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Sensitivity of *Ceriodaphnia dubia* of Different Ages to Sodium Chloride

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Young organisms are generally more sensitive to toxic substances than adults. For this reason it is recommended to use neonates or first instar organisms in all toxicity tests (Weber 1993).

Buikema et al. (1980), in experiments using *Daphnia*, mentioned that the advantage of using neonates or first instars is logical because of their smaller size and larger surface area and they molt more frequently (between three and five times in the first 48 hours). It has been suggested that ecdysis is the most sensitive life period of cladocerans.

The protocols of chronic toxicity tests using *Ceriodaphnia dubia* established an age of test organisms less than 24 hours old, and all released within an 8 h period (Environment Canada 1992; Lewis et al. 1994; ABNT 1995). At CETESB (Environmental Agency of São Paulo State, Brazil) the laboratory of Aquatic Ecotoxicology have been using test organisms with age ranging between 6 and 30 hours due to practical and functional reasons.

Thus, the aim of this study was to compare the sensitivity of *Ceriodaphnia dubia* considering ages different from that recommended in standardized methods.

MATERIALS AND METHODS

The culture water was collected at Ribeirão do Piraí Reservoir: hardness adjusted to 40-48 mg/L $CaCO_3$; pH: 7,2-7,6; conductivity: 172-212 μS/cm; DO: approximately 7,0 mg/L.

Individual organisms were reared in 15 mL of culture water in 30-mL glass beakers. They were fed daily with a solution of digested fish food and an algal suspension (*Selenastrum capricornutum*). The organisms were transferred to fresh medium three times a week, typically on monday, wednesday and friday (ABNT 1995).

The test organisms were separated in order to get two age ranges: 16 to 24 hours

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Table 1. Sodium chloride toxicity to *Ceriodaphnia dubia* of different ages.

Test number	Age of organisms (hs)	NOEC; 7d (g NaCl/L)	IC50; 7d (g NaCl/L)
1	16- 24	0,5	1,13
2	16-24	0,5	0,92 (1,02)*
3	6-30	0,25	0,75
4	6-30	0,5	0,96 (0,86)*
5	16-24	0,5	1,1
6	16-24	0,5	0,98 (1,04)*
7	6-30	<0,25	0,71
8	6-30	0,25	0,68 (0,69)*
9	16-24	0,25	0,57
10	16-24	<0,25	0,61 (0,59)*
11	6-30	0,25	0,72
12	6-30	0,5	0,67 (0,69)*

* values in parenthesis represent IC50 mean of toxicity tests run at the same date.

old and 6 to 30 hours old at test initiation.

Young organisms of *C. dubia* were exposed in a static renewal system to four concentrations (2,0; 1,0; 0,5; 0,25 g/L) of sodium chloride and a control during a period of seven-day. It were used 10 replicates for concentration. The organisms were put in 15 mL of test solution in 30-mL glass beakers and they fed daily like in cultures. The test results are based on the reproduction of test organisms (ABNT 1995).

Six tests were performed using organisms from 16 to 24 hours (ABNT 1995) and six tests using organisms from 6 to 30 hours old. The tests were conducted over three weeks, and for each age two tests per week were made. The organisms used in the same week, for both ages, were from the same stock culture.

At the end of the tests, the IC50 (concentration of toxicant that causes a 50% reduction in the reproduction of test organisms in relation to control) and the NOEC (the highest concentration of toxicant in which the reproduction of test organisms are not statistically significantly different from the control) were determined using Icpin (Norberg-King 1993) and TOXSTAT 3.5 (Gulley 1996) programs respectively.

The statistical test used to analyse the results (IC 50 means) was the non-parametric Wilcoxon T-Test (Gad and Weil 1991). This test was chosen due to the few number of paired observations.

RESULTS AND DISCUSSION

Based on the available data and using the Wilcoxon test, it was not possible to evidence significant difference between the IC50 means ($T = 6$; $p = 0,3454$) for

both ages tested (16-24 hs and 6-30 hs).

In addition, other approaches to analyzing the results were adopted. For example, Bertoletti (2000) mentions that a factor > 2.0 can be considered sufficient to detect differences among toxicity tests. So, for this criterion, and as can be observed in Table 1, the median values of IC50 for both age, in the same week, presented a difference of less than a factor of 1.5. Thus, it can be concluded that there are no expressive differences between the results obtained for the different ages tested.

Barera and Adams (1983) performed acute toxicity tests with six age groups of *Daphnia magna*, ranging from ≤ 6 h to 216 h, testing five chemicals and they observed a good agreement in the EC 50; 48 h values for the groups with less than 48 h of age. The average difference in the EC50 values between the 6 h and 48 h groups had a factor of 1.23.

The precision of chronic toxicity tests is considered acceptable if the NOECs vary by no more than one concentration interval above or below a central tendency (Lewis et al. 1994). As can be observed in Table 1, the NOECs obtained for both ages ranged from < 0.25 to 0.5 g/L. So there are no expressive differences between NOECs for tests run with both ages.

In regard to this last criterion, similar results were obtained by Cooney et al.(1992), who performed many experiments with sodium chloride, using neonates of *Ceriodaphnia dubia* in three different age categories: 0-4 h, 20-24 h and 0-24 h to begin the tests. Those authors concluded that the tests results were comparable, and the NOECs didn't present significant differences for the ages tested.

The data obtained in this study are in accordance with Lasier et al. (2000), who evaluated the toxicity of manganese in chronic toxicity tests using *C. dubia* in two different ages: < 24 hours old and between 24 and 48 hours old. The authors observed that manganese sensitivity of *C. dubia* was not significantly different between two ages tested.

The results show that there are no significant differences between toxicity tests run for both ages tested. This suggests that it is appropriate to use *Ceriodaphnia dubia* at ages of 6 to 30 hours to initiate chronic toxicity tests.

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REFERENCES

ABNT (1995) Assoc. Bras. de Normas Técnicas – Avaliação de toxicidade crônica, utilizando *Ceriodaphnia dubia* Richard, 1894 (Cladocera, Crustacea). NBR 13373. Julho/1995. 14 p

Barera Y, Adams, WJ (1983) Resolving some practical questions about Daphnia acute toxicity tests. Aquatic Toxicology and Hazard Assessment: Sixth Symposium, ASTM STP 802, Bishop WE, Cardwell RD, Heidolph BB (eds) American Society for Testing and Materials. Philadelphia, Pennsylvania.

Bertoletti E (2000) Estimativa de efeitos tóxicos crônicos com *Danio rerio* (Pisces, Cyprinidae). Tese de doutorado USP/FSP – São Paulo. 120 p

Buikema ALJr, Geiger JG, Lee DR (1980) *Daphnia* Toxicity tests Aquatic invertebrate bioassays, ASTM STP 715. Buikema ALJr, Cairns JJr (eds) American Society for Testing and Materials. Philadelphia, Pennsylvania. p 48-69

Cooney JD, De Graeve GM, Moore EL, Lenoble BJ, Pollock TL, Smith GJ (1992) Effects of environmental and experimental design factors on culturing and toxicity testing of *Ceriodaphnia dubia*. Environ Toxicol Chem 11: 839-850

Environment Canada (1992) – Biological Test Method: Test of reproduction and survival using the cladoceran *Ceriodaphnia dubia*. Environment Protection Series – EPS 1/RM/21.72p. Environmental Protection Publications. Ottawa, Ontario.

Gad SC, Weil CS (1991) (eds) Hypothesis testing of categorical and ranked data. In: Statistics and Experimental Design for Toxicologists. CRC Press, Inc.,. 2nd edition. Boca Raton, Florida. 376 p

Gulley D (1996) TOXSTAT Version 3.5, West Inc. University of Wyoming. Cheyenne, Wyoming.

Lasier PJ, Winger PV, Bogenrieder KJ (2000) Toxicity of manganese to *Ceriodaphnia dubia* and *Hyalella azteca*. Arch Environ Contam Toxicol 38: 298-304

Lewis PA, Klemm DJ, Lazorchak JM, Norberg-King TJ, Peltier WH, Heber MA (eds) (1994) U.S.EPA – Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Third edition EPA/600/4-91/002. July 1994. Environmental Monitoring Systems Laboratory. Cincinnati, Ohio. 341 p

Norberg-King TJ (1993) A linear interpolation method for sublethal toxicity: the inhibition concentration (ICp) approach. (version 2.0). U.S. Environmental Protection Agency. Environmental Research Laboratory. Duluth, Minnesota.

Weber CI (1993) (ed) U.S.EPA – Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth Edition EPA/600/4-90/027F. U.S.EPA. Cincinnati, Ohio. 293p