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**ORIGINAL ARTICLE**

# The Acute Toxicity of Zinc chloride on *Daphnia magna* Straus

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**ABSTRACT**

In this study the acute toxicity of zinc chloride ($ZnCl_2$) to *Daphnia magna* Straus was investigated in a static bioassay. After 24, 48, 72 and 96 h the mobility of daphnids were examined and immobile ones were counted. The 24, 48, 72 and 96 h $EC_{50}$ of zinc chloride to *D. magna* were found as 11.63, 6.03, 1.17 and 0.67 mg/L, respectively.

***Key Words****: Zinc, daphnid, toxicity, $EC_{50}$, probit*

## 1. INTRODUCTION

Although the basic role of nutritionally essential metals such as zinc ($Zn^{2+}$) is to provide some component of a vital biochemical or enzymatic reaction, they can be toxic at high levels [1-3]. Aquatic ecosystems are the final sink for all potentially toxic metals in the environment via transfer from natural and/or anthropogenic sources. The fact that the increasing use of contaminating chemicals in many industrialised parts of the world makes the development of ecotoxicity measurement techniques an absolute necessity [4]. The main objective of ecotoxicology is to evaluate the risk for an ecosystem exposed to environmental stress, including contamination. The current ecotoxicological requirements of the Directive 79/831/EEC for all new industrial chemicals are that acute toxicity tests must be carried out using fish and *Daphnia* [5]. *Daphnia magna* is one of the most used bioindicator organisms for both water and sediment toxicity bioassays [3,6,7]. The extremely fast growth and high reproductive rates and short life cycles associated with *Daphnia* were all perceived as positive features for an ideal test organism [3,8-10].

Zinc is one of the essential metals which take part in more than 300 enzymes [11]. Although it is essential, it can be toxic to many aqutic organisms at high levels [1,2,6,12-15] and it can be accumulated in aquatic ecosystems. The aim of this study is to investigate the acute toxicity (24, 48, 72 and 96h) of zinc chloride to laboratory cultured *Daphnia magna* Straus.

## 2. MATERIALS AND METHODS

All reagents were of analytical grade and all laboratory glassware were soaked in 10% $HNO_3$ for at least 48h and rinsed with distilled water at least 3 times prior to use. Deionised water from a Millipore Milli-Q ultra pure (Milli- Di, France) water system was used through out the study except for daphnid culture.

The test organism *Daphnia magna* was obtained from the Kepez Aquaculture Research Institute (Antalya, Turkey) and introduced to 30 L aquariums with de-chlorinated tap water, which serves as holding tanks, maintained with a 16 h light and 8 h dark photocycle at 21.4±2.3°C. The physico-chemical properties of the water were pH 7.71±0.49, electrical conductivity (EC) 217.4±16.95 µS/cm**,** dissolved oxygen 6.39±0.45 mg/L

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and $CaCO_3$ alkalinity and hardness was 80±2.3 and 77.5±1.2 mg/L, respectively. *D. magna* was cultured and handled according to the procedures outlined in the ISO-6341 [10]. Acute 24, 48, 72 and 96 h toxicity tests for zinc chloride $ZnCl_2$ (Merck) was examined under static non-renewal conditions in 100 mL reconstituted water in 250 ml erlenmayer flasks. Reconstituted water was used as dilution water ($CaCl_2.H_2O$ 290 mg/L, $MgSO_4.7H_2O$ 120 mg/L, $NaHCO_3$ 65 mg/L, KCl 6 mg/L). The toxicity is expressed by the initial concentration that inhibits the mobility of 50% of the daphnids during 24, 48, 72 or 96h period of exposure ($EC_{50}$).

Ten neonates (age<24 h) obtained from the original culture, were introduced into erlenmayer flasks having different concentrations of zinc. The solutions to be tested were prepared immediately before the tests were carried out. The tested concentrations were between 5-20 mg/L (increasing at a 1 mg scale) for 24 h, 1-15 mg/L (increasing at a 1 mg scale) for 48 h, 0.5-2.0 mg/L (increasing at a 0.1 mg scale) for 72 h and 0.1-1.5 mg/L (increasing at a 0.1 mg scale) for 96 h $EC_{50}$. There was no feeding during the bioassays and the containers were not aerated for not to disturb daphnids with air bubles. The mobile daphnids were counted at 12 h intervals by gently shaking the glass containers and the ones that could not move were regarded as immobile. All bioassays were run in duplicate and the results are given as mean. $EC_{50}$ (median effective concentration) values were calculated using a regression line obtained by plotting the concentration (on a logarithmic scale) against the immobilization percentage on a probit scale and the results were evaluated with probit analysis.

## 3. RESULTS

Acute toxicity tests indicated that zinc at higher concentrations had a detrimental effect on the survival of *D. magna*. The calculated 24, 48, 72 and 96 h $EC_{50}$ value of zinc chloride, using a static bioassay system for *D. magna* was 11.63, 6.03, 1.17 and 0.67 mg/L, respectively. Control mortality was zero and controls did not show any behavioural abnormalities. The results of acute toxicity test for zinc chloride are presented in Table 1 and expressed as median effective concentration ($EC_{50}$).

Table 1. EC values (Mean; Min-Max) (mg $Zn^{2+}$/L), 95% confidence limits of zinc chloride for 24, 48, 72 and 96 h on *D. magna.*

| Point | 24h | 48h | 72h | 96h |
|---|---|---|---|---|
| EC 1.00 | 4.81 (3.35-5.95) | 1.26 (0.58-1.94) | 0.53 (0.38-0.64) | 0.13 (0.06-0.21) |
| EC 5.00 | 6.23 (4.76-7.33) | 2.01 (1.11-2.77) | 0.67 (0.52-0.77) | 0.22 (0.12-0.30) |
| EC 10.00 | 7.15 (5.72-8.21) | 2.55 (1.57-3.37) | 0.76 (0.62-0.86) | 0.28 (0.17-0.37) |
| EC 15.00 | 7.85 (6.48-8.87) | 3.01 (1.97-3.85) | 0.82 (0.69-0.92) | 0.33 (0.22-0.42) |
| **EC 50.00** | **11.63 (10.58-12.72)** | **6.03 (4.96-7.07)** | **1.17 (1.07-1.27)** | **0.67 (0.56-0.79)** |
| EC 85.00 | 17.24 (15.44-20.39) | 12.08 (9.99-16.24) | 1.66 (1.51-1.93) | 1.36 (1.11-1.89) |
| EC 90.00 | 18.93 (16.71-23.04) | 14.24 (11.48-20.30) | 1.81 (1.62-2.15) | 1.60 (1.27-2.38) |
| EC 95.00 | 21.73 (18.73-27.70) | 18.17 (14.01-28.45) | 2.05 (1.79-2.53) | 2.05 (1.55-3.37) |
| EC 99.00 | 28.14 (23.10-39.28) | 28.69 (20.16-54.11) | 2.58 (2.17-3.45) | 3.27 (2.24-6.52) |

In the exposure groups, it was observed that daphnids showed lethargy. Daphnids in the higher exposure groups (higher than 10 mg/L) were moving only when the containers were shaken. Corrosions were observed in the carapax of dead daphnids, and in higher concentrations partial ruptures were observed.

## 4. DISCUSSION

Understanding the problems associated with the degradation of water quality requires detailed knowledge of the state of an aquatic system and the way in which it changes with time. Acute lethal toxicity bioassays are useful for providing info on the toxicity of substances and for assessing the sensivity of organisms to these substances [16]. The development of new methods that can be used to identify the presence of toxic susbtances that effect water quality is extremely important to guarantee a continuous supply of high-quality water suitable for human consumption. Several of these methods are based on the use of test organisms including fish and invertebrates. Among these, the cladoceran *D. magna*, is widely used as a test organism in a variety of ecological studies. *D. magna* is relatively easy to maintain in the laboratory, has a short life cycle, and can be maintained at high population densities in relatively small volumes [3,8,9]. Furthermore, it has been studied extensively in a wide range of ecotoxicological investigations [3,6,7]. It is also known to be sensitive to many chemicals that are commonly

found in the aquatic environment, and can respond to these susbtances with a variety of physiological and behavioural characteristics [17].

Zinc is known as an essential metal and takes part in more than 300 enzymes [11]. However it is shown that zinc may be toxic at high levels. Khangarot and Ray (1989), found 24 and 48h $EC_{50}$ values of $ZnSO_4$ for *D. magna* as 1.0 mg/L and 0.56 mg/L, respectively [1]. Persoone and Jansen (1993) found 24 and 48h $EC_{50}$ values for $ZnSO_4$ on D. *magna* as 7.6 and 2.1 mg/L, respectively [6]; Sorvari and Sillanpaa (1996) found 24h $EC_{50}$ values for $ZnCl_2$ on D. *magna* as 5.5 mg/L [13]; Guilhermino *et al*. (1997) found that 24h $EC_{50}$ values for $ZnSO_4$ were between 3.65 and 7.32 mg/L, 48h $EC_{50}$ were between 0.69 and 1.26 mg/L, depending on the medium used [14]. Villanueva-Canizares *et al*. (1999) found 48h $EC_{50}$ values for $ZnCl_2$ on *D. magna* as 11.48 mg/L [16]. Seco *et al*. (2003) found 24h $EC_{50}$ values for $ZnCl_2$ on *D. magna* as 11.56 mg/L, which is similar to our findings [2]. As it can be seen from the results mentioned above, there is a great discrepancy among the toxicity of zinc. This may simply result from the physical and chemical properties of the water and/or the medium used in the bioassays. For example, Berglind and Dave (1984) found that 24h $EC_{50}$ value of zinc was 3.0 mg/L in hard water (300 mg $CaCO_3$/L) and 5.3 mg/L for soft water (50 mg $CaCO_3$/L) [12]. Similar findings were reported also by Khangarot and Ray (1989) [1]. Furthermore, the $EC_{50}$ values for $ZnCl_2$ are high when compared to $ZnSO_4$, which may indicate that zinc sulphate is more toxic to *D. magna.* Also, the bioavailability of zinc as zinc sulphate may be higher than zinc chloride. It was impossible to compare our data to other studies since 72 and 96h $EC_{50}$ values were not studied for zinc on *D. magna* before. However, it was observed that there was a sharp decrease between the 48 and 72 h $EC_{50}$ values (Figure 1).

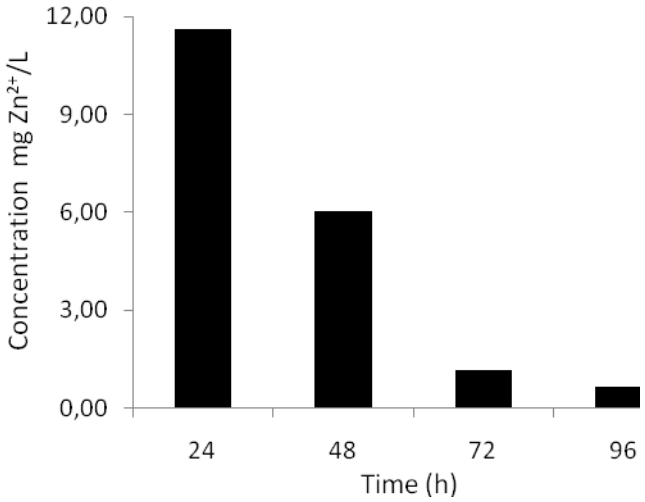


Figure 1. $EC_{50}$ values for 24, 48, 72 and 96 h for $ZnCl_2$ on *D. magna*.

Although, *D. magna* is the most common test organism in aquatic toxicology studies and there are a few standard methods developed to study the acute toxicity of toxicants, there are still discrepancies between different laboratories which may rise from the local tap water quality and differences in the resistance of different *D. magna* stocks, which should be investigated in future studies.

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