Investigation of acute toxicity and the effect of cadmium chloride (CdCl$_2$·H$_2$O) metal salt on behavior of the guppy (Poecilia reticulata)

Mehmet Yılmaz *, Ali Gül, Erhan Karaköse

Department of Biology Education, Gazi University, Teknikokullar, 06500 Ankara, Turkey

Received 20 March 2003; received in revised form 4 November 2003; accepted 26 November 2003

Abstract

In this study 96-h LC$_{50}$ value of cadmium chloride (CdCl$_2$·H$_2$O), a metal salt widely used in industry, was determined for the guppy (Poecilia reticulata, Pallas, 1859). The experiments were planned in four series of a total of 440 guppies employing the static test method of acute toxicity. 10 fish were placed in each replicate of each dose. The experiments were performed as four replicates, and behavioral changes in the guppy were determined for each cadmium chloride metal salt concentration. The data obtained were statistically evaluated by the use of EPA computer program based on Finney’s Probit Analysis Method and a 96-h LC$_{50}$ value for P. reticulata was found to be 30.4 mg/l in a static bioassay test system. This value was estimated to be 30.6 mg/l with Behrens–Karber’s method. The two methods were in good agreement. 95% lower and upper confidence limits for the LC$_{50}$ were 29.3 and 31.7 mg/l, respectively. The water temperature was kept between 21 and 23 °C. The behavioral changes observed in fish were, swimming in imbalanced manner, capsizing, attaching to the surface, difficulty in breathing and gathering around the ventilation filter.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Bioassay; Acute toxicity; LC$_{50}$; Cadmium chloride; Guppy; Poecilia reticulata

1. Introduction

The decision whether a certain xenobiotic is dangerous for the aquatic system and the food chain, can only be made after the (a) mammalian acute toxicity (b) bacteria acute toxicity (c) fish acute toxicity and (d) biological dissociation tests have been carried out in detail (Ardalı, 1990). The fact that increasing use of contaminating chemicals in many industrialised parts of the world makes the development of ecotoxicity measurement techniques an absolute necessity (Brandão et al., 1992).

The first step is the acute toxicity test on algae, fish, etc. in order to show the potential risks of these chemicals (OECD, 1993). Although the initial aquatic toxicity tests were carried out using bacteria, invertebrates like Cladocera and Rotifera and other groups, they can in no way replace the actual test performed on fish, which is the last chain in the aquatic food cycle (Castano et al., 1996). Cadmium chloride, heavy metal and potential threat to organisms due to high toxicity and tissue accumulation in tissues, contaminates aquatic media both from air and land. Organic and inorganic matter in household and industrial effluents are major contributors to pollution by cadmium (McCarty and Shugart, 1990). Potential for carcinogenesis, mutagenesis and teratogenesis through bioaccumulation also pose risk to
humans. Main target organs for cadmium toxicity are the kidneys, lungs, bones and the immune system (Alloway, 1990; ATSDR, 1992; Allen, 1994). Hutchinson and Manning (1996), investigated the effect of in vivo cadmium exposure on the respiratory burst of marine fish phagocytes. They observed significant reductions in the respiratory burst of phagocytes for all cadmium exposed marine fish, dab (Limanda limanda), compared with control. Sindhe et al. (2002), after exposing the fish Notopterus notopterus to heavy metals at sublethal concentrations, found that protein, lipid and cholesterol content of ovary and liver were reduced with the exposure of HgCl₂, and HgCl₂ was more toxic than CdCl₂.

Muley et al. (2000), found significant alterations in the DNA and RNA contents in gills, liver and brain of the common carp, Cyprinus carpio exposed to 96-h LC₅₀ (98 and 504 ppm) and LC₅₀ (121.8 and 594 ppm) concentrations of cadmium chloride and lead acetate, respectively. Both heavy metals decreased DNA content in all tissues. Cd and Pb toxicity decreased RNA content in liver and brain and increased it in gills. Krishnaja et al. (1987), examined the toxic effects mercuric chloride, phenyl mercuric acetate, cadmium chloride, selenium dioxide, arsenic trioxide and lead nitrate on Scylla serrata, the common edible Indian marine crab. They examined marked histopathological changes in the hepatopancreas and gill of S. serrata exposed to acute 96-h LC₅₀ values of these metal compounds. They found that 30 days long-term exposure to sublethal concentrations of mercury and cadmium brought about degenerative changes in the hepatopancreas and gills of exposed animals. Anam (1998), reported comparative damaging effects even at sublethal concentrations of cadmium and methyl parathion on the gonads, during the spawning stage of Cirrhinus miriga. The 96-h TL₅₀ values of adult (spawning; weight 220.76 ± 19.64 g) stage of C. miriga for cadmium chloride and Metacid-50 were found as 51.115 and 15.532 ppm by Anam.

Toxic effects of cadmium on Rana ridibunda have been recently investigated by Loubbourdis et al. (1999) and Selvi et al. (2003). Loubbourdis et al. found the 96-h LC₅₀ value of cadmium on growth of larvae of the frog R. ridibunda as 71.8 ppm. Selvi et al. found the 96-h LC₅₀ value of cadmium chloride on R. ridibunda as 51.2 mg/l. Extensive risk assessment and toxicity ranking on cadmium salts are documented by the EPA (EPA, 1993, 1994).

This study investigates the toxic effects of cadmium chloride on the guppy (Poecilia reticulata), standard aquatic test organism according to OECD (1993) and the Turkish national legislation (TSE, 1998) by the determination of 96-h LC₅₀ values and evaluates behavioral disorders of the guppy exposure to different concentration of the toxicant. Cadmium has gained wide interest in the scientific community in recent years due to its potential human health hazards.

2. Materials and methods

This study was carried out in the Hydrobiology Laboratories of Department of Biology, Gazi Faculty of Education, Gazi University. P. reticulata, Pallas, 1859 (Pisces, Poeciliidae) used in the study were supplied from the fish breeders in Ankara. They were transported to the laboratory within 30 min in plastic containers. In the laboratory they were acclimated to dechlorinated tap water in aquaria with 100 l capacity.

The study was carried out using 20 l aquaria with a width of 18 cm, depth of 22 cm and a length of 49 cm. The aquaria were aerated with a central system for a period of 48 h and the fish were exposed to 15 days conditioning period at room temperature. The fish were fed with commercial pelleted food at least once a day during this period. Acclimated fish were not fed 24 h before the start of the tests. Care was taken in order to keep the mortality rate of fish not more than 5% in the last four days before the experiment was started.

The toxicant used in static bioassays was Cadmium chloride (CdCl₂·H₂O, Merck) in tap water. The test organisms (i.e. fish) were randomly distributed in different concentrations of cadmium chloride. For the acute bioassay tests, 10 fish were used per concentration per replicate. A total of four replicates were carried out for each dose and the control group. The aquaria were not aerated at the time of dosing with cadmium chloride. Cadmium concentrations administered in the 20 l capacity test tanks were 22.5, 24, 25.5, 27, 28.5, 30, 31.5, 33, 34.5 and 36 mg/l. The amount of cadmium chloride to be added in each aquarium was calculated after the volume of each aquarium was accurately determined.

There was a simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the cadmium chloride, keeping all other conditions constant. The mortality rate in the control group did not exceed 10% and 90% of the fish looked healthy throughout the experiment.

Water quality parameters (temperature, dissolved oxygen (DO), salinity and pH) in the aquaria were determined before and periodically during the tests. The water temperature was kept between 21 and 23 °C and the experimental medium was aerated in order to keep the amount of oxygen not less than 4 mg/l. Average water temperature was 21.7 °C; average pH was 8.8; average salinity was 0.189% on day one and 0.290% on the last day; dissolved oxygen 8.3 mg/l and the experimental medium was aerated in order to keep the amount of oxygen not less than 4 mg/l.

All experiments were carried out for a period of 96 h. The number of dead fish was counted every 12 h and removed immediately from the aquaria. The mortality rate was determined at the end of 24, 48, 72 and 96 h. No food was given to the fish during the experiments. The
behavioral changes of the healthy fish and the fish exposed to various doses of cadmium chloride were photographed with camera and evaluated for behavioral anomalies.

The experiments were carried out with static acute experimental method. In this method the experimental solution and the samples (i.e. fish) are put in a suitable experimental cell (i.e. aquarium) and kept like that for a certain period. Since the decreased amount of oxygen and increased metabolic waste become a problem in long term experiments, the duration of such experiments are usually kept at 96 h or less (TSE, 1998).

In this study the acute toxic effect of cadmium chloride on the guppy (P. reticulata) was determined by the use of Finney’s Probit Analysis LC50 Determination Method (Finney, 1971). The computer analysis was carried on with LC50 1.00 software developed by EPA (1999). The data were also evaluated according to ‘Behrens–Karber method’ by the of use the following formula (Klassen, 1991).

\[
LC_{50} = \frac{ab + \cdots + ab}{n}
\]

Here \(LC_{50}\) and \(LC_{100}\) indicate the lethal doses for the 50% and 100% of the samples. “\(a\)” is the difference between the two consecutive doses, “\(b\)” the arithmetic mean of the mortality caused by two consecutive doses and “\(n\)” the number of samples in each group.

3. Results

Average water quality data have been given in Section 2. Table 1 shows the relation between the cadmium chloride concentration and the mortality rate of P. reticulata according to Finney’s Probit Analysis using EPA Computer Program. The results obtained from acute static 96-h toxicity experiments of cadmium chloride for guppy and estimated LC50 values and confidence limits are listed in Table 2.

The mean LC50 value of cadmium chloride on guppy individuals was found to be 30.4 mg/l by the use of EPA computer program based on Finney’s Probit Analysis Method. This value was estimated to be 30.6 mg/l with the Behrens–Karber’s method. The two methods are in good agreement. Fig. 1 shows the plot of Finney’s adjusted probits and LC50 results.

3.1. The change in behavioral patterns

Behavioral changes are the most sensitive indication of potential toxic effects. Optomotor responses are very useful in evaluating the behavioral changes of fish (Richmonds and Dutta, 1992).

In this study, P. reticulata exposed to various concentrations of cadmium chloride at ppm level displayed behavioral disorders. Behavioral changes were observable within the first hour. The magnitude and duration of behavioral changes increased with increased concentration. In the highest two doses, all 10 animals in the tank showed behavioral changes. The behavioral changes observed in fish are as follows:

Control group: There were no behavioral changes and deaths observed in the control group throughout the

Table 1
The relation between the cadmium chloride concentration and the mortality rate of Poecilia reticulata, Pallas, 1859

<table>
<thead>
<tr>
<th>Concentration (mg/l)</th>
<th>Number of exposed fish</th>
<th>Number of dead fish</th>
<th>Death in the bioassay (mg/l)</th>
<th>Expected death</th>
<th>Estimating death</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.5</td>
<td>10</td>
<td>0</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0072</td>
</tr>
<tr>
<td>24.0</td>
<td>10</td>
<td>1</td>
<td>0.1000</td>
<td>0.1000</td>
<td>0.0271</td>
</tr>
<tr>
<td>25.5</td>
<td>10</td>
<td>1</td>
<td>0.1000</td>
<td>0.1000</td>
<td>0.0758</td>
</tr>
<tr>
<td>27.0</td>
<td>10</td>
<td>1</td>
<td>0.1000</td>
<td>0.1000</td>
<td>0.1658</td>
</tr>
<tr>
<td>28.5</td>
<td>10</td>
<td>2</td>
<td>0.2000</td>
<td>0.2000</td>
<td>0.2971</td>
</tr>
<tr>
<td>30.0</td>
<td>10</td>
<td>4</td>
<td>0.4000</td>
<td>0.4000</td>
<td>0.4534</td>
</tr>
<tr>
<td>31.5</td>
<td>10</td>
<td>7</td>
<td>0.7000</td>
<td>0.7000</td>
<td>0.6096</td>
</tr>
<tr>
<td>33.0</td>
<td>10</td>
<td>7</td>
<td>0.7000</td>
<td>0.7000</td>
<td>0.7439</td>
</tr>
<tr>
<td>34.5</td>
<td>10</td>
<td>8</td>
<td>0.8000</td>
<td>0.8000</td>
<td>0.8451</td>
</tr>
<tr>
<td>36.0</td>
<td>10</td>
<td>10</td>
<td>1.0000</td>
<td>1.0000</td>
<td>0.9132</td>
</tr>
</tbody>
</table>

Table 2
Estimated LC values and confidence limits

<table>
<thead>
<tr>
<th>Point</th>
<th>Concentration (mg/l)</th>
<th>95% confidence limits Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC 1.00</td>
<td>22.8</td>
<td>19.7</td>
<td>24.7</td>
</tr>
<tr>
<td>LC 5.00</td>
<td>24.8</td>
<td>22.3</td>
<td>26.4</td>
</tr>
<tr>
<td>LC 10.00</td>
<td>25.9</td>
<td>23.8</td>
<td>27.3</td>
</tr>
<tr>
<td>LC 15.00</td>
<td>26.8</td>
<td>24.8</td>
<td>28.0</td>
</tr>
<tr>
<td>LC 50.00</td>
<td>30.4</td>
<td>29.3</td>
<td>31.7</td>
</tr>
<tr>
<td>LC 85.00</td>
<td>34.6</td>
<td>32.9</td>
<td>37.6</td>
</tr>
<tr>
<td>LC 90.00</td>
<td>35.7</td>
<td>33.8</td>
<td>39.3</td>
</tr>
<tr>
<td>LC 95.00</td>
<td>37.3</td>
<td>35.0</td>
<td>41.9</td>
</tr>
<tr>
<td>LC 99.00</td>
<td>40.6</td>
<td>37.4</td>
<td>47.5</td>
</tr>
</tbody>
</table>
experiment. The theoretical spontaneous response in the control group was zero.

22.5 mg/l: Abnormal swimming and the fish tended to gather at the surface.

24 mg/l: Swimming disorders such as vertical and downward manner increased and only one fish died at the end of 96-h period.

25.5 mg/l: Vertical and downward swimming patterns, suspending motionless on water surface and swimming around its own axis behavioral changes were observed. The fish tended to gather at the surface.

27 mg/l: Abnormal swimming behavior increased and the fish were observed to hit the aquarium walls and each other.

28.5 mg/l: The fish were observed to have breathing difficulties and tried to breathe air from the surface and their motility slowed down. Vertical and downward swimming patterns were observed. Swimming sideways and on the dorsal fin were also observed.

30 mg/l: Swimming disorders and loss of balance increased. Vertical and downward swimming patterns and gathering around the ventilation filter were observed.
31.5 mg/l: The motion of fish become extremely slow and displayed behavioral disorders such as capsizing in water, loss of balance, respiratory difficulty.

33 mg/l: The fish were observed to make sudden movements, display loss of balance and swimming disorders. Fish capsized in water and became motionless.

34.5 mg/l: The fish display loss of balance and swimming disorders as soon as the reagent was added. There were swimming problems and the fish were observed to have breathing difficulties and gather around the ventilation filter.

36 mg/l: The fish were observed to have breathing difficulties. Initially fish sank down to the bottom and became motionless. The first fish died within the first 24 h and the remaining at 48 h.

4. Discussion and conclusion

We employed two different methods of data evaluation for acute toxicity response. Our results were similar in two methods tested. Finney’s Probit Analysis gave 96-h LC50 value for the guppy exposed to different cadmium chloride concentrations as 30.4 mg/l. This value was estimated to be 30.6 mg/l with Behrens–Karber’s method. Control mortality was zero. 95% lower and upper confidence limits for the LC50 were 29.3 and 31.7 mg/l, respectively. The behavioral changes observed in fish were, swimming in imbalanced manner, capsizing, attaching to the surface, difficulty in breathing and gathering around the ventilation filter, slowness in motion and sinking down to bottom. These toxic effects increased with the dose. Our results are in agreement with the results reported by the following researchers:

The 96-h LC50 values of cadmium on Salmo gairdneri and Xenopus laevis larvae were reported to be between 80 and 100 mg/l by Woodal et al. (1988); while Muley et al. (2000) reported the 96-h LC50 value of cadmium on C. carpio as 121.8 ppm. The LC50 values of cadmium on rainbow trout (Oncorhynchus mykiss) for 24, 48, 72 and 96 h were found to be 7.76, 1.95, 0.5, and 0.45 mg/l, respectively, by Oryan and Nejatkhah (1997). Chambers (1995), investigated the effect of acute cadmium toxicity on marron, Cherax tenuimanus (Smith, 1912; Parastacidae). He found 96-h LC50 value with 95% confidence limits as 17.9 (13.4–23.9) mg/l. Asato and Reish (1988) found the LC50 value of cadmium on Holmesimysis costata (Crustacea: Mysidae) as 0.008 mg/l.

Sehgal and Pandey (1984) observed that LC100 and LC50 values on guppy were 300 and 250 ppm of CdCl2, respectively. In addition, they found that the cadmium chloride treatment 225 ppm for 30 days significantly inhibited the spermatogenesis in the exposed fish. They noticed that the changes included formation of less number of greatly vacuolated cysts of different stages and interstitium as well. Significant effects were seen in spermatogonia, spermatocyte, spermatic and sperm cyst.

Acute and chronic effects of cadmium have been widely described for different aquatic organisms and exposure routes. This metal is an important constituent in industrial effluents and municipal waste discharged into freshwaters and seas. The results obtained in this study clearly reveal the fact that it is necessary to control the use of a heavy metal such as cadmium.

References


EPA, 1994. US Environmental Protection Agency. Technical background document to support rulemaking pursuant to the clean air act—section 1122 (g). Ranking of pollutants with respect to hazard to human health. EPA 450/3-92-010 Emissions Standards Division Office of Air Quality Planning and Standards, Research Triangle Park, NC.


