely et al. (1977), except that 16 mL of concentrated nitric acid was used, instead of sulphuric acid, for digestion at 60 $^{o}$ C. Tissue samples from fish which had not been exposed to mercury were spiked with known quantities of mercury (II) chloride or methyl mercury chloride and run through the entire procedure to ascertain the percentage recovery of inorganic and organic mercury, respectively. The median recoveries for mercury (II) chloride and methyl mercury chloride were 97.1 and 89.9 % respectively. Analysis of total mercury (organic + inorganic) content, using a Perkin-Elmer (Model MAS 50B, USA) mercury analyzer system, was based on the method of Hatch and Ott (1968) as employed by Gergely et al. (1977). Acid-digested tissues and acidified standards were oxidized with excess potassium permanganate and made up to a volume of 100 mL in BOD bottles with double distilled water. Any remaining potassium permanganate was decolorized by the addition of 5 mL of 1.5 % hydroxylamine hydrochloride solution. Mercury was reduced to its elemental form by the addition of 5 mL of 10 % tin (II) chloride and measured by the cold-vapor atomic absorption principle.

As the majority of the data were not normally distributed, nonparametric statistical analyses were applied. For comparisons between one test group and one control group, the Mann-Whitney Test (two-tailed; $\alpha$ = 0.05) (Zar 1984) was used to test equality of medians. For comparisons involving 3 or more independent groups, the Kruskal-Wallis One-Way Analysis of Variance was used with the appropriate nonparametric multiple comparison test (Zar 1984; Equations 12.27 and 12.28).

## RESULTS AND DISCUSSION

Table 1 shows the effects of exposing O. aureus to 0.1 mg $L^{-1}$ mercury for 12 and 24 hr and for 1 wk on the mercury content of various tissues. All samples taken from treated fish, including plasma and bile, were significantly higher in mercury content ($P < 0.05$) than the respective tissues from control fish. Furthermore, each tissue exhibited a progressive increase in mercury concentration with increasing exposure time. For the 12 hr exposure, the mercury concentration in tissues was in the order kidney > spleen > gill filaments > intestine > brain > testes > liver > ovaries > caudal muscle. For tissue mercury concentrations compared by the Kruskal-Wallis One Way ANOVA, kidney contained a significantly higher mercury concentration when compared with caudal

Table 1. Tissue mercury levels ($\mu g\ Hg\ g^{-1}$ wet weight) in *O. aureus* exposed to 0.1 mg $L^{-1}$ mercury.

| | Control | | Test | |
|---|---|---|---|---|
| | Median(n) | [ Range ] | Median(n) | [ Range ] |
| Liver[1] | 0.00(12) | [0.00-0.01] | 0.31*(12)$^{CD}$ | [0.12-1.14] |
| Liver[2] | 0.02(12) | [0.01-0.05] | 3.74*(12)$^{BCDE}$ | [0.93-13.5] |
| Liver[3] | 0.01(12) | [0.00-0.03] | 11.3*(12)$^{BC}$ | [3.47-22.9] |
| Brain[1] | 0.00(12) | [0.00-0.13] | 0.65*(12)$^{BCD}$ | [0.20-0.86] |
| Brain[2] | 0.05(12) | [0.00-0.14] | 3.76*(12)$^{CDE}$ | [1.58-8.18] |
| Brain[3] | 0.05(12) | [0.00-0.14] | 10.6*(12)$^{BC}$ | [5.21-16.3] |
| Gills[1] | 0.01(12) | [0.00-0.02] | 4.58*(12)$^{AB}$ | [0.95-9.60] |
| Gills[2] | 0.02(12) | [0.01-0.05] | 24.3*(12)$^{AB}$ | [14.1-35.8] |
| Gills[3] | 0.01(12) | [0.00-0.02] | 21.4*(12)$^{AB}$ | [15.4-32.5] |
| Intestine[1] | 0.01(12) | [0.00-0.02] | 2.55*(12)$^{ABC}$ | [0.34-35.4] |
| Intestine[2] | 0.02(12) | [0.01-0.07] | 5.21*(12)$^{BCD}$ | [2.66-10.6] |
| Intestine[3] | 0.02(12) | [0.00-0.03] | 11.7*(12)$^{BC}$ | [4.30-20.3] |
| C.muscle[1] | 0.01(12) | [0.01-0.02] | 0.11*(12)$^{D}$ | [0.04-0.16] |
| C.muscle[2] | 0.01(12) | [0.00-0.05] | 0.30*(12)$^{E}$ | [0.16-0.88] |
| C.muscle[3] | 0.01(12) | [0.00-0.02] | 0.47*(12)$^{C}$ | [0.13-1.19] |
| Spleen[1] | 0.05(12) | [0.00-0.22] | 5.61*(12)$^{AB}$ | [1.73-9.46] |
| Spleen[2] | 0.10(12) | [0.00-0.52] | 16.0*(12)$^{ABC}$ | [7.67-28.9] |
| Spleen[3] | 0.11(12) | [0.00-0.24] | 23.4*(12)$^{AB}$ | [11.8-58.7] |
| Kidney[1] | 0.04(12) | [0.00-0.17] | 12.5*(12)$^{A}$ | [6.28-15.6] |
| Kidney[2] | 0.08(12) | [0.00-0.26] | 67.4*(12)$^{A}$ | [38.1-113 ] |
| Kidney[3] | 0.06(12) | [0.00-0.15] | 208*(12)$^{A}$ | [ 156-432 ] |
| Ovaries[1] | 0.00(13) | [0.00-0.02] | 0.21*(20) | [0.02-0.56] |
| Ovaries[2] | 0.05(19) | [0.00-0.20] | 2.45*(17) | [0.90-9.25] |
| Ovaries[3] | 0.04(17) | [0.00-0.12] | 1.48*(15) | [1.48-7.85] |
| Testes[1] | 0.02(11) | [0.00-0.20] | 0.34*(4) | [0.15-0.65] |
| Testes[2] | 0.11(7) | [0.07-0.60] | 4.99*(5) | [0.91-10.6] |
| Testes[3] | 0.00(6) | [0.00-0.05] | 4.79*(8) | [0.82-14.7] |
| Eye[2] | 0.02(12) | [0.00-0.03] | 0.67*(12)$^{DE}$ | [0.23-0.77] |
| Bile[1] | 0.01(11) | [0.00-0.07] | 0.07*(12) | [0.05-0.27] |
| Bile[2] | 0.02(12) | [0.00-0.05] | 1.73*(12) | [0.36-158 ] |
| Bile[3] | 0.01(12) | [0.01-0.03] | 3.92*(12) | [0.50-9.01] |
| Plasma[1] | 0.03(12) | [0.00-0.06] | 0.20*(12) | [0.13-0.37] |
| Plasma[2] | 0.00(12) | [0.00-0.05] | 0.76*(12) | [0.52-1.16] |
| Plasma[3] | 0.01(12) | [0.00-0.05] | 1.62*(12) | [0.72-2.47] |

* = Significantly different from respective control.
Superscript numbers: 1 = 12 hr; 2 = 24 hr; 3 = 1 wk.
Detection limit = 0.01 $\mu g\ Hg\ g^{-1}$ wet weight of tissue.
Similar superscripts denote no significant difference between test tissues at same time intervals.

muscle, liver and brain. Caudal muscle accumulated a significantly lower concentration of mercury than spleen, gill filaments and intestine.

After 24 hr exposure to 0.1 mg $L^{-1}$ mercury, tissue accumulation of mercury was in the order kidney > gill filaments > spleen > intestine > testes > brain > liver > ovaries > eye > caudal muscle. Kidney contained a significantly higher concentration of mercury than caudal muscle, eye, liver, brain and intestine while caudal muscle contained a significantly lower concentration of mercury than gill filaments, spleen and intestine.

After 1 wk exposure to 0.1 mg $L^{-1}$ mercury the order of mercury concentration in the tissues was kidney > spleen > gill filaments > intestine > liver > brain > testes > ovaries > caudal muscle. Amongst the tissues statistically compared for mercury concentration, kidney contained a significantly higher level of mercury than caudal muscle, brain, liver and intestine. Caudal muscle was found to have significantly less mercury than spleen and gill filaments.

The effects of 6, 12 and 24 hr exposure to 0.5 mg $L^{-1}$ mercury on tissue mercury concentrations of O. aureus are shown in Table 2. In all tissues and bile, there was a significant increase in mercury concentration above the control levels. The plasma showed significant elevations of mercury concentration only for exposure periods of 12 and 24 hr. In bile, plasma and all tissues except brain, there was a progressive increase in mercury concentration with increasing time of exposure. For 6 hr exposure to 0.5 mg $L^{-1}$ mercury, the order in which tissues accumulated mercury was gill filaments > kidney > spleen > brain > liver > intestine > caudal muscle. Gill filaments accumulated a significantly higher concentration of mercury than caudal muscle, intestine and liver. Caudal muscle mercury content was significantly lower than the mercury content of spleen.

After 12 hr exposure to 0.5 mg $L^{-1}$ mercury, tissue mercury accumulation was in the order kidney > gill filaments > spleen > testes > liver > intestine > brain > ovaries > caudal muscle. Kidney mercury content was significantly higher than the mercury content of caudal muscle, brain, intestine and liver. The mercury content of caudal muscle was significantly lower than the mercury content of the spleen and gill filaments.

After 24 hr exposure to 0.5 mg $L^{-1}$ mercury the accumulation profile was kidney > gill filaments > spleen > intestine > liver > brain > testes > ovaries > caudal muscle. There was a significantly higher mercury

Table 2. Tissue mercury levels (µg Hg $g^{-1}$ wet weight) in *O. aureus* exposed to 0.5 mg $L^{-1}$ mercury.

| | Control | Test | |
|---|---|---|---|
| | Median(n)[ Range ] | Median(n) | [ Range ] |
| Liver[1] | 0.06(12)[0.00-0.42] | 0.29*(12)$^{BC}$ | [0.13-1.06] |
| Liver[2] | 0.00(12)[0.00-0.01] | 0.57*(12)$^{BC}$ | [0.44-1.38] |
| Liver[3] | 0.02(12)[0.00-0.04] | 1.51*(12)$^{CD}$ | [0.42-26.6] |
| Brain[1] | 0.09(12)[0.00-1.50] | 1.14*(12)$^{ABC}$ | [0.61-4.52] |
| Brain[2] | 0.00(12)[0.00-0.19] | 0.38*(12)$^{BC}$ | [0.00-1.20] |
| Brain[3] | 0.07(12)[0.00-0.23] | 1.34*(12)$^{CD}$ | [0.75-3.61] |
| Gill[1] | 0.07(12)[0.01-0.27] | 7.90*(12)$^{A}$ | [3.55-45.0] |
| Gill[2] | 0.00(12)[0.00-0.03] | 12.8*(12)$^{A}$ | [8.75-36.4] |
| Gill[3] | 0.04(12)[0.00-0.09] | 30.2*(12)$^{AB}$ | [19.6-36.7] |
| Intestine[1] | 0.02(12)[0.00-0.21] | 0.27*(12)$^{BC}$ | [0.18-1.03] |
| Intestine[2] | 0.00(12)[0.00-0.07] | 0.44*(12)$^{BC}$ | [0.24-1.31] |
| Intestine[3] | 0.03(12)[0.00-0.06] | 4.75*(12)$^{BC}$ | [3.01-11.1] |
| C.muscle[1] | 0.02(12)[0.01-0.02] | 0.03*(12)$^{C}$ | [0.03-0.03] |
| C.muscle[2] | 0.05(12)[0.00-0.01] | 0.10*(12)$^{C}$ | [0.04-0.21] |
| C.muscle[3] | 0.02(12)[0.00-0.06] | 0.19*(12)$^{D}$ | [0.07-0.27] |
| Spleen[1] | 0.21(10)[0.01-0.82] | 1.43*(11)$^{AB}$ | [0.68-7.27] |
| Spleen[2] | 0.16(12)[0.00-0.83] | 3.90*(12)$^{AB}$ | [1.76-10.7] |
| Spleen[3] | 0.77(12)[0.04-1.42] | 8.27*(12)$^{ABC}$ | [3.26-14.0] |
| Kidney[1] | 0.50(10)[0.23-0.87] | 6.19*(11)$^{A}$ | [2.22-27.7] |
| Kidney[2] | 0.18(12)[0.00-0.35] | 35.0*(12)$^{A}$ | [22.7-111 ] |
| Kidney[3] | 0.26(12)[0.01-0.56] | 73.5*(12)$^{A}$ | [11.6-141 ] |
| Ovaries[2] | 0.00(9) [0.00-1.72] | 0.19*(12) | [0.01-0.94] |
| Ovaries[3] | 0.01(16)[0.00-0.55] | 0.50*(17) | [0.09-1.25] |
| Testes[2] | 0.25(4) [0.11-0.43] | 0.78*(4) | [0.69-0.92] |
| Testes[3] | 0.21(6) [0.07-0.33] | 1.01*(8) | [0.01-1.41] |
| Bile[1] | 0.02(18)[0.00-0.23] | 0.06*(19) | [0.02-0.16] |
| Bile[2] | 0.01(12)[0.00-0.27] | 0.10*(12) | [0.04-1.03] |
| Bile[3] | 0.03(12)[0.00-0.06] | 0.38*(12) | [0.08-1.40] |
| Plasma[1] | 0.00(6) [0.00-0.35] | 0.00 (7) | [0.00-2.86] |
| Plasma[2] | 0.06(12)[0.00-0.13] | 0.66*(12) | [0.09-4.83] |
| Plasma[3] | 0.04(12)[0.00-0.23] | 0.92*(11) | [0.58-1.91] |

* = Significantly different from respective control. Superscript numbers: 1 = 6 hr; 2 = 12 hr; 3 = 24 hr. Similar superscript letters denote no significant difference between test tissues at same time intervals. Detection limit = 0.01 µg Hg $g^{-1}$ wet weight of tissue.

content in the kidney than in intestine, liver, brain and caudal muscle. Caudal muscle had a significantly lower mercury concentration than gill filaments, spleen and intestine. Exposure to 0.1 or 0.5 mg $L^{-1}$ mercury resulted in similar mercury concentrations in the brain and liver at all exposure intervals. In mammals, the mercury content of the brain is usually much lower than the liver (Magos 1987; WHO 1989). These differences may be explained by a better developed blood-brain barrier in mammals.

Fresh water fishes drink little water in comparison with marine fishes (Eddy 1981), therefore the gills might be expected to be the primary route for the uptake of waterborne pollutants (Allen et al. 1988), the gut playing a secondary role; Kuroshima (1992) supports this hypothesis. In the present study, kidney appeared to be the target tissue for mercury during acute exposures, since the kidney accumulated median concentrations as high as 208 µg Hg $g^{-1}$ after a 1 wk exposure to only 0.1 mg $L^{-1}$ mercury. Even 12 hr of exposure to this concentration elevated kidney mercury levels to a far higher concentration than in any other organ. Only where the exposure periods were very short did other organs equal the mercury sequestration shown by the kidney, e.g., 6 hr exposure to 0.5 mg $L^{-1}$ mercury resulted in similar mercury concentrations in the gill and kidney. The gill and kidney also accumulated similar mercury concentrations when exposed to 1 mg $L^{-1}$ mercury for 2 hr (Allen et al. 1988). Hilmy et al. (1987) found a similar rapid increase in the mercury concentration of the kidney, liver and gills of Clarias lazera exposed to inorganic divalent mercury. In their study, kidney accumulated the highest concentration of mercury. Mercury is present in the kidney, liver and gills predominantly in the form of a complex with metallothionein (MT) (Marafante 1976). MT is responsible for the long-term stability of mercury in the kidney (Hilmy et al. 1987). Cuvin-Aralar and Furness (1990) also found the kidney of Phoxinus phoxinus accumulated mercury to a higher concentration than any other tissue after exposure to 0.1 µg $L^{-1}$ mercury for 1 wk, while the visceral remains and muscle contained the lowest concentrations of mercury.

Since the fish in the present study were not fed for the first 24 hr of exposure, any mercury entering the intestine could not have originated from food which adsorbed the mercury from the water. Therefore, after 24 hr exposure to 0.5 mg $L^{-1}$ mercury the intestinal concentration of 4.75 µg Hg $g^{-1}$ probably originated from mercury complexes in the bile entering the intestine. It is also feasible that the plasma could have contributed to the mercury content of the intestine. Bile plays an

important role in the distribution of heavy metals from the liver to the different tissues (Klaassen 1976). Many heavy metals form conjugates with reduced glutathione, cysteine or other thiols in the liver. These metal conjugates are then excreted into the bile and enter the intestine (Grahl et al. 1985; Allen et al. 1988). However, some of these metal conjugates are then reabsorbed into the body via the intestine (Klaassen 1976). Exposure to mercury at 0.5 and 0.1 mg $L^{-1}$ for different periods resulted in excretion of mercury in the bile, reaching 3.92 µg Hg $g^{-1}$ bile after exposure to 0.1 mg $L^{-1}$ mercury for 1 wk. Therefore, bile appears to play an important role in the interorgan distribution of mercury in fishes.

The safety level for fish, and fish products, intended for human consumption is 0.5 µg $g^{-1}$ wet weight (Sorensen 1991). In the present study the median caudal muscle concentration almost exceeded this safety level. However, in the Far East many cultures consume whole-fish, while others preferentially eat gonads, gills, eyes and other tissues. These dietary preferences could lead to dangerous dietary levels of mercury since the present study has demonstrated levels of mercury above the safety level in tissues other than caudal muscle. The mercury burden of fish meal could be reduced if the viscera, kidney and gill filaments were removed from fish prior to processing. Mercury was found to be preferentially concentrated in the gill filaments, spleen and kidney. These tissues had exceeded the safety level within 6 hr of exposure. It is conceivable that fish in contact with highly contaminated sediments from chloralkali plants could be exposed to very high mercury concentrations for short periods of time. Mercury concentrations ranging from 0.27 to 1.7 µg $g^{-1}$ dry weight have been reported from the sediments in the vicinity of chloralkali plants (WHO 1989).

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