Bull. Environ. Contam. Toxicol. (1984) 33:605-611
© 1984 Springer-Verlag New York Inc.

Bulletin of Environmental Contamination and Toxicology

# Selenium Effects on Antennal Integrity and Chronic Copper Toxicity in *Daphnia pulex* (deGeer)

Robert W. Winner

Department of Zoology, Miami University, Oxford, OH 45056

Although it is obviously important in aquatic toxicological research to maintain experimental animals in chemically defined test media which are both physiologically compatible to the experimental organism and similar to natural waters, such media have not yet been developed for daphnids. Most such research is conducted in media such as dechlorinated tap water (Buikema et al., 1976), well water (Lewis and Maki, 1981), or surface water (Goulden and Hornig, 1980) which are not completely defined chemically. In those cases where chemically defined media have been used the ionic concentrations and proportions usually differ markedly from those in natural waters and/or synthetic chelating chemicals and buffers have been added: Crow and Taub (1979), Tevlin (1978), U.S. EPA (1975). Also, experiments in such waters have not been sufficiently prolonged to document that normal life spans can be attained.

Murphy (1970) suggested that calcium was the only inorganic ion required by Daphnia magna and he maintained his cultures in distilled water to which only calcium acetate had been added. All other mineral requirements were supposedly provided by the algal food. Trace elements were probably also added as contaminants in the organic supplements (e.g. liver extract and choline). Moreover, in this system, D. magna survived a maximum of 30 days at 20$^{o}$C (Murphy, pers. commun.). Subsequently, however, Winner and Farrell (1976) showed that D. magna survived up to 120 days on Murphy's algal diet when maintained in pond water at 20$^{o}$C. Obviously something was lacking in Murphy's medium. Winner (1976) also cultured D. magna in a water reconstituted by adding reagent-grade salts to distilled water (Marking, 1969). The life span of animals in this water was only about half that of animals maintained in pond water. In addition, animals reared in reconstituted water lost the biramous portions of the second antennae at an age of about 20 days. Overnight aeration of the medium prior to introducing daphnids, or increasing the calcium concentration, delayed but did not prevent antennal damage. Taub and Dollar (1968) have also recorded antennal damage in *D. magna* maintained in reconstituted water.

Currently, I have been using reconstituted waters containing only four or five salts in chronic copper toxicity tests with *D. pulex*. Antennal damage has been rare among control animals and has occurred only in the softest of the waters. However, animals develop antennal damage at an age of about 25 days when exposed to copper concentrations sufficiently high to reduce longevity in soft water.

Kathleen Keating (Rutgers Univ.) has shown (unpubl. ms.) that such antennal damage in *D. pulex* may be caused by a selenium deficiency. The objective of the present research was to determine the effect of sodium selenite on the incidence of antennal damage in cohorts of *D. pulex* exposed to Cu in a soft, reconstituted water.

## MATERIALS AND METHODS

Each test was conducted with a cohort of ten newborn (<24-h old) *D. pulex* individually reared in 40 mls of reconstituted water in 50-ml pyrex beakers. Beakers were covered with watch glasses to minimize evaporation and contamination from the atmosphere. All tests were conducted in an environmental chamber at 20 +/- 1$^{o}$C on a 16-h photoperiod under white fluorescent lights at an illumination of 28 lux.

The reconstituted water was prepared by adding reagent-grade $CaCO_3$ (35 mg/l), $MgCO_3$ (19 mg/l), KCl (0.15 mg/l) and NaCl (11.5 mg/l) to distilled, charcoal-filtered, deionized, Organex-Q (Millipore Filter Corp.) filtered water which had been saturated with $CO_2$ to convert the carbonates to bicarbonates. After aerating to bring the pH up to above 8.0, 4.67 mg $NaHCO_3$/l was added to increase the alkalinity without increasing the hardness. The final product has a hardness of 50 and an alkalinity of 100 (as mg $CaCO_3$/l) and a pH of approximately 8.6. Organics were below detection limits when analyzed by fluorescent spectroscopy using a Spex Fluorolog corrected spectrophotofluorimeter. This is one of a series of waters being used to evaluate the effects of water hardness on metal toxicity.

Tests were run in water to which had been added (ppb): 0 Cu - 0 Se, 10 Cu - 0 Se, and 10 Cu - 5 Se. Copper concentrations were monitored by flameless atomic-absorption spectroscopy. Animals were transferred to freshly prepared media on M-W-F for 42 days. Mortality, reproductive condition, and antennal damage were noted daily when young were removed and each animal fed a ration of vitamin-enriched *Chlamydomonas reinhardtii* (Winner and Farrell, 1976).

Mean brood sizes, copper concentrations and pH were compared among cohorts by ANOVA and Duncan's New Multiple Range Test using the SAS "GLM" computer program (Helwig and Council, 1979). Survivorship curves and percentage antennal damage over time were compared by a Lee-Desu $Chi^2$ analysis using the SPSS computer program (Hull and Nie, 1983). Unless otherwise indicated, statements of statistical differences are based on accepting $P \leq 0.05$.

RESULTS AND DISCUSSION

Copper concentrations of control beakers averaged 0.8 ppb (N = 18, SE = 0.13). In the beakers containing a nominal Cu concentration of 10 ppb, the mean was 10.1 ppb (N = 20, SE = 0.24). The mean pH was not significantly different among treatments and averaged 8.58 (n = 44, SE = 0.04).

The cohort reared in water containing 10 ppb Cu, and no added Se, began to exhibit antennal damage on day 28 of the experiment and all animals exhibited antennal damage by day 32 (Fig. 1). By contrast, the cohort reared in water containing 10 ppb Cu and 5 ppb Se exhibited no antennal damage over the 42-day exposure. In the absence of both Cu and Se, only one animal developed antennal damage and that late in the experiment (day 40).

The addition of Se to the test water not only eliminated antennal damage, it also significantly reduced the chronic toxicity of Cu as evidenced by an increase in survival (Fig. 2). Only three animals died in the cohort exposed to Cu in the presence of Se and these deaths occurred very early in the experiment (days 10 and 11) and were probably not due to Cu. In the cohort exposed to Cu in the absence of Se all animals were dead by day 36.

We have previously shown that, even at concentrations which reduce longevity, copper increases the number of young per brood in daphnids which are fed an adequate diet (Winner et al., 1977). This conclusion has been substantiated in the present study where mean brood sizes were 7.0, 11.0, and 11.2 young/female for cohorts reared in Cu-Se concentrations of 0-0, 10-0, and 10-5 ppb, respectively. Brood sizes were not significantly

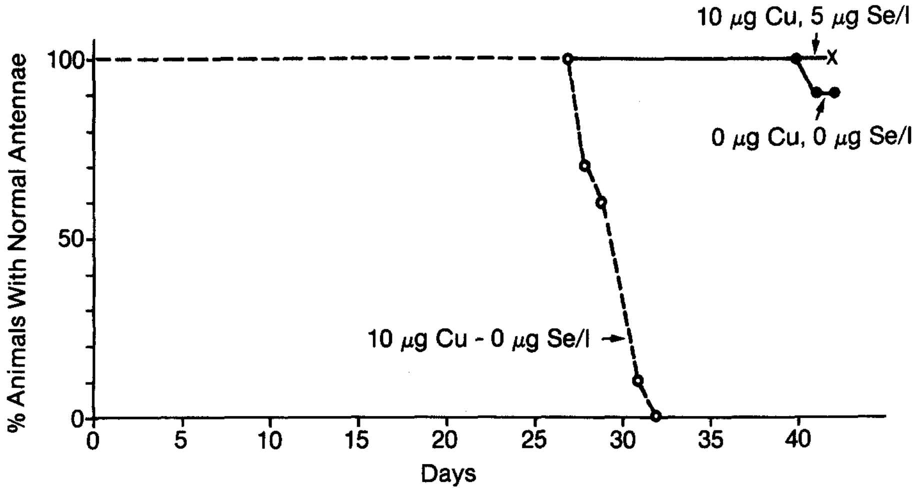


Fig. 1. The development of antennal damage in three cohorts of D. pulex exposed to three combinations of copper and selenium.

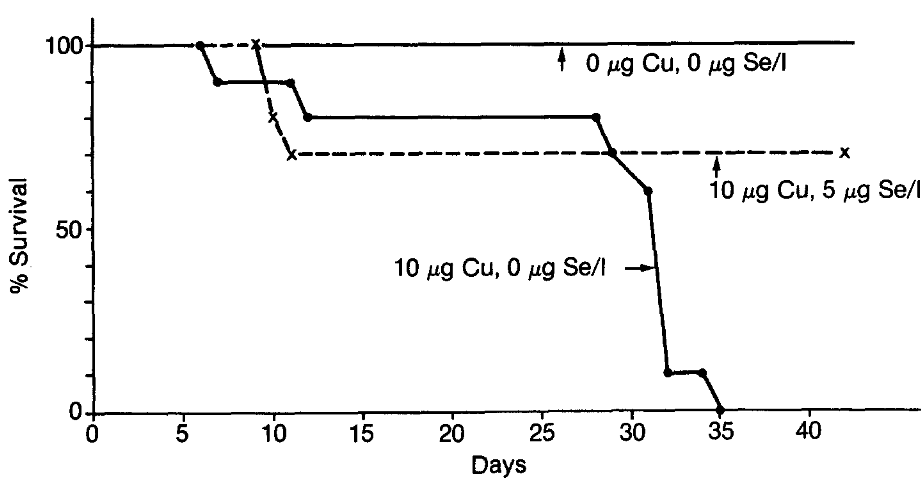


Fig. 2. Survivorship curves for three cohorts of D. pulex exposed to three combinations of copper and selenium.

different between the two cohorts reared in 10 ug Cu/l, but both of these were significantly larger than for the cohort reared in water to which no Cu had been added ($P \leq 0.01$).

Although it has been well documented that Se is an essential trace metal for mammals and birds (Shamberger, 1983), the only documentation of a Se requirement by invertebrates seems to be Keating's unpublished manuscript. However, considering that Se is known to be essential for the synthesis of glutathione peroxidase, it is highly probable that all organisms require this element. This need has probably not been recognized in maintaining laboratory populations of freshwater organisms because of accidental inclusion of Se in most laboratory waters and/or foods. In retrospect, the delay in onset of antennal damage which was induced by aeration or increasing calcium content of test waters (Winner, 1976) was probably a coincidental result of Se contamination from the laboratory atmosphere or the calcium salts.

The Se requirements of algae are poorly documented and this element has not been generally added to algal culture media. However, Lindstrom (1983) has determined that Se is an essential micronutrient for the dinoflagellate _Peridinium cinctum_ and possibly for the diatom _Stephanodiscus hautzschi_ and eight species of green algae. It has generally been believed that daphnids obtain most of their mineral requirements from their food resources (Murphy, 1970) rather than directly from the aqueous medium. It may, therefore, be possible to satisfy the requirements of daphnids by incorporating Se into the algal food and it seems quite probable that such an addition would benefit not only the herbivores but also the algae.

The aggravation of a Se deficiency by a Cu stress in _D. pulex_ agrees with published information on birds. Jensen (1975) has shown that the addition of Cu to a Se-marginal diet resulted in the development of Se-deficiency symptoms in chicks.

The protective effects of Se against a chronic Cu stress in _D. pulex_ also agrees with results from mammalian research. Kar _et al_. (1960) have shown that Se completely prevented the toxic effect of Cd in ovarian and testicular tissues of rats. Godwin et al. (1977) have shown that Se can prevent acute copper toxicity in rats. The only reference that I can find of Se protecting against metal toxicity in invertebrates is the work of Van Puymbroeck _et al_. (1982). They found that selenium reduced the acute toxicity of Cd to the freshwater snail, Lymnaea stagnalis L.

In conclusion, Se should be included as one of the essential nutrients in laboratory cultures of daphnids (and probably other freshwater organisms) and this metal will have to be added to the increasing list of water quality parameters which must be considered when deriving water-quality criteria and standards for metal toxicity.

Acknowledgments. This research was supported by cooperative agreement #809224010 with the Environmental Research Laboratory, U.S. EPA, Corvallis, Oregon and by equipment grants CDP-8004830 and PRM-8113375 from the National Science Foundation. I am grateful to W Houk for analyzing the organic content of the test water and to H Serazin, A Flickinger and J Gauss for technical assistance.

REFERENCES

Buikema AL Jr., Lee DR, Cairns J, Jr. (1976) A screening bioassay using Daphnia pulex for refinery wastes discharged into freshwater. J Test Eval 4:119-125

Crow ME, Taub FB (1979) Designing a microcosm bioassay to detect ecosystem level effects. Int J Environ Stud 13:141-147

Godwin KO, Patrick EJ, Fuss CN (1977) Adverse effects of copper, and to a lesser extent iron, when administered to selenium-deficient rats. In: Kirchgessner M (ed) Trace Element Metabolism in Man and Animals. 3:185-187. Freising-Weihenstephan, W. Germany

Goulden CE, Hornig LL (1980) Population oscillations and energy reserves in planktonic Cladocera and their consequences to competition. Proc Natl Acad Sci USA 77:1716-1720

Helwig JT, Council KA (1979) SAS Users Guide. SAS Institute Inc, Raleigh, NC, USA

Hull CH, Nie N (1983) SPSSX Users Guide. McGraw Hill Pub New York, NY

Jensen LS (1975) Modification of selenium toxicity in chicks by dietary silver and copper. J Nutr 105:769-775

Kar AB, Das RP, Mukerji B (1960) Prevention of cadmium induced changes in the gonads of rats by zinc and selenium - a study in antagonism between metals in the biological system. Proc Natl Inst Sci India Part B 26:40-50

Lewis MA, Maki AW (1981) Effects of water hardness and diet on productivity of Daphnia magna Straus in laboratory culture. Hydrobiol 85:175-179

Lindstrom K (1983) Selenium as a growth factor for plankton algae in laboratory experiments and in some Swedish lakes. Hydrobiol 101:35-48

Marking LL (1969) Toxicity of quinaldine to selected fishes. Invest Fish Control 23. Bur Sport Fish Wildl, Wash. DC 10 pp

Murphy JS (1970) A general method for the monoxenic cultivation of Daphnidae. Biol Bull 139:321-332

Shamberger RJ (1983) Biochemistry of selenium. Plenum Press. New York and London, 334 pp

Taub FB, Dollar AM (1968) The nutritional inadequacy of *Chlorella* and *Chlamydomonas* as food for *Daphnia pulex*. Limnol Oceanogr 13:607-617

Tevlin MP (1978) An improved experimental medium for freshwater toxicity studies using *Daphnia magna*. Water Res 12:1017-1024

US EPA (1975) Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecol Res Ser EPA-660/3-75-009. Corvallis, OR 97330 USA

Van Puymbroeck SLC, Stips Wouter JJ, Vanderborght OLJ (1982) The antagonism between selenium and cadmium in a freshwater molluscs. Arch Environm Contam Toxicol 11:103-106

Winner RW (1976) Toxicity of copper to daphnids in natural and reconstituted waters. Ecol Res Ser EPA-600/3-76-051. US EPA, Envir Res Lab, Duluth, MN 55804. 69 pp

Winner RW, Farrell MP (1976) Acute and chronic toxicity of copper to four species of *Daphnia*. J Fish Res Board Can 33:1685-1691

Winner RW, Keeling T, Yeager R, Farrell MF (1977) Effect of food type on the acute and chronic toxicity of copper to *Daphnia magna*. Freshw Biol 7:343-349

Received February 2, 1984; accepted March 7, 1984