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Comparison of the Effect of Metallic Copper and Copper Nitrate ($Cu(NO_3)_2 \cdot 3H_2O$) on *Ceriodaphnia dubia* Utilizing the Three-Brood Test

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The purpose of this paper was to discover whether a pure metal, such as copper, evoked a different toxic response from Ceriodaphnia dubia than a salt of that metal. The US EPA Water Quality Criteria for Copper (US EPA 1986) indicates that acute toxicity to Cu is reduced with increases in hardness, alkalinity and total organic carbon. In 1980 (US EPA 1980) they noted that hardness-alkalinity effects were negligible on the chronic toxicity of Cu, and in 1986 (US EPA 1986) they state that there is no difference in chronic response to Cu between fishes and invertebrates. In contrast to this, some workers (Chakoumakos et al 1979) have shown that increases in hardness-alkalinity reduced acute Cu toxicity to Salmo clarki while others (Miller and MacKay 1980) have noted that alkalinity affected the acute toxicity to Salmo gairdneri only at the highest hardness tested. Winner (1985) showed that hardness had little effect on either acute or chronic Cu toxicity but that the presence of humic acid reduced both the acute and chronic toxicity of Cu in waters having a hardness that ranged from 38-230 mg $CaCO_3$/L. Thus, this study compares the acute and chronic effect of metallic Cu and $Cu(NO_3)_2 \cdot 3H_2O$ in reconstituted water using the 3-brood test with Ceriodaphnia dubia.

MATERIALS AND METHODS

The methodology employed in carrying out the 3-brood Ceriodaphnia test has been previously described (Cowgill et al 1988, Cowgill and Milazzo 1989). C. dubia was procured from the US EPA Laboratory at Duluth, Minnesota but the origin is unknown. It was identified by D. Berner of Temple University, Philadelphia, Pennsylvania.

The length of the Ceriodaphnia test is generated by the amount of time it takes the control animals to produce 3 broods. The test was renewed every other day. Ten neonates represented each concentration and 20 animals were used for controls. Animals were

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Table 1. Water quality characteristics of all test solutions and controls. All tests were carried out in reconstituted water.

Measurement		Result
pH:	range	8.2-8.6
:	mean	8.4±0.1
Dissolved oxygen, mg/L:	range	7.9-9.1
:	mean	8.6±0.3
Salinity, part/1000:	mean	<1±0
Residual Cl, ppb:	mean	<1±0
Conductivity µmhos/cm:	range	350-650
:	mean	514±77
Hardness as mg $CaCO_3$/L:	range	84-140
:	mean	111±15
Alkalinity as mg $CaCO_3$/L:	range	64-96
:	mean	85±8

fed daily and progeny were enumerated daily. The test is carried out at 25±2°C which is roughly 10 degrees higher than these organisms are normally found in nature.

Bioassays were conducted in reconstituted water prepared by utilizing triple glass distilled water to which reagent grade salts were added, following the procedures outlined in Standard Methods (American Public Health Association 1985). Since our C. dubia population is maintained in Lake Huron water, we prepared the reconstituted water to mimic the water quality characteristics of this Lake. The reconstituted water population of C. dubia has been maintained for 3 years with no drastic changes in population. The water quality characteristics of the reconstituted water are presented in Table 1.

Metallic Cu was dissolved in 3 mL of concentrated HNO_3 and made up to volume with triple glass distilled water. Test concentrations were set at 1014 ppb followed by a decreasing progression of test solutions each 60% lower than the previous amount. Copper nitrate was dissolved in triple glass distilled water, beginning with a concentration of 2817 ppb followed by 60% cuts. The test was renewed every other day with fresh solutions.

Probit analysis was used to calculate the daily LC50 and their 95% confidence intervals. NOEL (no observable effect levels) were defined as the highest concentration in which no deaths were noted. EC50 values were calculated based on "Total Progeny", "Mean Brood Number" and "Mean Brood Size". These were arrived at by standard regression analysis. The NOEL was based on Dunnett's t-test and Bartlett's test for variability.

Table 2. Demographic characteristics of the control broods of two tests carried out with Ceriodaphnia dubia.

Subject	Test 1	Test 2	Mean
Total progeny	439	408	424±22
Total progeny in 3-broods	375	340	385±25
Total progeny/female	23.1	24	23.6±0.6
Total progeny/female in 3-broods	7.08	6.80	6.9±0.2
Mean brood size	7.08	7.42	7.2±0.2
Time first brood, h	96	120	108±17
Length of 3-brood test, h	240	240	240±0
Number of broods produced by 20 animals	62	55	58.5±4.9
Mean size of first brood	4.5	4.2	4.4±0.2
Mean size of second brood	7.4	6.4	6.9±0.7
Mean size of third brood	9.8	10.0	9.9±0.1
Mean size of fourth brood	7.1	13.6	10.4±4.6

RESULTS AND DISCUSSION

Table 2 contains the demographic characteristics of the control broods of the two tests carried out with C. dubia. It may be noted that out of 20 animals only 9 in the first test and 5 in the second test produced a fourth brood. These tests were carried out at 26°C and, therefore, it is unusual to find animals producing more than four broods. The results depicted in Table 2 are quite typical of the control broods in our population.

Table 3 presents the daily LC50 (based on survival) data for both tests. The results were calculated as µg Cu/L so that they are comparable. It is clear that both forms of Cu have a chronic effect on C. dubia but that the Cu salt is more toxic than the metallic form throughout the test. In addition, it should be noted that the salt is 3.6 times more toxic on day

Table 3. Daily LC50 data (based on survival) for metallic Cu and $Cu(NO_3)_2 \cdot 3H_2O$ reported as metallic Cu.

	Day number µg Cu/L (metallic Cu)									
	1	2	3	4	5	6	7	8	9	10
LC50	649	406	327	302	302	249	249	219	192	192
95% C.I.	608-1014	219-608	219-608	219-608	219-608	219-608	219-608	79-608	79-219	79-219
NOEL	219	219	79	79	79	79	79	79	79	79

	Day number µg Cu/L ($Cu(NO_3)_2 \cdot 3H_2O$									
	1	2	3	4	5	6	7	8	9	10
LC50	444	286	208	138	129	112	97	92	66	53
95% C.I.	267-7411	59-4741	124-449	87-245	82-225	72-186	63-155	34-155	29-187	29-109
NOEL	2.7	2.7	2.7	2.7	2.7	2.7	2.7	0.34	0.34	0.34

Table 4. EC50 results for total progeny, mean number of broods and mean brood size for the 3-brood Ceriodaphnia test exposed to metallic Cu and $Cu(NO_3)_2 \cdot 3H_2O$ expressed as metallic Cu.

	Total progeny	Mean brood number	Mean brood size
	µg Cu/L (metallic Cu)		
EC50	357	348	326
95% C.I.	0-1111	0-1032	0-878
NOEL	79	219	79
	µg Cu/L ($Cu(NO_3)_2 \cdot 3H_2O$)		
EC50	305	341	304
95% C.I.	0-707	0-871	0-805
NOEL	96	160	96

10 than is the metallic Cu, presumably due to the increased availability of the salt to the test cladoceran. The NOEL for the salt is 81 times lower than that for the metallic Cu at the beginning of the test and is 232 times lower at the end. According to Winner (1985) the accumulation of Cu by daphnids is affected by the age of the animal, water hardness and humic acid concentration. Apparently, more Cu is accumulated by animals living in soft water (<58 mg $CaCO_3$/L) than in medium or hard water (Winner, 1985). Older animals (<7 days) exhibit the same bioaccumulation of Cu in relation to water hardness as do neonates. The form of Cu used in Winner's (1985) study was $CuSO_4 \cdot 5H_2O$.

Table 4 shows the effect of the two forms of Cu on the total progeny, mean brood number and mean brood size in the 3-brood Ceriodaphnia test. It is clear that both forms of Cu have a less dramatic effect on the measures of reproductive success than on survival. The EC50 values are essentially the same for both forms of Cu. In the case of the salt, the NOEL values are considerably greater when reproductive variables are examined than for survival.

Thus, to summarize our findings, both forms of Cu studied are highly toxic (<1 mg/L) but the salt is more toxic than the metal (LC50 based on survival), presumably due to the cladoceran's increased ability to accumulate this form. Both forms of copper evoke both acute and chronic response from C. dubia. The EC50 values calculated from the various reproductive variables obtained from the 3-brood Ceriodaphnia test indicate by comparison that survival is by far the most sensitive endpoint and that these variables exhibit essentially the same order of magnitude response (<360 µg/L) to the two forms of Cu studied.

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