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Detection of acute toxicity of mercury chloride in Yellowfin sea bream (*Acanthopagrus latus*)

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ABSTRACT

Toxicity tests allow the determination of pollution effects, providing direct evidence of the biological responses of marine organisms to contaminants. The 96-h LC_{50} tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as heavy metals. Hg_{2+} tested concentrations were 20, 50, 100, 200, 500, 1000, 2000, 5000 and10000 µg/l, Groups of six male yellow fine sea bream (120 g) were exposed for 96 h to each of the Range Finding Test for LC50, in fiberglass tank equipped with aeration with 100 l of test medium. According to Range Finding Test (fifty percent of mortality between 500 and 1000) another tested concentration 550, 650, 750, 850 and 950 µg/l, Groups of six male yellow fine sea bream were exposed for 96 h to each of the LC_{50} 96h for test solutions. 24 h, 48 h, 72 h and 96 h LC_{50} were 962.75 886.48, 886.48 and 648.86 respectively. The 96 h NOEC, LOEC and LC_{50} were 500, 550 and 648.86 µg/l respectively. LC_{50} values indicated that mercury is more toxic to A. latus. LC50 obtained in the present study compare with corresponding values that have been published in the literature for other species of fish, show different LC_{50} of mercury in different species and even different time, but what is important, lower value of LC_{50} for *A. latus* compare with most species and confirm sensitively of *A. latus* to low mercury doses.

Key words: NOEC, LOEC, LC_{50}, Mercury Chloride, *Acanthopagrus latus.*

INTRODUCTION

Aquatic ecosystems are typically monitored for pollution of heavy metals using biological assays. Aquatic organisms have been reported to accumulate heavy metals in their tissues several times above ambient levels. Fishes have been used for many years to determine the pollution status of water, and are thus regarded as excellent biological markers of metals in aquatic ecosystems. Heavy metals have long been recognized as serious pollutants of the aquatic environment. They cause serious impairment in metabolic, physiological and structural systems when present in high concentrations in the milieu (Tort, 1987).

Mercury (Hg) is a liquid metal at ambient temperatures and pressures. It forms salts in two ionic states mercury (I) and mercury (II). Mercury (II), or mercuric salts, are much more common in the environment than mercury (I) or mercurous salts. These salts, if soluble in water, are bioavailable and considered toxic. Mercury also forms organometallic compounds, many of whichhave industrial and agricultural uses (Boening, 2000).

Mercury in fish was already recognized as a public health and ecological problem in the 1960's. It was commonly assumed that local point sources (industrial effluent, utility emissions, fungicide applications) were the main sources, and many

studies focused on waters with nearby point source contamination.

Although mercury chloride is not the most toxic mercury compound in the marine environment (Boudou and Ribeyre, 1997), it is the key form between the gaseous metal form transported through atmosphere and the methylmercury form that bioaccumulates in organism. Once it enters into the organism, mercury can draw various immunotoxic effects.

Toxicity tests allow the determination of these effects, providing direct evidence of the biological responses of marine organisms to contaminants. Due to the fact that organisms from different species vary in their sensitivity towards chemical substances, it is difficult to set standards for protection of species with regard to pollutants in the environment. Extrapolation from one species to another is, therefore, difficult if their relative sensitivities are not known (Van Straalen et al., 1994).

The 96-h LC50 tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as heavy metals. Higher LC50 values are less toxic because greater concentrations are required to produce 50% mortality in organisms (Eaton et al. 1995). The heavy metals that are toxic to many organisms at very low concentrations and are never beneficial to living beings are mercury, cadmium and lead (Hilmy *et al.* 1985).

The present study was conducted to determine the acute toxicity of the heavy metal compound $HgCl_2$ in a statistic system to the marine fish *Acanthopagrus latus*. This species was selected for bioassays because it can easily be raised under laboratory conditions. It fulfills most of the requirements of a model species and is available throughout the year.

MATERIALS AND METHOD

Ninety six yellow fine sea bream all immature male in same size (120 g final body weight average) were obtained from Mahshahr creeks with hooks in a Upon capture, (only healthy fish, as indicated by their activity and external appearance, were used in the experiments) the fish were maintained alive on board in a fiberglass tank and on return to shore transferred to a 300-L aerated vat filled with sea water for transport back to the nearby laboratory. In laboratory Fish maintained in a seawater re-circulatory system (300-L tanks) equipped with physical/biological filters and with aeration to the Mariculture Research Station of the South Iranian Aquaculture Research Center, Mahshahr, Iran from October to November.

All samples were acclimated for one weeks in a 15 aerated fiberglass tank containing 46 ppt saltwater maintained at 25 C under a constant 12:12 L:D photoperiod. Acclimatized Fish were fed daily with a live feed (fresh shrimp) and daily we check water quality and water parameters. Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of the water quality. LC50 is the ambient aqueous chemical activity causes 50% mortality in an exposed population. These calculations are based on two important assumptions. The first assumption is that the exposure time associated with the specified LC50 is sufficient to allow almost complete chemical equilibration between the fish and the water. The second assumption is that the specified LC50 is the minimum LC50 that kills the fish during the associated exposure interval. Fortunately, most reliable LC50 ' satisfy these two assumptions (Neely, 1984).

Hg^{2+} tested concentrations were 20, 50, 100, 200, 500, 1000, 2000, 5000 and10000 µg/l, Groups of six male yellow fine sea bream (120 g) were exposed for 96 h to each of the Range Finding Test for LC50, in fiberglass tank equipped with aeration with 100 l of test medium. The control group was exposed to filtered sea water in similar conditions.

The bioassay was performed in a temperature (25 ± 1 _C) and under a natural photoperiod (12hL: 12hD) controlled room. Test medium was not renewed during the assay and no food was provided to the animals. Values of pH, Temperature, and salinity were measured at time 0, 24, 48, 72 and 96 h.

At the end of the bioassay (Boyd and Tucker 1992), Range values were determined and according to that (fifty percent of mortality between 500 and 1000) another tested concentration 550, 650, 750, 850 and 950 µg/l, Groups of six male yellow fine sea bream (100 g) were exposed for 96 h to each of the LC50 96h for test solutions in same condition with Range Finding Test. At the end of the bioassay, LC50 96h values were determined (de Aguiar *et al.,* 2004).

LC value and standard error SE of LC were calculated following the probit procedure method as described by Wardlaw 1985. The $LC_{10,30,50,70,90}$ values are derived using simple substitution probit of 10,30,50,70 and 90 respectively for probit of mortality in the regression equations of probit of mortality vs. mercury. The 95% confidence limits for LC_{50} are estimated by using the formula LC_{50} (95% CL) = LC50 ± 1.96 [SE (LC50)]. The SE of LC_{50} is calculated from the formula: $SE(LC_{50}) = \frac{1}{b\sqrt{pnw}}$ Where: b=the slope of the mercury/probit response (regression) line; p=the number of mercury used, n = the number of animals in each group, w = the average weight of the observations (Hotos and Vlahos. 1998) (table 1). Acute toxicity tests were carried out in order to calculate the 96h-LC50 for mercury in yellow fin sea bream, based on OECD Guidelines (1998). Mortality was recorded after 24,48,72 and 96h, and LC50 values and its confidence limits(95%)were calculated by the Litchfield and Wilcox on Method (1949).The test was carried out in triplicate. Percentages of fish mortality were calculated for each mercury concentration at 24, 48, 72 and 96 h of exposure.

RESULTS

There was 100% mortality at 10000 µg/l concentration within the first 4h after dosing, and

Table1: The 95% confidence limits for LC_{50} of yellowfin sea bream

Concentration (µg/l)	24h	48h	72h	96h
b	0.012	0.002	0.002	0.009
p	5	5	5	5
n	6	6	6	6
W	120	120	120	120
SE	1.38	8.33	8.33	1.85
95% CL	3.5868	16.3268	16.3268	3.626

Table 2: Cumulative mortality of yellowfin sea bream (n=6, each concentration) at Range Finding Test

Concentration	No. of dead yellowfin sea bream			
(µg/l)	24h	48h	72h	96h
Control	-	-	-	-
20	-	-	-	-
50	-	-	-	-
100	-	-	-	-
200	-	-	-	-
500	-	-	-	-
1000	1	3	6	6
2000	2	6	6	6
5000	6	6	6	6
10000	6	6	6	6

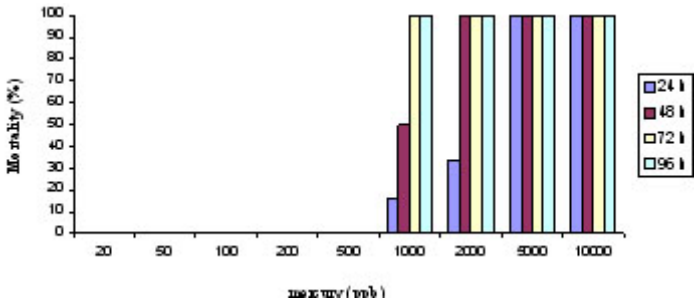

Fig. 1: The column mercury-response (mortality) for A. Latus in the Range Finding Test experiment

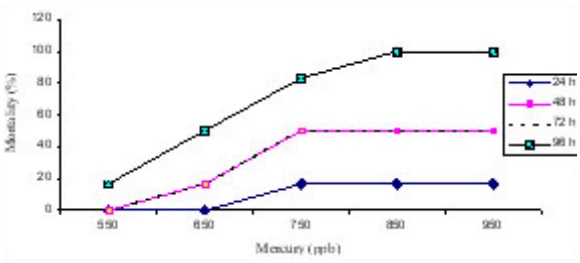

Fig. 2: The sigmoid mercury-response (mortality) curve for A. Latus in the LC_{50} experiment

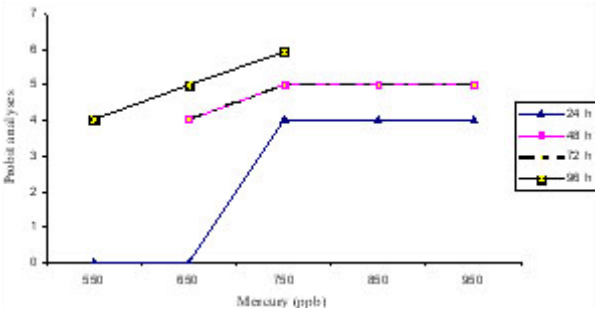

Fig. 3: The sigmoid probit analyses curve for A. Latus in the LC_{50} experiment

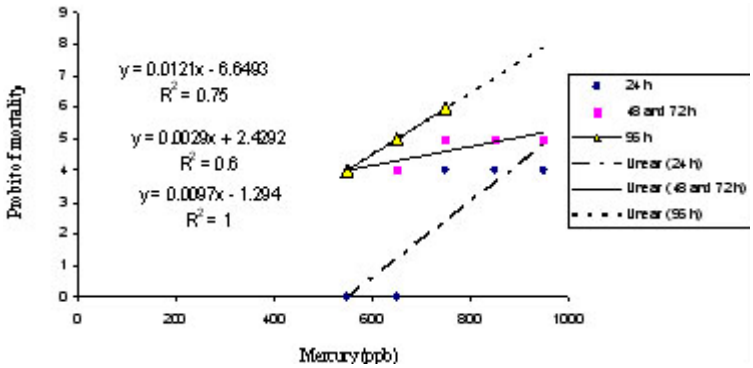

Fig. 4: Probit of mortality versus mercury regression lines for *A. latus* in the LC50 experiment. Also depicted are the regression equations and R_2 values. Probit values used are derived from Fig 3

Table 3: Cumulative mortality of yellowfin sea bream (n=6, each concentration) at LC50 test

Concentration (µg/l)	No. of dead yellowfin sea bream			
	24h	48h	72h	96h
Control	-	-	-	-
550	-	-	1	1
650	-	1	2	3
750	1	3	5	5
850	1	3	6	6
950	1	3	6	6

Table 4: Lethal concentrations (LC_{1-99}) of mercuric chloride depending on time (24-96h) for *A. latus*

Point	Concentration (µg/l) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_{30}	919.4132	705.6551	705.6551	594.8041
LC_{40}	941.8181	799.1379	799.1379	622.7525
LC_{50}	962.7520	886.4827	886.4827	648.8659
LC_{60}	983.6859	973.8275	973.8275	674.9793
LC_{70}	1026.0909	1007.3103	1007.3103	702.9278

Table 5: Physicochemical parameters of test water

	Parameters
Temperature (°C)	25 ± 1
pH	7.8 ± 0.1
Salinity	46±1

100% mortality at 5000 µg/l within the 14h whereas 100% mortality for 2000 µg/l was 42h and for 1000 µg/l was 54h.

The mortality of yellowfin sea bream for mercury chloride doses 20, 50, 100, 200, 500, 1000, 2000, 5000 and 10000 µg/l were examined during the exposure times at 24, 48, 72 and 96 h for Range Finding Test (table 2). Fish exposed during the period 24-96h had significantly increased number of dead yellowfin sea bream with increasing concentration. There were significant differences in number of dead fish between the duration 24-96 in each.

After finding this fact that main range is between 500-1000 (because of no mortality at 500 µg/l and 100% mortality at 1000 µg/l), the mortality of yellowfin sea bream for mercury chloride doses 550, 650, 750, 850 and 950 µg/l were examined during the exposure times at 24, 48, 72 and 96 h for LC50 Test (table 3).

Median lethal concentrations of 10%, 30%, 50%, 70% and 90% test are in table 4. Physicochemical parameters of test water are in table 5.

Mortality percentage of Range Finding Test and LC50 experiment are in figures 1 and 2 respectively, however sigmoid probit analyses and regression lines of probit are in figures 3 and 4 respectively.

Toxicity Testing Statistical Endpoints are in tow part

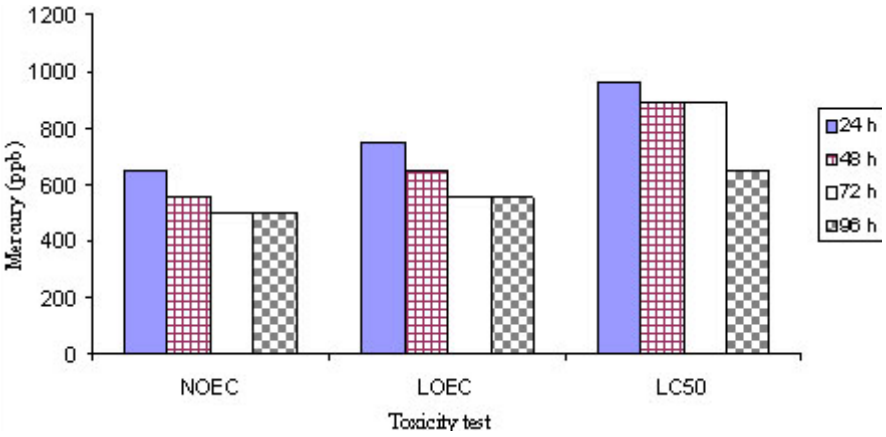

Fig. 5: Toxicity testing statistical endpoints in yellowfin sea bream

Hypothesis Testing: is there a statistically significant difference between the mean response in the treatments and mean response in control or reference sample? LOEC: Lowest Observed Effect Concentration; NOEC: No Observed Effect Concentration. 2- Point Estimates: what toxicant concentration will cause a specific effect on the test population? LC50: the median Lethal Concentration. Our result for Toxicity Testing Statistical Endpoints is in Fig 5.

DISCUSSION

Toxic effects of mercury and its compounds depend on the chemical form of mercury. Organic forms of mercury are generally more toxic to aquatic organisms than are inorganic forms. HgCl2 can be converted into highly toxic methyl mercury by methylation through chemical or biological processes.

Factors influencing mercury levels can be divided into exogenous (characteristics of the water body) and endogenous (characteristic of the individuals or species). Exogenous factors include pH, sulfur and organic matter (e.g., dissolved organic carbon). Endogenous factors include species, habitat and food preferences, metabolic rate, age, growth rate, size, mass, and diet.

According to the Gooley et al (2006), mercury is one of the concern metals in aquaculture and has 10-40 µg/l of LC50 with only 1µg/l for safe levels, whereas LC50 value for other heavy metals is higher than mercury (cadmium 80-420, cooper 20-100, zinc1000-10000, lead 1000-40000 µg/l). Chowdhury et al (2006) show the 96h LC50 for the juvenile trout as11 µg/l (95% CI = 9.2 – 11.9 µg/l). The 96-h LC_{50} value for catfish exposed to Hg2+ under static test was determined to be 570 µg/l (Elia et al., 2000). The 96-h LC50 value of mercury chloride for chub was found as 205 µg/l and 96-h LC_{50} for trout 814 µg/l (Verep *et al*. 2007). On the estuarine fish *Pomatoschistus microps,* LC_{50} of copper and mercury at 96 h were 568 µg/l and 62 µg/l, respectively (Vieira *et al*. 2009).

The concentrations of trace metals that resulted in mortality of *H.rubra* were investigated by exposing juveniles to acute concentrations of Cu, Zn, Hg and Cd for 96hr. Hg resulted in more sudden mortality rate after 24hr exposure compared to Cu yet produced a 96hr LC50 of 173µg Hg/L (Gorski. 2007).

EPA studies (1997) on many aquatic species show vast range of LC50 for mercury chloride, which for saltwater fish was 36 µg/l (juvenile spot) to 1678 µg/l (flounder), that was higher than saltwater invertebrate 3.5 µg/l (mysid shrimp) to 400 µg/l (soft clam). This result emphases that yellowfin sea bream is sensitive to mercury chloride and have low LC50 value.

According to FAO/UNEP (1991), the 96-h LC_{50} values of mercury chloride are for cat fish 350 µg/l, rainbow trout 220 µg/l, striped bass 90 µg/l and brook trout 75 µg/l.

The 96-h LC_{50} values of mercury chloride 37 µg/l for fathead minnow, 160 µg/l for bluegill sunfish, 903 µg/l for rainbow trout, 200 µg/l for rainbow trout and lower in invertebrate, 2 µg/l for crayfish, 5 µg/l for cladocera, 10 µg/l for Gammarus, 5 µg/l for blue mussel, 15 µg/l for prawn, and 3 µg/l for limpet (Eisler, 1987).

For mercury, 96 h LC50 values of 75 µg/l for the catfish (*Sarothrodon mossambicus*), 33 µg/l for the rainbow trout (*Salmo gairdneri*), 110 µg/l for the banded killifish (Fundulus diaphanous) and 90 µg/l for the striped bass (*Roccus saxatilis*) were found (Rehwoldt *et al.,* 1972; Hale, 1977; Das *et al*., 1980).

The susceptibility of fish to a particular heavy metal is a very important factor for LC50 values. The fish that is highly susceptible to the toxicity of one metal may be less or non-susceptible to the toxicity of another metal at the same concentration of that metal in the milieu. Similarly, the metal which is highly toxic to one organism at low concentration may be less or non-toxic to other organism at the same or even higher concentration, so the LC_{50} values reported in the present study for $HgCl_2$ were lower than the values reported by Agarwal (1991) for the *Channa punctatus* (Bloch) at 48, 72, and 96 h. He reported LC_{50} values of 2.512, 2.291, and 2.113 mg/L, respectively, at 48, 72, and 96 h. however, the present values, are higher than those of Khangarot (1981): 0.432 and 0.314 mg/L, respectively, at 72 and 96h in *Channa marulius*.

Rathore and Khangarot (2002) reported that the acute toxicity of $HgCl_2$ increases with increase in temperature. Cairns *et al*. (1981) reported similar trends for other metals. Khangarot and Ray (1987) also observed that the toxicity of copper abruptly decreased with an increase in pH of the Cu-containing medium. Acute toxicity studies are the very first step in determining the water quality requirements of fish. These studies obviously reveal the toxicant concentrations (LC_{50}) that cause fish mortality even at short exposure. Therefore, studies demonstrating the sensitivity of genotoxic effects of heavy metals in aquatic organisms, particularly in fish are needed. Thus, it can be concluded from the present study that fish are highly sensitive to $HgCl_2$ and their mortality rate is dose dependent.

Comparison of values reported earlier with those obtained in the present study may not be meaningful because various factors may influence bioassay techniques like differences in fish(e.g., species, weight, size) and other environmental factors (temperature, variations in pH of the water, total hardness of water, dissolved oxygen). Sprague (1969) observed variability in acute toxicity even in a single species and single toxicant depending on the size, age, and condition of the test species along with experimental factors. Gupta *et al*. (1981) reported that the differences in acute toxicity may be due to changes in water quality and test species. Chronic toxicity values are much lower than acute values and highlight the adverse effects of relatively low concentrations of mercury in water (i.e., < 1 µg/L).

In aquatic toxicology, if LC50 concentration is smaller than 1000 µg/l , the chemical is highly toxic, and if between 1000-10000 µg/l, then it is considered to be moderately toxic (Louis et al. 1996), therefore we report mercury chloride to be highly toxic to yellowfin sea bream and my cause many damage in this Fish.

The fish exposed to metal can compensate for the stressors. If it cannot successfully compensate for stressor effects, an altered physiological stage may be reached in which the organism continues to function and, in extreme cases, the acclimation response may be exhausted with a subsequent effect on fitness (Mayer *et al.* 1992). In the present study, LC50 values indicated that mercury is more toxic to *A. latus*. LC50 obtained in the present study (650 µg/l) compare with corresponding values that have been published in the literature for other species of fish, show different LC50 of mercury in different species and even different time, but what is important, lower value of LC50 for *A. latus* compare with most species and confirm sensitively of *A. latus* to low mercury doses.

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