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Artículo / Article

ACUTE TOXICITY OF CHROMIUM ON *Cnesterodon decemmaculatus* (PISCES: POECILIIDAE)

LA TOXICIDAD AGUDA DEL CROMO EN *Cnesterodon decemmaculatus* (PISCES: POECILIIDAE)

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ABSTRACT

The acute toxicity of Cr(VI) upon 96 h of continuous exposure of the ten-spotted live-bearing fish *Cnesterodon decemmaculatus* (Jenyns 1842) was evaluated ($LC_{50/96h}$) in the concentration from 0 to 45.5 mg L^{-1} Cr(VI) and found to be 21 mg L^{-1}; Upon 24, 48, 72, and 96 h of exposure, the LC_{50} values are 35.1, 27.5, and 24.0 and 21.4 mg L^{-1}, respectively. No mortality was observed for controls or for the fish exposed at 3.2 mg L^{-1}. Mortality at the highest Cr(VI) concentration within 24 h exposure was 63 % Cr(VI) is suggested to be used as positive control agent in piscine toxicity assessment, at least when *C. decemmaculatus* is involved.

Keywords: Chromium(VI); Mortality; $LC\text{-}_{50/24\text{-}96h}$; Ten-spotted live-bearer; C*nesterodon decemmaculatus.*

RESUMEN

Se evaluó la toxicidad aguda del Cr(VI) durante 96 h de exposición continua en ejemplares de madrecitas de agua, *Cnesterodon decemmaculatus* (Jenyns 1842), pez ovovivíparo de amplia distribución neotropical. Se evaluó la $CL_{50/24\text{-}96h}$ por exposición a concentraciones de 0-45,5 mg L^{-1} de Cr(VI). Los resultados mostraron que la CL-50 alcanzó valores de 35,1, 27,5, 24,0 y 21,4 mg L^{-1} luego de 24, 48, 72 y 96 h de exposición, respectivamente. No se observó mortalidad tanto en los controles como en aquellos peces expuestos a 3,2 mg L^{-1}. Sin embargo, dicho parámetro alcanzó valores del 63% en aquellos ejemplares expuestos a la mayor concentración luego de 24 h de exposición. Estos resultados sugieren que Cr(VI) podría ser utilizado control positivo en la evaluación de la toxicidad en peces, al menos cuando *C. decemmaculatus* es empleado como modelo experimental.

Palabras clave: Cromo(VI); Mortalidad; $CL\text{-}50_{24\text{-}96h}$; Madrecita de agua; C*nesterodon decemmaculatus*

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1. INTRODUCTION

The ten-spotted live-bearing fish *Cnesterodon decemmaculatus* (Jenyns 1842) (Pisces: Poeciliidae) is an endemic member of the fish family Poeciliidae with an extensive distribution in Neotropical America. It attains high densities in a large variety of water-bodies within the whole La Plata River and other South American basins (Menni The ten-spotted live-bearing fish *Cnesterodon decemmaculatus* (Jenyns 1842)

(Menni *et al.*, 1996). It is a small ovoviviparous, microomnivorous, benthic-pelagic, and non-migratory fish (maximum size, ≈25 mm and 45 mm for ♂♂ and ♀♀, respectively), often being the most abundant and sometimes the sole species present in small watercourses. This species is easy to handle and acclimatize to laboratory conditions. Its wide range of tolerance to many environmental parameters, e.g., temperature, salinity, and pH, means great advantages for its use in toxicity test (Menni *et al.*, 1996). Furthermore, several reports found this species suitable as a test organism in both acute and chronic toxicity bioassays (Carriquiriborde *et al.*, 2007; de la Torre *et al.*, 2005, 2007; Di Marzio *et al.*, 1996, 1998, 2001, 2005; García *et al.*, 1999; Gómez *et al.*, 1998; Gutierrez *et al.*, 2008; Marchese *et al.*, 2008; Parma *et al.*, 2008; Vera-Candioti *et al.*, 2010b; Villar *et al.*, 2000).

Chromium (Cr) is a metal most commonly found in oxidized, trivalent ionic state in the environment whereas its hexavalent form [Cr(VI)] is present in smaller quantities (WHO, 2000) which is, however, considerably more toxic. Due to anthropogenic activities, the primary sources of Cr(VI) in the atmosphere are, among others, chromate chemicals used as rust inhibitors in cooling towers and emitted as mists, particulate matter delivered during manufacture and use of metal chromates, chromic acid mist from the plating industry, waste from the manufacture of steel and other alloys, bricks in furnaces, dyes and pigments, and effluents from leather tanning industry and wood preserving (ATSDR, 1993d).

It is well known that Cr(VI) is a cancer-, mutation-, and malformation-causing agent classified as a Group I carcinogen by the International Agency for Research on Cancer (Eisler, 1986; IARC, 1990).

A wide range of adverse effects of Cr(VI) in aquatic organisms have been reported such as reduced fecundity and survival, growth inhibition, and abnormal movement patterns of benthic invertebrates (EPA, 1998), or reduced growth, disease resistance, morphological changes as well as chromosomal aberrations in fishes (Eisler, 1986). Furthermore, Cr(VI) inhibits growth in duckweed and algae and reduces growth of embryos and fingerlings of freshwater fish and amphibians (Eisler, 1986).

Fishes have been extensively used as test organisms for acute toxicity bioassays due to several advantages. According to U.S. Environmental Protection Agency, they are easily maintained under laboratory conditions, and they are sensitive to a variety of pollutants as well as generally available throughout the year from both commercial and natural sources (EPA, 2002).

In the present study we evaluated the acute toxicity of Cr(VI) to *C. decemmaculatus* exposed under laboratory conditions.

2. MATERIALS AND METHODS

1.1. Chemicals

Potassium dichromate ($K_2Cr_2O_7$, CAS 7778-50-9) was obtained from Merck KGaA (Darmstadt, Germany).

1.2. Quality control

Chemical analysis of Cr(VI) in test solutions was verified by atomic absorption spectrophotometry – direct air-acetylene flame method (Method 3111 B) (APHA, 1998) corresponding to solutions from the initial time and 24 h after treatment as reported elsewhere (Vera-Candioti *et al.*, 2010a). Experimental concentrations of test compound assessed throughout the study represent the

measured Cr(VI) concentrations (mg L^{-1}).

Water used for test solutions was La Plata tap water (pH 6.9–7.5, conductivity 994 µS cm^{-1}, alkalinity 259 mg $CaCO_3$ L^{-1}, hardness 143 mg $CaCO_3$ L^{-1}). Chemical parameters were determined according to standardized methods (APHA *et al.*, 1998).

1.3. Organisms

Specimens of *C. decemmaculatus* were collected from a permanent pond free from pluvial runoff from agricultural areas in the vicinity of La Plata City (Buenos Aires Province, Argentina). Adults were transported to the laboratory and then acclimatized for 30 days at 16:08 h light/dark cycles in aquaria at 20 ± 1°C with dechlorinated tap water (pH 6.9-7.5, conductivity 994 µS cm^{-1}, alkalinity 259 mg $CaCO_3$ L^{-1}, hardness 143 mg $CaCO_3$ L^{-1}) with artificial aeration and daily supply of fish food (TetraMin®, TetraWerke, Germany) until the beginning of the experiments.

1.4. Determination of LC-50

Acute toxicity assessment experiments were conducted following the recommendations proposed by the United States Environmental Protection Agency standardized methods (EPA, 1975). For each experimental point, 10 fishes were maintained in a 1L polypropylene jars filled with 1L of testing water and exposed to seven different concentrations of Cr(VI) (0, 3.2, 6.8, 11.4, 17.1, 22.7, 31.8, and 45.5 mg L^{-1}) over 96 h, reading mortality every 24 h (EPA, 1975). A negative control in dechlorinated tap water (pH 6.88-7.55, conductivity 994 µS cm^{-1}, alkalinity 259 mg $CaCO_3$ L^{-1}, hardness 143 mg $CaCO_3$ L^{-1}) was run simultaneously with Cr(VI)-exposed fish. Toxicity test type was semi-static and all test solutions were prepared immediately before using and were replaced every 24 h. Fish were not fed throughout the experiment. They were visually examined daily and considered dead when either no respiratory movement was observed or there was no sudden swimming in response to gentle touching in regard to control organisms. Each experimental point was performed in triplicate.

1.5. Statistical analyses

The median lethal concentration and 95% confidence limits were estimated by Probit analysis (Finney, 1971) using the Probit Analysis Program, version 1.5 (www.epa.gov). The different LC-50 values obtained at present study and LC-50 values from the literature registered on *C. decemmaculatus* were analyzed using one-way ANOVA followed by Tukey's HSD post-hoc test. Correlation analysis was also used to compared data of survival and tested Cr(VI) concentration. All data were analyzed with Statgraphics 5.1 Plus software. Significant differences were considered at $p < 0.05$.

3. RESULTS

Results of chemical analyses showed no changes in Cr(VI) concentration in treatments during the 24 h interval renewals of the testing solutions.

Probit analysis of mortality data allowed determination of toxicity values (mg Cr(VI) L^{-1}) and their respective 95% confidence limits: $LC\text{-}50_{24\,h}$ = 35.1, $LC\text{-}50_{48\,h}$ = 27.5, $LC\text{-}50_{72\,h}$ = 24.0 and $LC\text{-}50_{96\,h}$ = 21.4 (Table 1).

Table 1. Mean lethal concentration (LC-50) of Cr(VI) (mg L^{-1}) for *Cnesterodon decemmaculatus* (Pisces, Poeciliidae) after 24, 48, 72, and 96 h treatment.

Exposure time (h)	LC-50 (mg L^{-1})	95% confidence interval (mg L^{-1})
24	35.1	30.0-44.2
48	27.5	23.7-32.8
72	24.0	20.9-28.0
96	21.4	18.9-24.3

The no observed effect concentration (NOEC) was 3.2 mg Cr(VI) L^{-1} with no mortality throughout the experiment. At 24 h of initial treatment, a significant decrease in the survival rate was found when fishes were exposed to 45.5 mg Cr(VI) L^{-1}-the highest concentration tested-. At this experimental point, survival decreased 63% in regard to control values. Overall, correlation analysis revealed a significant and negative relationship between survival and tested Cr(VI) concentration ($r = -0.98$, $p < 0.001$) (Fig. 1).

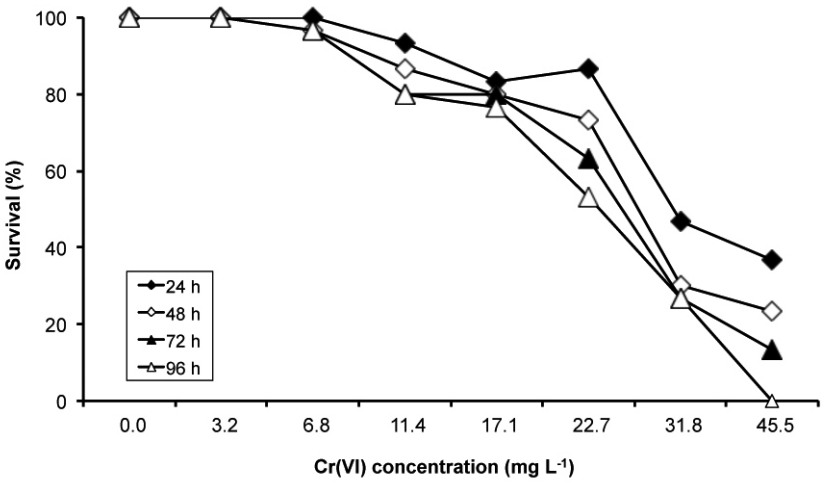

Figure 1. Survival (%) of *Cnesterodon decemmaculatus* (Pisces, Poeciliidae) specimens exposed to Cr(VI) (mg L^{-1}) for 24, 48, 72, and 96 h after initial treatment.

4. DISCUSSION

In the present study, we clearly demonstrated that Cr(VI) induced acute lethal effects on *C. decemmaculatus* exposed under laboratory conditions. According to our findings on the species selected for this study and following the criteria proposed by FAO/UNESCO/WHO committee (Sprague, 1973) and the Office of Pollution Prevention and Toxic Substances of the USEPA (Smrchek *et al.*, 1993; Wagner *et al.*, 1995), the test compound could be ranked from toxic to slightly toxic in the scale of toxicity, respectively.

Our current findings demonstrated that *C. decemmaculatus* is sensitive to Cr(VI), decreasing its survival capacity when increasing the exposure time. Similar results have been previously reported by Gutierrez and collaborators (Gutierrez *et al.*, 2008), suggested that this effect must indicate that Cr(VI) is accumulated by this Poeciliidae. Our results accords with this observation.

To the best of our knowledge, Cr(VI) LC-50 values have been reported for a limited number of species (Al-Akel and Shamsi, 1996; Brungs, 1978; Carriquiriborde and Ronco, 2006; Di Marzio, 1999; Dorn *et al.*, 1987; EPA, 1985; 2004; Mishra and Mohanty, 2008; Nath and Kumar, 1988; Prabakaran *et al.*, 2006; Svecevicius, 2006; Velma *et al.*, 2009). When comparing the LC-$50_{96\ h}$ values found in the current study with those aforementioned values, *C. decemmaculatus* could be considered as one of the most sensitive species to Cr(VI). This Poeciliidae member can be located within the range between the most and least sensitive species studied so far: *Odontesthes bonariensis* (Atherinopsidae) (Carriquiriborde and Ronco, 2006) and *Puntius conchonius* (Cyprinidae) (Pant and Gill, 1982) showing LC-$50_{96\ h}$ values of 8.2 and 331.4 mg Cr(VI) L^{-1}, respectively. Available data on Cr(VI)-induced fish lethality is scarce. However, it could be suggested that members of the Poeciliidae family are, in general, more sensitive to Cr(VI) than other families, e.g., Cyprinidae and Characidae. In addition, the sensitivity of *C. decemmaculatus* to Cr(VI) was higher than that reported to other metals like Zn with a LC-$50_{96\ h}$ = 102.2 mg L^{-1} (Di Marzio, 1999), but lower than that reported to Cu (LC-$50_{96\ h}$ = 0.16 µg L^{-1}) (Villar *et al.*, 2000).

Years ago, Di Marzio (1999) reported the LC-$50_{96\ h}$ values obtained on *C. decemmaculatus* for several pollutants, including Cr(VI). He reported a LC-$50_{96\ h}$ value of 13.7 mg Cr(VI) L^{-1} (8.4-23.2 mg Cr(VI) L^{-1}). When comparing our findings with those reported by the latter, our results suggest that the specimens of *C. decemmaculatus* we analyzed appear less sensitive being 1.5 orders of magnitude lower (Table 1). Although confidence intervals overlap, an analysis of variance revealed significant differences ($F = 20.16$, $p < 0.01$) between the LC-$50_{96\ h}$ value from the present study and that obtained by Di Marzio (1999).

Several laboratory and toxicity assay variables can be mentioned as putative factors for explaining the aforementioned discrepancy. These differences may be due to different physicochemical parameters of test water used in the laboratory studies. It is well known that Cr(VI)-induced toxic effects on aquatic organisms vary upon water pH (Carriquiriborde and Ronco, 2002; Svecevicius, 2006; Velma *et al.*, 2009). An increase in the $K_2Cr_2O_7$ concentration in test water and a concomitant decrease in the corresponding water pH has been recently demonstrated (Svecevicius, 2006; Velma *et al.*, 2009). Averages pH for test solutions employed in the study reported by Di Marzio (1999) was 8.2 while a lower value char-

acterized the water employed in the current study (pH = 7.2). Assuming this possibility and taking into account the experimental conditions of our study, for a given Cr(VI) concentration a higher mortality should have been registered than that found for the same concentration at a higher pH, e.g., the experimental conditions employed by Di Marzio (1999). However, we observed even at a lower pH than that of the water employed by Di Marzio, that it was necessary to increase the Cr(VI) concentration to achieve the same results previously found (Di Marzio, 1999). Then, the possibility that only a lower pH of the test water could be responsible for the lower sensibility of the specimens we studied could be ruled out. Recently, it has been reported that Cr(VI) and Cu(II) induced mortality on the aquatic invertebrate *Daphnia magna* depends upon the hardness of the test water (Gutierrez *et al.*, 2008; Marchese *et al.*, 2008). However, the possibility that the hardness of the experimental water could explain the discrepancies found between our current observations and those reported by Di Marzio (1999) could be ruled out since both studies were performed in water with a similar hardness. Most probably, such discrepancies should be committed to inherent characteristics/properties of the individuals from the population of *C. decemmaculatus* we employed and/or laboratory conditions rather than being only related to variations in the pH of the experimental test water. Among them, purity of the xenobiotics tested in the bioassays, general organisms' health, age and/or food quality, can be included.

Our results give further support that *C. decemmaculatus* provides a suitable and useful experimental model for biomonitoring aquatic ecosystems and environmental pollutants. Furthermore, they also reveal that chromium could be considered as an appropriate agent to be used as positive control in short term toxicity bioassays to assess mortality at least when this neotropical native species as *C. decemmaculatus* is employed as target organisms.

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