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Assessment of Mercuric Chloride Toxicity on Rainbow Trouts (*Oncorhynchus mykiss*) and Chubs (*Alburnoides bipunctatus*)

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Abstract: Acute toxicity of mercury-II chloride ($HgCl_2$), one of most toxic pollutants for aquatic ecosystems, in chub (*Alburnoides bipunctatus*) and rainbow trout (*Oncorhynchus mykiss*) was investigated in the present study. Actual concentration of $HgCl_2$ was ranged from 0.10 to 8.00 mg L^{-1}. Concentrations of $HgCl_2$ that killed 50% of the chub and rainbow trout within 96 h (96 h LC_{50}) were estimated as 0.205 and 0.814 mg L^{-1}, respectively. Acute toxicity tests were evaluated by the Finney's Probit Analysis. Chub (*Alburnoides bipunctatus*) was appeared to be more sensitive than trouts (*Oncorhynchus mykiss*) to $HgCl_2$.

Key words: Mercury-II-cloride, acute toxicity, European chub, *Alburnoides bipunctatus*

INTRODUCTION

The determination of toxic compounds present in the aquatic environment and its effect on fish is a basic issue in aquatic toxicology (Bolis *et al*., 2001; Datta and Kaviraj, 2003). Toxic substances may be determined with chemical analysis of water but its effects on fish and ecological risks for aquatic ecosystem could not be determined by chemical analysis. That's why, the preferred way to evaluate the ecological influence of toxic compounds is mortality or bioassay experiments in general (Başer *et al*., 2003; Svobodova *et al*., 2003).

Mercury compounds are very toxic and all forms of mercury are possible human carcinogens (ATSDR, 1999). Mercuric chloride has been used in agriculture as fungicide, in medicine as a topical antiseptic and disinfectant. Mercury-II-clorur is commonly used both for industrial, scientifically and agricultural purposes around the world as well as in Turkey.

In this study, mercury-II-clorur was selected for the bioassay experiment. The aim of this study was to determine the acute toxicity (96 h LC_{50}) of mercury-II-clorur formulation on rainbow trouts (*Oncorhynchus mykiss*) and chubs (*Alburnoides bipunctatus*) and variation of toxicity of mercuric chloride between two species.

Chub is (Eurasia) widely distributed in the basins of Biscay Bay, the North, Baltic Sea, Black, Caspian and Aral seas, in Vardar and Struma rivers of the Aegean basin, in the Tigris-Euphrates river system, in the Rezaiyeh Lake basin and in some other localities in Iran. This species is a member of protected fauna according to Appendix III of the Bern Convention (URL 1). Trouts is a common culture fish for aquaculture in the world.

The toxicity of many metal compounds to the rainbow trout, *Oncorhynchus mykiss*, was first studied in detail and chosen a widely available species, amenable to life in the laboratory, a fish of considerable commercial importance and sensitive to most toxic pollutants and reacts more quickly than most species to adverse environmental conditions (Abel, 1996).

MATERIALS AND METHODS

Two species; rainbow trouts (*Oncorhynchus mykiss*) and chubs (*Alburnoides bipunctatus*) were selected for the bioassay test study. Chubs (*Alburnoides bipunctatus*) (weights: 18.14±4.53 g, lengths: 12.43±1.06 cm) were obtained from Iyidere stream in Rize. Fish were caught by electrofishing and brought to the laboratory within 20 min in plastic buckets with sufficient air. Trouts (*Oncorhynchus mykiss*) (weights: 28.02±9.23 g, lengths: 12.75±1.53 cm) were taken from a local trout breeding farm near laboratory. After 15 days acclimatization, the fish were placed into the maintenance tanks 250 L capacity. Temperature was regulated at

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Table 1: Physicochemical properties of test water

Parameters	*Oncorhynchus mykiss*	*Alburnoides bipunctatus*
Tempetature (°C)	15±1	20±1
pH	7.3-7.5	7.3-7.5
Dissolved oxygen (mg L^{-1})	7.2-8.6	5.7-6.9
Conductivity (µS cm^{-1})	158-182	158-182
Total hardness (as $CaCO_3$)	61-65	61-65

20±1°C by using heaters for chubs and at 15±1°C for trouts. Except for the dosing instance, all tanks were aerated. Test tanks were filled with 200 L of tap water. Some characteristics of these tanks water were dissolved oxygen 7.35-7.86 mg L^{-1}, pH 7.3-7.5, conductivity 158-182 µS and total hardness 61-65 mg $CaCO_3$ L^{-1} (Table 1).

Following the preliminary experiments, all determinations were repeated three times. Groups of experimental animals, each consisting of 10 individuals were selected at random and placed into aerated tank. After 48 h of adaptation, different concentrations of mercury-II-clorur were added to the experimental tanks. Mercury-II-clorur was prepared by diluting it in pure water to give the stock material. Dosing solutions were prepared from this stock by diluting with pure water to give the dosing concentrations of 0.000, 0.100, 0.125, 0.150, 0.175, 0.200 and 0.400 mg L^{-1} for chubs. For trouts, the dosing concentrations are 0.000, 0.280, 0.400, 0.560, 0.840, 1.000, 1.120, 1.400, 2.000, 4.000 and 8.000 mg L^{-1}. During the last 24 h of adaptation and throughout the duration of the experiment, animals were not fed. Mortality was controlled at 24, 48, 72 and 96 h after the start of the tests. Dead individuals were removed immediately. Behavioral changes were recorded in detail at 1-6 h and every 12 h during tests.

The bioassay system was as described in standardized methods (APHA, AWWA, WEF, 1998) and the national regulation (Turkish Official Gazette, 1991). The LC_{50} and 95% confidence limits were calculated by a computer program (US EPA, 1999; Finney, 1971).

RESULTS

The physicochemical characteristics of test water are listed in Table 1. Temperature ranged from 14 to 16°C during experimentation. The pH of the water ranged from 7.3-7.5 and dissolved oxygen ranged from 7.2 to 8.6 mg L^{-1} for rainbow trout, from 5.7 to 6.9 mg L^{-1} for chubs. The calculated 96 h acute LC_{50} value (95% confidence limits) of mercury-II-clorur using a static bioassay system to chub (*Alburnoides bipunctatus*) and rainbow trout (*Oncorhynchus mykiss*) were 0.205 and 0.814 mg L^{-1}, respectively. Control mortality was zero. The results show that mercury-II-clorur is very toxic to fish (Table 2).

Table 2: LC_{50} values (with 95% confidence limit) of $HgCl_2$ (in mg L^{-1}) estimated by Finney for *O. mykiss* and *A. bipunctatus*

Species	LC_{50} value	95% confidence limit	Slope
Oncorhynchus mykiss	0.814	0.695-0.906	10.402
Alburnoides bipunctatus	0.205	0.185-0.346	12.095

Table 3: Cumulative mortality of fry rainbow trout (n = 10, each concentration)

Concentrations (mg L^{-1})	No. of dead fry rainbow trout			
	24 h	48 h	72 h	96 h
Control	-	-	-	-
0.28	-	-	-	-
0.40	-	-	-	-
0.56	-	1	1	1
0.84	1	4	4	4
1.00	4	8	8	8
1.12	7	10	10	10
1.40	10	10	10	10
2.00	10	10	10	10
4.00	10	10	10	10
8.00	10	10	10	10

- : Absent

Fish behaviour in mercuric chloride bioassay test on *Oncoynchus mykiss*: The control group showed normal behavior during the test period. The changes in behavioral response started 3 h after dosing mercury-II-clorur concentrations of 0.28, 0.40 and 0.56 mg L^{-1} concentrations had close to normal behavior. But there was 100% mortality at 1.40, 2.00, 4.00 and 8.00 mg L^{-1} concentrations within the first 30 min after dosing. At 0.84 mg L^{-1} concentration, less general activity was recorded when compared with the control group and loss of equilibrium was observed. The responses of loss of equilibrium and hanging vertically in the water were observed above 0.84 mg L^{-1} concentration. 1.12, 1.40, 2.00, 4.00 and 8.00 mg L^{-1} showed all responses at high levels and the onset was within the first hour after dosing with mercury-II-clorur. Rapid gill movement, erratic swimming, swimming at the water surface and gulping for air and prolonged and motionless laying down on the aquarium bottom were other responses observed at all concentrations above 0.84 mg L^{-1} concentration.

The mortality of rainbow trout for mercuric chloride doses 0.280, 0.400, 0.560, 0.840, 1.000, 1.120, 1.400, 2.000, 4.000 and 8.000 mg L^{-1} were examined during the exposure times at (24, 48, 72 and 96 h) (Table 3 and Fig. 1). The lowest concentrations of mercuric chloride (0.20, 0.40 and 0.56 mg L^{-1}) showed the lowest fish mortality at 96 h. The fish exposed during the period 24-96 h had significantly increased number of dead rainbow trout with increasing concentrations. There were significant differences in number of dead fish between the durations 24-48 h in each concentrations but no differences in 72-96 h. The highest concentrations of 1.12-8.00 mg L^{-1} showed the highest

Table 4: Letal concentrations (LC_{1-99}) of mercuric chloride depending on time (24-96 h) for fry rainbow trout

	Concentration (mg L^{-1}) (95% confidence limits)							
Point	24 h		48 h		72 h		96 h	
LC_1	0.738	(0.488-0.841)	0.487	(0.279-0.602)	0.487	(0.279-0.602)	0.487	(0.279-0.602)
LC_5	0.813	(0.602-0.900)	0.566	(0.369-0.671)	0.566	(0.369-0.671)	0.566	(0.369-0.671)
LC_{10}	0.857	(0.671-0.935)	0.613	(0.427-0.713)	0.613	(0.427-0.713)	0.613	(0.427-0.713)
LC_{15}	0.887	(0.722-0.960)	0.647	(0.471-0.743)	0.647	(0.471-0.743)	0.647	(0.471-0.743)
LC_{50}	1.028	(0.945-1.118)	0.814	(0.695-0.906)	0.814	(0.695-0.906)	0.814	(0.695-0.906)
LC_{85}	1.191	(1.100-1.462)	1.024	(0.920-1.232)	1.024	(0.920-1.232)	1.024	(0.920-1.232)
LC_{90}	1.233	(1.130-1.572)	1.081	(0.966-1.348)	1.081	(0.966-1.348)	1.081	(0.966-1.348)
LC_{95}	1.299	(1.174-1.754)	1.172	(1.033-1.550)	1.172	(1.033-1.550)	1.172	(1.033-1.550)
LC_{99}	1.431	(1.256-2.162)	1.363	(1.159-2.034)	1.363	(1.159-2.034)	1.363	(1.159-2.034)

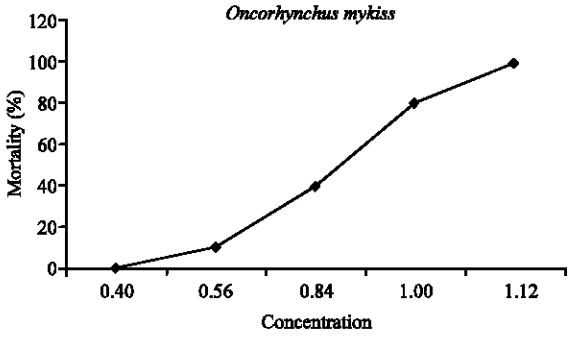

Fig. 1: Percentage of mortality of *O. mykiss* after 96 h exposure to different concentrations of mercuric chloride

fish mortality (Table 3 and Fig. 1). The calculated 24, 48, 72 and 96 h LC_{50} values (95% confidence limits) using a static bioassay system for fry rainbow trouts were determined as 1.028 (0.945-1.118), 0.814 (0.695-0.906), 0.814 (0.695-0.906), 0.814 (0.695-0.906) mg L^{-1}, respectively (Table 4). There were not significant differences between LC1-99 values obtained different time of exposure (Table 4).

Fish behaviour in mercuric chloride bioassay test on *Alburnoides bipunctatus*: The control group showed normal behavior during the test period. The changes in behavioral response started 3 h after dosing mercury-II-clorur concentrations of 0.100, 0.125 and 0.150 mg L^{-1} concentrations had close to normal behavior. But there were 100% mortality at 0.400 mg L^{-1} and 50% mortality at 0.200 mg L^{-1} concentrations within the first 6 h after dosing. At 0.175 mg L^{-1} concentration, less general activity was recorded when compared with the control group and loss of equilibrium was observed. The responses of loss of equilibrium and hanging vertically in the water were observed above 0.175 mg L^{-1} concentration. 0.200 and 0.400 mg L^{-1} showed all responses at high levels and the onset was within the first 6 h after dosing with mercury-II-clorur. Rapid gill movement, erratic swimming, swimming at the water surface and gulping for air and prolonged and motionless laying down on the aquarium bottom were other responses observed at all concentrations above 0.175 mg L^{-1} concentration.

Table 5: Cumulative mortality of Chub (n = 10, each concentration)

Concentrations (mg L^{-1})	No. of dead chub			
	24 h	48 h	72 h	96 h
Control	-	-	-	-
0.100	-	-	-	-
0.125	-	-	-	-
0.150	-	-	-	1
0.175	1	1	1	1
0.200	5	5	5	5
0.400	10	10	10	10

- : Absent

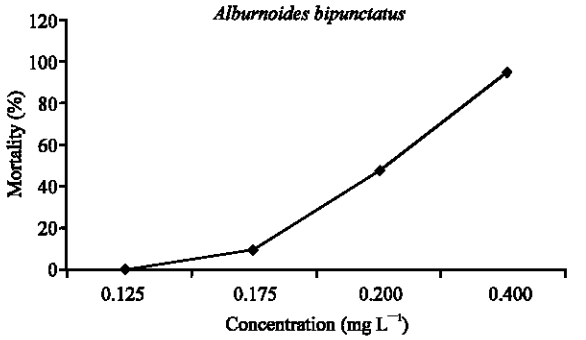

Fig. 2: Percentage of mortality of *A. bipunctatus* after 96 h exposure to different concentrations of mercuric chloride

The mortality of chub for mercuric chloride doses 0.100, 0.125, 0.150, 0.175, 0.200 and 0.400 mg L^{-1} were examined during the exposure times at (24, 48, 72 and 96 h) (Table 5 and Fig. 2). The lowest concentrations of mercuric chloride (0.100, 0.125 and 0.150 mg L^{-1}) showed the lowest fish mortality at 96 h. The fish exposed during the period 24-96 h had significantly increased number of dead chub with increasing concentrations. There were not significant differences in number of dead fish between the durations 24-96 h in each concentrations. The highest concentration of 0.400 mg L^{-1} showed the highest fish mortality (Table 5 and Fig. 2). The calculated 24, 48, 72 and 96 h LC_{50} values (95% confidence limits) using a static

Table 6: Letal concentrations (LC_{1-99}) of mercuric chloride depending on time (24-96 h) for chub

	Concentration (mg L^{-1}) (95% confidence limits)							
Point	24 h		48 h		72 h		96 h	
LC_1	0.158	(0.045-0.175)	0.158	(0.045-0.175)	0.158	(0.045-0.175)	0.132	(0.041-0.154)
LC_5	0.169	(0.076-0.182)	0.169	(0.076-0.182)	0.169	(0.076-0.182)	0.150	(0.075-0.168)
LC_{10}	0.176	(0.100-0.187)	0.176	(0.100-0.187)	0.176	(0.100-0.187)	0.161	(0.101-0.178)
LC_{15}	0.180	(0.120-0.192)	0.180	(0.120-0.192)	0.180	(0.120-0.192)	0.168	(0.123-0.187)
LC_{50}	0.200	(0.187-0.290)	0.200	(0.187-0.290)	0.200	(0.187-0.290)	0.205	(0.185-0.346)
LC_{85}	0.222	(0.203-0.632)	0.222	(0.203-0.632)	0.222	(0.203-0.632)	0.250	(0.212-1.841)
LC_{90}	0.227	(0.206-0.763)	0.227	(0.206-0.763)	0.227	(0.206-0.763)	0.262	(0.218-1.042)
LC_{95}	0.235	(0.211-1.009)	0.235	(0.211-1.009)	0.235	(0.211-1.009)	0.280	(0.227-1.433)
LC_{99}	0.252	(0.219-1.708)	0.252	(0.219-1.708)	0.252	(0.219-1.708)	0.319	(0.245-2.607)

bioassay system for chub were determined as 0.200 (0.187-0.290), 0.200(0.187-0.290), 0.200(0.187-0.290), 0.205 (0.185-0.346) mg L^{-1}, respectively (Table 5). There were not significant differences between LC_{1-99} values obtained different time of exposure (Table 6).

DISCUSSION

The 96 h LC_{50} value of mercury-II-clorur in chubs was found as 0.205 mg L^{-1} and 96 h LC_{50} for fry rainbow trout was 0.814 mg L^{-1} in the present work. In aquatic toxicology, if LC_{50} (mg L^{-1}) concentration is smaller than 1 mg L^{-1}, the chemical is highly toxic and if between 1-10 mg L^{-1}, then it is considered to be a moderately toxic (Louis *et al*., 1996). Therefore we report mercury-II-clorur to be highly toxic to fish species used. According to FAO/UNEP (1991), The 96 h LC_{50} values for $HgCl_2$ are for catfish, 0.35 mg L^{-1}; rainbow trout, 0.22 mg L^{-1}; striped bass, 0.09 mg L^{-1}; brook trout, 0.075 mg L^{-1} and mummichog, 2.0 mg L^{-1}.

The LC_{50} value of mercury-II-clorur in other aquatic organisms was reported as 0.037 mg L^{-1} for fathead minnow, 0.16 mg L^{-1} (96 h EC_{50}) for bluegill sunfish (size: 0.6 g) and 0.903 mg L^{-1} for rainbow trout (URL 2). The 96 h LC_{50} values of inorganic mercury was reported as 0.002 mg L^{-1} (30 day) for crayfish (*Orconectes limosus*), 0.005 mg L^{-1} for cladoceran (*Daphia magna*), 0.01 mg L^{-1} for scud (*Gammarus pseudolimnaeus*), 0.2 mg L^{-1} for juvenile rainbow trout, 0.44 mg L^{-1} for *Notopterus notopterus*, 0.0073 mg L^{-1} for leopard frog (*Rana pipiens*), 0.107 mg L^{-1} for marbled salamander (*Ambystoma opacum*), 0,0058 mg L^{-1} for blue mussel (*Mytilus edulis*), 0.33 mg L^{-1} for adult slipper limpet (*Crepidula fornicata*), 0.089 mg L^{-1} for bay scallop (*Argopecten irradiance*), 0.0035 mg L^{-1} for juvenile mysid shrimp (*Mysidopsis bohia*), 0.015 mg L^{-1} for adult copepod (*Acartia tonsa*), 0.0153 mg L^{-1} for prawn (*Penaeus indicus*), 0.014 mg L^{-1} for polychaete larva (*Capitella capitata*), 0.098 mg L^{-1} for haddock (*Melanogrammus aeglifinus*) (Eisler, 1987).

Sastry and Gupta (1978) reported the mercury (LC_{50}: 1.8 mg L^{-1}) inhibits the activities of phosphates in the liver but no significant effect on the digestive enzymes within the experimental period of 96 h. According to present study, chubs (*Alburnoides bipunctatus*) are more sensitive than rainbow trout (*Oncorhynchus mykiss*).

Mercury-II-clorur has slightly toxicity to fish and other aquatic organisms as is common with many metalloids, pesticides and other chemicals. However, because of lack of tight controls during marketing in many countries, there may be unexpected potential risks on the environment. Special consideration should be given to residues in food and bioaccumulation in non-target organisms including man.

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