Determination of The Lethal Concentration 50% (Lc50) of Cadmium Chloride in mosquito fish *Gambusia holbrooki*.

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Abstract

In this study 24,48 and 96-h exposure of male mosquito fish *Gambusia holbrooki* to cadmium chloride ($CdCl_2.H_20$) was accomplished to determine the cadmium lethal concentration 50 (LC_{50}) in mosquito fish *G. holbrooki*, The data obtained were statistically evaluated by the use of EPA computer program based on Finney's Probit Analysis Method and a (24,48 and 96h) LC_{50} values for *Gambusia holbrooki* were found to be(37.29 , 42.733 and 50.178) mg/l respectively .

Keywords: Acute toxicity; $_{LC50}$; Cadmium chloride; *Gambusia holbrooki.*

Introduction

Effects encounted with acute toxicity commonly consist of mortality or morbidity form a quantitative standpoint these effects are measured as the LC_{50}.The LC_{50} represente the dose of the material that causes mortality LC_{50} or some otherdefined effect (EC_{50}) in 50% of trated population. The LC_{50} and EC_{50} represent the concentration of the material to which the organisms were exposed that causes mortality (LC_{50}) or some other defined effect (EC_{50}) in 50% of an exposed population.(1). The decision whether a certain xenobiotic is dangerous for the aquatic system and the food chain, can be made after the fish acute toxicity (2). Metals, acts as catalyst in the oxidative reaction of biological macromolecules ; thus metal toxicities might be associated with oxidative tissue damage. The major challenge in experimental ecotoxicological studies is to cope with the relative paucity of literature on toxicity induced by. environmental pollution may elicit both adaptive and adverse response in animals at different structural levels, i.e, cells, tissues and organs. These reactions depend on a variety of factors, such as the type of contaminant and its concentration, the rate of exposure and the susceptibility, of the organisms (3).

For this purpose we compared LC_{50} of cadmium in different exposure periods (24,48 and 96 h) and cadmium accumulation in different organs of *G. holbrooki* in acute treatment.

Materials and Methods

Test organisms, fish were collected from north Mosul / Iraq in June 2012 (Mendan Bridge area). The reference site chosen for this study is where fish from the species *Gambusia holbrooki* have been recorded. Fish were captured using a hand net, kept in maintenance plastic tanks and immediately transported alive to the laboratory. First phase of laboratory maintenance involved a period of quarantine in which the fish were acclimated to the laboratory conditions for at least two weeks (15–30 days) prior to the experiment. The animals were kept in glass aquaria (20×25 ×40) filled with dechlorinated tap water, continuous aeration and temperature of 20 ± 1◦C. The photoperiod was 16:8 (16 h. light/8 h darkness) and fish were fed twice daily with commercially balanced fish food.

LC_{50} determination

Technical-grade Cadmium chloride, monohydrate, (CdCl2; 98.0% EC, maximum limits of impurities, iron 0.0005% and sulfate 0.005%) used for this study. The acute toxicity bioassay for the test chemical CdCl2 was conducted in static system to determine the 24,48 and 96-h LC_{50} in the test species *G. holbrooki*. The range finding test was carried out first to determine the concentration of the test solution for definitive test. In definitive test, ten fish were used for each group. Groups 1, 2,3 and 4 were exposed to increasing concentrations of cadmium whereas group 1 was maintained in cadmium-free water to serve as control. The nominal concentrations of cadmium tested were: 0, 20, 40, 60, 80and 100 mg/l. Fish were not fed for 24 h. prior to the experiments and no food was provided during the tests. The duration of exposure was 24, 48 and 96 h. The experiment was repeated thrice and the number of dead fish was recorded at 12, ,24,36,48, 72 and 96 h. The criteria for death were no gill movement and no reaction to gentle prodding, dead fish were removed and discarded after each observation The 24,48 and 96-h LC_{50} determination in this study the toxicity data of cadmium chloride upon the mosquito fish *G. holbrooki* were evaluated using probit analysis technique (4).For Finney's probit analysis LC_{50} 1.00 software developed by EPA was employed (5). Determining LC_{50} value of mortality for each exposure period was recorded .

The 24h, 48h. and 96 h. LC_{50} values were graphed by plotting the log concentration against percentage mortality occurred at 24, 48and 96 hrs. respectively, using Microsoft Excel program.

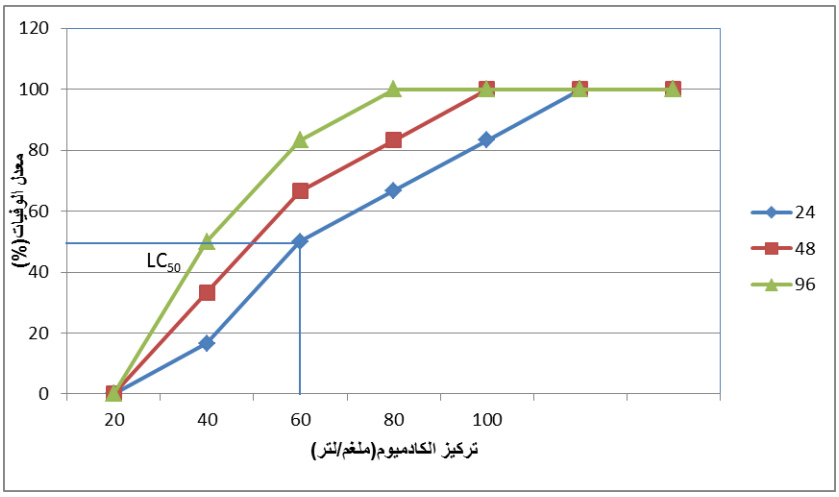

Fig.1 The mean LC_{50} values of 24 ,48 and 96 hours of cadmium chloride on *G.holbrooki*

Results

The LC_{50} values of 24 ,48 and 96 hours of cadmium were 50.178, 42.733and 37.298 µg/L respectively, no death was recorded among the control group (Fig1).

The mean LC_{50} values of 24 ,48 and 96 hours of cadmium chloride on *G.holbrooki* individuals were found to be 50.178, 42.733 and 37.298 µg/l respectively , by the use of EPA computer program based on Finney's Probit Analysis Method for the three test periods Fig (1). Figures (2,3and4) showed the plot of Finney's adjustedprobits and LC_{50} results. no death was recorded among the control group.

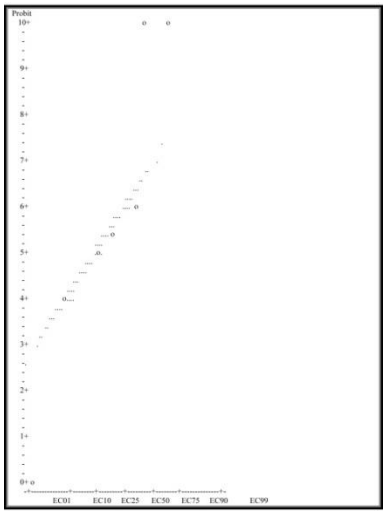

Fig. 2. Plot of adjusted probits and predicted regression(24h)

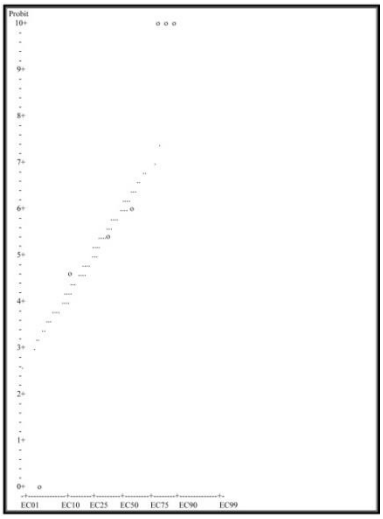

Fig. 3. Plot of adjusted probits and predicted regression(48h).

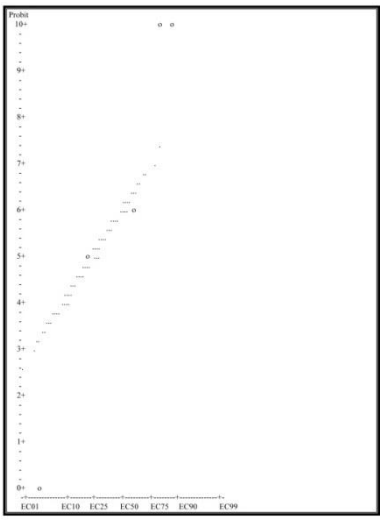

Fig. 4. Plot of adjusted probits and predicted regression(96h)

Tables (1, 2 and 3) shown the relation between the cadmium chloride concentration and the mortality rate of *G. holbrooki* according to Finney's Probit Analysis using EPA Computer Program. The results obtained from acute static 24, 48 and 96-h toxicity experiments of cadmium chloride for mature G. *holbrooki* and estimated LC_{50} values and confidence limits are listed in Tables (1.2. and 3.)

Tables(1). The relation between the cadmium chloride concentration and the mortality rate of *G.holbrooki*(24h).

Concentration (mg/l)	Number of exposed fish	Number of dead fish	Death in the Bioassay	Expected death	Estimating death
25	10	0	0.0000	0.0000	0.0114
35	10	2	0.1667	0.1667	0.1196
50	10	6	0.5000	0.5000	0.4954
60	10	7	0.6667	0.6667	0.7204
75	10	5	0.8333	0.8333	0.9055
80	10	8	1.0000	1.0000	0.9363
100	10	10	1.0000	1.0000	0.9879

Table(2) .The relation between the cadmium chloride concentration and the mortality rate of *G.holbrooki*(48h).

Concentration (mg/l)	Number of exposed fish	Number of dead fish	Death in the bioassay	Expected death	Estimating death
25	10	0	0.0000	0.0000	0.0260
35	10	3	0.3333	0.3333	0.2346
50	10	6	0.6667	0.6667	0.7155
60	10	5	0.8333	0.8333	0.8907
75	10	9	1.0000	1.0000	0.9793
80	10	10	1.0000	1.0000	0.9885
100	10	10	1.0000	1.0000	0.9990

Table(3). The relation between the cadmium chloride concentration and the mortality rate of *G.holbrooki*(96h).

Concentration (mg/l)	Number of exposed fish	Number of dead fish	Death in the bioassay	Expected Death	Estimating Death
25	10	0	0.0000	0.0000	0.0354
35	10	5	0.5000	0.5000	0.3870
50	10	8	0.8333	0.8333	0.9072
60	10	10	1.0000	1.0000	0.9841
75	10	10	1.0000	1.0000	0.9992
80	10	10	1.0000	1.0000	0.9997
100	10	10	1.0000	1.0000	1.0000

Discussion

Heavy metals from anthropogenic sources have been recognized as important contaminants in aquatic ecosystems(6). In polluted areas, exposure of fish to heavy metal leads to interactions between these chemicals and biological systems resulting in biochemical disturbances. Oxidative stress is an adverse reaction resulting from the exposure of molecules, cells or tissues to excess levels of free radical oxidants, especially reactive oxygen species (7,8). It is induced by the presence of molecules having unpaired electrons, usually derived from oxygen and its various reactive intermediates and also from metabolic reactions. Aquatic organisms have evolved antioxidant defense mechanisms that prevent and intercept ROS, as well as repair mechanisms for oxidized components(9).

Results of the current study showed that the LC_{50} values of 24, 48 and 96 hours of cadmium exposurein Gambusia were 50.178, 42.733and 37.298 µg/L respectively, no death was recorded among the control group (Fig.1).

The designer of LD_{50} test in 1927 acknowledged its serious inadequacies intending it only for certain narrow medical purposes (10). Inadequacies was due to the continuous changes in different factors affected Cd toxicity so it has been well documented that species, age, weight, sex, temperature, pH, animal susceptibility, food in addition to method of by which chemical administrated have marked effect on LD_{50} results (11). Nevertheless , use of the LD_{50} test has become widespread as general measure of chemical toxicity and has been challenged for decades as both unreliable and informative criteria. Thereby it is useful to reconsider the repeat determination of the LD_{50} before carrying out laboratory experiments. The mechanisms of acute toxicity are: Nacrosis, Acetylcholinestrase inhibition, Ion channel modulators, Inhibitors of cellular (12).

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تحديد التركيز المميت الوسطي للكادميوم في اسماك البعوض *Gambusia holbrooki*

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الملخص

في هذه الدراسة عرضت ذكور اسماك البعوض *Gambusia holbrooki* للفترات (24 و 48 و 96) ساعة الى كلوريد الكادميوم ($Cdcl_2.H_2O$) لتحديد التركيز القاتل للكادميوم لنصف العدد الكلي (LC_{50}) لاسماك البعوض *Gambusia holbrooki*. البيانات التي احرزت حللت احصائيا بواسطة برنامج الحاسوب EPA وذلك بالاعتماد على طريقة Finney's probit Analysis وان قيم (LC_{50}) للفترات (24 و 48 و 96) ساعة لاسماك البعوض كانت كالاتي (37.298 و 42.733 و 50.178) ملغم/لتر على التوالي.