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Acute and Subchronic Toxicity of the Heavy Metals Copper, Chromium, Nickel, and Zinc, Individually and in Mixture, to the Freshwater Copepod *Mesocyclops pehpeiensis*

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Hong Kong's inland water system consists of hundreds of streams, rivers and ponds. In recent years, the Hong Kong government has implemented effective pollution control measures to tackle the pollution problem of inland waters, but many are still polluted by heavy metals discharged from factories and vehicle repair shops in unsewered, rural areas. Because aquatic risk assessments in Hong Kong have focused on coastal marine systems (Blackmore 1998), there are fewer toxicity data available for freshwater organisms than for marine organisms. For freshwater organisms, aquatic toxicity data are restricted to cladocerans (Wong and Wong 1989). An important step to improve environmental risk assessment is to obtain sensitivity data for more relevant species. The genus *Mesocyclops* is one of the most common cyclopoid copepods in freshwater habitats worldwide. Ecologically, cyclopoid copepods play an important role in aquatic food webs as both primary and secondary consumers and also as food for planktivorous invertebrates and fish (Wetzel 1975). The purpose of this study was threefold. First, 48-h acute tests and 9-day subchronic tests were carried out to determine the toxicity of Cr, Cu, Ni and Zn to the nauplii of the freshwater copepod *Mesocyclops pehpeiensis*. Second, species sensitivity distributions for Cr, Cu, Ni and Zn were constructed using acute toxicity data for planktonic crustaceans and the sensitivity of *M. pehpeiensis* to individual metals was compared to those of other species. Finally, 48-h acute toxicity tests were conducted to determine the interactive effect between metals when they were applied in mixtures.

MATERIALS AND METHODS

Nauplii used in the present study were derived from a single egg-bearing female from a reservoir in the northern part of Hong Kong. Taxonomic identification of *M. pehpeiensis* was based on description of the species by Guo (2000). The copepods were cultured in filtered (125 μm) water from a large (150 L) aquarium. Algae, rotifer and *Paramecium* raised in water enriched with extracts from chicken manure were provided as food. Prior to each experiment, several egg-bearing adult females were isolated in small beakers. The beakers were inspected regularly and newly hatched (<12 h) nauplii were transferred to beakers without adults. After 2 days, the unicellular green alga *Chlorella pyrenoidosa* was added to provide a food medium of ~10^5 cells/mL.

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Stock solutions of metal ions were prepared by dissolving the metallic salts $CuSO_4 \cdot 5H_2O$ (1,000 mg/L), $K_2Cr_2O_7$ (5,000 mg/L), $NiCl_2 \cdot 6H_2O$ (8,000 mg/L), and $ZnSO_4 \cdot 7H_2O$ (5,000 mg/L) in distilled water. Test solutions were prepared just before use by diluting stock solutions in moderately hard synthetic water solution (APHA, 1995). Preliminary tests revealed that *M. pehpeiensis* grew similarly well in both aquarium water and synthetic water.

The 48-h acute toxicity tests were carried out using 3–4 day old nauplii. The nauplii were sorted individually into multidish (Nunclon™) wells. Each dish consisted of six wells. Each well contained 10 mL of test solution and *C. pyrenoidosa* (~10^5 cells/mL). The dishes were placed in an environmental chamber adjusted to 25°C and a light/dark cycle of 12L/12D. Each metal was tested at five concentrations plus a control. Range finding tests were conducted for each metal to ensure that the test concentrations would bracket the LC50 values. Thirty nauplii were tested for control and each test concentration. The test solution was not changed. The endpoint of the tests was mortality. Nauplii that did not move after gentle probing with a fine pin were considered dead.

Newly hatched nauplii (< 12 h) were used for the 9-day subchronic tests. The test conditions were similar to those used for the 48-h acute tests. The easily detectable morphological change from the last nauplius to the first copepodite instar was used as the endpoint. Food consisted of *C. pyrenoidosa* at 10^5 cells/mL. Preliminary tests revealed that the nauplii of *Mesocyclops* could reach the first copepodite instar on an exclusively algal diet. The 9-day test duration covered the development time for > 90% of the control nauplii to reach the first copepodite instar. Each metal was tested at five concentrations plus a control. The test concentrations were selected based on the results of the 48-h acute toxicity tests. Thirty nauplii were used for control and each test concentration. The test solution was changed daily and the number of nauplii and copepodites in each treatment and control was counted.

The toxicity of metal mixtures was studied using 3–4 day old nauplii. The test conditions were similar to those used for the study of single metals. Nauplii were exposed to six metal mixtures (Cr + Cu, Cr + Ni, Cr + Zn, Cu + Ni, Cu + Zn and Ni + Zn) and the toxicities of metal mixtures (50% of 48-h LC50 for metal A + 50% of 48-h LC50 for metal B) were compared with those of single metal (100% of 48-h LC50 for metal A or 100% of 48-h LC50 for metal B). The test duration was 48-h and the test endpoint was mortality. Thirty nauplii were used for each treatment.

The 48-h LC50 (median lethal concentration) and the 9-day EC50 (median effective concentration) of each metal were calculated using the Probit analysis program from USEPA. One-factor ANOVA was used to test for significant differences in mortality rate among metal mixtures and individual metals. The sensitivities of *M. pehpeiensis* to Cr, Cu, Ni and Zn were compared to those of other species of planktonic crustaceans. Single-species toxicity data (48-, 72- and 96-h LC50 values) for freshwater planktonic crustaceans from the USEPA

ECOTOX aquatic database were ranked, and the ranks were plotted against heavy metal concentrations to obtain a species sensitivity distribution. The 5- and 50-percentiles of the species sensitivity distribution for each metal were estimated using the method described in van der Hoeven (2001). These percentiles are called the HC5 (hazardous concentration for 5% of the species) and HC50 (hazardous concentration for 50% of the species). The rank of *M. pehpeiensis* on the species sensitivity distribution for each heavy metal was examined.

RESULTS AND DISCUSSION

The effects of Cr, Cu, Ni and Zn on the mortality and development of the nauplii of *M. pehpeiensis* are summarized in Table 1. Mortality among control nauplii ranged between 6.3–13.3%. Based on the values of the 48-h LC50, the order of toxicity was Cu > Zn > Cr > Ni. All four metals inhibited the naupliar development of *M. pehpeiensis*. Among control animals, change from the last nauplius to the first copepodite instar began after 5 days and survival rate after 9 days was from 83.3–96.7%. Compared to the value of the 48-h LC50, the 9-day EC50 for the inhibition of development to the first copepodite instar was lower for Cu, Cr and Ni and higher for Zn. Based on the 9-day EC50 values, the order of toxicity was Cu > Cr > Zn > Ni.

Table 1. 48-h LC50 and 9-day EC50 based on inhibition of larval development for nauplii of *M. pehpeiensis*.

Heavy metals	48-h LC50 (μg/L)	9-day EC50 (μg/L)
Cr	510 (362–668)	268 (152–336)
Cu	75 (39–121)	25 (20–33)
Ni	1191 (721–1674)	410 (375–517)
Zn	240 (128–375)	378 (246–482)

(95% confidence limits are given in parentheses)

Figure 1 presents the species sensitivity distribution for Cr, Cu, Ni and Zn. Acute toxicity data (48-, 72- and 96-h LC50) for freshwater planktonic crustaceans were obtained from the USEPA ECOTOX aquatic database. The datasets were based on the total metal concentrations used in the experiments and were dominated by species of *Daphnia*. The sample size ranged from 24 for Cr to 121 for Cu. Planktonic crustaceans showed wide variations in their sensitivity to heavy metals. The calculated HC5 values for Cu, Cr, Zn and Ni were 9.39, 22.0, 66.5 and 89.0 μg/L, respectively. The order of toxicity based on the HC5 values was Cu > Cr > Zn > Ni and was similar to that derived from subchronic tests based on the nauplii development of *M. pehpeiensis*. For all four metals, the 48-h LC50 value for the nauplii of *M. pehpeiensis* was higher than the value of the HC5 for planktonic crustaceans. Similarly, the 9-day EC50 values for the inhibition of development to the first copepodite instar were higher than the HC5 values. The HC50 values for Cu, Cr, Zn and Ni were 51.0, 2512, 438 and 2042

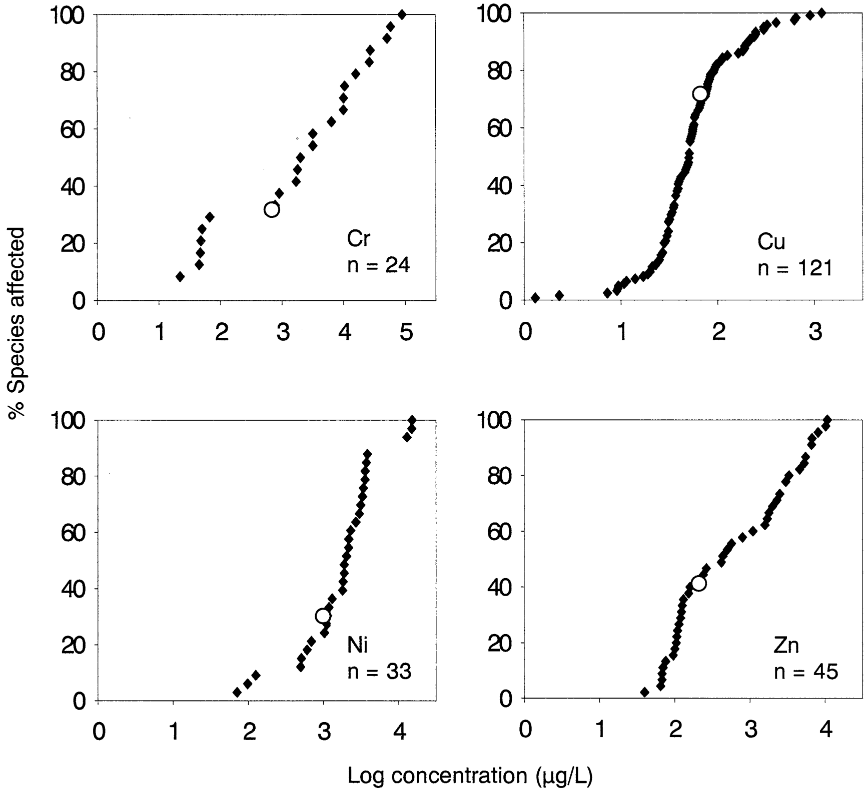

Figure 1. Species sensitivity distributions for Cr, Cu, Ni and Zn based on acute toxicity data for planktonic crustaceans. The 48-h LC50 values for the nauplii of *Mesocyclops pehpeiensis* are represented by open circles.

µg/L, respectively. Accordingly, the order of toxicity based on HC50 values was Cu, >Zn > Ni > Cr. This was different from those derived from the HC5 values and the *M. pehpeiensis* nauplii tests, but was similar to that based on the marine copepod *Tisbe holothuriae* (Verriopoulos and Dimas 1988). Compared to the HC50, the 48-h LC50 for *Mesocyclops* were higher for Cu and lower for Cr, Ni and Zn. Overall, these results suggest that the sensitivity of *M. pehpeiensis* to heavy metals was within the range of those exhibited by other planktonic crustaceans.

The effects of metal mixtures on the mortality of *M. pehpeiensis* are presented in Figure 2. In all experiments, the toxicity of a solution containing 50% of the 48-h LC50 of two metals was comparable to that of a solution containing 100% of the 48-h LC50 of a single metal, suggesting the interaction between metals was additive. Additive interaction is frequently observed when pollutants such as heavy metals are tested together in equitoxic concentrations (Moraitou-Apostolopoulou and Verriopoulos 1982; Verriopoulos and Dimas 1988). Additive toxicity of eight metals to *Daphnia magna* has been demonstrated in the

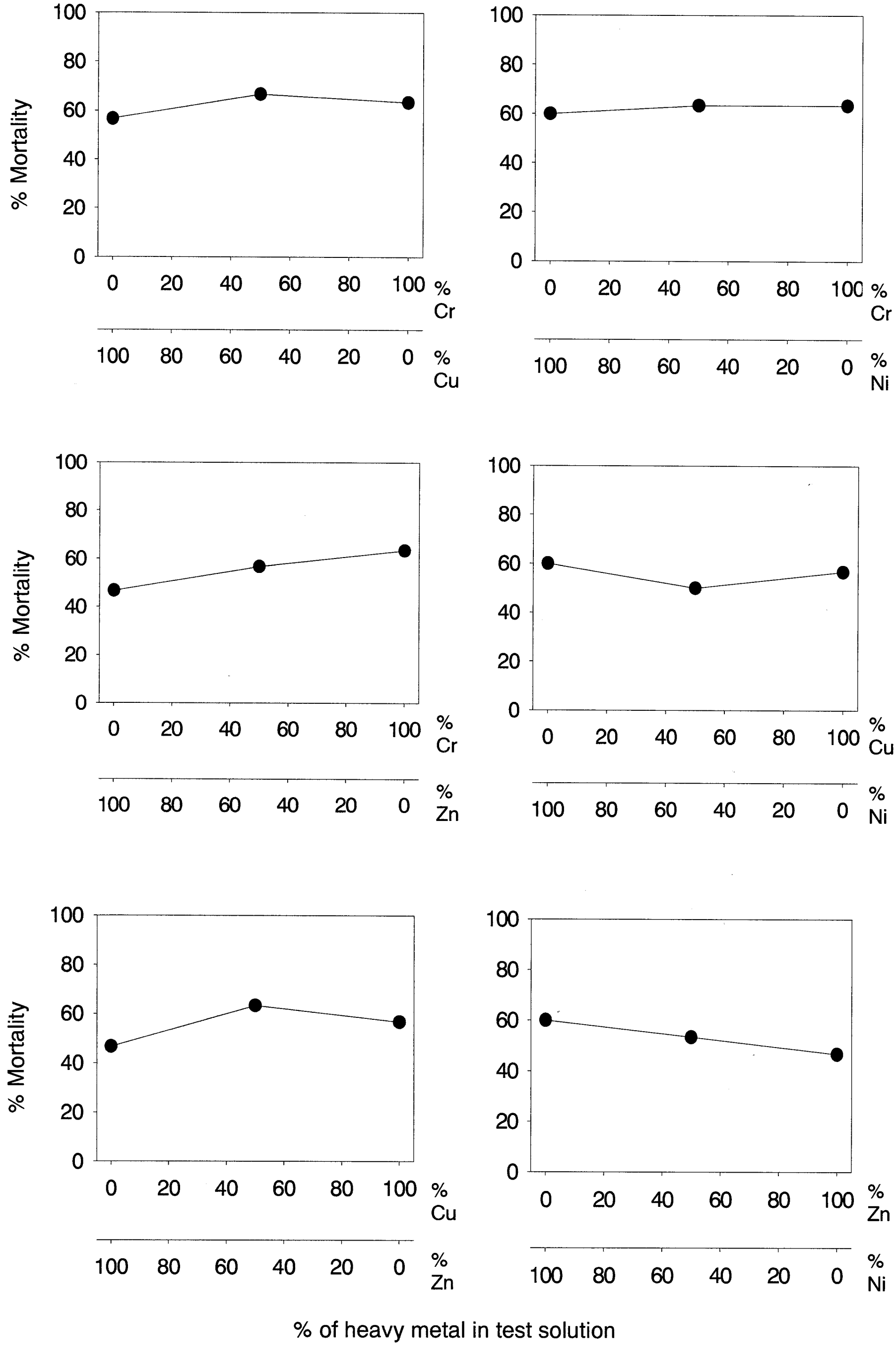

Figure 2. Mortality of the nauplii of *Mesocyclops pehpeiensis* after 48-h of exposure to metal mixtures. Each point represents the average value of 30 nauplii.

laboratory (Enserink *et al.*, 1991). The interaction among pollutants is complex and depends on the components of the mixture as well as the organisms affected. Cd, Cu, Ni, Zn and Pb produced synergistic toxicity to the estuarine copepod *Amphiascus tenuiremis* (Hagopian-Schlekat *et al.* 2001). The combined toxicity of Cd, Cr, Cu, Ni, Pb and Zn on the marine copepod *Tisbe holothuriae* was synergism (Verriopoulos and Dimas 1988). Break *et al* (1976) reported that the toxic action of Cu and Zn was synergistic against the dinoflagellate *Amhidirium carteri* and the diatom *Thalassiosira pseudonana*, but antagonistic against the diatom *Phaeodactylum tricornutum* (Break *et al.* 1976). Antagonism between Cu and Zn with reference to *P. tricornutum* has also been reported by Gudmund and Arne (1976). The combined effect of Cu and Zn was antagonistic on the fish *Pimephales promelas* (Parrott and Sprague 1993) and the gastropod *Tympanotonus fuscatus* (Otitoloju 2002), but synergistic on the snail *Lymnaea acuminate* (Khangarot *et al.* 1982) and the copepod *T. holothuriae* (Verriopoulos and Dimas 1988). The effect of Cu and Cd on cell growth of the green alga *Chlorella vulgaris* appeared synergistic at low metal concentrations and antagonistic at high metal concentrations (Lam *et al.* 1999). Clearly, the mechanism of interaction between metals requires further investigation.

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