Effect of cadmium and copper on survival and reproduction of *Daphnia pulex*

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Abstract

Acute and chronic toxicity tests were performed using *Daphnia pulex* with copper and cadmium as toxicants. The mean concentration causing 50% mortality in a 48 h acute test was 21 $\mu g \cdot t^{-1}$ for copper and 78 $\mu g \cdot t^{-1}$ for cadmium. In the 21 d chronic test, stimulation, recorded as increased offspring per animal (relative to that in the control), was observed for added copper concentrations of 0,003 $\mu g \cdot t^{-1}$ to 0,3 $\mu g \cdot t^{-1}$. Impairment of reproduction occurred at an added copper concentration of 3 $\mu g \cdot t^{-1}$. In the case of the chronic test on cadmium, reproductive impairment at 21 d was observed over the added concentration range of 0,003 $\mu g \cdot t^{-1}$ to 3 $\mu g \cdot t^{-1}$. The no observed adverse effect level (NOAEL) for chronic toxicity for copper was approximately 0,4 $\mu g \cdot t^{-1}$ and for cadmium <0,003 $\mu g \cdot t^{-1}$.

Introduction

The Department of Water Affairs and Forestry is in the process of establishing quality guidelines for the aquatic environment. Related to this activity is the need to develop and interpret toxicity bioassays for use in the whole effluent approach for the management of toxic effluents. For this purpose bioassays need to be tested and evaluated locally. Such evaluation also serves a purpose in placing overseas environmental quality criteria in perspective. Decisions will have to be made whether international environmental quality criteria should be adopted in South Africa, and to what extent they need modification appropriate to local conditions.

An important concept in toxicology is that the occurrence of poisoning is a matter of dose and exposure to a potentially toxic substance. The factor that determines whether a chemical agent is potentially harmful or safe is the relationship between the concentration of the chemical to which an organism is exposed, together with the amount absorbed, and the duration of the exposure (Rand and Petrocelli, 1985).

Daphnids are widely used in acute and chronic toxicity bioassays, both to quantify the toxic effects of single (Khangarot and Ray, 1989) or multiple (Enserink et al., 1991) substances, and to serve as biological indicators of effluent and receiving water quality (EPA, 1991a). Results from daphnid chronic toxicity tests are also used in estimating the no observed effect level (NOEL) (Kühn et al., 1989) and the maximum acceptable toxicant concentration (MATC) (Gersich and Milazzo, 1990). The advantages of using these organisms as test species include their short life cycle, the ease of laboratory culturing, their wide distribution and ecological significance, their low space and water volume requirements, and their sensitivity to chemicals (Elnabarawy et al., 1986). A considerable body of information exists on the effects of chemicals on the survival, growth and reproduction of various Daphnid species (Sheedy et al, 1991).

Particularly relevant ecologically are the effects on growth and reproduction of aquatic organisms, as even small changes in the levels of some variables can disturb the balance in a biocoenosis quite drastically (Schober and Lampert, 1977). Survival and population growth of aquatic organisms exposed over longer time intervals are usually affected at concentrations much lower than the levels of specific chemicals that cause acute effects (Savino and Tanabe, 1989). Acute concentrations thus do not represent concentrations that are completely safe in aquatic habitats subject to pollution (*Standard Methods*, 1989). The establishment of a MATC or NOEL (more strictly NOAEL; the no observed adverse effect level) of chemical substances to aquatic organisms, has relevance to the setting and evaluation of criteria for the protection of aquatic life.

In the characterisation of toxicants one can often distinguish between pure toxicants showing α-type toxicity, as would be expected of e.g. cadmium, and substances which act as growth stimulants at low concentrations (ß-type toxicity), such as exhibited by micronutrients, e.g. copper. The α-curve is the pattern commonly observed for the effect of a toxic substance, showing no departure from the organisms' response or state from normal at low concentrations, to a progressive inhibition above a threshold concentration. The ß-curve shows a single stimulatory peak at concentrations immediately below those that are inhibitory. The term hormesis was used by Stebbing to describe stimulatory effects caused by low levels of potentially toxic agents, although the concept was described by Schulz as early as 1888 (Stebbing, 1982).

In order to allow thorough evaluation and interpretation of bioassay results in actual cases of pollution, the response of indicator organisms to specific microcontaminants must be established. This study investigated 2 common industrial pollutant metals, copper and cadmium, as they affect survival and reproduction of *Daphnia pulex*. Both acute and chronic toxic effects were evaluated, this being essential to the interpretation particularly with respect to environmental criteria for these 2 metals.

Materials and methods

Culture technique

Test organisms were from a *D. pulex* culture which has been maintained at the Hydrological Research Institute, South Africa, for more than 3 years. Stock cultures were maintained in reconstituted moderately hard water (total hardness of 80 to 90 $mg \cdot t^{-1}$ $CaCO_3$) as recommended by EPA (1991b). *Daphnia pulex* cultures were fed on a suspension of commercial trout pellets, alfalfa and yeast, prepared according to the technique described by EPA (1985). The cultures were maintained in a controlled environment at 20°C, which has been recommended as the culture and test

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TABLE 1
DAPHNIA PULEX 48 h LC50 VALUES WITH 95% CONFIDENCE LIMITS OF THE TRIPLICATE TESTS WITH $CuCl_2$ AND $CdCl_2$. RESULTS ARE EXPRESSED IN $\mu g \cdot \ell^{-1}$ AS Cu AND Cd.

Test chemical	Replicate 1	Replicate 2	Replicate 3	Mean 48 h LC50
$CuCl_2$	18 (16-20)	24 (21-27)	22 (19-25)	21
$CdCl_2$	66 (55-78)	99 (82-119)	70 (58-85)	78

temperature for *Daphnia* (Lewis and Horning, 1991). Fluorescent lighting provided a 16 h:8 h light:dark cycle. As culture vessels 3 ℓ glass beakers, filled with approximately 2,5 ℓ culture medium, were used. The medium in each stock culture vessel was replaced in total each week, during which the population was thinned to prevent over-crowding.

Test organisms

Adult females bearing embryos were removed from the stock cultures 24 h preceding the initiation of a test. To ensure the use of healthy organisms in tests, females were not taken from cultures producing ephippia (resting eggs that are produced during unfavourable conditions). Less than 24 h-old daphnids were removed from these subcultures by pipette and used as test organisms in both acute and chronic tests.

Test procedure

All tests were conducted at the same controlled conditions (temperature and light regime) as described for the culture technique.

Acute tests

Daphnia pulex test organisms were exposed for 48 h under static conditions to selected concentrations of $CuCl_2$ and $CdCl_2$. Test organisms were placed in 50 mℓ beakers containing 25 mℓ test solution. Five test organisms were placed in each beaker, with 4 replicate beakers for each concentration - giving a total of 20 test organisms per test concentration. Moderately-hard reconstituted water was used to prepare the dilutions as well as for the control. A test was only regarded as valid if the control mortality did not exceed 10%. Tests were conducted in triplicate.

Test solutions were not aerated and organisms were not fed during the tests. Mortality or immobility of the test organisms was the toxic response measured. The 48 h acute LC50 values and the associated 95% confidence intervals, based on added concentrations of the chemicals in the test solutions, were estimated by means of the Spearman-Karber method (EPA, 1991b).

Chronic tests

Static-renewal, chronic (21 d exposure period) toxicity bioassays were conducted to test the chronic effects of cadmium and copper on the survival and reproduction of *D. pulex*. Stock solutions of 1 $mg \cdot \ell^{-1}$ concentration were made from Titrisol (Merck) $CuCl_2$ and Titrisol (Merck) $CdCl_2$. Each test consisted of a control and 4 concentrations (3,0; 0,3; 0,03 and 0,003 $\mu g \cdot \ell^{-1}$) of each metal. Reconstituted culture water was used for the controls as well as the dilutions.

For each concentration a total of 20 test organisms were distributed among 4 test vessels - giving 20 test animals per concentration level. As test vessels 400 mℓ beakers, filled with 250 mℓ test solution, were used. Each test was done in triplicate.

The static-renewal procedure meant that the parent animals in the test and control vessels had to be transferred 3 times a week (Mondays, Wednesdays and Fridays) into freshly prepared control and test solutions. During each transfer the dead parent animals were removed and offspring were counted and the total number in each test vessel was recorded. A 0,5 mℓ aliquot of food was added after parent animals had been transferred. Reproductive impairment was defined as a reduction in the cumulative number of young per adult.

Analysis of *Daphnia* food and culture water

The copper and cadmium content of the *Daphnia* food and culture water were determined by inductively coupled plasma (ICP) emission spectrometry. Total metals in the food were determined after an aqua regia digestion of the mixture. In addition the relatively readily bioavailable metal content of the food mixture was determined as the dissolved metals after 0,45 µm membrane filtration.

Test results

Acute tests

The estimated 48 h LC50 values of copper and cadmium for *D. pulex* are presented in Table 1.

Chronic tests

Copper

The number of survivors (of the original individuals, not including offspring produced) are shown in Fig. 1. Where 0,003 and 0,03 $\mu g \cdot \ell^{-1}$ copper was added, survival was higher than the control up to 16 d. For 0,3 and 3 $\mu g \cdot \ell^{-1}$ copper added, survival was initially (up to 7 d) higher than in the control, whereafter greater mortalities occurred.

Normalised (relative to the control) cumulative number of young per surviving animal, shown for copper in Fig. 2, indicated that only at the highest added copper concentration of 3 $\mu g \cdot \ell^{-1}$ did impairment of reproduction occur. At lower copper concentra-

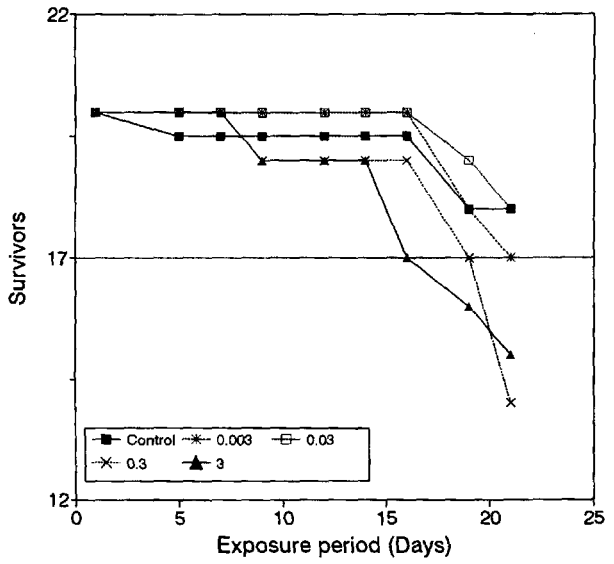

Figure 1
The number of survivors (of the original test organisms) at the various added copper concentrations. Copper concentrations are given in $\mu g \cdot l^{-1}$

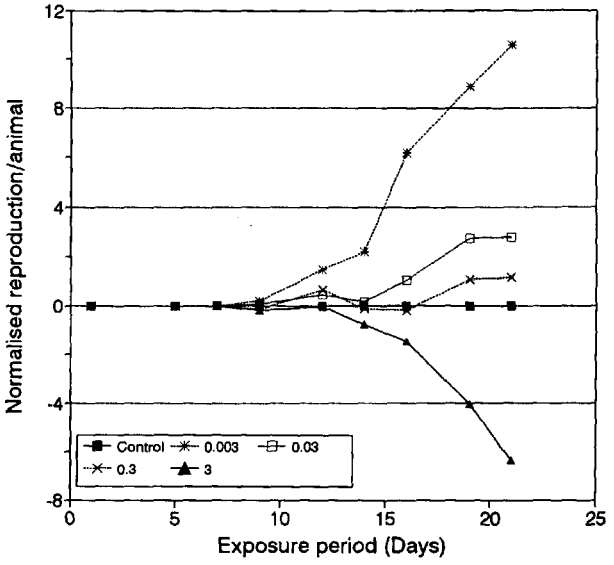

Figure 2
Normalised cumulative number of young per surviving animal at the various copper test concentrations (copper concentrations in $\mu g \cdot l^{-1}$)

tions, the number of young per animal after 21 d was greater than that of the control.

Figure 3 shows the normalised number of young per surviving animal after 21 d of exposure to the various copper test concentrations. The maximum, median and minimum values obtained in the triplicate tests have been normalised relative to the median value obtained in the controls. Figure 3 shows a typical ß-type toxicity curve, with a beneficial or stimulating effect occurring at added copper concentrations below approximately 0,3 $\mu g \cdot l^{-1}$, and inhibition at 3 $\mu g \cdot l^{-1}$. The greatest stimulation was obtained at 0,003 $\mu g \cdot l^{-1}$ added copper.

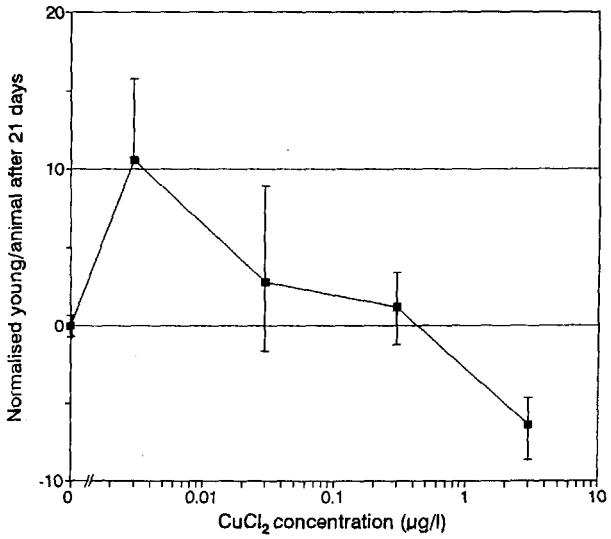

Figure 3
Normalised cumulative number of young per surviving animal after 21 d of exposure to various copper concentrations. Maximum, median and minimum values obtained in the triplicate tests are indicated, all normalised relative to the median value obtained in the controls

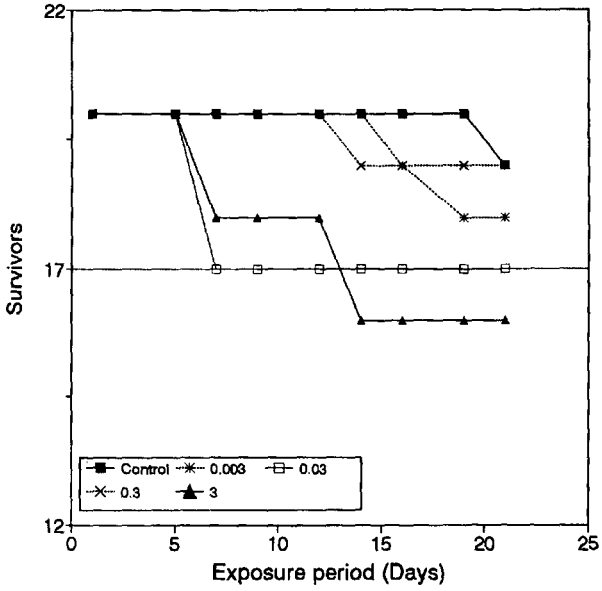

Figure 4
The number of survivors (of the original test organisms) at the various added cadmium concentrations. Cadmium concentrations are given in $\mu g \cdot l^{-1}$

Cadmium

Figure 4 indicates the number of survivors for the different cadmium concentrations during the 21 d exposure period. For 0,003 $\mu g \cdot l^{-1}$ and 0,3 $\mu g \cdot l^{-1}$ added cadmium survival was initially (respectively up to 14 and 12 d) the same as in the control, whereafter higher mortalities occurred. Where 0,03 and 3 $\mu g \cdot l^{-1}$ cadmium was added, survival was less than that of the control from day seven.

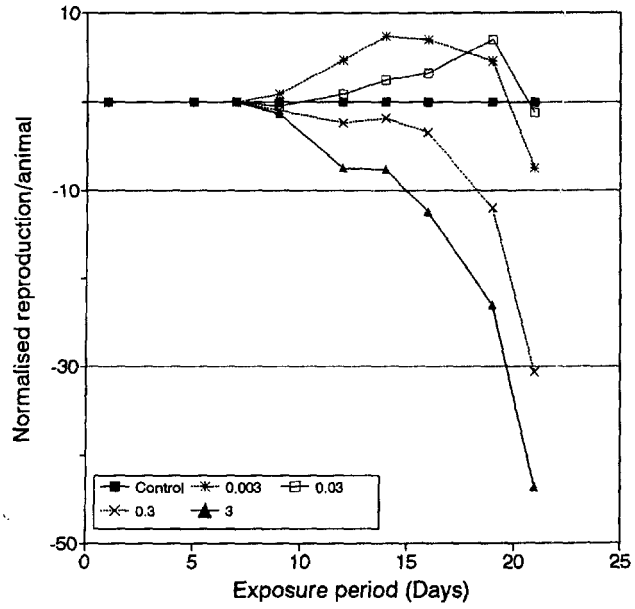

Figure 5
Normalised cumulative number of young per surviving animal at the various cadmium test concentrations (cadmium concentrations in $\mu g \cdot l^{-1}$)

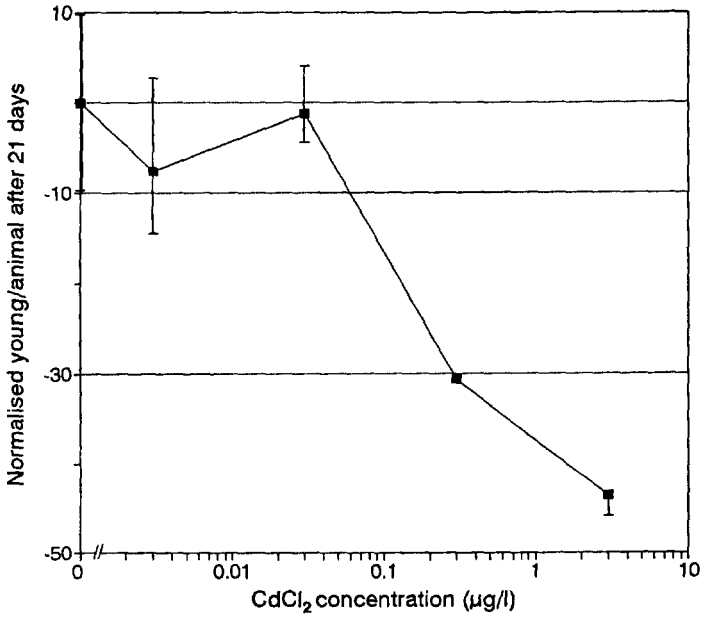

Figure 6
Normalised cumulative number of young per surviving animal after 21 d of exposure to various cadmium concentrations. Maximum, median and minimum values obtained in the triplicate tests are indicated, all normalised relative to the median value obtained in the controls

Normalised cumulative number of young per surviving animal, shown for cadmium in Fig. 5, indicates that only at 0,003 and 0,03 $\mu g \cdot l^{-1}$ added cadmium did stimulation of reproduction occur, and only up to day 19. After the full exposure period reproductive impairment occurred at all the tested cadmium concentrations. The impairment was greater with increasing added cadmium concentrations.

The normalised number of young per surviving animal after 21 d, shown in Fig. 6 for the cadmium test, shows an α-type toxicity curve, with inhibition of reproduction occurring at all tested cadmium concentrations.

Food and culture water

Total concentrations of copper and cadmium of 30 $\mu g \cdot g^{-1}$ and <5 $\mu g \cdot g^{-1}$ respectively were found in the food. With each feeding a total copper concentration of 1 $\mu g \cdot l^{-1}$ and cadmium concentration of $<0,2$ $\mu g \cdot l^{-1}$, contained in the food, was added to the test solutions. Ten percent, i.e. 0,1 $\mu g \cdot l^{-1}$, of the total copper was in a dissolved form.

Discussion

Acute versus chronic toxicity

It is interesting to note that while cadmium in the acute toxicity test was almost 4 times less toxic than copper (Table 1), quite the reverse situation was found in the chronic toxicity test. In the 21 d test cadmium caused reproductive impairment even at the lowest

concentration added of 0,003 $\mu g \cdot l^{-1}$ (Fig. 5). Copper on the other hand only caused reproductive impairment at the highest added concentration of 3 $\mu g \cdot l^{-1}$ (Fig. 2).

The mean LC50 found for copper of 21 $\mu g \cdot l^{-1}$ is comparable with reported values of 20 $\mu g \cdot l^{-1}$ (Qureshi et al., 1982) and 31 $\mu g \cdot l^{-1}$ (Elnabarawy et al., 1986). LC50 values of 42 $\mu g \cdot l^{-1}$ (Lewis and Horning, 1991) and 319 $\mu g \cdot l^{-1}$ (Elnabarawy et al., 1986) were reported for cadmium; a mean LC50 value of 78 $\mu g \cdot l^{-1}$ was obtained in the present study.

Hormesis

From the writings of Paracelcus it is evident that even in the 16th century it was realised that many substances which are toxic may be beneficial in small amounts. Apart from inhibiting some biological processes above a threshold sublethal level, some toxic agents may be expected to stimulate these processes at still lower (sub-inhibitory) levels (Stebbing, 1982).

The hormesis phenomenon is not surprising in the case of essential micronutrients, as indicated in this study for copper (Fig. 3), since it is assumed that conditions for growth are optimal when growth is maximal. Hormesis, however, also occurs in non-essential elements, as indicated with cadmium, although not for the full exposure period (Fig. 5). Stebbing (1982) suggests that this may be the consequence of regulatory overcorrections by basal control mechanisms to low levels of inhibitory challenge, resulting in growth that is greater than normal.

Bioavailability and toxicity

When carrying out the 21 d *Daphnia* reproduction test, chemical quantification of substance concentrations should be conducted in order to ensure reliable interpretation of results (Kühn et al., 1989). It is apparent that the optimum copper concentration for *D. pulex* was acquired when an additional 0,003 $\mu g \cdot l^{-1}$ copper was added. In other words the copper content in the food was near optimum. It is to be expected that since the food is of biological origin, it already contained copper at appropriate concentration from the natural bio-uptake in the source material (fish, alfalfa and yeast). What is significant is that only a slight increase in the copper content results in a dramatic beneficial effect, whereas inhibition occurred when the copper concentration added was 3 $\mu g \cdot l^{-1}$ (or increased fourfold). Of course, it is possible that not all the copper found by total digestion of the food is biologically available, since only 10% of the copper in the food was in a readily soluble form.

As it is not known what fraction of the total copper present in the food is biologically available, it places an uncertainty on the observed NOAEL figure obtained. The US EPA has indicated that their permit regulations are only intended to regulate bioavailable metals (Hall et al., 1992). Borgmann et al. (1991) tested the chronic toxicity of cadmium to the amphipod *Hyalella azteca* and found that toxicity expressed as a function of cadmium bioaccumulated, rather than the cadmium concentrations added or measured in the water, was more constant.

It is important to realise that this is only a benchmark type experiment. As described in the literature the initial points will change where 2 or more metals are present together in the growth medium.

Environmental quality criteria

It is important to note that LC50 values found for copper and cadmium are considerably higher than the lowest concentrations found to cause reproductive impairment in the 21 d test. The difference in the case of copper was almost one order of magnitude and in the case of cadmium 5 orders of magnitude. Consequently, environmental criteria based on acute toxicity results only, will not provide adequate protection for aquatic life.

Cappon (1991) indicated that the environmental quality objectives in the Netherlands for copper and cadmium in water are 3 $\mu g \cdot l^{-1}$ and 0,2 $\mu g \cdot l^{-1}$ respectively, which are defined as the NOAEL values. This study with *D. pulex* indicates a NOAEL value of added copper at approximately 0,4 $\mu g \cdot l^{-1}$ (Fig. 3). If the copper contained in the food is taken into consideration, the NOAEL for copper was approximately 1,4 $\mu g \cdot l^{-1}$. For cadmium adverse effects occurred at all added concentrations (Fig. 6), and it appears as if the NOAEL for chronic toxicity was below 0,003 $\mu g \cdot l^{-1}$.

The NOAEL for copper found in this study was one order of magnitude less than the Netherlands environmental quality objective, while the NOAEL found for cadmium was at least 2 orders of magnitude less than the Netherlands environmental quality objective. It should be noted, however, that this applies to the ionic species of the tested metals. If the total copper present in the food is included then the NOAEL for copper is very similar to the Netherlands criterion. While Daphnia are known to be sensitive indicators of trace metal pollution, the Netherlands environmental quality objectives do not necessarily represent the lowest NOAEL for the most sensitive species, but are in fact compromise objectives aimed at protecting 95% of the species in an ecosystem (Van de Meent et al., 1990).

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