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Acute Toxicity of Organic and Inorganic Compounds on the Freshwater Cyclopoid Copepod *Eucyclops neumani neumani* (Pesta, 1927)

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Copepods are recognized as fundamental links in the dynamics of trophic chains in marine and freshwater ecosystems. Their role as food for juveniles of several fish species has been stressed by Alheit and Scheibel (1982). Despite the wide distribution of these organisms in different aquatic ecosystems, ecotoxicity studies have been focused mainly on marine species (Verriopoulos and Moraietou-Apostolopoulou, 1981; Moraitou-Apostolopoulou and Verriopoulos, 1982; Williams, 1992; Larrain et al.1998). Specifically, the cyclopoids are among the copepod species relatively untested for their sensitivity to xenobiotics. The habitats of these crustaceans extend to the water column and to the meiobenthos, providing them with the potential of being useful for water and sediment quality assessment (Willis, 1999). Keeping them in the laboratory demands little effort and they have a short life cycle – both desirable features for test species in ecotoxicology. The aim of this study is to evaluate the sensitivity to organic and inorganic compounds of *Eucyclops neumani neumani*, a laboratory cultured freshwater cyclopoid which might become another convenient species to work with, representative of Chilean aquatic fauna in toxicity assessment.

The expression of toxicity with an index like EC50 has a source of variation in the specification of the exposure concentration that is worth to take in consideration and try solve it in order to reduce the differences in toxicity assessment of the same product by ecotoxicologists using dilution media of different origins. The same nominal concentration of a salt dissolved in water will generate different responses, as proportion of exposed organisms manifesting an effect, according to the chemical composition of the water. The actual proportion of dissolved salts that interact with biological membranes is dependent on the chemical speciation that takes place in the solution, on physicochemical factors like pH and water hardness and the occurrence of other molecules that compete with the toxicant free ions for the binding sites in the membranes (Zirino and Yamamoto, 1972). The manifestation of toxicity under exposure to heavy metals will depends finally in the proportion of ions that are still free for reacting with or passing through cell membranes after chelating agents from the dissolved organic fraction reach an equilibrium capturing ions in the form of organo-metallic complexes (Meador, 1991). When standard reconstituted water is used, as with *Daphnia* species bioassays, we can assume that variation in metallic ion bioavailability tends to

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minimize for the same compound from one test to another. On the contrary, lake water as we used in our study, although more representative of the natural environment where species live, changes its composition seasonally and between years (Dellarossa et al., 1994), therefore toxicity assessment in such medium requires determination of free ion availability in the solution before reliable comparisons of toxicity in waters of different origins can be done. Most toxicity data published in the past, however, were calculated based on nominal concentration of toxicants without no mention about the proportion of the ions which were effectively bioavailable. To cover both kind of situations we exposed the freshwater copepod to toxicants in lake water of unknown chemistry composition and then we estimated the same toxicity index (EC50) for one of the metals, copper, in reconstituted water of known chemistry. The available free copper ion concentration in this water was estimated through a metal dissolution chemical equilibrium model and the EC50 was expressed in terms of that portion of the dissolved salt.

MATERIALS AND METHODS

The laboratory stock of *E. neumani* was reared from specimens collected in sediment from wetlands around Concepción (Central Chile) and kept in an aquarium of 2.5 L. They were fed with laboratory cultures of the microalga *Scenedesmus spinosus* and *Daphnia* food (US EPA, 1993). Adult male organisms were selected for acute toxicity bioassays and exposed for 48 h independently to the fungicides pentachlorophenol (PCP), tribromophenol (TBP) and the inorganic salts $CuSO_4$ or $K_2Cr_2O_7$. The experiments were performed at 21 °C, with a photoperiod of 16:8 L:D, in filtered natural water from Lake Chica of San Pedro, Concepción, Chile, with a hardness of 4,76 mg/L of $CaCO_3$ and 1.45 mg/L of total organic carbon (TOC). Solutions were prepared 24 hr before testing. Glass vials of 20 ml with five individuals each and four replicates per concentration were used for the toxicity evaluations. The animals were not fed during the tests. The copepod sensitivity to copper was also tested in reconstituted water of known chemical composition, with a hardness of 125 mg/L $CaCO_3$ and pH 7.7 (ISO,1996). The TOC from the beginning to the end of the exposure varied from 1.51 to 1.70 mg/L. These data was used to determine the proportion of the dissolved copper which was effectively bioavailable to generate toxicity on the copepod. The obtained LC50 and the basic chemistry of the reconstituted water was input into a software (Biotic Ligand Model (BLM) (http://www.hydroqual.com/blm/)) that calculates – among other variables – the proportion of the total cupric salt dissolved which is available as free metal ion to react with the active sites of biological membranes involved in toxic effects. The BLM is based on equilibrium speciation of dissolved copper including the formation of inorganic and organic complexes and interaction with a site of acute toxicity in the organism referred to as the biotic ligand (International Copper Association, 2000).

After preliminary screenings with wide concentration ranges of the toxicants, the definitive exposure ranges (nominal concentrations) and the dilution factors were as follows: Cu^{++} from 1,000 to 31.25 μg/L (x 0.5); $K_2 Cr_2O_7$ from 17,143 to 90 μg/L (x 0.35); TBP from 1,500 to 190 μg/L (x 0.5) and PCP from 100 to 6.25 μg/L (x 0.5). At the end of each experiment the number of dead organisms was recorded. These data were used to estimate the EC_{50} of each compound by means of the Probit statistical analysis (US EPA, 1988).

RESULTS AND DISCUSSION

The copepod mortality derived from exposure to different nominal concentrations of organic and inorganic compounds is depicted in Figure 1. The EC50 ± SD of PCP for *Eucyclops neumani* was 33 ± 15 μg/L, which reveals a higher sensitivity of this species compared to other adult copepods like *Calamoecia lucasi* and *Mesocyclops leuckarti.* Willis (1999) found EC50 of the same pesticide for those species at 106 and 173 μg/L, respectively. Therefore, so low levels of concentration for a xenobiotic that still causes deleterious effects on a very important structural group of aquatic ecosystems is a fact that should be considered at the time of proposing its environmental safe concentration. This kind of information is crucial if an appropriate protection of the local flora and fauna is intended. These results become more important when standard ecotoxicity laboratory species like *Daphnia magna* and *Daphnia pulex* neonates, recognized by their high sensitivity to many substances, are at least one order of magnitude less sensitive than *E. neumani* adults. Pentaclorophenol EC50 determinations for both cladocerans were from 1000 to 143 and from 1100 to 246 μg/L respectively (Versteeg *et al*, 1997).

Table 1. Sensitivity of *Eucyclops neumani neumani* to different compounds expressed as EC50 and its standard deviation from three bioassays.

Toxicant	PCP	TBP	Cu SO_4	$K_2Cr_2O_7$
EC50 ± SD (μg/L)	33 ± 15	685 ± 172	549 ± 107	1,955 ± 62

As in many other countries, Chile has recently banned the commercial use of PCP. The action spectra of PCP on biological material has been related to interference with the metabolic pathways generating high energy phosphate compounds, essential for cell respiration (Eisler, 1989). A substitute for its application as a fungicide in wood preservation is TBP. Its EC50 ± SD to *E. neumani* was 685 ± 172 μg/L, which compared to the 33 μg/L of PCP, is indeed an improvement from an environmental point of view, although it still has acute effects at ppb levels on non target, freshwater fauna.

The responses of *E. neumani* to inorganic salts of Cu and Cr reveal, contrary to the organic compounds tested, a lower sensitivity for these metals when compared with several cladoceran species. The Cu^{++} EC50 for the cyclopoid was 549 ± 107 μg/L when dissolved in lake water and 479 (374 – 595) μg/L in reconstituted water, while Cu^{++} EC50s for cladocerans have been found at levels of 10 to 50 μg/L (Versteeg *et al*, 1997; Mark & Solbé, 1998). The similar value of the EC50 obtained in lake water and reconstituted water in our case is probably consequence of the equally low dissolved organic content in both waters. When the copepod response to copper is expressed in terms of the concentration of free available ion – as provided by the BLM computer program – the EC50 in reconstituted water becomes 92 μg/L.

Hexavalent chromium EC50 for *E. neumani* was 1,955 ± 62 μg/L, also higher than the immobilization index for daphnids (22 to 195 μg/L) cited by Versteeg *et al.* (1997). The same compound tested in aquatic larvae of the midge *Chironomus petiolatus* (= *C. piger*) – another organism from the same environment as the copepod – gave EC50 values between 19,600 and 96,300 μg/L (Larrain *et al*, 1997) which set the cyclopoid copepod in an intermediate position between Cladocera and Diptera with respect to metal sensitivity.

In conclusion, *E. neumani's* sensitivity is high enough to recommend its inclusion on the list of species to be tested in risk assessment studies of contaminants in continental waters. Further testing with a wider type of compounds will help to discern whether *E. neumani* is more sensitive than *Daphnia* for all toxic organic compounds or only for certain chemical structure groups.

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