Bull. Environ. Contam. Toxicol. (1987) 38:722–726

Bulletin of Environmental Contamination and Toxicology

Correlation Between Heavy Metal Acute Toxicity Values in *Daphnia magna* and Fish

B. S. Khangarot and P. K. Ray*

Preventive Toxicology Division, Industrial Toxicology Research Centre, Post Box No. 80, Mahatma Gandhi Marg, Lucknow-226 001, India

In the toxicant bioassays, invertebrates with special reference to aquatic arthropod species have been of recent interest as test models due to the need for developing nonmammalian test system (Turner et al 1983a, 1983b; Richie et al 1984). The cladoceran Daphnia magna bioassays have several practical advantages. For example, they are easy to culture in laboratory conditions and inexpensive, short life cycle, discrete growth, aging period and size which allows large number of test species can be used for statistical design and analysis. The additional advantages of small volumes, ease in handling, high fecundity minimized test apparatus make this invertebrate an extremely desirable test species for aquatic toxicologists (Maciorowski and Clarke 1980). D. magna has been used as a useful test species (Anderson 1944; Biesinger and Christensen 1972; Adema 1978) and its sensitivity to environmental pollutants have been recognized as a general representative of other freshwater zooplankton species(Buikema et al 1980). The objectives of this study were to determine the acute toxicity of various heavy metals to Daphnia magna for 48 h of exposure and to compare these values with the existing LC50 values for rainbow trout (Salmo gairdneri); which is commonly used as a test animal in aquatic bioassay studies.

MATERIALS AND METHODS

Daphnia magna were collected from natural pond situated at Gheru Campus of Industrial Toxicology Research Centre, Lucknow and stock culture were made in tubewell water. Bioassays were conducted between December 1984 and January 1985 i.e. during winter months. Acute bioassay procedure was followed during this study (APHA 1976). Animals were fed during the acclimatization and culture period but were starved during exposure time. At the begining of test 10 daphnids were placed randomly in 200 mL beakers containing 100 mL of test water. A series of test concentrations (7-10) of toxicant and control in tubewell water were used in this study. All tests were performed in two replicate sets.

*Correspondence and reprint author.

Stock solutions of metallic salts were made in distilled water. Various metallic salts included in the study were $3CdSO_4.8H_2O$; $(CH_3COO)_2Pb$; $CuSO_4.5H_2O$; $AgNO_3$; $NiCl_2.6H_2O$; $SnCl_2$; $K_2Cr_2O_7$; $ZnSO_4.7H_2O$. The concentrations are given as ppm of metal. The immobilized daphnids were removed and counted. The criterion for determining the immobilization was no response to gentle prodding with a blunt glass rod. Young daphnids produced at 12 h and 24 h were removed. The EC50 values and 95% confidence limits (C.L.) and significance difference between EC50 values were calculated according to Harris (1959). The regression equations were obtained between the 48 h EC50 for D. magna and 96 h LC50 for S. gairdneri (Snedcore and Cochran 1967). The physico-chemical properties of test water were determined by Standard Methods (APHA 1976).

RESULTS AND DISCUSSION

The physico-chemical characteristics of tubewell water are given in Table 1. Decreased pH values were observed at some of the higher test concentrations of Zn, Ni and Cr, but these values were never greater than 0.5 pH unit.

Table 1. Physico-chemical properties of test water

Parameters	Unit	Mean	Range
Air temperature	°C	15	14-17
Water temperature	°C	13	11.5-14.5
pH		7.6	7.4-7.8
Dissolved oxygen	ppm	5.6	5.2-6.5
Total hardness	ppm as $CaCO_3$	240	235-260
Total alkalinity	ppm as $CaCO_3$	400	390-415
Calcium	ppm	152	145-165
Magnesium	ppm	92	85-96
Chloride	ppm	7	5-10

A comparison of acute toxicity values for 24 h and 48 h demonstrated that there is an increase in toxicity following longer exposure of period (Table 2). However, the magnitude of this difference is not grate for chromium since there was no significance difference between 24 h and 48 h EC50 values, thus suggesting that cu mulative toxicity is

Table 2. EC50 values (ppm of metal) and their 95% confidence limits for various metals tested against Daphnia magna

Metal	EC50's and 95% confidence limits		Significance difference
	24 h	48 h	
Ag	0.023 (0.017-0.034)	0.01 (0.0054-0.0156)	0.3229*
Hg	0.0081 (0.0067-0.0099)	0.0052 (00.0042-0.0072)	0.0982
Cu	0.536 (0.466-0.643)	0.093 (0.081-0.112)	0.6521
Zn	1.0**	0.56**	0.2516
Cr	2.2 (1.82-3.26)	1.79 (1.24-2.49)	- 0.1270
Cd	4.66 (3.93-5.72)	1.88 (1.09-3.29)	0.3430
Pb	4.89 (4.19-5.89)	3.61 (2.83-4.4)	0.0451
Ni	10.9 (8.2-13.2)	7.59 (6.05-9.26)	0.2172

*The two EC50 values will be judged significantly different if number is 0

**95% confidence limits cannot be calculated

apparently not significant for this heavy metal (Table 2.).

The toxicity data observed for D. magna and published data of rainbow trout are compared to each other (Table 3). The decreasing

Table 3. Comparison of acute toxicity values for D. magna and rainbow trout (S. gairdneri) for eight heavy metals

Test metal	Daphnia 48-h EC50 (in ppm)	Rank order	Rainbow trout 96-h LC50 (in ppm)	Rank order
Hg	0.0052	1	0.033[a]	1
Ag	0.01	2	0.029[a]	2
Cu	0.093	3	0.253[a]	3
Zn	0.56	4	0.55[a]	4
Cr	1.79	5	11.2[b]	7
Cd	1.88	5	2.5[c]	5
Pb	3.61	5	8.0[a]	6
Ni	7.59	8	35.5[a]	8

a- From Hale (1977)
b- From Bills et al (1977)
c- From Ball (1967)

order of toxicity of tested heavy metal ions in Daphnia and rainbow trout (S. gairdneri) are as follows:

Daphnia magna
Hg Ag Cu Zn Cr= Cd= Pb Ni
Salmo gairdneri
Ag= Hg Cu Zn Cd Pb Cr Ni

In case of D. magna no significance difference was observed between the 48 h EC50 values of Cr, Cd and Pb, therefore, same rank order was given for these metals. There are some differences between the two animals in relative orfer of toxicity for metal ions. In both organisms Ag^{+}, Hg^{2+}, Cu^{2+} and Zn^{2+} are most toxic and Ni^{2+} is among the least toxic. The position of chromium, however, is noticeably different; in Daphnia it is next to Zn^{2+} whereas chromium is below to lead in rainbow trout. A toxicity ranking is approximate, because so many physical, chemicals and biological factors may influences a pollutant's toxicity (Eisler and Hennekey 1977). A strong correlation between Daphnia and ranibow trout acute toxicity values is obtained for eight heavy metallic ions. A regression analysis suggests a good correlation exists (Fig 1); $r^2 = 0.814$ for straight line equation. Using the regression equation it is theoritically possible to calculate acute toxicity values for rainbow trout. A statistical comparison of the relative orders of toxicity for Daphnia and rainbow trout LC50 values for various heavy metal ions were performed by evaluating the Spearman rank correlation coefficient, r_s. This comparison gave value of r_s=0.929 for EC 50 values of Daphnia and rainbow trout. This clearly indicate that the acute toxic responses of Daphnia is strongly correlated to the LC50 of fish. Therefore, the screening of metal compounds and untested compounds might well be done using D. magna. Since it is well known that metal toxicity is changed by hardness of

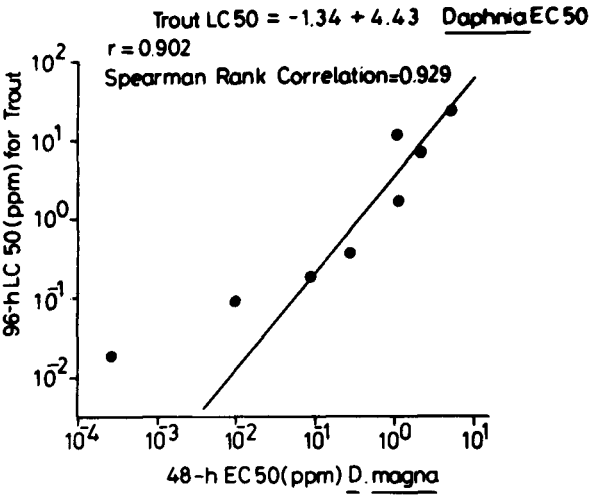

Fig 1 Correlation between acute toxicity values of Daphnia magna and rainbow trout for various heavy metals.

water, being most toxic in soft water, it is reasonable that good comparison between rainbow trout and Daphnia acute toxicity data for these metals would agree more positively if water of comparable hardness had been used for both studies.

We believe that the correlation between Daphnia and fish toxicity values will be useful for generating toxicity in the absence of other data and it also serves to demonstrate that aquatic safety data would be developed for the protection of aquatic organisms. However, it is now very important to examine the significant of this correlation with several organic and inorganic compounds, pesticides, detergents and industrial effluents potentially reaching to aquatic ecosystem.

Acknowledgments. B.S. Khangarot, greatfully acknowledges CSIR, New Delhi for providing financial assistance in the form of Scientist's Pool. Thanks are also due to L.K. Goswami for typing the manuscript.

REFERENCES

Adema DMM (1978) Daphnia magna as a test animal in acute and chronic tests. Hydrobiologia 59: 122-134

Anderson BG (1944) The toxicity thresholds for various substances found in industrial wastes as determined by the use of Daphnia magna. Sewage Work J 16: 1156-1165

APHA (1976) Standard methods for the examination of water and waste waters. American Public Health Association, 14th ed, Washington, DC

Ball IR (1967) The toxicity of cadmium to rainbow trout (Salmo gairdenri Rich.) Water Res 1: 805-806

Biesinger KE, Christensen GM (1972) Effect of various metals on growth survival, reproduction and metabolism of Daphnia magna. J Fish Res Bd Can 29: 1691-1700
Bills TD et al (1977) Effects of residues of polychlorinated Aroclor 1254 on sensitivity of rainbow trout to selected environmental contaminants. Prog Fish-Cult 39: 150 (Cited by WA Brungs, RW Carltron, WB Horning II, JH McCormick, RL Spehar and JD Lee Yount (1978) Effects of Pollution of fresh water fish. J Water Pollut Control Fed 50: 1582-1637
Buikema AL, Geiger JG, Lee DR (1980) Daphnia toxicity tests. In: Buikema AL, Cairns Jr J (eds) Aquatic invertebrate bioassays. ASTM STP 715: 48-69
Eisler R, Hennekey RJ (1977) Acute toxicity of Cd^{2+}, Cr^{+6}, Hg^{2+}, Ni^{2+} and Zn^{2+} to estuarine macrofauna. Arch Environ Contam Toxicol 6: 315-323
Hale JG (1977) Toxicity of metal mining wastes. Bull Environ Contam Toxicol 17: 66-73
Harris EK (1959) Confidence limits for the LD50 using the moving average-angle methods. Biometrics 15: 422-432
Macriorwaski HD, Clarke R (1980) Advantages and disadvantages of using invertebrates in toxicity testing. In: Buikema AL, Cairns Jr J (eds) Aquatic Invertebrate Bioassays ASTM STP 715: 36-47
Maki AW (1979) Correlation between Daphnia magna and fathead minnow (Pimephales promelas) chronic toxicity values for several classes of test substances. J. Fish Res Bd Can 36: 411-421
Richie JP Jr, Mills BJ, Lang CA (1984) The verification of a mammalian toxicant classification using a mosquito screening methods. Fundam Appl Toxicol 4: 1029-1035
Snedcore GW, Cochran WB (1967) Statistical methods. Iowa State University Press, Ames, IA p 595
Turner JE, Lee EH, Jacobson K, Christie HT, Williams MW, Hoeschele JD (1983) Investigation of correlation between chemical parameters of metal ions and acute toxicity in mice and Drosophila. The Sci Total Environ 28: 343-354
Received June 30, 1986; accepted November 20, 1986