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The Acute Toxicity of Copper to *Gammarus fasciatus* Say, A Freshwater Amphipod

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The purpose of this toxicity test was to determine the reliability and comparability of Cu++ toxicity on Gammarus fasciatus. The study was conducted to compare toxicity results for Rainbow trout, Salmo gairdneri Richardson, another coldwater poikilotherm. Since G. fasciatus comprises a major portion of the diet of many freshwater fishes, it can be stated to be an "important" species (PATRICK et al. 1968).

MATERIALS AND METHODS

G. fasciatus, a freshwater amphipod, was tested for its acute response to toxicity of Cu++. It is an important food web organism comprising greater than 10% of the diet of trout, coldwater poikilotherms, and warmwater fishes (GECKLER et al. 1976). G. fasciatus is found in wide distribution along with G. linnaeus, Smith and Crangonyx gracilus, Smith. They are the majority of amphipods taken (PENNAK 1953). These organisms are widely distributed and are found in many habitat types from cold to warm waters (PENNAK 1953).

A standard screening bioassay in hard water was run to determine the range for the definitive tests (STANDARD METHODS 1965). Two definitive tests of 48 hr duration were conducted to determine reproducibility and reliability of the test organism. Also, 48 hr LC50 values were calculated to determine the response of G. fasciatus to Cu++ as $CuSo_4$. Results of these tests were evaluated using the Litchfield and Wilcoxin Nomograph Analysis (LITCHFIELD and WILCOXIN 1949) for $\alpha = 0.05$ confidence limits on the slope and LC50 values obtained.

A screening test using a control group and dilutions of 1.0, 10.0, 100, and 1000ppm for Cu++ was used to determine broad range toxicity to G. fasciatus. Each battery jar was acid rinsed and contained 3.0L of solution and 10 test organisms picked randomly from a stock group. The organisms were collected from a cold spring near Alanson, Michigan on state highway M31. The temperature of the spring ranged from 12 to 14°C and the organisms were transported back to the laboratory where they were kept for 48 hr without feeding prior to testing.

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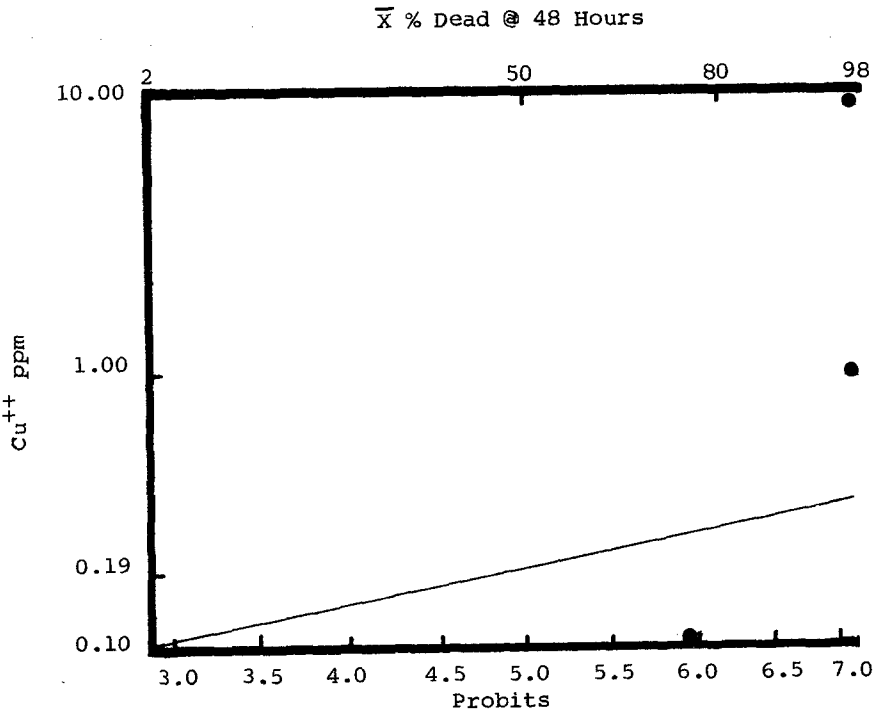

Fig. 1. Preliminary LC50 Determination

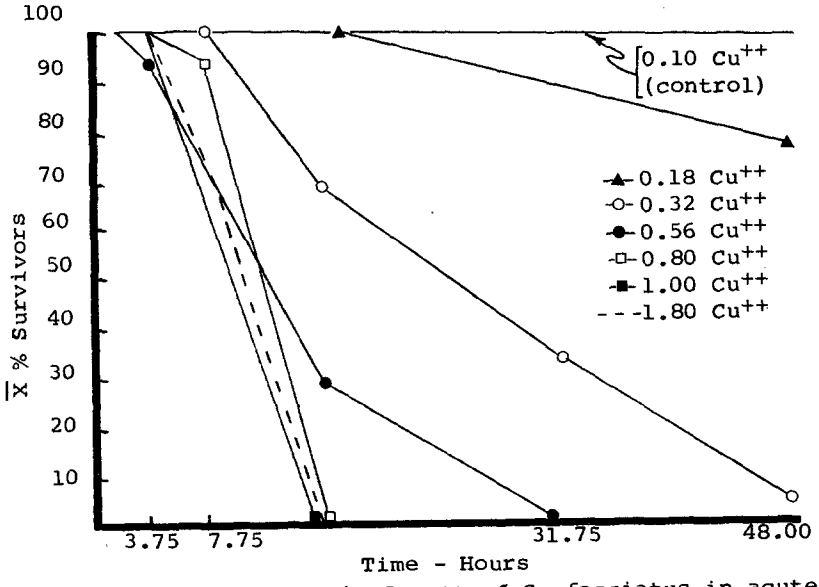

Fig. 2. Survivorship Curves of G. fasciatus in acute bioassays with Cu^{++}

All tanks were cooled in a flow-through water bath with temperature maintained at 11.8 to 12.1°C throughout acclimation and testing. Well water from the University of Michigan Lakeside Laboratory was used as dilution water and cooling water. All battery jars were fully aerated for 24 hr prior to testing to drive off CO_2. Mean values for dissolved oxygen were maintained at 12.0ppm at 11.8°C. (114% of saturation). Aeration was continued throughout the test procedure as Cu++ is a non-volatile material (STANDARD METHODS 1965). Dissolved oxygen, hardness, and pH were determined at the beginning, middle, and end of the 48 hr periods. Mean pH values were 7.75 throughout the test and hardness, expressed as mg/L $CaCo_3$, was 206. Lakeside laboratory water fits the definition of very hard water. Other characteristics of the well water are given in Table 1.

Table 1

Well Water Data*

	June	July
NO_3-N(ppm)	0.00	0.00
NH_4-N(ppb)	702	1700
PO_4(ppb)	218	204
Fe(ppb)	347	843
Cl(ppm)	1.01	1.53
Na(ppm)	2.8	2.6
K(ppm)	0.5	0.7
Mg(ppm)	13.0	13.0
Ca(ppm)	37.0	38.0

*From Dr. John Gannon (1976), University of Michigan Biological Station, Unpublished Data.

The organisms were selected randomly from a multi-aged stock group and time till death was recorded at 0.25 hr, 0.75 hr, 1.75 hr, 3.75 hr, 7.75 hr, 15.75 hr, 31.75 hr, and 48 hr. The number dead in each battery jar was recorded for the particular time and plotted on linear graph paper to determine death curves as percent survivors. These data were replotted on probit scale paper to give a straight line relationship and approximate LC50 values to use in the definitive tests. See Figure 1. Death was determined when the organism gave no response to a mechanical stimulus or showed no respiratory movement.

The LC50 value from the screening test was used as the middle concentration in the definitive test procedure. These values were:

Cu++ as $CuSo_4$--Control, 0.1, 0.18, 0.32, 0.56, 0.80, 1.0, 1.8ppm

Replicates were run simultaneously so that no variation occurred due to water chemistry, temperature fluctuation, or dissolved oxygen. The test times for death of the organisms were identical to those for the screening test, and the data were recorded similarly (See Figure 2).

The Litchfield and Wilcoxin Nomograph Analysis was used to determine the significance of lines drawn for best fit on a probit scale graph. The results of this analysis will be discussed later.

To ensure that no contamination occurred in any of the tanks, separate dip nets were used for the control groups and the Cu++ solutions. A stock solution of 100ppm Cu++ was used to formulate dilutions. All chemicals were weighed on a Mettler Balance ± 0.1mg to insure accuracy. Dead organisms were removed when visibly dead to insure no contamination due to metabolic waste products and oxidation.

RESULTS

Preliminary screening tests of G. fasciatus indicated good toxicity curves and response of the organism to Cu++. Preliminary values for Cu++ toxicity indicated a 48 hr LC50 values of 0.19ppm.

The definitive tests also gave good results. Mean Cu++ toxicity values were plotted for 2 consecutive runs of 48 hr each as percent survivors against time with good curves resulting. These values were converted to percent dead after 48 hr and plotted on 3 cycle probit paper to determine the definitive 48 hr LC50. The value of 0.21ppm Cu++ was calculated as the concentration that killed 50% of the test organisms. The line was analyzed using the Litchfield and Wilcoxin Nomograph Analysis method for good fit, and a X^2 value of 1.16 was obtained for the analysis with 1 degree of freedom. The table X^2 value was 3.41 so the line was not a bad fit. The Upper Limit of the LC50 value was 0.25ppm; and the Lower Limit was 0.17ppm. An analysis of the slope gave an F_{slope} value of 1.05, with an Upper Limit on the slope of 1.50 and a Lower Limit of 1.36. The toxicity of Cu++ at 0.21ppm, $\alpha = .05$, was therefore significant (see Figure 3).

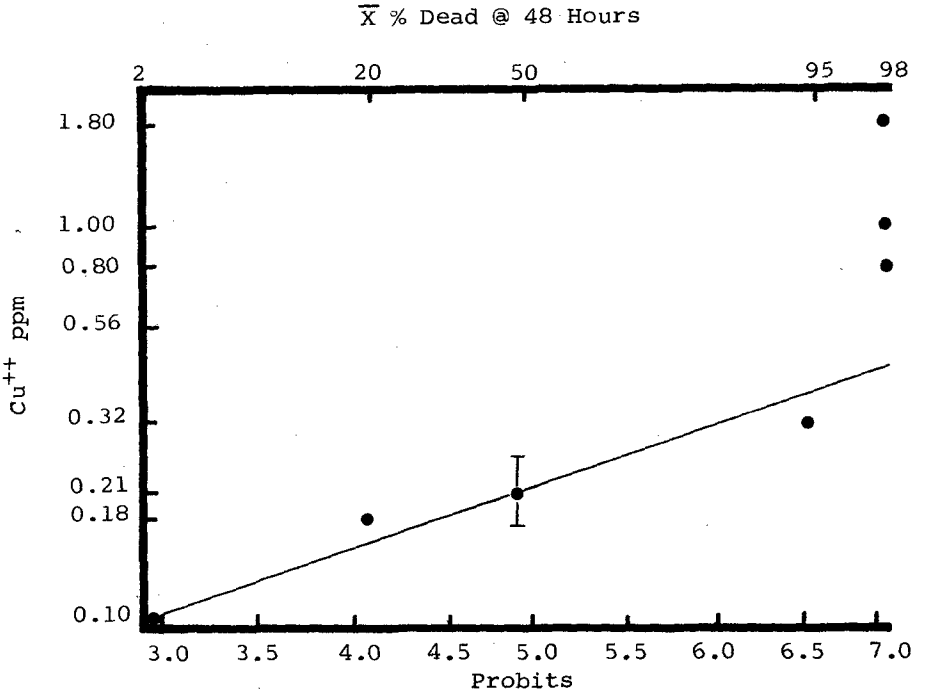

Fig. 3. Definitive LC50 Determination with 95% C.I.

DISCUSSION

The results of the 48 hr acute bioassay for Cu++ were significant. The response of G. fasciatus to Cu++ toxicity was reproducible. The results of the tests for Cu++ toxicity are also significant when compared with published values for Rainbow trout, Salmo gairdneri. HERBERT and VAN DYKE (1964) published data giving a 48 hr LC50 value of 0.20ppm Cu++ for Rainbow trout in hard water. GECKLER et al. (1976) reported similar values of 0.15-0.20ppm Cu++ (48 hr) in water with 125-175mg/L $CaCo_3$ and a pH of 7.7.

Since the results of the 48 hr acute bioassay for Cu++ on G. fasciatus are significant, $\alpha = .05$, it is reasonable to suggest that these organisms could be used as the experimental organism of importance in areas where they are found in abundance.

CONCLUSIONS

Gammarus fasciatus could, and possible should, be used as the experimental organism in conjunction with other organisms being used in laboratory bioassays.

It is more economical to keep in the laboratory than Rainbow trout, Salmo gairdneri, and since experimental values for Cu++ toxicity are identical, or nearly so, it would seem to be a good practice. Admittedly, more tests need to be run with this organism to determine if its response to toxic compounds, on the whole, are comparable with results for other test organisms. Also, it should be determined if life stages influence toxicity results. G. fasciatus should be included in any bioassay program where they are an important aspect of the aquatic ecosystem under consideration.

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