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Acute and Chronic Toxicity of Copper to the Estuarine Copepod *Eurytemora affinis*

Final Report

Chesapeake Bay Program

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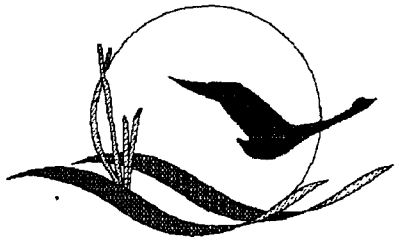

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Acute and Chronic Toxicity of Copper to the Estuarine Copepod *Eurytemora Affinis*

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ABSTRACT

The objectives of this study were to conduct acute (48 and 96h) and chronic (8d) copper toxicity tests with the estuarine zooplankter, *Eurytemora affinis* and develop an acute to chronic ratio (ACR) for this species. Total dissolved copper, copper speciation and organic complexation were measured on selected samples during these toxicity tests. Determination of organic complexation was critical to determine the bioavailability of copper to the test species. Concentrations of total dissolved copper at selected test conditions displayed a loss of 20 to 35% over the course of the acute and chronic experiments with the most significant loss occurring during the first 48 hours. Due to the reported loss of copper during exposures, a decay model was developed to calculate the concentrations that were used to determine the final toxicity values (adjusted copper concentrations). The 48h LC50, 96h LC50 and 8d chronic values were 83.0, 69.4 and 64 μg/L dissolved copper, respectively, using the adjusted copper concentrations. An acute to chronic ratio of 1.3 was calculated using the 48 h and 8 d toxicity values. Voltammetric analysis of selected samples indicated greater than 99% complexation of copper in all samples with complexing capacity increasing with both time and copper concentration. The inorganic copper concentration was therefore a very small fraction of the total copper added during these experiments. Inorganic copper (II) speciation predicted by a model identified seven significant species of copper. Of these seven species, $CuCO_3$ was the dominant species accounting for approximately 78% of the total copper. The free cupric ion (Cu^{2+}) accounted for only 8% of the total dissolved copper.

ACKNOWLEDGMENTS

We would like to acknowledge the U.S. Environmental Protection Agency's Chesapeake Bay Program Office for funding this study through grant number CB993438010. Special consideration is extended to the following programs for their contributions to the study design and support: Maryland Department of the Environment, Chesapeake Bay Program Office, and the Toxics of Concern Workgroup of the Chesapeake Bay Program's Toxics Subcommittee.

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INTRODUCTION

Copper occurs in natural waters primarily as the divalent cupric ion in free and complexed forms (Callahan et al., 1979). This trace metal is a minor nutrient for both plants and animals at low concentrations but is toxic to aquatic biota at concentrations only slightly higher. Copper has been identified as a "Toxic of Concern" in the Chesapeake Bay watershed and is therefore given priority for water quality criteria development in the 1988 Chesapeake Bay Basin wide Toxics Reduction Strategy (U. S. EPA, 1991). National water quality criteria for protection of marine life from copper were developed by the U. S. Environmental Protection Agency (EPA) in 1980 and revised in 1984 (U. S. EPA, 1980; U. S. EPA, 1984). The existing EPA acute criterion for protection of marine life is 2.9 μg/L. There is no saltwater EPA chronic criterion for copper. Available surface water data for copper in the Chesapeake Bay suggests that background concentrations of 0.4 to 2.0 μg/L total copper are at or near the EPA criterion (Maryland Department of the Environment, 1991).

The U. S. Environmental Protection Agency (1984) acknowledged the similarity between background concentrations of copper and the marine criterion. The EPA further recognized that national water quality criteria may be under protective or over protective at specific sites due to differences in sensitivities between test species at a particular site and those species used for deriving the national criteria. Due to these issues, Maryland Department of the Environment (MDE) established an estuarine acute water quality criterion of 6.1 μg/L using toxicity data for estuarine species resident in Chesapeake Bay that were tested at salinities ranging from 1 to 35 ppt (Maryland Department of the Environment, 1991). Insufficient copper toxicity data exists for MDE to derive an estuarine chronic criterion for Maryland waters of Chesapeake Bay. One requirement of EPA's "Guidelines for Deriving Numeral Water Quality Criteria for the Protection of Aquatic Organisms

and their Uses" for development of chronic criteria for saltwater species is a minimum of three acute to chronic ratios (Stephan et al., 1985). No such data are currently available that meet all EPA criteria.

The primary objective of this study was to generate data that can be used by MDE along with other toxicity data to develop a chronic copper criterion for the protection of aquatic life in estuarine waters of the Chesapeake Bay and tributaries. *Eurytemora affinis* was selected as a test species because it is one of the most dominant zooplankton species in the Chesapeake Bay, it has high ecological importance and it is an essential component of trophic structure in the Bay (Ziegenfuss and Hall, 1994). Acute (48 and 96 h) and chronic (8 d) copper toxicity tests were conducted with the *Eurytemora affinis*. An acute to chronic ratio (ACR) was also developed for this zooplankton species. Total dissolved copper, copper speciation, and organic complexation were measured on selected samples measured during toxicity experiments. Organic complexation measurements were particularly critical to determine the bioavailability of copper to the test species.

METHODS

Culture Procedures

Eurytemora affinis cultures were maintained at 14-16 ppt salinity and 20-25 °C in our laboratory. Culture water was autoclaved, filtered (1 μm) estuarine water (14-17 ppt salinity) obtained from the Choptank River at the University of Maryland, Horn Point Laboratory (Cambridge, MD). Salinity was adjusted with H-W Marinemix or deionized water. The copepod cultures were fed equal volumes of two phytoplankton species, *Thalassiosira weissflogii* and *Isochrysis galbana*, each maintained in log-phase growth. Phytoplankton were cultured in autoclaved, filtered, and salinity adjusted Choptank River water supplemented with F/2 media (Gillard, 1975).

Test Procedures

Test conditions used for these experiments are summarized in Table 1 and methods for *Eurytemora* toxicity tests are described in detail in Ziegenfuss and Hall (1994). Copper chloride (copper (II) chloride dihydrate) used in these experiments was obtained from J.T. Baker Inc. (Phillipsburg, NJ, lot number J06342). Autoclaved, filtered and salinity adjusted (15 ppt) Choptank River water was used as the diluent and control water for the acute and chronic toxicity tests. The acute toxicity tests were 48 and 96 h static non-renewal exposures. The 8-d static-renewal chronic test had a fifty percent renewal at each treatment on day 4. All test conditions were held in a biological incubator to maintain a constant temperature of 25 C and a photoperiod of 16 h light:8h dark. Standard water quality parameters (temperature, salinity, pH and dissolved oxygen) were recorded initially and at the end of the exposure period for each test condition. Selected test

Table 1. Test conditions used for *Eurytemora affinis* copper toxicity tests.

1. Temperature:	25 ± 2°C
2. Lighting:	100-150 fc
3. Photoperiod:	16 L: 8h D
4. Size of Test Vessel:	150 mL beaker
5. Volume of Test Solution:	100 mL
6. Age of Test Copepods:	≈ 24 h
7. No. of Copepods per Test Vessel:	12-16
8. No. of Concentrations:	6 - 7 (including controls)
9. No. of Replicates per Concentration:	4
10. Feeding Regime:	Daily algal mixture 10^4 cells/ml for *I. galbana* 10^3 cells/ml for *T. weissflogii*
11. Aeration:	None, unless DO concentration falls below 40% saturation
12. Dilution Water:	Autoclaved filtered natural estuarine water (salinity adjusted)
13. Test Duration:	Acute test - 48 and 96 h Chronic test - 8 d
14. Effect Measured:	Mortality (acute tests), mortality, fecundity and maturation (chronic test)

treatments were sampled initially and on the final day of each test and analyzed for total dissolved copper and copper complexation to organic constituents.

The following nominal copper concentrations were used for the 48 h tests: 0, 50, 88, 158, 280 and 500 μg/L. For the 96-h acute toxicity experiment, the following nominal copper concentrations were used: 0, 16, 28, 50, 90, 160 and 284 μg/L. Nominal copper concentrations used for the 8-d chronic test were: 0, 16, 26, 40, 64, 100 and 160 μg/L. Each test concentration was prepared by diluting a working stock solution (100 mg/L) of copper chloride with salinity adjusted Choptank River water. Stock solutions were prepared by dissolving 2.6828 g copper chloride dihydrate into 1 L deionized water for the primary stock solution (1g/L copper) and diluting 50 ml of the primary stock solution with 450 ml deionized water to make the working stock solution.

Acute and chronic toxicity tests were initiated with initial introductions of *Eurytemora* nauplii (~ 24-h old). Nauplii were obtained by isolating adult copepods (202 μm sieve) in polycarbonate jars containing salinity adjusted estuarine water for 24-h and harvesting the recently hatched neonates using a 53 μm sieve. The adult copepod isolation chambers were supplied with equal volumes of *Thalassiosira weissflogii* and *Isochrysis galbana* algae at a ratio of 40 ml algae mix/L of culture water. Four replicate 150 ml glass beakers containing 100 ml of test solution were used for each condition. A test chamber was suspended within each beaker to contain the organisms. Chambers were constructed from 3.8 cm diameter rigid polycarbonate tubing cut to a length of 5.7 cm and suspended to provide an interior volume of approximately 40 ml. The bottom of each chamber was covered with 53 μm mesh Nitex screen. Nauplii were counted by drawing small aliquots of nauplii and water into a wide-bore glass pipet and examining under a dissecting microscope (7.5 x magnification). The initial number of copepods (12-16) placed into each chamber

was recorded on data sheets. *Eurytemora* were fed daily with 1.0 ml of a two-species phytoplankton mixture comprised of equal volumes of *Isochrysis galbana* and *Thalassiosira weissflogii*. Algal counts were made with a hemacytometer at least once during each acute test and twice during the chronic test. The mean algal densities per test chamber were 3.6×10^4 *Isochrysis* cells/ml and 3.4×10^3 *Thalassiosira* cells /ml. Survival was evaluated in each condition after 48-h and 96-h for the acute tests and after an 8-d exposure period for the chronic test. Copepods were counted in the acute experiments by first lowering the volume of solution in the test chamber, then removing the remaining copepods and water in small aliquots with a pipet. Each aliquot within the pipet was examined with the aid of a dissecting microscope for the presence of live copepods. The same methods were used to count copepods in the chronic test except after they were counted the copepods were deposited into individually marked (one vial per treatment replicate) 20 ml vials with 5 ml of diluent. After each replicate sample had been counted and deposited into the vials, 2 ml of 10% buffered formalin was added to each vial for storage of test species.

Preserved copepods were examined under a dissecting microscope and categorized as: gravid female; non-gravid female; male and immature (See Ziegenfuss and Hall, 1994). The proportion of gravid females (gravid/total females = fecundity) and proportion of copepods reaching maturity (maturation) were the reproductive endpoints used.

General Procedures for Copper Analysis

Total dissolved copper and copper complexation measurements were conducted by the College of Marine Studies, University of Delaware (Lewes, DE). Samples for analyses were filtered (0.4 μm polycarbonate membrane) into precleaned (acid rinsed) polyethylene containers on the

initial and final day of each experiment. Total dissolved copper samples were preserved with 0.2 ml Ultrex II nitric acid (J.T. Baker Inc., Phillipsburg, NJ) per 100 ml of sample. Samples for complexation measurements were collected in precleaned polyethylene bottles and immediately frozen. All samples were transported from the Wye Research and Education Center to the analytical lab at the University of Delaware. The following test conditions from the 48 h acute test were measured for dissolved copper at the initiation of the test and 48 h later : 0, 50, 158, and 500 μg/L. The following test conditions were measured for dissolved copper at test initiation and termination from the 96 h acute test: 0, 50, and 160 μg/L. During the 8d chronic tests, dissolved copper was measured at test initiation and termination at the following conditions: 0, 16, 40, and 160 μg/L.

Total Dissolved Copper Analyses

Total dissolved copper concentrations were measured by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). Concentrations were determined by comparison of sample emission values with a linear calibration curve. Working standards were prepared by serial dilution of a 1000 ppm commercial ICP copper standard (Inorganic Ventures, Inc., Toms River, NJ). Standards were prepared in an organic-free (ultraviolet-irradiated) natural seawater matrix, diluted to 15 ppt salinity and acidified to 0.1 N with high purity quartz-distilled nitric acid. The matrix of the standards was equivalent to that of the samples, correcting for any signal suppression due to seasalts in the samples. Analytical sensitivity was 0.0957 emission units/(μg/L-Cu), with a detection limit of approximately 3 μg/L and precision of 2-4%.

Organic Complexation

The "copper complexation capacity" and the conditional stability constant for copper binding (Log K'_{CuL}) were determined for selected samples by anodic stripping voltammetry, using an EG&G

Princeton Applied Research (PAR) Model 384B-4 polarographic analyzer with a PAR Model 303A mercury drop electrode. Analyses were performed in the square wave anodic stripping voltammetry (SWASV) mode with a hanging mercury drop electrode (HMDE). Copper was reduced and deposited in the mercury drop for one to three minutes at a deposition potential of -0.30 V. Instrumental parameters for reoxidation and stripping of the copper from the mercury drop were: scan rate = 200 mV sec^{-1}, pulse amplitude = 20 mV, pulse frequency = 100 Hz, scan range = -0.3 to -0.05 V. Copper complexation capacity (CuCC) is defined as the total concentration of all metal-binding sites or ligands in solution (e.g. Robinson and Brown, 1991). The CuCC values and the conditional stability constants (K'_{CuL}) were determined by complexometric titration with a Ruzic-type linearization, after the manner of Coale and Bruland (1988, 1990).

The complexometric titration method included the titration of excess binding ligand in solution with copper. When the measurements were obtained by ASV, it was assumed that the current measured at a fixed deposition potential was due solely to the free metal ion and/or weak inorganic complexes. Metal-ligand organic complexes were assumed to be electroactively inert at that same deposition potential. The formation of a complex is described by the reaction:

$$M' + L' = ML \qquad (1)$$

where M' is the sum of the dissolved inorganic metal species and L' is the excess "free" binding ligand. The equilibrium expression for the formation of the complex was:

$$K'_{ML} = [ML]/[M'][L'] \qquad (2)$$

where K'_{ML} is the conditional stability constant of the metal-organic complex. For a sample containing a moderate to strong complexing ligand, the data from a titration was linearly transformed

to allow calculation of the total concentration of the ligand, [L'], and the conditional stability complex for the metal-ligand complex (Ruzic, 1982).

Coale and Bruland (1988, 1990) have described the application of the linearization technique to the determination of copper complexation in seawater. For the formation of a single strong zinc-ligand species, the linearization equation is:

$$[Cu']/[CuL] = [Cu']/[L] + 1/(K'_{CuL} \times [L]) \quad (3)$$

A plot of $[Cu']/[CuL]$ versus $[Cu']$ for each titration point will yield a straight line with a slope of $[L']^{-1}$ ($\cong$ 1/CuCC)and intercept of $(K'_{CuL} \times [L])^{-1}$.

Assuming that there are no electroactive organic copper complexes in solution, the measured current was directly proportional to the concentration of Cu', where [Cu'] was defined as the total concentration of electroactive dissolved inorganic copper species present (i.e. the Cu^{2+} ion plus inorganic complexes). [Cu'] was calculated from equation 2, and was related to the concentration of the free ion, $[Cu^{2+}]$, by an inorganic side reaction coefficent, $\alpha_{Cu'}$, such that $[Cu'] = [Cu^{2+}]\alpha_{Cu'}$ (Ringbom and Still, 1972). The organically bound fraction, CuL, was the difference between the total dissolved Cu (determined by ICP-AES) and [Cu']. In the absence of total inorganic carbon or alkalinity measurements, the samples were assumed to be in equilibrium with the atmosphere with respect to the carbonate system ($p_{CO_2} = 10^{-3.5}$ atm, pH = 8.2, t = 25°C., Ionic strength = 0.3). Under these conditions, $\alpha_{Cu'} \cong 13.1$.

Titrations were conducted using an acidified 1000 μg/L Cu^{2+} standard, which reduced the final pH of the samples to $\cong$ 2. Acidification prevented loss of added copper by precipitation or adsorption. The lower pH, however, resulted in an underestimation of the CuCC and an

overestimate of the Log K'_{CuL} relative to the values at the *in situ* pH (e.g. Kozarac et al., 1989; Skrabal et al. 1992). To account for this offset, two samples were titrated with an unacidified Cu^{2+} standard at the sample pH of about 8.2. The ratio of the 8.2 CuCC to that at pH 2 was approximately 1.71 ± 0.4. This value was similar to the ratio observed by Skrabal et al., 1992 for samples collected in Indian River Bay, DE (CuCC ratio ≅ 1.86). The Log K'_{CuL} value at pH 8.2 was approximately 7.67 ± 0.38. Then the concentration of Cu′ was:

$$[Cu'] = [CuL]/(K'_{CuL}[L]) \cong [Cu]_{TOTAL}/(K'_{CuL}\,[CuCC]) \qquad (4)$$

Complexation was further verified by analysis of copper by square wave voltammetry (SWV) before and after UV-irradiation to oxidize organic matter and by measurement of the copper "pseudopolarogram". A pseudopolarogram is plot of ASV stripping peak current versus deposition potential. The resulting curve is equivalent to a DC polarogram. For a given metal, a pseudopolarogram will display one or more relatively sigmoid-shaped polarographic waves, corresponding to the free ionic and complexed species. The position and shape of each wave is dependent upon the binding strength of the metal-ligand complex and upon the reversibility of the chemical and electron transfer processes at the electrode (Lewis et al., 1995).

Equilibrium Modeling

The solution speciation of copper with respect to free hydrated ions and inorganic complexes in the sample solutions was estimated using the program EASEQL, an interactive PC version of the original MINEQL equilibrium modeling program. MINEQL (Westall et al., 1976), and its predecessor REDEQL (Morel and Morgan, 1972), utilizes equilibrium constants to solve mass balance expressions, using a modified Newton-Raphson iterative procedure. The model was run

assuming pH = 8.2, T = 25°C, I = 0.3, and an open system in equilibrium with the atmosphere (p_{CO2} = $10^{-3.5}$ atm.

Statistical Analysis

The 48 and 96-h LC50 values with 95% confidence limits (acute tests) were generated from the mortality data using the Trimmed Spearman-Karber method. Statistical analysis for the chronic experiment was conducted by using ANOVA procedures with subsequent means testing (Dunnett's test) with copper concentrations (adjusted with decay rate (s)) to determine the No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) values. Endpoints used were mortality, fecundity and maturation. The chronic value was determined by calculating the geometric mean of the NOEC and LOEC for the most sensitive endpoint.

Due to the loss of copper during the experiments an exponential decay model was used to determine the acute and chronic copper concentrations used for analysis. The model used was as follows:

$Cu(t) = Cu(0) \exp(rt)$

where

Cu(t) is copper concentration at time t
Cu(0) is initial copper concentration
exp is the exponential function
r is the rate of copper absorption
t is time

For these experiments integrating Cu(t) over the exposure time and dividing by the exposure time yielded an estimate of the average exposure concentration for the period. Eight observations were available to estimate copper absorption (r) in these experiments. Data from the different test durations were analyzed by a GLM procedure from SAS to determine if test duration influenced

decay rates.

RESULTS

Water Quality

Ranges of water quality conditions measured at test initiation and test termination for the 48 h, 96 h and 8d tests are presented in Table 2. Water quality conditions for the 8-d chronic test were also measured on day 4 in addition to the initial and final day of the experiment. All water quality conditions were adequate for survival of test species.

Total Dissolved Copper Analyses

The results of the total dissolved copper analyses are presented in Table 3. Measured values for the control samples were all below the detection limit of 3 μg/L. The experimental samples collected on Day 0 of each test generally displayed good agreement (less than 15% deviation) between nominal and measured values. All experimental samples displayed a mean loss of approximately 31% dissolved total copper over the course of the experiment. Most of the copper loss was reported during the first 48 hours.

Due to the reported loss of copper during the acute and chronic experiments a decay model (see methods section) was used to determine the concentrations used for statistical analysis of the toxicity data. The following eight observations were used to estimate the rate of copper loss.

Table 2. Ranges in water quality conditions during acute and chronic copper toxicity tests with *Eurytemora affinis*.

Test Type	Temperature (°C)	Salinity (ppt)	pH	D.O. (mg/L)
Acute 48-h	24.1 - 24.6	14	7.9 - 8.7	7.5 - 8.8
Acute 96-h	24.0 - 25.1	15 - 16	7.9 - 8.8	7.4 - 9.8
Chronic 8-d	24.1 - 25.1	14 - 16	7.9 - 8.8	7.2 - 8.9

Table 3. Total dissolved nominal and measured copper concentrations (μg/L) during the 48 and 96 h acute test and the 8 d chronic test. Concentrations were measured in selected conditions during test initiation and termination. Detection limit was 3 μg/L.

Test	Nominal Conc. D = 0	Measured Conc. D= 0	Measured Conc. Test Termination	% Loss
48 h	0	< 3	< 3	0
	50	50.4	39.4	21.8
	158	155.7	117.3	24.6
	500	436	351	19.4
96 h	0	< 3	< 3	0
	50	54.6	36.6	32.9
	160	165	114.5	30.6
8 d	0	< 3	< 3	0
	16	18.4	13	29.3
	40	43.1	20.4	52.6
	160	161.9	103.8	35.8

Obs	Test	T	Nom	Cu0	CuT	P	L
1	48h	48	50	50.4	39.4	0.78175	0.21825
2	48h	48	150	155.7	117.3	0.75337	0.24663
3	48h	48	500	436.0	351.0	0.80505	0.19595
4	96h	96	50	54.6	36.6	0.67033	0.32967
5	96h	96	160	165.0	114.5	0.69394	0.30606
6	8d	96	16	18.4	13.0	0.70652	0.293448
7	8d	96	40	43.1	20.4	0.47332	0.52668
8	8d	96	160	161.9	103.8	0.64114	0.35886

Two different exposure times were represented: 48 hours for the 48 hour test and 96 hours for both 96 hour and 8 day tests. Copper concentrations were renewed at day 4 during the 8-d test. These data were first analyzed to determine if test duration influenced loss of copper. The results were as follows:

Source	DF	Type III SS	Mean Square	F value	Pr>F
T	1	0.8962	0.8962	49.71	0.0009
T*Test	2	0.0213	0.0106	0.59	0.5879

These results showed that there was no significant interaction of decay rate and exposure duration (p=0.58). Therefore all eight observations were used to estimate a single decay rate of -0.0049 as shown below.

Parameter	Estimate	T for HO	Pr > T	Std Error Est.
T	-0.0048521	-8.85	0.0001	0.000548

The average exposure concentration for each nominal concentration (NOMADJ) was calculated using a decay rate of 0.0048522 as presented in Table 4. These adjusted concentrations were used to calculate the toxicity values presented below in the Toxicity Data section.

Voltammetric Analyses

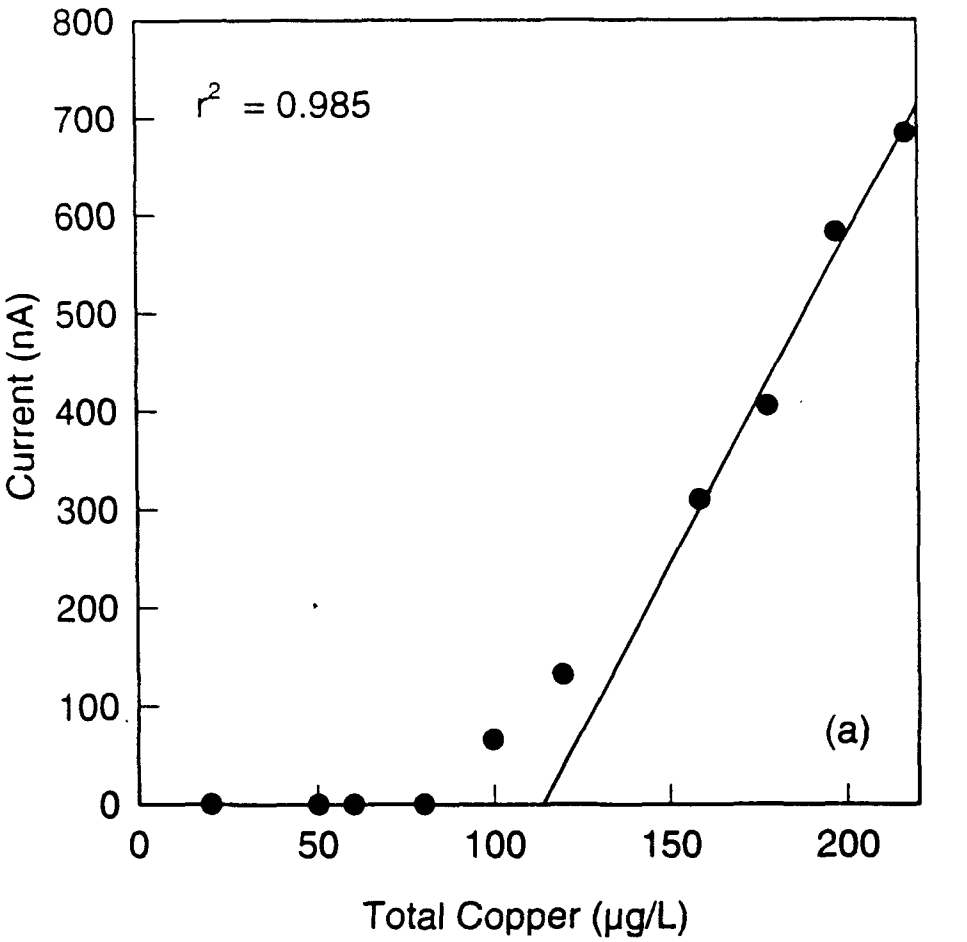
Ten samples from the chronic test, including the stock water and two controls, were selected for speciation analysis. A typical complexometric titration curve and the corresponding linear transformation plot are shown in Figure 1. The results are summarized in Table 5. In each instance, the estimated copper complexing capacity at pH 8.2 exceeded the total copper concentration, with≥ 99% complexation in all instances. The inorganic copper concentration ([Cu′]) was therefore a very small fraction of the total copper added, ranging from about 0.15 to 1.2 μg/L for the experiment. The corresponding cupric ion concentrations would be approximately a factor of 13 lower, from about 0.01 to 0.09 μg/L. Assuming a total copper concentration of 1 μg/L, the Cu′ concentration for the controls would be <0.06 μg/L Cu^{2+}

The complexation of copper by organic compounds was verified by analysis of selected samples before and after UV-irradiation (Figure 2) and by the construction of a copper pseudopolarogram (Figure 3). The 160 μg/L sample from the chronic test was analyzed by Square Wave Voltammetry without stripping. Figures 2a and 2b display the resulting current vs. potential scans for the day 0 and the day 8 samples, respectively, while 2c shows the scan for the day 8 sample following UV-irradiation. On day 0 of the chronic test, the sample displayed peaks at -0.16 V for labile copper (Cu′) and at -1.35 V for a strong Cu(II)-organic complex. For day 8 of the test, scan

Table 4. Nominal adjusted copper concentrations (NOMADJ) for various test conditions using a decay rate of R of -0.0048522. These values were used for analysis of toxicity data.

OBS	Test	Duration (h)	Nominal	R	NOMADJ
1	48h	48	50	-.0048522	44.604
2	48h	48	88	-.0048522	78.504
3	48h	48	150	-.0048522	133.813
4	48h	48	280	-.0048522	249.784
5	48h	48	500	-.0048522	446.043
6	96h	96	16	-.0048522	12.791
7	96h	96	28	-.0048522	22.384
8	96h	96	50	-.0048522	39.971
9	96h	96	90	-.0048522	71.947
10	96h	96	160	-.0048522	127.906
11	96h	96	284	-.0048522	227.033
12	8d	96	16	-.0048522	12.791
13	8d	96	26	-.0048522	20.785
14	8d	96	40	-.0048522	31.976
15	8d	96	64	-.0048522	51.162
16	8d	96	100	-.0048522	79.941
17	8d	96	160	-.0048522	127.906

Figure 1. (a).SWASV complexometric titration for copper in 15 ppt estuarine water at pH 8.2. (Chronic 40 μg/L at day 8). The solid line is the linear regression taken through the last four data points. (b). Linear transformation of the data (Ruzic, 1982). Solid and dotted lines represent the linear regression through the data and the 95% confidence interval, respectively.



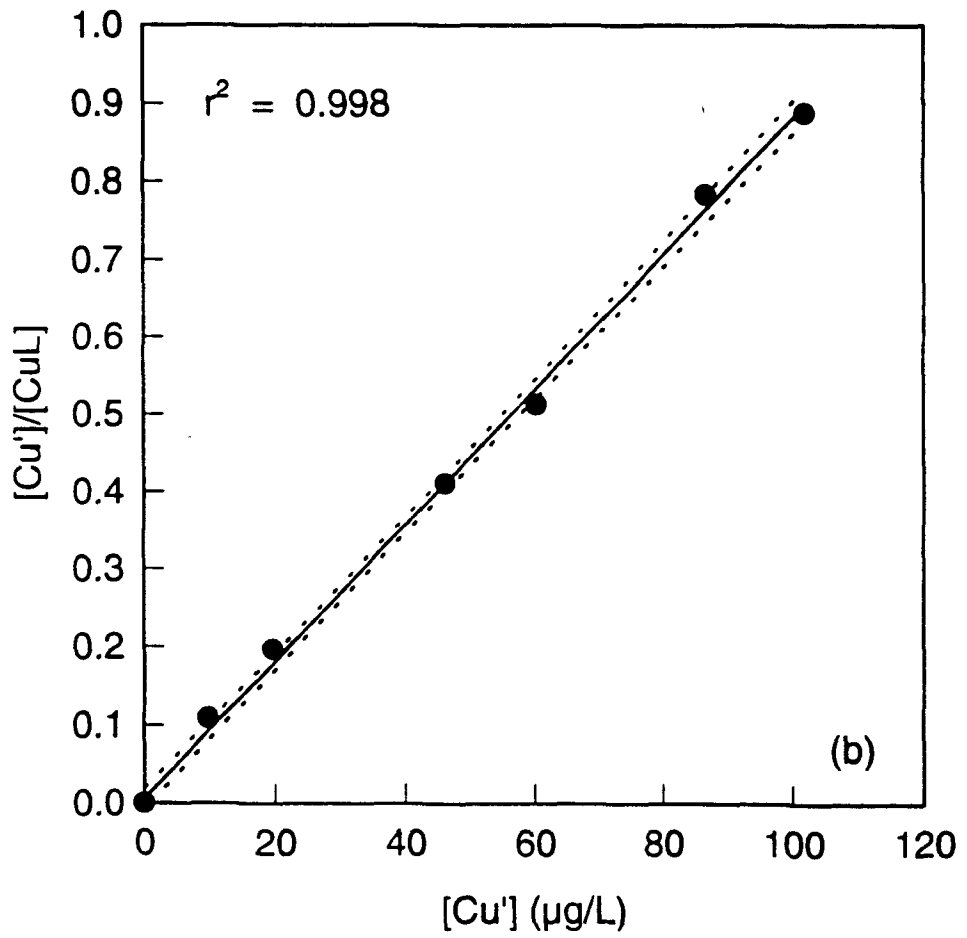


Table 5. Measurements of copper complexation capacity (CuCC) and estimated free cupric ion concentrations for test conditions during chronic tests.

Sample	Day of Test	Total [Cu] (μg/L)	CuCC pH 8.2 (μg/L) (meas.)	CuCC pH 8.2 (μg/L) (meas.)	CuCC pH 8.2 (μg/L) (est.)#	Log K (cond.) pH 8.2	[Cu^{1}] (μg/L)	[Cu2+] (μg/L)
Control, stock water	-	<D.L.*	14.4		24.6		***	***
Conrtol	0	<D.L.	16.4	33.7	28.0	7.73 ± 0.16	***	***
Control	8	<D.L.	24.4 ± 9.1		41.7 ± 16		***	***
16 μg/L	0	18.4	44.1 ± 1.1		75.4 ± 1.9		3.31E-01	2.52E-02
16 μg/L	4	12.8	53.7		91.8		1.89E-01	1.44E-02
16 μg/L	8	13.0	69.2 ± 1.3		118 ± 2.2		1.50E-01	1.14E-02
40 μg/L	0	43.1	67.4 ± 3.8		115 ± 6.5		5.09E-01	3.87E-02
40 μg/L	8	20.4	83.8	116	143	7.61 ± 0.35	1.94E-01	1.47E-02
160 μg/L	0	162	113 ± 11		193 ± 18		1.14E+00	8.66E-02
160 μg/L	8	104	131 ± 6.1		224 ± 10.4		6.29E-01	4.78E-02

* D.L. = detection limit of ICP-AES Cu analysis; approx. 3μg/L.

Based upon ratio of measured values at pH 8.2 vs. pH 2: values at pH 2 are multiplied by a factor of 1.71.

Figure 2. SW voltammograms of copper: (a) chronic test condition of 160 μg/L at day = 0, (b, c) 160 μg/L chronic test condition on day = 8 before and after UV - irradiation.

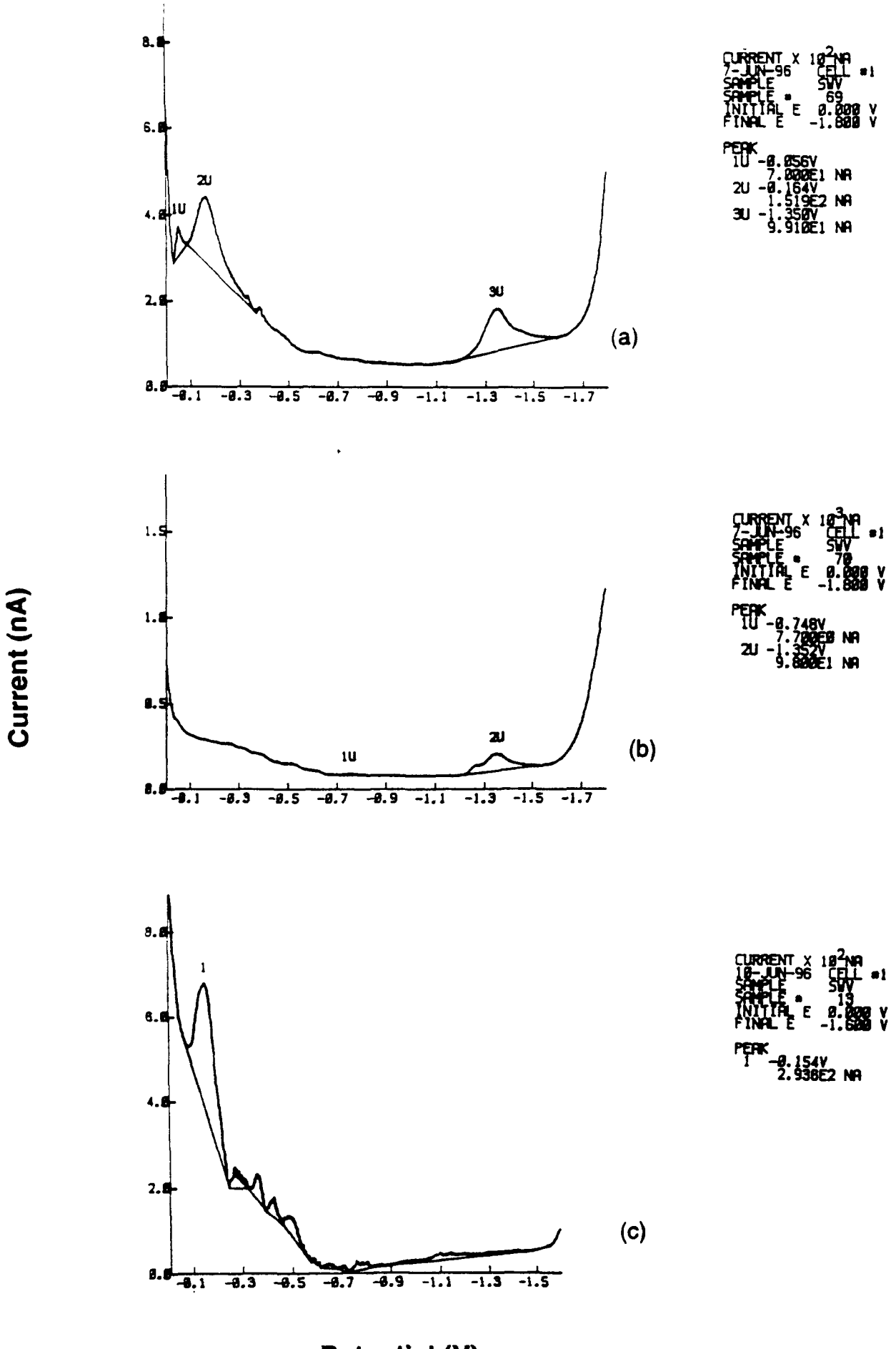
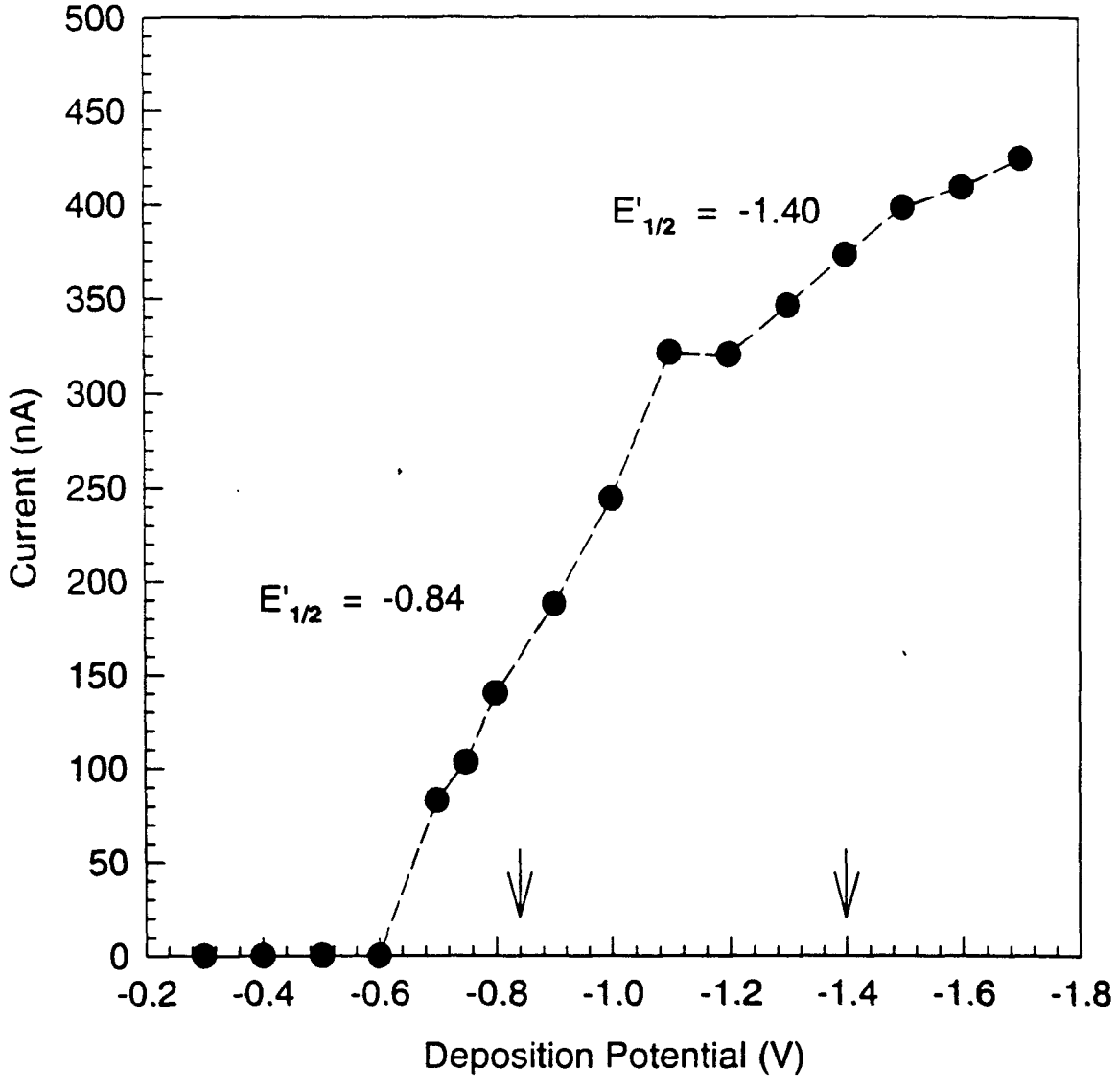


Figure 3. SWASV pseudopolarogram for copper in 15 ppt estuarine water at pH 8.2 (120 sec deposition) at chronic test condition 40 μg/L on day 8.



displayed only the latter peak, indicating that essentially all of the copper was now strongly complexed. The complex peak did not increase in size, therefore, suggesting that additional complexes were formed, but were apparently either electrochemically inert or below the detection limit for SWV without stripping. Upon UV-irradiation, the complex peak disappeared and the signal for labile copper reappeared, indicating breakdown of organic copper complexes. The titration plot for the UV-irradiated aliquot (not shown) still exhibited some degree of nonlinearity, indicating the presence of residual complexing material in the sample. A pseudopolarogram (Figure 3) performed on the 40 μg/L sample on day 8 of the chronic test indicated the presence of at least two classes of copper chelating ligands, with $E'_{1/2}$ values of -0.84 and -1.40 V. The latter agrees well with the complex peak observed in the 160 μg/L samples from Figures 2a and 2b.

Equilibrium Modeling

The inorganic copper(II) speciation as predicted by EASEQL is given in Table 6. Seven species were identified as significant (>0.1%); the free hydrated ion, Cu^{2+}, carbonate and sulfate complexes, $CuCO_3$, $Cu(CO_3)_2^{2-}$ and $CuSO_4$, two chloride complexes, $CuCl^+$ and $CuCl_2$, and the hydroxide species $Cu(OH)^-$. Of these seven, the speciation is dominated by the $CuCO_3$ species, which accounts for approximately 78% of the total copper. The free cupric ion, Cu^{2+}, accounts for only about 8% of the total dissolved copper.

Toxicity Data

Toxicity values were determined using the adjusted copper concentrations presented in Table 4. The 48h LC50, 96 h LC50 and 8 day chronic value of 83, 69.4 and 64.0 μg/L, respectively are

Table 6. The inorganic speciation of Cu(II) in estuarine water as predicted using the EASEQL thermodynamic equilibrium program (pH = 8.2, T = 25C, I = 0.3, p_{co2} = $10^{-3.5}$ atm, [Cu'] < 1.2 ppb).

Copper Species	Percentage of Total Cu'
Cu^{2+}	7.6
$CuC0_3$	78.2
$Cu(C0_3)_2^{2-}$	3.8
$CuS0_4$	0.6
$CuCl^+$	1.9
$CuCl_2$	0.1
$CuOH^-$	7.7

reported in Table 7 (see Appendix A for raw data). The chronic test endpoints of percent survival, percent gravid females and percent immatures all resulted in adjusted copper NOEC values of 51.1 μg/L and LOEC values of 79.9 μg/L. The 8-d chronic value of 64 μg/L in conjunction with the 48-h acute value (83 μg/L) were used to calculate an acute to chronic ratio of 1.3. Also shown in Table 7 are the control group survival values for all three tests. These values, ranging from 85.7 to 94.1%, are typical for *Eurytemora* control survival based on our previous studies (Ziegenfuss and Hall, 1994).

Table 7. Forty-eight and ninety-six hour LC50 values (μg/L) and 95% confidence limits for acute copper toxicity tests with the estuarine copepod *Eurytemora affinis*. The chronic value in μg/L (no confidence limits) for the 8-d chronic test is also given. Mean % control survival for each test duration is included.

Test Type	Mean % Control Survival (S.E.)	LC50/Chronic Value (95% C.L.)[1]
48-h Acute	85.7 (7.0)	83.0 (75.21 - 91.68)
96-h Acute	94.1 (3.3)	69.4 (60.70 - 79.45)
8-d Chronic	90.9 (4.6)	64.0

[1]LC50 values are given for the acute tests. A chronic value is given for the chronic test and no confidence limits are associated with this value.

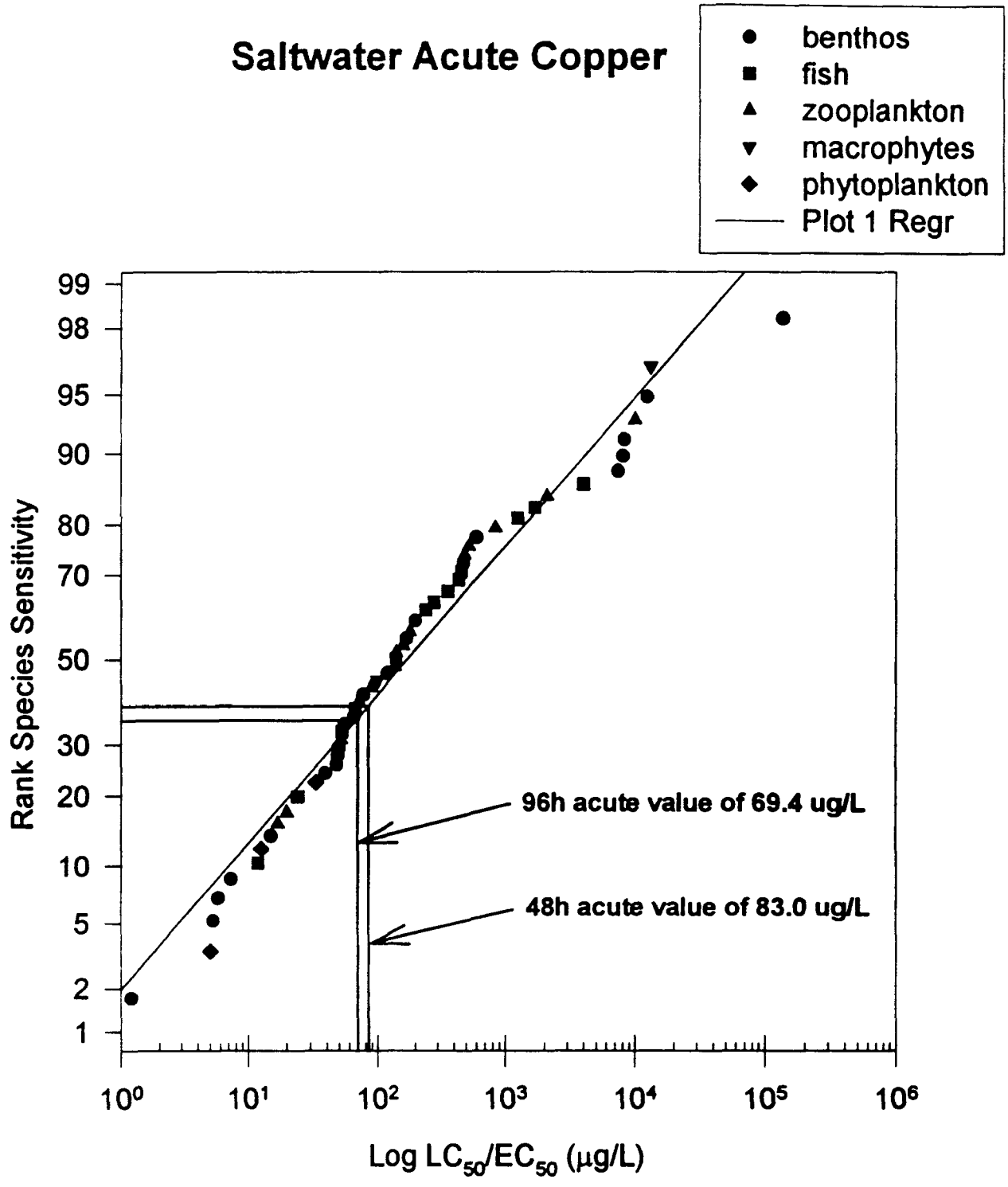
DISCUSSION

The chemistry measurements for total dissolved copper and copper complexation capacity were important in determining the concentrations of copper available to the test species. Over the course of the experiments, approximately 20 to 35% of the copper was lost with the most significant loss during the first 48 hours. Possible reasons for loss of copper during these experiments were adsorption to the vessel walls or particulate matter in the test chambers and/or biological uptake by *Eurytemora* or the phytoplankton used as a food source.

Voltammetric analysis of selected samples indicated greater than 99% complexation of copper in all samples, with copper complexing capacities increasing with both time and total copper concentration. The copper complexing capacity increased with time by a factor of approximately 1.2 to 1.6 for each total dissolved copper treatment, and with total copper concentration by nearly ten-fold relative to the stock water. While the source of the additional complexing capacity cannot be determined with certainty, it is likely that it resulted from the production and release of strongly binding copper chelators from the phytoplankton utilized as a food source. Moffett and Brand (1996) have recently described the production by the marine cyanobacteria *Synechococcus* spp. of strongly binding extracellular copper chelators, in response to increasing copper stress. The results observed in the present study suggest a similar response. The production of chelating compounds from *Eurytemora* cannot be ruled out, but is probably less likely.

The acute toxicity data reported in this study can be compared with a distribution of species response data determined from an analysis of existing copper toxicity data from various trophic groups (Figure 4). For both the 48 and 96 h acute values, the rank in species response is in the 35 to 40% range. These data suggest that *Eurytemora* has a moderate to sensitive response to copper

Figure 4. Distribution of acute copper toxicity data from various trophic groups. Arrows show how our 48 and 96 h LC50s compare and rank with these data.



b[0] = -2.05976
b[1] = 0.92002
r^2 = 0.96

stress. Two other zooplankton species , the copepod *Acartia tonsa* (Sosnowski and Gentile, 1978; Gentile, 1982)) and the rotifer, *Brachionus plicatilis* (Snell and Personne, 1989) had acute LC50 values of 17 to 52 μg/L which are lower than we reported in our experiments. In a previous acute copper toxicity experiment with *Eurytemora affinis*, Gentile (1982) reported an LC50 of 928 μg/L which is significantly higher that the two acute values reported in this study. In contrast, Sullivan et al. (1983) reported 96 h LC50's for larval *E. affinis* ranging from 28.7 to 33.7 ug/L which compare favorably with our data.

Chronic saltwater toxicity data with copper were limited. The chronic value of 64 μg/L reported for *Eurytemora* in our study is similar to the chronic value of 54 μg/L reported by Lussier et al. (1985) for the mysid, *Mysidopsis bahia*. Acute to chronic ratios from our study (1.3) and the Lussier et al. (1985) study (3.3) are both very low thus indicating a narrow range between acute and chronic responses. This is not a surprising result since low acute to chronic ratios have also been reported for other heavy metals (Lussier et al., 1985).

A comparison of the acute and chronic *Eurytemora* toxicity values (64 to 83 μg/L dissolved copper) with environmental concentrations of copper measured in the Chesapeake Bay watershed can be conducted to provide insight on possible ecological risk. The Toxics of Concern Workgroup of the U.S. Environmental Protection Agencies Toxics Subcommittee assembled available data on environmental concentrations of copper in the Chesapeake Bay (U.S. EPA, 1996). Based on 764 copper measurements ranging from < 1 to 990 μg/L, a mean value of 4.7 μg/L was determined. Using a very simplistic quotient method, this mean exposure value is significantly lower than any of the three toxicity values reported above. This comparison would generally suggest minimal ecological risk to *Eurytemora* from copper exposure although effects would be possible at some of

the higher environmental concentrations if these exposures existed for extended periods of time. Future efforts are planned for conducting a probabilistic ecological risk assessment with copper using a distribution of both effects and exposure data for the Chesapeake Bay (SETAC, 1994). This probabilistic approach has a number of advantages over assessments based on single measures of effects and exposure because it uses all relevant single species toxicity data and when combined with exposure distributions allows for a quantitative estimation of risks to aquatic organisms.

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APPENDIX A

Survival and reproduction data from the

Eurytemora 48 h, 96 h and 8 d copper toxicity tests

ACUTE /CHRONIC COPPER TOXICITY TEST DATA

Test	Nominal Cu (μg/L)	Final Copepod Survival / Initial Nauplii Per Replicate				Mean % Survival
		A	B	C	D	
48-h Acute	0	14/14	9/13	11/14	14/15	85.3
	50	14/14	11/12	11/15	13/15	87.5
	88	8/13	11/15	7/13	9/14	63.6
	158	0/14	0/12	0/12	0/12	0.0
	280	0/16	0/15	0/12	0/13	0.0
	500	0/15	0/13	0/13	0/12	0.0
96-h Acute	0	13/15	12/12	12/12	11/12	94.6
	16	12/12	12/12	11/12	10/13	91.8
	28	9/12	10/12	13/13	13/14	88.2
	50	13/13	11/13	9/13	11/13	84.6
	90	8/13	7/14	11/12	5/13	59.6
	160	0/13	0/12	0/13	0/14	0.0
	284	0/14	0/12	0/12	0/14	0.0
8-d Chronic	0	13/15	12/14	15/15	-----[1]	90.9
	16	12/12	12/12	13/13	10/12	95.9
	26	11/13	10/12	10/12	14/15	86.5
	40	10/12	11/13	11/12	11/14	84.3
	64	8/13	11/14	13/13	10/12	84.0
	100	8/13	4/14	3/13	8/12	44.2
	160	0/12	0/12	0/13	0/13	0.0

[1]Replicate broken during course of experiment.

CHRONIC COPPER TOXICITY % GRAVID FEMALES

Test	Nominal Cu (μg/L)	Final Gravid Females / Initial Nauplii Per Replicate A	B	C	D	Mean % Gravid
8-d Chronic	0	6/15	4/14	6/15	-----[1]	39.8
	16	2/12	3/12	-----[2]	3/12	23.9
	26	7/13	-----[2]	4/12	-----[2]	51.8
	40	2/12	5/13	3/12	6/14	36.8
	64	2/13	1/12	6/13	5/12	32.6
	100	0/13	0/14	0/13	0/12	0.0
	160	0/12	0/12	0/13	0/13	0.0

[1]Replicate broken during course of experiment.
[2]Replicates where the number of preserved individuals did not correspond with the number of individuals counted at end of test.

CHRONIC COPPER TOXICITY % IMMATURE

Test	Nominal Cu (μg/L)	Final Immature / Initial Nauplii Per Replicate A	B	C	D	Mean % Immature
8-d Chronic	0	1/15	0/14	3/15	-----[1]	9.2
	16	2/12	1/12	-----[2]	3/12	18.3
	26	0/13	-----[2]	1/12	-----[2]	5.0
	40	2/12	1/13	0/12	2/14	11.8
	64	1/13	2/12	1/13	0/12	9.6
	100	7/13	4/14	2/13	4/12	76.0
	160	0/12	0/12	0/13	0/13	0.0

[1]Replicate broken during course of experiment.
[2]Replicates where the number of preserved individuals did not correspond with the number of individuals counted at end of test.