United States
Environmental Protection
Agency

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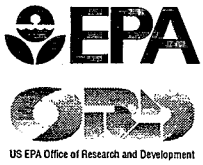
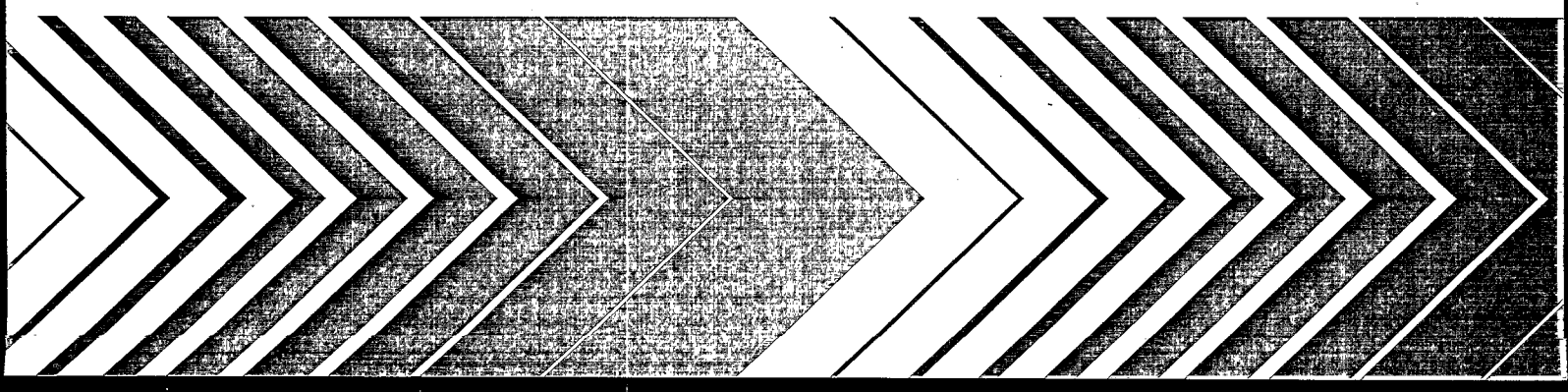
Washington DC 20460

EPA/600/R-01/051
June 2001



Early Life-Stage Toxicity of Copper to Endangered and Surrogate Fish Species



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Early Life-Stage Toxicity of Copper to Endangered and Surrogate Fish Species

by

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EPA Project No. DW14937809-01-0

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Notice

The U.S. Environmental Protection Agency, Office of Water funded this research through an Interagency Agreement between the Columbia Environmental Research Center (CERC), as part of U.S. EPA Project No. DW14937809-01-0, "Biological and Chemical Evaluation of Contaminants." It has been subjected to the Agency's peer and administrative review and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Abstract

Water quality criteria (WQC) for the protection of aquatic life have not explicitly considered the degree of protection afforded to aquatic species listed as endangered or threatened under the U.S. Endangered Species Act (listed species) . Most WQCs are based primarily on responses of a limited number of surrogate species, which are easily cultured and tested in the laboratory. Little information is available about the relative sensitivity of listed species to toxic chemicals, especially with respect to chronic toxicity. We conducted a series of chronic, early life-stage toxicity tests with two listed species, fountain darter (*Etheostoma fonticola*) and spotfin chub (*Cyprinella monacha*), and two surrogate species, fathead minnow (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*), exposed to copper (Cu).

Data from the tests with the four species, which included repeated tests with three species, were used to evaluate the suitability of test endpoints and toxicity metrics. Endpoints measured included survival, growth (total length and average dry weight of surviving fish), and biomass (total dry weight of survivors). Toxicity metrics were established by hypothesis testing to determine no-observed-effect concentrations (NOEC) and lowest-observed-effect concentrations (LOEC), and by a linear interpolation technique, to estimate inhibition concentrations associated with 10% and 25% reductions of test endpoints (IC_{10} and IC_{25}). The hypothesis testing and linear interpolation methods generally gave similar results, as all calculable IC_{10} values fell within the NOEC-LOEC range. The 'chronic value' calculated from these studies (ChV = geometric mean of NOEC and LOEC) corresponded closely to the IC_{10} for most species and endpoints.

For three of the four species tested, growth and/or biomass endpoints were more sensitive than survival. For fountain darters, no significant effects on growth occurred at concentrations less than LOECs for survival and biomass, and IC_{10} values indicated that reductions in growth (both dry weight and total length) only occurred at concentrations greater than those affecting survival. For the other three species, reductions in growth, expressed as individual dry weight, occurred at concentrations at least as low as those affecting other endpoints. However, growth in dry weight showed wide variation among three tests with fathead minnows, with ChVs ranging from 2.8 to 15.9 μg/L. Results from tests with fathead minnows and other species suggested that growth in dry weight was affected by differences in fish density caused by differential survival among replicates and between treatments. Growth in total length was less variable than dry weight and LOECs for total length were close to those for dry weight, but IC_{10} values for total length were consistently greater than those for dry weight. Biomass, which reflects combined toxic effects of Cu on both survival and individual growth, was nearly as sensitive as growth in dry weight and was less variable among tests.

Sensitivity to Cu toxicity did not differ substantially between listed and surrogate species. Lowest average ChVs for the four species tested ranged from 7.7 μg/L for the fountain darter (for reduced survival and biomass) to 15.9 μg/L for the spotfin chub (for reduced growth and biomass). The average ChV for growth of fathead minnows from three tests (7.8 μg/L) was nearly equal to that for fountain darters, although this value was strongly influenced by the low ChV of 2.8 μg/L determined from one of the three tests. Evaluation of relative species sensitivities with IC_{10} produced similar results, with values ranging from <8.0 μg/L for fountain darters to 23 μg/L for spotfin chubs.

Toxicity thresholds (either ChVs or IC_{10}s) estimated from our chronic, early life-stage toxicity tests indicated that the current chronic Cu WQC would protect the endangered spotfin chub, but may not adequately protect the endangered fountain darter or the two surrogate species tested. This finding contrasts with results of previous acute toxicity tests in our laboratory, which concluded that current acute WQC for Cu would adequately protect fountain darters. These results suggest that protection of fountain darters from chronic toxicity of Cu would require application of a safety factor of about 0.5 to the current chronic Cu WQC. This safety factor would be consistent with that estimated from previous acute toxicity studies conducted at our laboratory with surrogate and listed species.

Introduction

Federal environmental laws, including the Clean Water Act, the Federal Insecticide, Fungicide and Rodenticide Act, and the Toxic Substances Control Act require the testing and regulation of toxic chemicals to prevent hazards to the environment or human health. The Endangered Species Act further requires Federal agencies to insure that any action authorized, funded, or carried out is not likely to jeopardize the continued existence of endangered or threatened (listed) species. The U.S. Environmental Protection Agency (USEPA), the U.S. Fish and Wildlife Service (USFWS), and the U.S. Geological Survey (USGS) have conducted research to determine the acute sensitivity to several classes of toxic chemicals for 13 freshwater species that are listed are listed by USFWS or state agencies (Dwyer et al 1995; 1999a,b; 2000). These studies found that the common surrogate species, rainbow trout (*Oncorhynchus mykiss*), was generally as sensitive as the listed species in acute tests. Across all the species and chemicals tested, median lethal concentrations (LC_{50}s) for listed species differed from those for rainbow trout by no more than a factor of three. Additional comparisons, based on 7-day effluent toxicity tests, also found that the sensitivity of the surro-

gate species, fathead minnow (*Pimephales promelas*) was similar to the listed species tested (Dwyer et al. 1999b). These acute toxicity data are being used to evaluate potential risks to listed fish species during consultations on state water quality standards and in pesticide spray programs. However, acute toxicity tests may not establish safe exposure levels for listed organisms that may be exposed to contaminants throughout their life cycle. Few suitable data are available to evaluate the chronic sensitivity of listed species to toxic chemicals, or to compare the acute and chronic toxicity of chemicals to listed species (Beyers et al. 1994). Acute-chronic ratios for surrogate species, based on acute LC_{50}s and thresholds for chronic effects on survival, growth, and/or reproduction, vary widely among species (USEPA 1996a). Because of this uncertainty about the sensitivity of listed species to chronic toxicity, it is not known whether current chronic water quality criteria (WQC) adequately protect listed species.

Chronic WQC are based on the toxicity thresholds determined by statistical hypothesis testing, typically analysis of variance (ANOVA). This approach estimates the lowest-observed-effect concentration (LOEC) and no-observed-effect concentration (NOEC), based on statistically significant differences between treatment groups and controls. USEPA uses the geometric mean of the NOEC and LOEC to derive a 'chronic value' (ChV), which is used to compare the sensitivity of species and to calculate acute-chronic ratios (USEPA 1985a). The principal criticisms of ANOVA-based metrics for analysis of chronic toxicity data (Stephan and Rogers 1985; Crane and Newman 1999) are that (1) results of ANOVA-based data analysis are affected by differences in the power of statistical analyses, and (2) these metrics are not continuous variables (i.e., they can only be assigned to the discrete exposure concentrations selected for a given test) and therefore have no definable statistical confidence interval. As a result, the degree of reduction in a given endpoint that is associated with the LOEC can vary widely among tests depending on the experimental design and the power of the statistical test selected. Another criticism of ANOVA-based toxicity metrics is that concentration-response data are more appropriately analyzed by regression methods (Stephan and Rogers 1985, Beyers et al. 1994). Regression techniques, such as probit analysis and logistic regression, use data from all exposure concentrations to make point estimates of effects thresholds, such as the LC_{50}. However, unlike LC_{50}s, point estimates for 'biologically significant' effects tend to be far from the midpoint of the regression thresholds (i.e., reductions of less than 50% in test endpoints), where confidence intervals for point estimates become wider.

An alternative (or adjunct) to both ANOVA and regression analysis is the use of linear interpolation to estimate inhibition concentrations (ICP) associated with specific percent inhibition of biological responses (Norberg-King 1993; USEPA 1994). The ICP methods estimates threshold concentrations associated with a level of impact on a biological response (e.g., 10% or 25% reductions) that are assumed to be biologically significant. The IC_{25}, or 25% inhibition concentration, has been suggested as a biologically meaningful toxicity metric, although few studies have compared NOECs, LOECs, and ICPs in chronic tests (Marchini et al. 1992). The ICP procedure assumes that short sections of the dose-response curve are approximately linear, but it does not require assumptions about the overall shape of the concentration-response curve. Confidence intervals for ICP estimates can be estimated by a nonparametric bootstrap technique, which reflects the variation in the test endpoint at exposure concentrations adjacent to the interval of interest (Norberg-King 1993).

We conducted a series of toxicity tests to address three objectives: (1) evaluate the suitability of different test endpoints and toxicity metrics for comparing chronic toxicity between listed and surrogate species; (2) compare the sensitivity of listed species and surrogate species, using the toxicant copper (Cu); and (3) determine whether the species tested are adequately protected by the existing chronic WQC for Cu. Early life-stage tests, which typically start before or shortly after egg hatching and last for at least 30 days, were selected because they are good predictors of toxicity in full life-cycle chronic tests (ASTM 2000a). We evaluated data on survival, individual growth (in dry weight and total length), and total biomass during these tests, using metrics derived by ANOVA (NOEC; LOEC, and ChV) and by the linear interpolation technique (IC_{10} and IC_{25}). Toxicity tests with Cu were conducted with two endangered fish (fountain darter, *Etheostoma fonticola*; and spotfin chub, *Cyprinella monacha*) and two surrogate test species (fathead minnow and rainbow trout).

Materials and Methods

Test organisms

Toxicity tests were conducted at the Columbia Environmental Research Center (CERC), Columbia, Missouri. Tests with fountain darter, spotfin chub, and fathead minnow (Tests 1, 2, and 3) were started with newly-hatched larvae (typically one day after hatching) obtained from the National Fish Hatchery and Technology Center (San Marcos, TX), Conservation Fisheries, Inc. (Knoxville, TN), and Aquatic BioSystems, Inc. (Fort Collins, CO), respectively. Rainbow trout embryos were obtained from the Ennis National Fish Hatchery (Ennis, MT). The first test with rainbow trout (Test 4A) was started with eyed eggs. The second rainbow trout test (Test 4B) was started with eggs that had been held in a vertical-tray incubation box at 10°C in

CERC well water (alkalinity 258 mg/L as $CaCO_3$, hardness 286 mg/L as $CaCO_3$, pH 7.8) until the swim-up life stage. Trout larvae were acclimated to test waters over a period of 48 hours before being added to test chambers.

Exposure systems

Tests were conducted in an intermittent-flow proportional diluter system (Lemke et al. 1978). Stock solutions of Cu ($CuSO_4 \cdot 5H_2O$) were prepared in deionized water. The diluter dispensed five Cu concentrations with a dilution factor of 0.5, plus a control, and provided about 250-ml of water to each replicate exposure cup or chamber every 20 minutes. Glass incubation cups (350 mL, with stainless steel screen bottoms) were used for holding rainbow trout eggs. Plastic cups (1000 mL, with 40-mesh stainless steel screen window) were used for holding larvae of the other three species for the first two weeks of the exposures. Test solution flowed directly into the cups, which were suspended in the test chambers. Four test chambers (10-L glass aquaria, with stainless steel screen window) were submerged in each of 12 large glass aquaria held in a water bath, which controlled test temperatures within ±1°C of the target temperatures (Table 1). Water depths in the aquaria were controlled by stand-pipes to produce a volume of 6 liters in individual test chambers. Each Cu treatment was delivered to two aquaria and each species was stocked into two of the four replicate test chambers in each aquarium, resulting in four replicate chambers for each species.

Water samples for Cu analysis were collected biweekly from one test chamber for each concentration and preserved with 1% (v/v) ultra-pure nitric acid. Water samples were analyzed by inductively-coupled plasma-mass spectroscopy (ICP-MS) without further sample preparation (May et al. 1997; Appendix 1). Total hardness, total alkalinity, conductivity, pH, and dissolved oxygen were measured weekly, and ammonia was monitored periodically during each test.

Early life-stage toxicity tests

Four sets of early life-stage toxicity tests were conducted in general accordance with ASTM (2000a) and USEPA (1996b) guidelines, as summarized in Table 1. Tests were conducted under a photoperiod of 16 h light and 8 h darkness, with moderately-hard reconstituted water: hardness, 160 to 180 mg/L as $CaCO_3$; alkalinity, 110 to120 mg/L as $CaCO_3$; and pH, 7.6 to 8.0 (2000b). Tests with two listed species, fountain darters and spotfin chubs, were conducted concurrently with tests with the surrogate species, fathead minnow, in the same diluters. Ten newly-hatched larvae of each species (<24 hr post-hatch for fathead minnows; <72 h post-hatch for listed species) were placed in each of four repliate egg cups for each concentration.The only exception to this experimental design was for fountain darters in Test 2, where only three replicates were stocked at the three highest Cu concentrations, due to the limited number of fry available. After about two weeks of exposure, fish were released to the surrounding chambers. Throughout the tests, fish were fed *ad libitum* three times a day with live <24 h-old brine shrimp nauplii. Water temperature for Test 1 (fountain darters and fathead minnows) and Test 3 (spotfin chub and fathead minnows) was maintained at 25 ±1 °C. Test 2 (fountain darters and fathead minnows) was conducted at 23°C, which is reported to be the optimum temperature for survival and growth of fountain darters (Bonner et al. 1998). These tests ended after 30 days of exposure.

Tests with rainbow trout were conducted starting with two different life stages, eyed embryos (Test 4A) and swim-up larvae (Test 4B). These two tests were conducted concurrently, to allow a direct comparison of the sensitivity of the full ELS test (embryo-alevin-fry) to the shorter test with swim-up fry. Treatment groups and replicates were distributed as described above. For Test 4A,

Table 1. Summary of test conditions for early life-stage chronic toxicity tests, conducted in general accordance with guidance in ASTM (2000a).

Parameter	Conditions
1. Exposure system	Intermittent flow proportional diluter
2.Temperature	Test 1 (fathead minnow, fountain darter): 25°C Test 2 (fathead minnow, fountain darter): 23°C Test 3 (fathead minnow, spotfin chub): 25°C Test 4 (rainbow trout): 10°C
3.Toxicant	Copper sulfate (pentahydrate)
4. Dilution	Control and 5 copper concentrations (dilution factor = 0.5)
5. Photoperiod	16 h light:8 h darkness
6. Test chamber	6-L with egg/fry cup
7. Water renewal rate	3 volume-replacements/day (0.75 L/hour)
8. Age of fish	Rainbow trout: eyed embryos and swim-up fry (2 test) Fathead minnows: <24 hr old larvae Listed species: <72 hr old larvae
9. Fish or eggs/chamber	25 for rainbow trout; 10 for other three species
10. Replicates	4 replicate chambers per concentration
11. Duration:	30 d; except 58 d for rainbow trout embryo test (test ended after 30-d swim-up life stage);
12. Feeding	3 times a day with <24 h live brine shrimp nauplli
13. Test water	ASTM hard water (hardness 160 to180 mg/L as $CaCO_3$ alkalinity 110 to 120 mg/L as CaCO3, pH 7.6 to 8.0)
14. Endpoints	Survival, growth (mean dry wt.), biomass (total dry wt.)
15. Test acceptability	>70% average survival in controls

25 eyed embryos were held in each of four replicate egg cups at each concentration. Embryos were incubated in darkness, under black plastic that blocked about 90% of incident light. When fish hatched, they were transferred into the surrounding chambers. Test 4A ended 30 days after fish reached the swim-up stage, resulting in a total test duration of 58 days. For Test 4B, 25 fish at the swim-up stage were added to each test chamber, and the test ended after 30 days. Water temperature was maintained at 10 ±1 °C for both tests. Trout were fed *ad libitum* three times a day with live <24 h-old brine shrimp nauplii after they reached the swim-up stage.

During all four tests, dead fish were counted, recorded, and removed daily. Fish were not fed for 24 h before the end of the tests. At the end of tests, surviving fish in each replicate chamber were euthanized, counted, placed in a tared aluminum weigh boat, and dried at 60 °C for 36 h for determination of dry weight. Dry weights were not corrected for initial weights of fish at the beginning of the test, which was assumed to be equal for fish in each test. Dry weight data were used to determine growth (mean dry weight per individual), and biomass (total dry weight per replicate) of surviving fish. In tests with fathead minnows, fountain darters, and spotfin chubs, total lengths of freshly-euthanized fish were measured with a digital caliper as an additional growth endpoint.

Data Analysis

Toxicity thresholds were estimated from test data for survival, growth (total length and dry weight), and biomass by statistical hypothesis testing and by the linear interpolation technique, using TOXSTAT statistical software (WEST Inc. 1996). Hypothesis testing to determine LOEC and NOEC followed the statistical flow-chart recommended by USEPA (1994), with testing for normality and homogeneity of variance followed by statistical analysis by parametric ANOVA and Dunnett's test or by the nonparametric Steel's many-one rank test. For the sake of simplicity, both types of tests are referred to as 'ANOVAs' for the remainder of this report. Statements of statistical significance refer to a probability of a type 1 error of no greater than 5% ($p \leq 0.05$). The NOEC for each endpoint was defined as the highest exposure concentration in which the endpoint was not significantly reduced relative to controls and the LOEC was determined as the lowest concentration above the NOEC. In order to focus on effects on growth that occurred at concentrations less than those affecting survival, test concentrations above the NOEC for survival were excluded from the statistical analysis for growth data (USEPA 1994). If no significant reductions in growth occurred at or below the NOEC for survival, the LOEC for growth was undefined and was assigned the value of '≥[LOEC for survival]' for comparisons. In addition, the data were analyzed by the linear interpolation procedure (Norberg-King 1993) to determine 10% and 25% inhibition concentrations (IC_{10} and IC_{25}). Data from all concentrations were used for ICP calculations, except in cases where a trend for decreased growth was reversed at the highest test concentration, coincident with survival less than 30%. Based on these criteria, growth data from the highest exposure concentrations were excluded from ICP calculations for fathead minnows in Test 3, and for rainbow trout in Tests 4A and 4B.

Results and Discussion

Test Conditions

Test conditions and performance indicators for toxicity tests corresponded closely to the guidelines in Table 1. Cu concentrations in Test 1 showed evidence of background contamination, with elevated Cu concentrations in the control treatment (5.4 µg/L) and concentrations consistently above nominal concentrations in the four lower Cu treatments (Table 2). The source of the problem, a pump with a worn impeller, was identified and corrected before subsequent tests. Tests 2 through 4 had low Cu concentrations in controls (<2 µg/L), and measured Cu concentrations closely reflected nominal diluter concentrations, except for slight deficits in the highest Cu treatments, which probably reflected losses of Cu to sorption or to formation of particulate species.

Quality assurance for Cu analyses met all data quality objectives (Appendix 1). The detection limit for Cu for four analytical runs ranged from 0.2 to 0.6 µg/L. Precision of duplicate analyses ranged from 0.3% to 3.7% relative percent difference. Recovery of Cu from two standard reference solutions averaged 100%, recoveries from analysis spikes ranged from 95% to 99%, and recoveries of Cu spiked into interference check solutions ranged from 90% to 108%.

Water quality in the four toxicity tests was within expected ranges. Routine water quality measurements corresponded to expected characteristics of ASTM hard water (Table 3). Measured hardness averaged 164 mg/L, within the range cited by ASTM (2000b). Dissolved oxygen ranged from 8.8 to 10.5 for rainbow trout test (at 10°C), and from 6.5 to 8.3 mg/L for tests with the other three species (at 23 to 25°C). Ammonia concentrations measured during the tests did not exceed 0.12 mg/L as total ammonia or 0.002 mg/L as unionized ammonia.

Control survival in all tests met the minimum acceptable level of 70% established for early life-stage testing by ASTM (2000a; Tables 4-6). Lowest control survival (78%) occurred for fountain darters in Test 2, but control survival ranged from 89% to 100% in other tests. Elevated background Cu concentrations in Test 1 had no obvious effect

Table 2. Nominal and measured copper concentrations in water from early life-stage toxicity tests. Nominal values for each test are underlined. SD=standard deviation.

	Copper Concentration (ug/L)					
Study 1	Control	3.13	6.25	12.5	25	50
Day 0	5.9	–	12.1	16.7	28.5	42.4
Day 2	5.8	8.6	10.9	16.1	28.6	53.7
Day 20	4.6	9.9	11.4	17.2	27.7	55.3
Mean	5.4	9.3	11.5	16.7	28.3	50.5
(SD)	(0.7)	(0.9)	(0.6)	(0.6)	(0.5)	(7.00)
Study 2	Control	2.5	5	10	20	40
Day 0	1.7	5.0	6.4	10.4	19.2	39.0
Day 15	1.8	3.9	6.8	11.6	20.8	41.1
Day 29	2.2	4.4	6.3	9.9	17.6	39.4
Mean	1.9	4.4	6.5	10.6	19.2	39.8
(SD)	(0.3)	(0.5)	(0.3)	(0.90)	(1.6)	(1.1)
Study 3	Control	2.5	5	10	20	40
Day 0	1.9	4.0	6.7	11.8	22.4	47.2
Day 13	2.0	3.4	5.3	9.1	18.5	34.7
Day 19	1.5	4.2	6.7	11.0	19.8	44.0
Day 27	2.3	3.8	6.9	12.3	30.2	42.5
Mean	1.9	3.9	6.4	11.1	22.7	42.1
(SD)	(0.3)	(0.3)	(0.8)	(1.4)	(5.2)	(5.3)
Study 4	Control	3.13	6.25	12.5	25	50
Day 0	1.5	3.1	4.8	10.0	18.1	41.7
Day 9	1.9	3.8	6.3	11.9	22.2	44.4
Day 21	1.2	3.4	6.7	12.1	25.2	47.5
Day 40	1.7	4.8	7.3	13.0	24.1	45.5
Day 56	1.4	4.1	6.8	12.6	26.0	47.5
Mean	1.6	3.7	6.2	11.8	22.4	44.8
(SD)	(0.3)	(0.7)	(1.0)	(1.3)	(3.1)	(2.)0

on control performance for either fathead minnows or fountain darters, but background Cu concentrations effectively obscured the two lowest exposure concentrations (nominal concentrations: 3.1 and 6.3 μg/L).

Copper toxicity

The sensitivity of the two listed species to Cu differed widely, with fountain darters more sensitive than spotfin chubs (Table 4). In Tests 1 and 2, survival and biomass of fountain darters were significantly reduced, relative to controls, at Cu concentrations of 9 μ g/L. and 11 μg/L, respectively. No darters survived at Cu concentrations of 28 μg/L and greater. Growth of darters (mean dry wt. or total length of individuals) was not significantly reduced at concentrations less than those affecting survival. The similar results of these two tests indicate that the difference in water temperature (25°C in Test 1, 23°C in Test 2) did not affect the toxicity of Cu to fountain darters. In contrast, survival of spotfin chubs (Test 3) was not significantly reduced at Cu concentrations less than 42 μg/L, but both growth (in dry wt. and total length) and biomass were significantly reduced at 23 μg/L.

Table 3. Water quality characteristics of test water (ASTM hard) during early life-stage toxicity tests. Values are means, with standard deviation in parentheses.

Test (N)	Temperature (°C)	Conductivity (µmhos/cm)	pH	Alkalinity (Mg/L as $CaCO_3$)	Hardness
1 (4)	25 ± 1	575 (30)	8.1 (0.1)	114 (3)	163 (5)
2 (4)	23 ± 1	604 (10)	8.3 (0.1)	120 (14)	162 (12)
3 (4)	25 ± 1	604 (10)	8.3 (0.1)	120 (14)	162 (12)
4 (6)	10 ± 1	588 (13)	8.1 (0.2)	111 (7)	167 (5)

Table 4. Survival, growth (dry weight and total length), and biomass of fountain darters *(Etheostoma fontalis)* and spotfin chubs *(Cyprinella monacha)* in early life-stage toxicity tests with copper. Values are means, with standard deviation in parentheses (N = 4 unless indicated otherwise). Growth data in treatments with significant reductions in survival (Means below dotted lines)were excluded from ANOVAs.

Test	Cu (ug/L)	Survival (%)	Dry Weight (mg)	Total Length (mm)	Biomass (mg)
			Fountain Darter		
1	5.4	90 (8)	6.4 (1.0)	15.3 (0.8)	58 (14)
	9.3	33 (22)*	7.0 (0.5)	16.1 (0.5)	22 (15)*
	12	18 (13)*	9.0 (1.5)[3]	17.0 (1.2)[3]	16 (12)*
	17	3 (5)*	4.5 (0)[1]	13.6 (0)[1]	1 (3)*
	28	0 (0)*	-- --		0 (0)*
	51	0 (0)*	-- --		0 (0)*
2	1.9	78 (10)	7.1 (0.3)	15.9 (0.2)	55 (5)
	4.4	65 (39)	7.0 (0.4)	15.4 (0.2)	45 (26)
	6.5	88 (13)	6.9 (0.3)	15.9 (0.3)	60 (8)
	11	27 (6)*[3]	7.5 (0.9)[3]	16.1 (0.7)[3]	20 (2)*[3]
	19	10 (10)*[3]	4.8 (1.1)[2]	13.2 (0.3)[2]	5 (4)*[3]
	40	0 (0)*[3]	-- --		0 (0)*[3]
			Spotfin Chub		
3	1.9	100 (0)	25.5 (0.5)	26.5 (0.3)	256 (5)
	3.9	98 (5)	25.1 (0.9)	26.2 (0.4)	245 (15)
	6.4	100 (0)	25.8 (0.5)	27.1 (0.5)	257 (6)
	11	95 (10)	27.0 (2.2)	27.1 (0.2)	254 (12)
	23	100 (0)	23.2 (1.6)*	26.2 (0.9)	232 (16)*
	42	82 (13)*	19.0 (1.3)	24.2 (0.7)	155 (14)*

[1,2,3] Superscripts indicate reduced number of replocates, due to mortality or to limited numbers of available test organisms.
* significant reductions relative to controls (ANOVA/ Dunnett's test (P<0.05).

Growth and biomass were the most sensitive endpoints in three tests with fathead minnows, which were conducted concurrently with tests with the listed species (Table 5). Survival of fathead minnows was not significantly reduced at Cu concentrations less than 28 μg/L in any of the three tests. Significant reductions in growth (in dry wt. and total length) and biomass of fathead minnows occurred at concentrations

less than those affecting survival in all three tests. LOECs for biomass and growth in total length were equivalent for each test, ranging from 11 to 23 μg/L. LOECs for growth in dry weight were the same as those for total length and biomass in two of the three tests, but the LOEC for growth in Test 2 (4 μg/L) was much lower than the other endpoints. Reduced growth of fathead minnows in Test 2 may reflect the lower test temperature, as is suggested by reduced growth in the control group. Alternatively, reduced within-treatment variation in Test 2 may have increased the statistical power of the ANOVA. A 13% reduction in growth of fathead minnows was found to be statistically significant in Test 2, compared to reductions of 17% at LOECs in both Tests 1 and 3.

The two tests with rainbow trout, started with different life stages, showed similar sensitivity to Cu toxicity (Table 6). LOECs for survival and biomass (22 μg/L) were identical for Test 4a, with exposure from eyed eggs through swim-up, and Test 4b, which included only the swim-up stage. This similarity reflects the fact that most mortality occurred after swim-up in both tests. Effects of Cu on growth (in dry wt) of rainbow trout were very similar between the embryo-alevin-fry test (Test 4A) and the test with swim-up fry (Test 4B); however effects on growth at 11μg/L were statistically significant inTest 4a, but not 4B. This difference may reflect a subtle, cumulative effect of Cu on growth of trout during the longer exposure period in Test 4A. Alternatively, the significant reductions in growth at the LOEC and the other intermediate Cu concentration may have been influenced by greater densities of surviving fish in these treatments, relative to controls (97-99% vs 89%). However, treatment means for growth showed a consistent decreasing trend with increasing Cu concentrations across a wide range of survival, except for the greater average dry weight of the few surviving fish at the highest Cu concentration.

Evaluation of test endpoints and toxicity metrics

Reduced growth and biomass of larval fish, which reflect the physiologic or behavioral costs of Cu exposure, were the most sensitive responses to chronic Cu exposure during early life stage toxicity tests. Statistically significant reductions in growth and biomass of three of the four species occurred at Cu concentrations lower than those affecting survival. The sensitivity of dry weight, total length, and biomass endpoints were essentially equal, whether expressed as LOECs or ICPs (Table 7). Biomass and total length endpoints showed less among-test variation than dry weight. Lesser variation in growth in total length, compared to dry weight, may indicate that total length is inherently less variable than dry weight, it may reflect the technical and statistical advantages of multiple length measurements of individual animals, compared to single measurement of dry weight per replicate. The sensitivity and low variability of the biomass endpoint reflect the fact that biomass toxic effects on bothsurvival and growth, while minimizing the possible influence of density-dependent responses.

The susceptibility of the dry weight endpoint to among-test variation led to greater uncertainty about thresholds for growth effects for fathead minnows and rainbow trout. Among the potential causes for among-test variation are differences in the statistical power of the ANOVA and density-dependent differences in growth. Although the level of statistical significance and the number of replicates were consistent in the current study, the statistical power of ANOVA may have varied among tests due to differences in within-treatment variation. Low within-treatment variation could have contributed to LOECs that correspond to low percent reduction in dry weight relative to controls. Exclusion of treatments with low survival from ANOVAs for growth effects, which was intended to reduce the influence of density differences on growth, also tended to increase statistical power by reducing within-treatment variance (Tables 5 and 6). Despite the exclusion of low-survival treatments, differences in growth responses among treatments could have affected toxicity thresholds for growth. For example, the lower survival of fathead minnows in the control group in Test 2 (93%), relative to the lowest Cu concentration (98% survival), could have contributed to the determination of a statistically-significant reduction in growth in this treatment group, which produced the lowest LOEC for this species. Conversely, density-dependent increases in growth in treatment groups with reduced survival could contribute to greater LOEC values. This density-dependent response was observed in treatments with very low survival (<30%) in tests with fountain darters, fathead minnows, and rainbow trout (Tables 4-6). In most cases, this bias was avoided by disqualifying treatments with significant reductions in survival from ANOVAs for growth effects.

Our results indicate that estimates of toxicity thresholds based on the ICP linear interpolation technique do not differ greatly from ANOVA-based toxicity metrics. Some of the presumed negative characteristics of LOECs were evident in our tests, as LOECs corresponded to a wide range in percent reductions in survival, growth, or biomass relative to controls (9% to 76%), despite consistent experimental designs. The range of effect concentrations estimated by IC_{10}s and IC_{25}s differed somewhat from the NOEC-LOEC range established by ANOVA (Table 7). In most cases, IC_{25}s were greater than LOECs, suggesting that the ANOVAs had sufficient power to detect reductions of less than 25% relative to controls. However, LOECs for total length were substantially less than IC_{25}s, and approached IC_{10} values (Table 7). This trend is consistent with the lower

within-treatment variation of total length data and associated higher power of ANOVAs for this endpoint. Despite these differences in the influence of statistical power on LOECs, almost all IC_{10}s fell within the ranges defined by NOECs and LOECs. As a result, IC_{10}s corresponded closely to ChVs, as these metrics differed by 2 μg/L or less in most tests (Figure 1).

Sensitivity of listed species to Cu toxicity

The two listed species represented the extremes of Cu sensitivity for the four species tested. Species chronic values, (geometric means of the NOEC and LOEC), and IC_{10} values generally indicated that the fountain darter was the most sensitive species tested and the spotfin chub was the least sensitive species tested (Figure 1). The chubs had the highest ChVs and IC_{10}s for survival, growth (in dry weight and total length), and biomass (Table 7). In contrast, the endangered fountain darter was at least as sensitive to chronic Cu toxicity as either of the surrogate species. The darter was more sensitive to effects of Cu on survival than other species. However, lowest chronic values and IC_{10}s for fountain darters (ChVs for growth and biomass, 7.7; IC_{10} for biomass, 8.0 μg/L) were essentially equal to those for surrogate species (lowest ChV for dry weight of rainbow trout, 8.1 μg/L; IC_{10} for dry weight of fathead minnows, 8.6 μg/L).

Table 5. Survival, growth (dry weight and total length), and biomass of fathead minnows (*Pimephales promelas*) in early life- stage toxicity tests with copper. Values are meanswith standard deviation in parentheses (N = 4). Growth data in treatments with significant reductions in survival (means below dashed lines) were excluded from statistical analyses.

Test	Cu (ug/L)	Survival (%)	Dry Weight (mg)	Total Length (mm)	Biomass (mg)
1	5.4	100 (0)	32.7 (2.0)	26.0 (0.7)	326 (20)
	9.3	95 (6)	30.2 (3.1)	25.2 (1.2)	285 (19)
	12	92 (10)	27.1 (1.5) *	23.8 (1.5)*	251 (32)*
	17	95 (6)	21.9 (3.5) *	20.4 (0.5*	207 (22) *
	28	78 (13)*	24.0 (4.3)	20.2 (0.5)	182 (13)*
	50	70 (8)*	12.2 (10.7)	15.6 (2.9)	80 (60) *
2	1.9	92 (5)	28.9 (2.3)	25.4 (0.6)	267 (12)
	4.4	98 (5)	25.2 (0.3)*	24.7 (0.2)	245 (11)
	6.5	100 (0)	24.4 (1.8)*	24.1 (0.5)	243 (18)
	11	98 (5)	22.0 (1.5)*	23.2 (0.5)*	214 (16)*
	19	88 (5)	18.7 (0.6)*	20.3 (1.5)*	164 (9)*
	40	55 (6)*	11.3 (4.0)	15.3 (1.3)	62 (20)*
3	1.9	100 (0)	32.0 (0.9)	25.6 (0.5)	320 (9)
	3.9	95 (6)	32.6 (0.9)	26.0 (0.1)	309 (14)
	6.4	100 (0)	32.1 (0.3)	26.0 (0.3)	320 (3)
	11	100 (0)	31.0 (0.9)	25.4 (0.9)	310 (9)
	23	95 (6)	26.5 (2.8)*	22.1 (1.0)*	251 (31)*
	42	22 (15)*	32.3 (7.2)	23.4 (2.6)	65 (31)*

* indicate significant reductions relative to controls (P <0.05)

Previous studies also found that the fountain darter is highly sensitive to Cu toxicity, relative to other listed and surrogate species. Dwyer et al. (1999a) found that fountain darters were the most sensitive of 14 species tested in acute toxicity tests with Cu in ASTM hard water (170 mg/L as $CaCO_3$). Median lethal concentrations of Cu in 96-h tests were 57μg/L for the fountain darter and 90 μg/L for the spotfin chub, compared to 80 μg/L for rainbow trout and 470 μg/L for fathead minnow. The high LC50 for fathead minnows is consistent with the relative insensitivity of the survival endpoint in our early life-stage tests with fathead minnows. Fountain darters are apparently more sensitive to Cu toxicity than other darters (Percidae: Etheostominae). Dwyer et al. (1999a) found that greenthroat darters (*E. lepidum*), were substantially less sensitive to Cu than fountain darters, with a 96-hr LC of 260 μg/L. Previous acute toxicity tests with Cu in a water of similar hardness (200 mg/L as $CaCO_3$) found 96-hr LC50s of 320 and 850 μg/L for rainbow darters (*E. caeruleum*) and orangethroat darters (*E. spectabile*), respectively, and 440 to 490 μg/L for fathead minnows (Geckler et al. 1976). One-year chronic tests with adult Johnny darters (*E. nigrum*) and fantail darters (*E. flabellare*) in stream water with average hardness of 271 mg/L found no effects on survival and growth of these species (or fathead and bluntnose minnows) at Cu concentrations ranging from 91 to 107 μg/L (Geckler et al. 1976).

Protectiveness of chronic WQC

Our results indicate that the endangered fountain darter may not be adequately protected from chronic Cu toxicity by the current national WQC for Cu. Populations of fountain darters, and other aquatic species listed for recovery under the authority of the Endangered Species Act, are protected from any significant 'take' associated with exposure to toxic chemicals. In contrast, guidelines for development of numeric WQC have the goal of prevention of 'unacceptable effects' (defined as protection of 95% of aquatic taxa tested) and knowledge that adverse effects on some species may occur at concentrations below these criteria (USEPA 1985a). Chronic WQC are frequently derived from acute criteria using acute-to-chronic toxicity ratios (ACR), to take advantage of the larger data sets available from acute toxicity testing. The chronic criterion for CU was derived from the acute CuWQC, using an average ACR of 2.823 (USEPA 1985b, 1996a). Although Dwyer et al. (1999a) concluded that the fountain darter would be adequately protected by the national acute criterion for CU, thresholds for chronic toxicity of Cu to fountain darter in our early life-stage tests (both chronic values and IC_{10}s) are less than the current national water quality criterion of 14.7 μg/L (for water hardness of 170 mg/L, USEPA 1985b; Figure 1). This lack of protectiveness of the chronic WQC reflects the relatively large ACR for fountain darters, 7.125, calculated from the results from our chronic tests and from the acute tests conducted by Dwyer et al. (1999a).

Our results also suggest that the current chronic WQC for Cu is not adequately protective of the surrogate species

Table 6. Survival, dry weight, and biomass of rainbow trout (*Onchorhynchus mykiss*) in embryo-alevin-fry test (Test 4A) and swim-up fry test (Test 4B) with copper. Values are means, with standard deviation in parentheses (N=4). Growth data in treatments with significant reductions in survival (means below dashed lines) were excluded from statistical analyses.

Test	Cu (µg/L)	Survival (%) pre-hatch		pre-swim up		swim up		overall		Dry Weight (mg)		Biomass (mg)	
4A	1.6	100	(0)	98	(2)	92	(5)	89	(5)	55.3	(2.4)	1228	32)
	3.7	100	(0)	99	(2)	91	(7)	90	(7)	54.0	(2.5)	1224	(59)
	6.2	100	(0)	99	(2)	98	(2)	97	(4)	50.8	(2.5)	1231	(19)
	12	99	(2)	100	(0)	100	(0)	99	(2)	49.1	(1.5) *	1214	(57)
	22	99	(2)	95	(5)	80	(6) *	75	(10) *	47.8	(3.8)	892	(86) *
	44	98	(4)	28	(13) *	40	(7) *	13	(8) *	55.7	(16.9)	159	(36) *
4B	1.6		NA		NA	98	(2)	98	(2)	52.7	(1.5)	1291	(58)
	3.7		NA		NA	96	(6)	96	(6)	53.0	(2.2)	1269	(45)
	6.2		NA		NA	97	(2)	97	(2)	52.5	(1.5)	1272	(20)
	12		NA		NA	98	(2)	98	(2)	50.3	(1.0)	1232	(4)
	22		NA		NA	88	(3) *	88	(3) *	48.7	(1.7)	1071	(60) *
	44		NA		NA	24	(3) *	24	(3) *	55.3	(2.1)	333	(54) *

* indicate significant reductions relative to controls ($P < 0.05$)

Table 7. Thresholds for chronic toxicity of Cu to four fish species in early life-stage toxicity tests. NOEC and LOEC = no observed effect concentration and lowest observed effect concentration; IC_{10}, IC_{25} = 10% and 25% inhibition concentrations. All values, including 'less than' and 'greater than' values were used to calculate (geometric) means.

Cu toxicity thresholds (ug/L)

Test	Survival NOEC-LOEC	IC10	IC25	Dry Weight NOEC-LOEC	IC10	IC25	Total Length NOEC-LOEC	IC25	IC10	Biomass NOEC-LOEC	IC10I	IC25
					Fountain Darter							
1	5.4 - 9.3	<9.3	<9.3	5.4 - >9.3	12	13	5.4 - >9.3	15	17	5.4 - 9.3	<9.3	<9.3
2	6.5 - 11	7.0	8.0	6.5 - >11	12	15	6.5 - >11	15	19	6.5 - 11	6.9	7.9
Mean	5.9 - 9.9	<8.1	<8.6	5.9 - >10	12	14	5.9 - >10	15	18	5.9 - 10	<8.0	8.6
					Spotfin Chub							
3	23 - 42	32	>42	11 - 23	23	40	11 - 23	>42	>42	11 - 23	23	33
					Fathead Minnow							
1	17 - 28	19	36	9.3 - 12	10	15	9.3 - 12	12	32	9.3 - 12	<9.3	12
2	19 - 40	19	29	1.9 - 4.4	<4	11	7 - 11	11	24	7 - 11	6.9	13
3	23 - 43	24	28	11 - 23	16	>23	11 - 23	20	>42	11 - 23	15	24
Mean	20 - 36	21	31	5.8 - 10	<8.6	>16	8.8 - 15	14	28	8.8 - 15	9.9	15
					Rainbow Trout							
4A	12 - 22	17	24	6.2 - 12	9.5	>22				12 - 22	15	21
4B	12 - 22	22	27	12 - >22	>22	>22				12 - 22	16	25

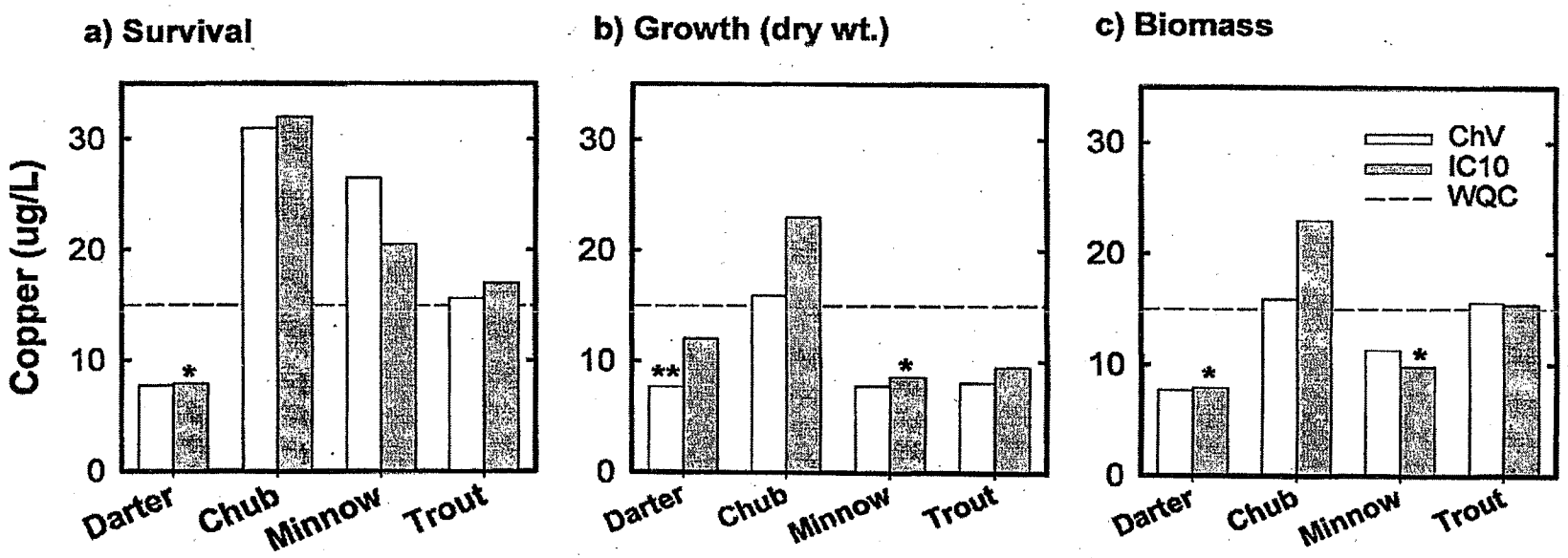

Figure 1. Toxicity thresholds for early life-stage toxicity of Cu to listed and surrogate fish species. Bars indicate chronic value (ChV; geometric mean of LOEC and NOEC) and 10% inhibition concentration (IC10). Values are geometric means of multiple tests for fathead minnow (3 tests) and fountain darter (2 tests) and from individual tests for spotfin chub and rainbow trout (Test 4A). Horizontal reference line indicates chronic water quality criterion for Cu (WQC; USEPA 1996). Bars with one asterisk contain one or more 'less than' value; bars with two asterisks include one or more 'greater than' values (see Table 7).

tested. Chronic values and IC_{10}s for reduced growth and biomass of fathead minnows and reduced growth of raninbow trout in our studies are also lower than the current chronic WQC for Cu (Figure 1). However, high variation in LOECs and ICPs from repeated tests with fathead minnows resulted in some uncertainty about the threshold for effects on growth. Among-test variation was greatest for LOECs based on growth in dry weight, primarily due to the extreme LOEC value for one test (4 μg/ L in Test 2; Table 4). Among-test variation was less for IC_{10}s based on dry weights, and the IC_{10} for growth in Test 2 was less xtreme than the growth LOEC. Among-test variation was also lower for biomass and for growth in total length (Tables 4 and 5), and LOECs and IC_{10}s for these endpoints suggested lesser sensitivity to Cu toxicity. However, mean thresholds for all three growth-related endpoints for fathead minnows (dry wt., total length, and biomass), whether expressed as ChVs or IC_{10}s, ranged from 8 to 14 μg/L, less than the chronic WQC (Figure 1). The sensitivity of rainbow trout to Cu effects on growth in embryo-alevin-fry test (Test 4A) was similar to that of fat head minnows, with ChVs ranging from 8.1 to 15.5 μg/L and IC_{10}s ranging from 9.5 to 17 μg/L. The ranges of these ELS thresholds for both surrogate species are somewhat lower than the ranges of ChVs previously reported for these species across a range of water hardness: 11-27 for fathead minnows and 11-22 μg/L for rainbow trout (Great Lakes Environmental Research Laboratory [GLERL] 1998). "Our results suggest that the current national chronic WQC for Cu is not adequately protective of either the listed or the surrogate species tested. A draft revision of the WQC forCu would establish much lower chronic criteria for Cu (3.36 μg/L at 170 mg/L hardness) that would be protective of all species we tested (GLERL 1998)."

Generation of the additional toxicity data required to revise all the existing WQC to assure adequate protection of listed species would be a difficult task. An alternative mechanism for increasing protection of listed species would be to apply site- and species-specific 'safety factors' that would apply to areas where listed species occur or to designated critical habitats. Dwyer et al. (1999a) concluded that a safety factor of 0.3, applied to existing acute criteria, would provide adequate protection for listed species. Our results suggest that a safety factor of 0.5, applied to the current chronic WQC for Cu, would provide an adequate margin of protection for the species we tested. Further research in our laboratory will repeat this series of studies with additional chemicals. These studies will help determine if chronic WQCs for other chemicals are protective of listed species, and whether the safety factor approach may be more broadly applicable.

Conclusions

1. One of the two listed species tested, the fountain darter, was at least as sensitive to early life-stage toxicity of Cu as the two surrogate species tested, fathead minnow and rainbow trout. In contrast,the listed spotfin chub was the least sensitive species tested.

2. Reduced average growth, especially growth in dry weight, and reduced biomass of surviving fish were more sensitive to Cu toxicity than survival for three of the four species tested.

3. Results of three tests with fathead minnows suggested that growth in dry weight may be subject to high among-test variation, which may reflect density-dependent differences in growth among replicates and among treatments.

4. Lowest-observed-effect concentrations (LOECs) reflected a wide range of reductions in survival, growth, and biomass relative to controls (9% to 76%).

5. Estimates of 10% and 25% inhibition concentrations (IC_{10} and IC_{25}), determined by a linear interpolation technique, tended to show less among-test variation in Cu toxicity than LOECs.

6. Toxicity thresholds for the endangered fountain darter and for the two surrogate species tested (LOECs and IC_{10}s were lower than current chronic WQC for Cu at the water hardness used in these studies.

7. A safety factor of 0.5, applied to the current chronic water quality criterion, would be necessary to protect the endangered fountain darter and the surrogate species we tested.

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Acknowledgments

The authors thank Eugene Greer for culturing the test organisms and Douglas Hardesty, David Whites, James Kunz, Eric Brunson for their technical assistance during these tests. We also thank Tom Brandt of the National Fish Hatchery and Technology Center (San Marcos, TX), and Pat Rakes of Conservation Fisheries, Inc. (Knoxville, TN) for providing the fountain darters and spotfin chubs, respectively. We thank Tom May, Ray Wiedmeyer, and William Brumbaugh of the CERC Environmental Chemistry Branch for conducting the copper analysis. We appreciate the thorough reviews of the manuscript by Denny Buckler of CERC and Dr. Foster Mayer of the USEPA, Office of Research and Development, Gulf Ecology Division (Gulf Breeze, FL).

U. S. Department of the Interior

U.S. Geological Survey

DETERMINATION OF Cu IN WATER FROM TOXICITY STUDIES WITH ENDANGERED SPECIES

Final Report FY00-32-04

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Studies 99-20-05 and 99-20-14

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DETERMINATION OF Cu IN WATER FROM TOXICITY STUDIES WITH ENDANGERED SPECIES

SAMPLE HISTORY:

From 2/26/99 to 10/4/99, various groups of water samples were received by the Inorganic Chemistry section of the Columbia Environmental Research Center (CERC). The samples were generated from toxicity studies investigating the effects of copper on endangered and surrogate species of salmonids. In addition to samples collected during the toxicity tests, other samples served as "pre-test" samples to check out all aspects of diluter operation before actual testing began. These "pre-test" samples required "quick turn around" and thus minimal quality control as compared to toxicity study samples. The toxicity study samples were designated Batch and CERC ID #s as follows: Batch 521 (CERC 19293 - 19302; Batch 524 (CERC 19361 – 19372; Batch 528 (CERC 19414 – 19433); Batch 557 (CERC 20012 – 20025); Batch 563 (CERC 20143 – 20167); Batch 565 (CERC 20183 – 20190); Batch 570 (CERC 20488 – 20500); Batch 575 (CERC 20709 – 20720). For each batch, analyses requested were for samples to be analyzed for Cu. The samples (60 or 100 mL each) were acidified with nitric acid prior to receipt (1% v/v).

METHODS:

No further preparation was conducted on the samples prior to instrumental analysis. Analysis was performed with a PE/SCIEX Elan 6000 ICP-MS, which was set up and optimized according to the manufacturer's specifications and described in CERC SOP P.241. Samples were automatically delivered to the ICP-MS by means of a software-controlled CETAC ASX-500/ADX-100 autosampler/autodilutor system, which also conducted all dilutions. All samples were prediluted 10X by the autodiluter, and any samples over the upper calibration standard of 20 ng/mL were automatically diluted 10X in a serial fashion until concentrations were within the confines of the standard line. The internal standard was Ge (50ppb), which was metered into the sample line via peristaltic pump. Calibration standards for analysis were 2, 5, 10, and 20 ng/mL for the element. Two masses were monitored for Cu (Cu-63 and Cu-65), but only Cu-63 was reported.

RESULTS AND DISCUSSION:

Concentrations of Cu in water samples determined by ICP-MS are presented in the Table 2 of the report. In most cases, copper concentrations agreed well with nominal values.C

QUALITY CONTROL:

The samples were generally analyzed within a few weeks after receipt. The samples were divided into eight groups for instrumental analysis, with each group identified by a separate date (BID or block initiation date). In each of these instrumental runs, the following quality control was included for the determination of Cu by ICP-MS: duplicate samples, dilution checks, reference solutions, analysis spikes, and calibration checks. All quality control results were tabulated to provide an overview of quality assurance and to facilitate interpretation.

A calibration blank and an independent calibration verification standard were analyzed every 10 samples to confirm the calibration status of the ICP-MS (Table A1). Results from the analysis of two reference solutions are indicated in Table A2, where recoveries of Cu were 100%. Recovery of the element from analysis spikes ranged from 95% to 99% (Table A3). Precision from the duplicate analysis of water samples for Cu is indicated in Table A4 and ranged from 0.3 to 3.7 relative percent difference (RPD). As a check for potential interferences, water samples were manually diluted 10X, analyzed, then diluted an additional 5X (Table A5). Dilution percent differences for both elements were ≤ 10%. A synthetic solution containing high concentrations of Al, Ca, Fe, Mg, Na, P, K, S, C, Mo, and Ti was analyzed to observed the effects of these interferences on Cu (Table A6). Copper recoveries ranged from 90% to 108%. Finally, the instrument detection limits, method detection limits, and limits of quantitation for Cr are indicated in Table A7. Overall, the quality control was considered within acceptable limits as specified by CERC.

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Table A1. Concentrations of elements in a continuing calibration blank (CCB) and independent calibration verification standard (ICVS) ran every 10 samples. Results expressed as ng/mL.

BIDa	Element	CCB[b]	ICVS	% Rec[c]	BID[a]	Element	CCB[b]	ICVS	% Rec[c]
2/26/99	Cu	0.00258	25.3	101	8/9/99	Cu	-0.0018	6.14	102
2/26/99	Cu	0.00745	25.1	100	8/9/99	Cu	-0.0047	5.68	95
3/11/99	Cu	0.00146	6.02	100	8/9/99	Cu	0.00113	5.91	98
3/11/99	Cu	0.00219	6.10	102	8/9/99	Cu	0	6.07	101
3/11/99	Cu	0	6.15	103	8/26/99	Cu	0.00021	12.33	103
3/11/99	Cu	0.0002	6.12	102	8/26/99	Cu	-0.00435	12.36	103
3/29/99	Cu	0.0008	5.97	100	8/26/99	Cu	-0.00443	12.25	102
3/29/99	Cu	0.00129	6.07	101	8/26/99	Cu	-0.00423	12.13	101
3/29/99	Cu	0.00334	6.29	105	8/26/99	Cu	-0.00481	12.04	100
3/29/99	Cu	0.00224	6.05	101	8/26/99	Cu	-0.00741	12.18	101
3/29/99	Cu	0.00641	6.00	100	8/26/99	Cu	-0.00806	12.07	101
6/1/99	Cu	-0.00226	6.01	100	8/26/99	Cu	-0.00847	12.13	101
6/1/99	Cu	-0.00300	6.11	102	8/26/99	Cu	-0.01020	12.09	101
6/1/99	Cu	-0.00309	6.18	103	8/26/99	Cu	-0.00994	12.01	100
7/13/99	Cu	0.00092	6.15	103	10/5/99	Cu	0.00233	12.30	103
7/13/99	Cu	0.00018	6.03	101	10/5/99	Cu	0.00253	12.34	103
7/13/99	Cu	0.00127	6.14	102	10/5/99	Cu	0.00366	12.30	102
7/13/99	Cu	0.00021	6.04	101	10/5/99	Cu	0.00304	12.79	107
7/13/99	Cu	0	6.28	105					

[a] BID = Block Initiation Date: a date assigned to each member of a group of samples
[b] Acceptance criteria for CCB is +/- 3 X IDL for each element.
[c] Acceptance criteria for ICVS = +/- 10% (90% - 110%). ICVS = 6, 12, or 25ppb.

Table A2. Recoveries of Cu from reference solutions used as laboratory control samples in the quantitative analysis of water samples.

Reference Material	Element	Actual Conc.	n	Meas. Conc.	SD	Mean % Rec	ISOP	Operator Initials
NIST 1643[a]	Cu	20.5 +/- 3.8	6	20.5	0.728	100	P.241	RHW/TWM
TMDW [b]	Cu	20 +/- 2	13	19.3	0.601	100	P.241	RHW/TWM

[a] NIST 1643d = National Institute of Standards and Technology Standard Reference Material Trace Elements in Water 1643d. Concentration results expressed as ng/mL. Solution used as laboratory control sample.

[b] TMDW = Trace Metals in Drinking Water laboratory control solution, Cat # CRM-TMDW; concentration results in ng/mL. Solution used as laboratory control sample.

Table A3. Percent recovery of Cu from a spiked water sample.

BID[a]	Ele.	Spk Type	Units	Spike	Vol	Effective	Bkgd.	Total	% Rec.[f]	ISOP	Oper. Init.
7/13/99	Cu	20155 - Analytical	ng/mL	80	10	8	0.20	7.83	95	P.241	RHW/TWM
7/13/99	Cu	20162 - Analytical	ng/mL	80	10	8	0.13	7.96	98	P.241	RHW/TWM
8/9/99	Cu	20185 - Analytical	ng/mL	80	10	8	0.15	7.98	98	P.241	RHW/TWM
8/26/99	Cu	20185 - Analytical	ng/mL	100	10	10	0.16	10.0	99	P.241	RHW/TWM
8/26/99	Cu	20233 - Analytical	ng/mL	100	10	10	1.53	11.3	97	P.241	RHW/TWM
8/26/99	Cu	20246 - Analytical	ng/mL	100	10	10	0.49	9.96	95	P.241	RHW/TWM
10/5/99	Cu	20709 - Analytical	ng/mL	150	10	15	0.23	14.7	97	P.241	RHW/TWM

[a] BID = Block Initiation Date: a date assigned to each member of a group of samples that will identify the sample as a member of the group or "block."

[b] Spike Amt. ng = the absolute microgram (ng) amount of the spike which was added to a sample (ECRC#s 18001 and 8033 were samples used for spiking).

[c] Effective Conc. = the Spike Amt (ng) divided by the total solution volume, units ng/mL.

[d] Bkgd Conc. = the measured concentration of the sample prior to spiking, units ng/mL.

[e] Total Conc. = the measured concentration of the spiked sample (spike + background, units ng/mL).

[f] % Rec. = percent recovery: [(Total Conc. - Bkgd Conc.)/Effective Conc. * 100]

Table A4. Relative percent difference from the duplicate analysis of water.

BID [a]	Matrix	Element	Sample	Dup 1	Dup 2	Diff[b]	Mean	RPD[c]	ISOP[d]	Oper. Init.
7/13/99	water	Cu	20154	4.4	4.3	0.05	4.4	1.1	P.241	RHW/TWM
7/13/99	water	Cu	20166	4.37	4.41	0.04	4.39	0.8	P.241	RHW/TWM
8/9/99	water	Cu	20190	3.91	3.96	0.05	3.94	1.3	P.241	RHW/TWM
8/26/99	water	Cu	20190	4.19	4.04	0.15	4.12	3.7	P.241	RHW/TWM
8/26/99	water	Cu	20236	7.96	8.06	0.10	8.01	1.2	P.241	RHW/TWM
8/26/99	water	Cu	20500	4.68	4.70	0.01	4.69	0.3	P.241	RHW/TWM
10/5/99	water	Cu	20714	4.76	4.68	0.08	4.72	1.6	P.241	RHW/TWM

[a] BID = Block Initiation Date: date assigned to identify a sample as a member of a group or "block".
[b] Diff = Dup 1 - Dup 2.
[c] RPD = relative percent difference, calculated as Diff/Mean X 100; acceptance criteria +/- 10%.
[d] ISOP = standard operating procedure used for instrumental analysis of sample (C5.212).

Table A5. Interference check using dilution percent difference.

BID [a]	Run Date	Sample	Matrix	Element	Concentration (ug/L)		% Diff [c]
					Undiluted	Diluted [b]	
7/13/99	7/15/99	20166	water	Cu	4.5	0.9	0.7
7/13/99	7/15/99	20167	water	Cu	9.1	1.9	3.6
8/9/99	8/9/99	20190	water	Cu	8.9	1.8	0.6
8/26/99	8/26/99	20228	water	Cu	10.51	2.1	1.3
8/26/99	8/26/99	20243	water	Cu	19.97	4.0	1.0
8/26/99	8/26/99	20500	water	Cu	5.27	0.9	10.0
10/5/99	10/5/99	20709	water	Cu	14.83	3.1	3.3

[a] BID = Block Initiation Date: date assigned to identify a sample as a member of a group or "block".
[b] Dilution factor = 5 (1+4).
[c] Dilution % difference acceptance criteria = +/- 10%; concentrations exceeding +/- 10% indicative of suspect interferent.

Table A6. Recovery of Cu from an interference check solution[a].

BID	Element	Concentration (ppb)		Dilution	
		measured	actual	Factor	% Recovery [b]
2/26/99	Cu	93.6	100	5	94
3/29/99	Cu	90.4	100	5	90
7/13/99	Cu	108	100	10	108
8/9/99	Cu	100	100	10	100
8/26/99	Cu	92.3	100	5	92
10/5/99	Cu	94.3	100	5	94

[a] High Purity ICP-MS Solution AB in 2% nitric acid, Charleston, SC.; CAT # ICP-MS-ICS.
[b] Suggested acceptance tolerance 80% - 120%.

Table A7. Instrument detection limits, method detection limits, and limits of quantitation.

BID	Date [a]	Element	Type	Conc. [b]	SDb [c]	SDst [d]	IDL [e]	MDL [f]	LOQ [g]	ISOP	Oper. Init.
7/13/99	3/5/99	Cu	diluter	2.0	0.01984	0.19612	0.015	0.6	1.9	P.241	RHW/TWM
8/9/99	3/5/99	Cu	diluter	2.0	0.04084	0.03894	0.015	0.2	0.6	P.241	RHW/TWM
8/26/99	3/5/99	Cu	diluter	2.0	0.05961	0.08043	0.015	0.3	1.0	P.241	RHW/TWM
10/5/99	3/5/99	Cu	diluter	2.0	0.05315	0.03292	0.015	0.2	0.6	P.241	RHW/TWM

[a] Date of 3rd non-consecutive day analysis, following which IDL was computed.
[b] Concentration of low level standard diluted used in MDL computation.
[c] Standard deviation from analysis of reagent blank (n=3).
[d] Standard deviation from analysis of low level standard diluted 10X (n=3).
[e] IDL = instrument detection limit (ng/mL), computed as 3 X mean of standard deviations of a low level standard analyzed 7 times on 3 separate days.
[f] MDL = method detection limit (ng/mL), computed as 3 X (SDb2 + SDs2)1/2 where SDb = standard deviation of a blank (n = 3) and SDs = standard deviation of a low level sample or spiked sample (n = 3).
[g] LOQ = limit of quantitation ng/mL, computed as 3.3 X the MDL.

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