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Toxicity of copper on rainbow trout: Lethal concentration or lethal dose evaluation?

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ABSTRACT

A 96-h copper acute toxicity for rainbow trout was investigated through static method. The toxicity test was conducted at a pH of 7.4 and temperature of 15 ÚC. Concentrations of copper ranged from 0.0 to 1.5 mg/L. The concentrations of copper that killed 50% of two sizes of rainbow trout (1.5 g and 3.8 g) within 96-h was 0.530 and 0.440 mg/L, respectively. Survival number of fish increased with increasing size of fish, but lethal dose was 0.28 g/g and 0.30 g/g for 1.5 g and 3.8 g of fish, respectively.

KEYWORDS

Rainbow trout;
LC50;
LD50;
Copper.

INTRODUCTION

Lethal Concentration (LC) values usually refer to the concentration of a chemical in air but in environmental studies it can also mean the concentration of a chemical in water. According to the U.S. Environmental Protection Agency, LC50 is defined as the concentration of a chemical in air or water which is expected to cause death in 50 percent of test animals living in that air or water. According to the OECD (Organisation for Economic Cooperation and Development) Guidelines for the Testing of Chemicals, a traditional experiment involves groups of animals exposed to a concentration (or series of concentrations) for a set period of time (usually 4 hours). The animals are clinically observed for up to 14 days. The concentration of the chemical in air that kills 50% of the test animals during the observation period is the LC_{50} value. Other durations of exposure (versus the traditional 4 hours) may apply depending on specific laws. Toxic substances dissolved in water increase often the sensitivity of aquatic organisms to temperature variations, changes in dissolved O2 and vice-versa. Also the growth performance can be impaired and reproduction capacity can be reduced. Metabolic effects of heavy metal exposure (i.e. oxygen consumption) can be explained by the accumulation of heavy metals on the gill surface impairing O2 diffusion capacity.

Rainbow trout (*Oncorhynchus mykiss)* was studied here as one of the most sensitive fish against pollution[3, 4], and it is a non-native fish in Iran.

Ammonium sulfate or copper sulfate was used successfully to control *P. parvum* in some ponds in past years, but the hazards and pathological effects of excess amounts of copper are reasonably well

known in fish[5, 1, 2]. The toxic effects of waterborne exposure to dissolved Cu include gill injury, osmoregulatory disturbances and oxidativestress. Interference with sodium homeostasis in the animal and inhibition of Na^+, K^+-ATPase is a particular feature of Cu toxicity and raises the concern that Cu is also neurotoxic to fishes. The aim of this study was to investigate the 96-hour lethal concentration (LC50) of copper sulfate on the survival of two sizes of rainbow trout.

MATERIALS AND METHODS

Analysis of the water physico-chemical variables

Exposure solution copper (CuSO4.5H2O) were prepared daily from stock solutions, and 9 different Cu concentrations (0, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 1.0, and 1.5 mg/L) with a series of exposure concentrations prepared by serial dilutions. Metal concentrations in exposure solutions were verified by ICP-OES. pH value was measured using a pH-meter (CORINING-pH Meter, model 220, Corning Incorporated, USA).

Test organisms

Rainbow trout with an average weight of 2–3 g were selected and bought from a fish farm in Varamin (a City in Worth of Iran) and transported to Toxicology Research Center in University of Tehran. All fish were kept in a 500 L PVC tank equipped with a refrigerator and one powerful air compressors aerated the water. Fish are maintained for two weeks before the start of the experiments in continuously aerated, dechlorinated, recirculation, city tap water. Tank was cleaned carefully before and after each experiment while, and tank was covered with netting to prevent fish escapement. Light period was 8 h light and16 h dark and were fed twice daily with a commercial trout diet. This study was conducted indoors using eight of 9 L poly ethylene (PET). At least 24 h before starting the test, due to the fish adoption, they were introduced to the eight of 9 L poly ethylene (PET) tanks containing five fish in PET tank which hanging in 500 L PVC tank. The fish in 9 L were not fed during an experiment. The experiments were performed in static water. There were five individuals per replicate, with each treatment performed in dublicate. Basic water chemistry such as dissolved oxygen, pH, temperature, conductivity, and hardness were monitored daily. The number of dead fish was recorded every 24 hours and the mortality rate was documented at 96 h. Test temperatures were approximately 15 ^{o}C. The heavy metal Cu in the form of copper sulfate 5.H_2O (Merck, Germany) was used in the present study. The entire experiment was under ethical approval and fish were subject to independent health checks during the work.

RESULTS AND DISCUSSION

The acute toxicity test was performed for 4 days in which two replicates of 9 different Cu concentrations (0, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 1.0, and 1.5 mg/L) were used and five rainbow trout were placed into each tank. At 24, 48, 72, and 96 h, fish dead were counted in the different Cu concentrations along with the control group. No fish died during the acclimation period prior to copper exposure, and no control fish died during the toxicity tests. During the 24 h all fish in three replicates of 0.75, 1.0 and 1.5 mg/L of copper died. Mean survival of rainbow trout in copper sulfate treated tanks was 0% for 1.0 and 1.5 mg/L, 40% for 0.50 mg/L, 100% for 0.025-0.25 mg/L and no mortality occurred in the control tank.

Probit Analysis is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms. Probit analysis assumes that the relationship between number responding (not percent response) and concentration is normally distributed. If data are not normally distributed, logit is preferred. In this study, the acute toxic effects of Cu on Rainbow trout were determined by the use of Logit Analysis, LC50 determination method (It transforms the sigmoid dose-response curve to a straight line that can then be analyzed by regression either through least squares or maximum likelihood). The concentration values causing 50% mortality at the end of the 96-h period were analysed and the results were displayed in TABLE 1, 2. LC50 was found only in the concentrations of 0.53 and 0.44 mg/L Cu. The No Observed Acute Effect Concentration (NOAEC),

TABLE 1 : The cumulative mortalities, acute 96 h LC50 and acute 96 h LD50 of Cu in rainbow trout (weight of fish = 1.5 g and biomass = 1.67 g/L) according to logit analysis

Cu dose (mg/L)	No. of exposed fish	No of dead fish				Overall deaths within 96 h
		D1	D2	D3	D4	
0.00	5	0	0	0	0	0
0.025	5	0	0	0	0	0
0.050	5	0	0	0	0	0
0.075	5	0	0	0	0	0
0.100	5	0	0	0	0	0
0.250	5	0	0	0	0	0
0.500	5	1	2	2	2	2
1.00	5	1	5	5	5	5
1.50	5	5	5	5	5	5

TABLE 2 : The cumulative mortalities and acute 96 h LC50 of Cu in rainbow trout (weight = 3.8 g and biomass = 2.11 g/L) according to logit analysis

Cu dose (mg/L)	No. of exposed fish	No of dead fish				Overall deaths within 96 h
		D1	D2	D3	D4	
0.00	10	0	0	0	0	0
0.025	10	0	0	0	0	0
0.050	10	0	0	0	0	0
0.075	10	0	0	0	0	0
0.100	10	0	0	0	0	0
0.250	10	0	0	0	0	0
0.500	10	0	0	0	0	0
0.75	10	10	10	10	10	10
1.50	10	10	10	10	10	10

or the highest concentration tested that did not exhibit acute toxicity was 0.25.

$$96hLC50 = \frac{0.530mg}{L} \text{ and } LD50 = \frac{0.30mg}{gfish}$$

$$96hLC50 = \frac{0.440mg}{L} \text{ and } LD50 = \frac{0.28mg}{gfish}$$

Accor ding to the Environmental Protection Series (Canada) or Organisation for Economic Cooperation and Development (OECD) guideline for testing of chemicals, for ex: loading of fish into each test vessel must not exceed a density of 0.5 g/L *and their average wet weight must be between 0.3 and 2.5*, but it is not done in many of laboratory. Thus, several researchers reported different LC50 values for rainbow trout fed at different water conditions containing copper or other toxicant: high mortality rate and lower growth performance in water. However, in the present study, the LC50 value of 0.440-0.530 mg/L Cu. This may be caused by the fish size used in the study supporting the varying effects of copper, depending on different water conditions, different fish sizes used and different copper salts in the experiments. The concentrations at which a compound is lethal can depend upon many contributing factors including species and water quality. When comparing the 96-hr LC50 values with the size of fish exposed, it appears that copper toxicity increased as biomass (size) increased (TABLE 1, 2), but Specific LC 50 (lethal dose) was not significantly differ from each other. It can see if we calculate LD50 instead of LC50, the toxicity of rainbow has more validity. I this work we report LD50 for two sizes of fish. Although, LC 50 for 1.5 g and 3.5 g is 0.530 mg/L and 0.44 mg/L respectively, but the LD50 is approximately equal to each other (0.30 mg/g and 0.28 mg/g respectively).

TABLE 3 : The acute 96-h LC50 values of copper and their confidence limits in Rainbow trout according to Logit Analysis

	95% Confidence Limits for C			95% Confidence Limits for log(C)[a]		
Probability	**Estimate**	**Lower Bound**	**Upper Bound**	**Estimate**	**Lower Bound**	**Upper Bound**
LOGIT.010	.209	.000	.345	-.680	-5.310	-.462
.020	.241	.000	.376	-.618	-4.546	-.425
.030	.262	.000	.396	-.582	-4.095	-.403
.040	.278	.000	.411	-.556	-3.773	-.386
.050	.292	.000	.424	-.535	-3.520	-.372
.060	.303	.000	.436	-.518	-3.312	-.361
.070	.314	.001	.446	-.504	-3.134	-.351
.080	.323	.001	.455	-.491	-2.979	-.342
.090	.331	.001	.464	-.480	-2.841	-.333
.100	.339	.002	.473	-.469	-2.716	-.325
.150	.373	.006	.512	-.429	-2.224	-.291
.200	.400	.014	.550	-.398	-1.858	-.260
.250	.424	.027	.591	-.373	-1.562	-.228
.300	.446	.049	.641	-.351	-1.311	-.193
.350	.467	.081	.707	-.331	-1.094	-.150
.400	.487	.124	.803	-.312	-.906	-.095
.450	.508	.179	.953	-.294	-.747	-.021
.500	.529	.240	1.202	-.277	-.620	.080
.550	.551	.300	1.629	-.259	-.523	.212
.600	.574	.353	2.366	-.241	-.452	.374
.650	.600	.399	3.669	-.222	-.399	.565
.700	.628	.438	6.073	-.202	-.358	.783
.750	.661	.475	10.846	-.180	-.324	1.035
.800	.700	.510	21.474	-.155	-.293	1.332
.850	.751	.547	49.889	-.124	-.262	1.698
.900	.825	.591	155.168	-.084	-.228	2.191
.910	.845	.602	206.804	-.073	-.220	2.316
.920	.867	.614	284.288	-.062	-.212	2.454
.930	.893	.627	406.459	-.049	-.203	2.609
.940	.923	.641	611.839	-.035	-.193	2.787
.950	.960	.659	988.181	-.018	-.181	2.995
.960	1.006	.679	1767.621	.003	-.168	3.247
.970	1.068	.706	3716.391	.029	-.151	3.570
.980	1.162	.743	10496.214	.065	-.129	4.021
.990	1.340	.809	60991.148	.127	-.092	4.785

a. Logarithm base = 10.

CONCLUSION

Because the toxicity of copper is influenced by water quality and sizes of fish, it seems that in acute toxicology test, LD50 is more suitable than LC50.

Conflict of interest

We certify that this study involving human subjects is in accordance with the Helsinky declaration of 1975 as revised in 2000 and that it has been approved by the relevant institutional Ethical Committee.

REFERENCES

[1] G.A.Al-Bairuty, B.J.Shaw, R.D.Handy, T.B.Henry; Histopathological effects of waterborne copper nanoparticles and copper sulphate on the organs of rainbow trout (Oncorhynchus mykiss), Aquatic Toxicology, **126**, 104– 115 **(2013)**.

[2] E.Bagdonas, M.Z.Vosylienë; A study of toxicity and genotoxicity of copper, Zinc and their mixture to rainbow trout (*Oncorhynchusmykiss*), Biologia., **1**, 8–13 **(2006)**.

[3] M.Boran, U.Altinok, E.Apkin, H.Karaam, V.Buer; Acute toxicity of carbaryl, Methiocarb, and Carbosulfanto the Rainbow Trout (Oncorhynchus mykiss) and Guppy (Poecilia reticulata), Turk.J.Vet.Anim.Sci., **31(1)**, 39-45 **(2007)**.

[4] J.L.Miller, M.J.Miller, V.Vlaming, K.Larsen, E.Smith, K.Reece; Selection and application of a rainbow trout toxicity testing procedure for screening Sacramento River Watershed, California samples, Environ Monit Assess, **150**, 333–349 **(2009)**.

[5] T.Sovová, D.Boyle, K.A.Sloman, C.V.Pérez, R.D.Handy, Impaired behavioural response to alarm substance in rainbow troutexposed to copper nanoparticles, Aquatic Toxicology, **152**, 195–204 **(2014)**.