Arch. Environ. Contam. Toxicol. 48, 155–165 (2005)
DOI: 10.1007/s00244-003-0039-z

ARCHIVES OF
Environmental
Contamination
and Toxicology


# Assessing Contaminant Sensitivity of Endangered and Threatened Aquatic Species: Part II. Chronic Toxicity of Copper and Pentachlorophenol to Two Endangered Species and Two Surrogate Species

J. M. Besser,[1] N. Wang,[2] F. J. Dwyer,[3] F. L. Mayer, Jr.,[4] C. G. Ingersoll[1]

[1] Columbia Environmental Research Center, United States Geological Survey, 4200 New Haven Rd., Columbia, Missouri 65201 USA
[2] AScI Corporation, Columbia, Missouri 65211 USA
[3] United States Fish and Wildlife Service, Columbia, Missouri 65201 USA
[4] United States Environmental Protection Agency, Gulf Breeze, Florida 32561 USA

Received: 28 March 2003/Accepted: 21 April 2004

**Abstract.** Early life-stage toxicity tests with copper and pentachlorophenol (PCP) were conducted with two species listed under the United States Endangered Species Act (the endangered fountain darter, *Etheostoma fonticola*, and the threatened spotfin chub, *Cyprinella monacha*) and two commonly tested species (fathead minnow, *Pimephales promelas*, and rainbow trout, *Oncorhynchus mykiss*). Results were compared using lowest–observed effect concentrations (LOECs) based on statistical hypothesis tests and by point estimates derived by linear interpolation and logistic regression. Sublethal end points, growth (mean individual dry weight) and biomass (total dry weight per replicate) were usually more sensitive than survival. The biomass end point was equally sensitive as growth and had less among-test variation. Effect concentrations based on linear interpolation were less variable than LOECs, which corresponded to effects ranging from 9% to 76% relative to controls and were consistent with thresholds based on logistic regression. Fountain darter was the most sensitive species for both chemicals tested, with effect concentrations for biomass at $\leq$ 11 μg/L (LOEC and 25% inhibition concentration [IC25]) for copper and at 21 μg/L (IC25) for PCP, but spotfin chub was no more sensitive than the commonly tested species. Effect concentrations for fountain darter were lower than current chronic water quality criteria for both copper and PCP. Protectiveness of chronic water-quality criteria for threatened and endangered species could be improved by the use of safety factors or by conducting additional chronic toxicity tests with species and chemicals of concern.

Environmental laws of the United States—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 decrease hazards to the environment or human health. The Endangered Species Act further requires federal agencies to ensure that any action authorized, funded, or carried out is not likely to jeopardize the continued existence of threatened and endangered (listed) species or adversely modify their critical habitat. Recent research in our laboratory, supported by the United States Environmental Protection Agency (USEPA) and the United States Fish and Wildlife Service (USFWS), has evaluated the acute toxicity of several classes of chemicals to listed species relative to responses of two commonly tested species, rainbow trout (*Oncorhynchus mykiss*) and fathead minnows (*Pimephales promelas*), which may serve as appropriate surrogate species for the contaminant sensitivities of listed cold- and warm-water fish species, respectively (Wolf and Rumsey 1985; Beyers 1995). These studies found that the rainbow trout was generally as sensitive to the 5 chemicals tested (copper, pentachlorophenol, 4-nonylphenol, carbaryl, and permethrin) as were 13 listed species in acute tests (Dwyer *et al.* 2004; Sappington *et al.* 2001). Across all the species and chemicals tested, LC50s for listed species differed from those for rainbow trout by no more than a factor of 3. Additional studies found that the sensitivity of the fathead minnow was similar to that of listed species tested in 7-day effluent toxicity tests (Dwyer *et al.* 1999). These short-term 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, short-term toxicity tests may not establish safe exposure concentrations for listed organisms that may be exposed to contaminants for extended time periods. Few suitable data are available to evaluate the chronic sensitivities 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 commonly tested species—based on acute lethality (LC50s) and thresholds for chronic effects on survival, growth, or reproduction—vary widely among species and chemicals (USEPA 1996a). Because of this uncertainty about the sensitivities of listed species to chronic toxicity, most of which have never been tested with any chemicals, it is not

*Correspondence to:* J. M. Besser; *email:* jbesser@usgs.gov

known whether current chronic water-quality criteria (WQCs) adequately protect listed species.

Chronic WQCs are typically based on effect concentrations determined by statistical hypothesis testing using analysis of variance (ANOVA), a term used here to refer both to the parametric ANOVA and nonparametric alternatives (USEPA 2000). This approach estimates the LOEC and the no–observed effect concentration (NOEC) based on statistically significant differences between treatment groups and controls. The USEPA uses the geometric mean of the NOEC and the LOEC to derive the "chronic value" (ChV), which is used to compare the sensitivities of species and to calculate acute–chronic ratios (USEPA 1985). The principal criticisms of ANOVA-based effect concentrations for analysis of chronic toxicity data (Stephan and Rogers 1985; Crane and Newman 1999) are that (1) results of ANOVA are affected by differences in the power of statistical analyses and (2) NOECs, LOECs, and ChVs 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 decrease in a given end point associated with an 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 effect concentrations 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 determine point estimates of effects levels such as the LC50. However, unlike LC50s, point estimates for "biologically significant" effect concentrations tend to be far from the midpoint of the regression, where confidence intervals for point estimates based on commonly used regression models become wider. In addition, iterative curve-fitting algorithms may not produce useful estimates of regression parameters if data are inadequate or if the shape of concentration–response curves do not fit typical sigmoidal models.

An alternative to ANOVA or regression analysis is the use of linear interpolation to estimate inhibition concentrations (IC*p*), which estimate chemical concentrations associated with a specific percent inhibition (*p*) of biologic responses (USEPA 1994). Starting with a monotonic concentration–response curve (or a curve that is smoothed using moving averages), the IC*p* method makes linear interpolations between treatment means to estimate the concentration associated with *p* percent inhibition. This method allows estimation of chemical concentrations associated with low levels of effects (e.g., 10% or 25% decreases) that may be judged to be "biologically significant" rather than a statistically significant level of effect that varies among tests with different statistical power. The IC25, or 25% inhibition concentration, has been suggested as a biologically meaningful effect concentration, although few studies have compared NOECs, LOECs, and IC*p*s in tests of chronic exposure (Marchini *et al.* 1992). Confidence intervals for IC*p* estimates can be estimated by a nonparametric bootstrap technique, which reflects the variation associated with points adjacent to the interval of interest (USEPA 1994).

We conducted a series of chronic toxicity tests with two federally listed fish species (the endangered fountain darter, *Etheostoma fonticola*, and the threatened spotfin chub, *Cyprinella monacha*) and two commonly tested test species (fathead minnow and rainbow trout). The listed species were selected based primarily on their availability from established laboratory cultures, although the fountain darter has been reported by Dwyer *et al.* (2004) to have relatively high sensitivity to the acute toxicity of several chemicals. Two toxicants, copper and pentachlorophenol (PCP), were selected because of their wide occurrence in contaminated aquatic environments and because they represent two broad classes of aquatic contaminants (cationic metals and water-soluble organic compounds) that are amenable to chronic water-phase toxicity testing. Early life-stage tests, which typically start before or shortly after egg hatching and last for at least 30 days, were selected as good predictors of toxicity in full life-cycle chronic tests (American Society for Testing and Materials [ASTM] 2003a). We evaluated data on survival, growth (average dry weight of survivors), and biomass (total dry weight of survivors per replicate) during these tests based on effect concentrations derived by hypothesis testing (ANOVA), by logistic regression, and by linear interpolation. The objectives of these tests were (1) to compare the toxicity of two chemicals to two listed and two commonly tested fish species using the most appropriate test end points and effect concentrations for evaluating chronic toxicity and (2) to determine whether the species tested are adequately protected by existing WQCs for chronic exposure to copper and PCP.

## Materials and Methods

### *Toxicity Tests*

Toxicity tests were conducted at the Columbia Environmental Research Center (CERC), Columbia, MO. Tests were conducted with newly hatched fountain darter (National Fish Hatchery and Technology Center, San Marcos, TX), spotfin chub (Conservation Fisheries, Knoxville, TN), and fathead minnow (Aquatic BioSystems, Fort Collins, CO). Tests with rainbow trout (Ennis National Fish Hatchery, Ennis, MT) were started with fish at two different life stages: eyed embryos (approximately 2 weeks before hatch) and swim-up fry. Trout eggs were held in a vertical-tray incubation box at 10°C in CERC well water (alkalinity 260 mg/L as $CaCO_3$ and hardness 290 mg/L as $CaCO_3$, pH 8.3) until the start of embryo tests, and swim-up fry were transferred to flow-through holding tanks and acclimated to test waters during 24 hours before the start of tests.

Tests were conducted in an intermittent-flow proportional diluter system. Stock solutions of copper sulfate ($CuSO_4 \cdot 5H_2O$, 99%; Sigma-Aldrich, St. Louis, MO) were prepared in deionized water, and stock solutions of reagent-grade PCP (99+%; Sigma-Aldrich, St. Louis, MO) were prepared in acetone for test PCP1 and in triethylene glycol for subsequent tests. Maximum concentrations of both solvents were 0.03% (v/v) at the highest exposure concentration. The diluter dispensed five chemical concentrations with a dilution factor of 0.5 plus a control: water control for copper test and solvent control for PCP tests. Glass incubation cups (350-mL with stainless steel screen bottoms) were used for holding rainbow trout eggs. Larvae of the other three species were held in glass test chambers (6-L water volume with stainless steel screen windows). Four replicate test chambers were held in each of 12 large glass aquaria in a water bath, which controlled test temperatures within ±1°C of the target temperatures (Table 1). Test solution flowed directly into cups or test chambers, and excess water overflowed into the aquaria through the screen windows, so there was no exchange of test water among replicates. The diluter provided three volume replacements per day for each test chamber.

**Table 1.** Summary of test conditions for early life-stage chronic toxicity tests with four fish species

| Parameter | Description |
|---|---|
| Exposure system | Intermittent flow proportional diluter |
| Temperature | 25°C (except rainbow trout = 10°C and fountain darter = 23°C, in test Cu2) |
| Toxicant | Copper sulfate (pentahydrate) or PCP (99%) |
| Dilutions | Control and five concentrations (dilution factor = 0.5) |
| Photoperiod | 16 hours light and 8 hours darkness |
| Test chamber | 6-L |
| Water renewal | Three volume replacements/d (0.75 L/h) |
| Age or life stage | Listed species <72 h; fathead minnows <48 h; trout, eyed embryos or swim-up fry |
| Individuals/chamber | 15 or 25 for rainbow trout and 10 or 15 for other species |
| Replication | 4 chambers/concentration |
| Test duration | 30 d (except tests with rainbow trout eggs = 30 d after swim-up) |
| Feeding | 3 times/d with <24-hours-live brine shrimp nauplli |
| Test water | ASTM (2003a) hard-hardness 170 mg/L, alkalinity 115 mg/L, and pH 8.3 |
| End points | Survival, growth (mean dry weight/surviving fish), and biomass (total dry weight/replicate) |
| Test acceptability | >70% average control survival |

PCP = Pentachlorophenol.

Early life-stage toxicity tests were conducted in general accordance with ASTM (2003a) and USEPA (1996b) guidelines as listed in Table 1. Tests were conducted under a photoperiod of 16 hours of light and 8 hours of darkness with moderately hard reconstituted water (ASTM 2003b; average hardness 170 mg/L as $CaCO_3$). Tests with the listed species, fountain darters and spotfin chubs, were conducted concurrently and in the same diluter system with tests with fathead minnows. Ten or 15 larvae of each species were placed in each of 4 replicate chambers for each concentration, except in test copper (Cu)2, where 3 replicates were stocked with fountain darters at the 3 highest copper concentrations because of the limited number of fish available. Water temperature for tests with fountain darters, spotfin chubs, and fathead minnows was maintained at 25 ± 1°C; except Test Cu2 (with fountain darters and fathead minnows), which was conducted at 23 ± 1°C, reported to be the optimum temperature for survival and growth of fountain darters (Bonner *et al.* 1998). Throughout the tests, fish were fed *ad libitum* 3 times/d with live <24-hour-old brine shrimp nauplii. The duration of these tests was 30 days.

Tests starting with different life stages of rainbow trout were conducted concurrently at temperatures of 10 ± 1°C. For series A tests, 15 (PCP5A) or 25 (Cu4A) eyed embryos were added to each of 4 replicate incubation cups and held in darkness under black plastic that blocked approximately 90% of incident light. As eggs hatched, they were transferred into the surrounding test chambers. Tests ended 30 days after fish reached the swim-up stage (test duration was 58 days for copper and 59 days for PCP). Series B tests were started with 25 swim-up fry added to each test chamber and ended after 30 days. After reaching swim-up stage, trout were fed *ad libitum* 3 times/d with live <24-hours-old brine shrimp nauplii. During all tests, dead fish were counted, recorded, and removed daily. Fish were not fed for 24 hours before the end of the tests. At the end of the tests, surviving fish in each replicate chamber were killed with tricaine methanesulfonate (Argent Chemical Laboratories, Redmond, WA), counted, placed in a tared aluminum weigh boat, and dried at 60°C for 36 hours for determination of dry weight. For each replicate, dry-weight data were used to determine growth (mean dry weight per survivor) and biomass (total dry weight of survivors).

Water samples for chemical analysis were collected biweekly. Samples for copper analysis were preserved with 1% (v/v) ultrapure nitric acid, and samples for analysis of PCP were refrigerated until analysis. Samples were analyzed for copper by inductively coupled plasma-mass spectroscopy without further sample preparation (May *et al.* 1997). Analysis for PCP was conducted by direct injection, reverse-phase, high-performance liquid chromatography with ultraviolet detection (Orazio *et al.* 1983). Total hardness, total alkalinity, conductivity, pH, and dissolved oxygen were measured weekly, and ammonia was monitored periodically during each test using standard methods (APHA *et al.* 1995).

## *Data Analysis*

Effect concentrations were estimated from toxicity data and measured chemical concentrations by three methods: hypothesis testing, linear interpolation, and logistic regression. Hypothesis testing to determine LOEC and NOEC for each end point was conducted by ANOVA with mean comparisons made by one-tailed Dunnett's test or by alternative methods as described by USEPA (1994, 2000). These analyses were performed using ToxStat software (version 3.5; Lincoln Software, Bisbee AZ). Determinations of statistical significance refer to a 5% probability of a type I error. The NOEC for each end point was defined as the highest exposure concentration in which the end point was not significantly decreased relative to controls, and the LOEC was determined as the lowest concentration above the NOEC. Chronic values were calculated as the geometric means of LOEC and NOEC values. To focus on effects on growth that occurred at concentrations less than those affecting survival, and to decrease possible influence of density-dependent growth, exposure concentrations above the NOEC for survival were excluded from the ANOVAs for growth data (USEPA 1994). Therefore, if no significant decreases in growth occurred at concentrations less than the LOEC for survival, the LOEC for growth was defined as equal to the LOEC for survival for calculations.

The linear interpolation method (USEPA 1994, 2000) was used to estimate concentrations causing 25% and 10% inhibition of test end points (IC25 and IC10, respectively) using ToxStat. Data from all test concentrations were used for IC*p* calculations, except in several cases where a trend for decreased growth with increasing test concentration was reversed at the highest concentration tested, coincident with low survival (< 30%). Based on these criteria, growth data from the highest exposure concentrations were excluded from IC*p* calculations for fathead minnows in test Cu3 and for rainbow trout in tests Cu4A and Cu4B. In cases where the 10% or 25% effect concentrations were outside the range of concentrations tested, IC10 or IC25 were estimated as either "less than [lowest concentration tested]" or "greater than [highest concentration tested] and the lowest or highest concentrations tested were used for calculations.

Logistic regression models of concentration–response curves were

derived using SigmaPlot software (version 7.0; SPSS, Chicago, IL). Effect concentrations (EC10 and EC25) were determined from regression models that were judged to be acceptable fits based on statistical significance ($p \leq 0.05$), goodness of fit ($r^2 \geq 0.80$), and visual inspection of fit in the range of interest (< 50% effect).

Chemical and biologic measurements were characterized by standard quality assurance measures. Accuracy of chemical analytes was expressed as percent recovery relative to verified mean concentrations. The data-quality objective for accuracy of chemical analyses was recovery from standard reference material within 10% of stated mean value. Precision of repeated measurements was expressed as relative percent difference (RPD = {difference mean}*100) for duplicate measurements and as relative RSD (also known as coefficient of variation; RSD = {standard deviation mean}*100) for ≥3 measurements. The data-quality objective for precision of multiple analyses was RPD or RSD < 20%. Limits of detection and quantitation for chemical analyses were defined as 3 times and 10 times the average blank concentration, respectively.

## Results and Discussion

### *Test Conditions*

Measured concentrations of copper and PCP closely reflected nominal exposure concentrations with few exceptions (Table 2). Results of test Cu1 showed evidence of background contamination of copper with increased concentrations in the controls (5 μg/L) and concentrations above nominal in most treatment groups. There was no detectable effect on control performance for fountain darters and fathead minnows in test Cu1: Survival was ≥ 90%, and growth and biomass were comparable with controls in subsequent tests. The source of contamination was identified and corrected (brass pump impeller replaced with plastic) before subsequent tests were performed. Measured concentrations of pentachlorophenol were approximately 20% greater than nominal in the three highest treatments in study PCP1 (Table 2). The source for this error was not determined, but all PCP concentrations were close to nominal in subsequent studies.

Analyses of copper and PCP had adequate sensitivity and met or exceeded our quality-assurance objectives. The detection limit for copper in four analytical runs ranged from 0.2 to 0.6 μg/L. Precision of duplicate analyses ranged from 0.3% to 3.7% RPD. Recovery of copper from two standard reference solutions were 97% and 100% of stated mean concentration (20 μg/L); recoveries from analysis spikes ranged from 95% to 99%; and recoveries of copper spiked into interference check solutions ranged from 90% to 108%. The average detection limit for PCP analyses was 1.1 μg/L. Precision of triplicate PCP analyses averaged 3.5% RSD. Recovery of PCP from a quality assurance standard (200 μg/L) averaged 101%.

Water quality in toxicity tests was consistent with the nominal composition of the ASTM moderately hard reconstituted water and was consistent over time and among tests. Average water-quality characteristics were (means ± SDs) as follows: pH 8.30 ± 0.13; conductivity 580 ± 16 μS/cm; hardness 170 ± 9 mg/L as $CaCO_3$; and total alkalinity 120 ± 10 mg/L as $CaCO_3$). Dissolved oxygen was > 70% of saturation in all tests. Ammonia concentrations did not exceed 0.12 mg/L as total ammonia or 0.002 mg/L as un-ionized ammonia.

### *Toxicity of Copper*

The sensitivities of the four species to copper toxicity differed widely, with fountain darter the most sensitive species (Table 3). LOECs for survival in two tests with fountain darters were 9.3 and 11 μg/L compared with LOECs ranging from 22 to 42 μg/L for the other species. Neither growth nor biomass of fountain darters was decreased significantly at copper concentrations less than those affecting survival, but both growth and biomass of spotfin chubs and fathead minnows were consistently more sensitive to copper than survival. Growth and biomass of spotfin chubs were significantly decreased at 23 μg/L. LOECs for sublethal end points for fathead minnows were similar both within and among tests (11 to 23 μg/L), except for a much lower LOEC for growth in test Cu2 (4.4 μg/L). Several previous studies of chronic toxicity of copper to fathead minnows reported chronic values ranging from 11 to 28 μg/L (Great Lakes Environmental Center 1998). Significant lethal and sublethal responses of rainbow trout to copper occurred at 22 μg/L in both tests, except growth was significantly decreased at 12 μg/L in test Cu4A. Chronic values of 9–16 μg/L were similar to those derived from two previous chronic tests with rainbow trout: 19 μg/L at a hardness of 45 mg/L (McKim *et al.* 1978) and 22 μg/L at a hardness of 120 μg/L (Seim *et al.* 1984).

Linear interpolation indicated a slightly broader range of effect concentrations for copper among the four species, with more consistent results among tests and among different end points for individual species (Table 3). For fountain darters, IC25s for both survival and biomass were <9.3 μg/L in test Cu1 and approximately 8 μg/L in test Cu2. Biomass of spotfin chubs had an IC25 33 μg/L. Results of IC*p* calculations did not corroborate the low LOECs for growth of fathead minnows in test Cu2 and for rainbow trout in test 4A. For fathead minnows, IC25s for sublethal end points were relatively consistent in all three tests (11 to 24 μg/L). For rainbow trout, all defined IC25s fell within a narrow range, i.e., 21 to 27 μg/L.

### *Toxicity of Pentachlorophenol*

Results of toxicity tests with PCP indicated a different ranking of the sensitivities of the four species (Table 4). Fountain darters were again the most sensitive species tested with LOECs of 39 μg/L for growth and biomass. The sensitivity of spotfin chubs was similar to that of rainbow trout, with LOECs for growth and biomass of both species ranging from 68 to 72 μg/L. The chronic value for PCP effects on biomass of rainbow trout was 51 μg/L for tests starting with eggs or swim-up fry—substantially higher than that determined from a previous early life-stage study with this species (14.5 μg/L at pH 7.4 by Dominguez and Chapman 1984). LOECs for biomass of fathead minnows from three of the four tests were similar to or greater than those for the other three species (68 to 152 μg/L), but the LOEC for biomass in test PCP2 (15 μg/L) was the lowest single LOEC for PCP for any of the species. The geometric mean of four chronic values for effects of PCP on biomass of fathead minnows (47 μg/L) was comparable with chronic values from previous tests of this species exposed to PCP

**Table 2.** Measured concentrations of copper and PCP in water from early life-stage toxicity tests with four fish species[a]

| Test | Exposure Concentration (μg/L) | | | | | |
|---|---|---|---|---|---|---|
| | Control | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
| Copper | | | | | | |
| Nominal | 0 | 3.1 | 6.3 | 12.5 | 25 | 50 |
| Cu1 | 5.4 (0.7) | 9.3 (0.9) | 12 (0.6) | 17 (0.6) | 28 (0.5) | 51 (7.0) |
| Nominal | 0 | 2.5 | 5 | 10 | 20 | 40 |
| Cu2 | 1.9 (0.3) | 4.4 (0.5) | 6.5 (0.3) | 11 (0.9) | 19 (1.6) | 40 (1.1) |
| Cu3 | 1.9 (0.3) | 3.9 (0.3) | 6.4 (0.8) | 11 (1.4) | 23 (5.2) | 42 (5.2) |
| Cu4 | 1.6 (0.3) | 3.7 (0.7) | 6.2 (1.0) | 11 (1.3) | 22 (3.1) | 45 (2.4) |
| PCP | | | | | | |
| Nominal | 0 | 13 | 25 | 50 | 100 | 200 |
| PCP1 | ND[b] | 15 (3) | 32 (8) | 59 (3) | 129 (13) | 240 (31) |
| Nominal | 0 | 19 | 38 | 75 | 150 | 300 |
| PCP2 | ND | 15 (6) | 39 (2) | 71 (11) | 143 (15) | 297 (13) |
| PCP3 | ND | 16 (3) | 36 (4) | 74 (15) | 152 (12) | 307 (10) |
| PCP4 | ND | 15 (5) | 35 (2) | 68 (13) | 148 (6) | 275 (35) |
| PCP5 | ND | 18 (3) | 36 (4) | 72 (4) | 149 (6) | 290 (7) |

[a] Means, with standard deviation, N = 3 to 5 measurements per test.
[b] ND = Not detected (detection limit = 1.1 μg/L).

**Table 3.** Effect concentrations (μg/L) for chronic toxicity of copper to four fish species in early life-stage toxicity tests

| Species | Survival | | | | Growth | | | | Biomass | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NOEC-LOEC[a] | ChV | IC10[b] | IC25[b] | NOEC-LOEC | ChV | IC10 | IC25 | NOEC-LOEC | ChV | IC10 | IC25 |
| Fountain Darter | | | | | | | | | | | | |
| Cu1 | 5.4–9.3 | 7.1 | <9.3 | <9.3 | 5.4–9.3 | 7.1 | 12 | 13 | 5.4–9.3 | 7.1 | <9.3 | <9.3 |
| Cu2 | 6.5–11 | 8.5 | 7.1 | 8.1 | 6.5–11 | 8.5 | 12 | 15 | 6.5–11 | 8.5 | 7.1 | 7.9 |
| Spotfin chub | | | | | | | | | | | | |
| Cu3 | 23–42 | 31 | 32 | >42 | 11–23 | 16 | 23 | 40 | 11–23 | 16 | 23 | 33 |
| Fathead Minnow | | | | | | | | | | | | |
| Cu1 | 17–28 | 22 | 19 | 36 | 9.3–12 | 11 | 10 | 15 | 9.3–12 | 11 | <9.3 | 12 |
| Cu2 | 19–40 | 28 | 19 | 29 | 1.9–4.4 | 2.9 | <4 | 11 | 6.5–11 | 8.5 | 6.9 | 13 |
| Cu3 | 23–42 | 31 | 24 | 28 | 11–23 | 16 | 16 | 23 | 11–23 | 16 | 15 | 24 |
| Rainbow Trout | | | | | | | | | | | | |
| Cu4A | 12–22 | 16 | 17 | 24 | 6.2–12 | 8.6 | 8.4 | >22 | 12–22 | 16 | 15 | 21 |
| Cu4B | 12–22 | 16 | 22 | 27 | 12–22 | 16 | >22 | >22 | 12–22 | 16 | 16 | 25 |

[a] NOEC (no–observed effect concentration) = highest concentration tested without significant difference from control; LOEC (lowest–observed effect concentration) = first concentration above NOEC.
[b] IC10 (10% inhibition concentration) = concentration associated with 10% decrease in test endpoint relative to control; IC25 = 25% inhibition concentration.
ChV = Chronic value.
Cu = Copper.

(57 μg/L at pH 7.2 to 7.9 by Holcombe *et al.* 1982 and 1993 μg/L at pH 7.4 by Hamilton *et al.* 1986).

Linear interpolation of data from PCP tests showed less variation among tests and end points and more consistent rankings among species. For fountain darters, IC25s for all three end points were lower than the corresponding LOECs and lower than IC25s for the other three species. As was observed for copper, IC25s did not support the more extreme LOEC values. For fathead minnows, the IC25 for biomass in test PCP2 (151 μg/L) was much greater than the LOEC for this test (15 μg/L), but the range of biomass LOECs for the other three tests (68 to 152 μg/L) overlapped broadly with all four biomass IC25s for this species (102 to 172 μg/L).

### *Test End Points and Effect Concentrations*

Growth was generally a more sensitive end point than survival in tests with both copper and PCP. Fountain darters were the only species that showed decreased survival at copper concentrations similar to those affecting growth (Table 3). Although the LOEC for survival of fountain darters exposed to PCP was greater than the LOEC for growth, IC25s indicated that survival was the more sensitive end point. For spotfin chub, fathead minnow, and rainbow trout, growth was a more sensitive end point than survival based on LOECs or IC25s from 8 of 13 tests with copper and PCP (Tables 3 and 4). Based on measurements made in a subset of these tests (6 tests with

**Table 4.** Effect concentrations (μg/L) for chronic toxicity of PCP to four fish species in early life-stage toxicity tests

| | Survival | | | | Growth | | | | Biomass | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | NOEC-LOEC | ChV | IC10[b] | IC25[b] | NOEC-LOEC | ChV | IC10 | IC25 | NOEC-LOEC | ChV | IC10 | IC25 |
| Fountain darter | | | | | | | | | | | | |
| PCP2 | 39–71 | 53 | 17 | 26 | 15–39 | 24 | 22 | 36 | 15–39 | 24 | <15 | 21 |
| Spotfin chub | | | | | | | | | | | | |
| PCP4 | 148–275 | 201 | 81 | 120 | 35–68 | 49 | 55 | 120 | 35–68 | 49 | 48 | 75 |
| Fathead Minnow | | | | | | | | | | | | |
| PCP1 | 129–240 | 176 | 133 | 161 | 129–240 | 176 | 98 | >240 | 59–129 | 87 | 88 | 143 |
| PCP2 | 143–297 | 206 | 90 | 164 | 143–297 | 206 | 168 | >297 | <15–15 | >15 | 73 | 151 |
| PCP3 | 74–152 | 106 | 83 | 112 | 74–152 | 106 | 131 | >307 | 74–152 | 106 | 76 | 102 |
| PCP4 | 148–275 | 202 | 155 | 197 | 148–275 | 202 | 61 | 235 | 35–68 | 49 | 55 | 172 |
| Rainbow Trout | | | | | | | | | | | | |
| PCP5A | 72–149 | 104 | 75 | 88 | 36–72 | 51 | 47 | 71 | 36–72 | 51 | 40 | 65 |
| PCP5B | 72–149 | 104 | 80 | 91 | 36–72 | 51 | 48 | >72 | 36–72 | 51 | 49 | 74 |

[a] NOEC (no–observed effect concentration) = highest concentration tested without significant difference from control; LOEC (lowest–observed effect concentration) = first concentration above NOEC.
[b] IC10 (10% inhibition concentration) = concentration associated with 10% decrease in test end point relative to control; IC25 = 25% inhibition concentration.
ChV = Chronic value.

copper), Besser *et al.* (2001) concluded that growth measured as total length was somewhat less sensitive than growth measured as dry weight. LOECs for dry weight were equal to or less than those for total length, and IC25s for dry weight were always less than those for total length. However, thresholds for effects of copper on growth of fathead minnows showed relatively high variation among repeated tests. Some of these tests suggested unusually low effect concentrations for growth (e.g., test Cu2; Table 3). Reversals of concentration–response curves in low-survival treatments suggested a compensatory response of growth to decreased density, decreased competition, or increased food supply (Besser *et al.* 2001). Although the influence of density on the growth end point was decreased by excluding growth data from low-survival treatments, density-dependent responses could have contributed to among-test variation in the growth endpoint. A critical review of early life-stage toxicity tests (Woltering 1984) also noted high variability of the growth end points and attributed this variation to several factors including density-dependent effects and variable power of statistical analyses. Arthur and Dixon (1994) also reported that toxic effects of PCP were affected by stocking density of fathead minnows, although we did not observe either density-related responses or high among-test variation of the growth end point in tests with PCP.

Biomass of surviving fish in a replicate group was a sensitive and consistent response of all 4 fish species in tests with both chemicals. LOECs for biomass were at least as sensitive as those for survival or growth for fountain darters and spotfin chubs in copper tests and for all four species in PCP tests (Tables 3 and 4). The sensitivity of the biomass end point was even more apparent when effects levels are expressed as IC25s. The IC25 for biomass was less than the IC25 for growth, often by wide margins, in 12 of the 15 tests. In only 1 test with 1 species (fathead minnow in test Cu2), the IC25 for growth was marginally less than that for biomass (11 vs. 13 μg/L). In repeated tests with copper, biomass of fathead minnows did not show the same high among-test variation as growth (Fig. 1a). Although LOECs for biomass were more variable than those for growth in tests with PCP, IC*p*s for biomass were generally less variable than those for growth (Fig. 1b). The sensitivity of the biomass end point reflects the fact that decreased biomass can result from decreased survival, decreased growth of survivors, or some combination of these responses. The greater consistency of the biomass end point in our studies suggests that it is less sensitive to density-related effects than the growth end point.

Effect concentrations estimated by the linear interpolation method provided a useful tool to evaluate the reliability of LOECs derived by hypothesis testing. Although most IC25 values were similar to LOECs, the IC*p* method provided a more consistent interpretation of results from several studies. The principal advantage of the IC*p* method was that it estimated toxicant concentrations associated with fixed percent decreases of toxicity end points relative to controls. In contrast, statistically detectable differences between treatment and control groups depend on the statistical power of ANOVA, which differs among studies because of factors such as experimental design and within-treatment and within-control variance. Despite the consistent experimental designs of our studies, LOECs corresponded to a wide range in percent decreases in survival, growth, or biomass relative to controls (9% to 76%). Approximately one half (48%) of LOECs fell within the range of 10% to 25% effects relative to controls defined by IC*p* calculations, but a substantial fraction of LOECs (44%) were greater than corresponding IC25s. The IC*p* method helped identify apparently aberrant LOECs that suggested extreme among-test variation in tests with fathead minnows. The LOEC for growth in test Cu2 and the LOEC for biomass in test PCP2 were 63% and 88% lower than the next lowest LOECs for these end points, but the corresponding IC25s differed from results of other tests by no more than 27%.

Nonlinear regression models, such as probit or logistic regression, have also been suggested as an alternative to ANOVA. Regression can generate point estimates of a desired level of effect (e.g., EC25 or LC50) and estimate the statistical uncertainty associated with these estimates (Stephan and Rog-

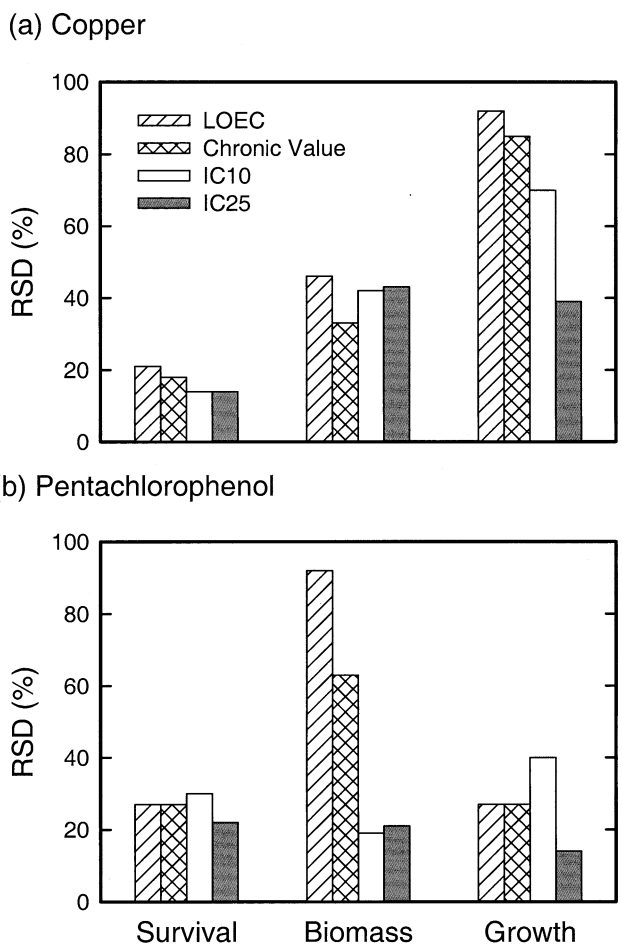


**Fig. 1.** Variation of effect concentrations based on three test end points among repeated early life-stage tests with fathead minnows (a) Copper (b) Pentachlorophenol. Mean RSD: n = 3 for copper and n = 4 for pentachlorophenol. RSD = relative standard deviation as percent of mean

ers 1985). However, successful use of nonlinear models requires a high-quality data set such that iterative curve-fitting procedures can converge on a solution that adequately fits the data. Even regression models that provide a good overall fit to the data may fail to adequately define the response in the low-effect portion of the curve that is usually of greatest interest, especially for listed species. We were able to derive logistic regression models that adequately fit the low-effect portion of concentration–response curves for > 20% of possible end points from our tests (Table 5), whereas IC25s were calculable for nearly 80% of end points. For end points where both methods could be used successfully, EC25s based on logistic regressions were consistent with IC25s derived by linear interpolation as indicated by an average RPD of 6.6% (Table 5).

There is considerable interest in estimates of chemical concentrations associated with low-level effects (i.e., < 25% inhibition of toxicity end points) for listed species. Concentrations associated with 10% inhibition of test responses (IC10s) could be calculated by the linear interpolation method for approximately 75% of our test end points (Tables 3 and 4) and by the nonlinear regression methods for approximately 20% of end points (Table 5). The ability to estimate IC10s was limited primarily by the inability of the algorithm to estimate IC10s in the range between the two lowest concentrations tested (USEPA 1994). One possible limitation of using IC10s to estimate thresholds for low-level toxic effects is the greater degree of uncertainty associated with these estimates. Comparison of IC10s with EC10s produced a greater average RPD (16%) than comparisons of IC25s with EC25s (Table 5), suggesting that the uncertainty associated with these estimates increases as the percent effect decreases. Among-test variation of IC10s for fathead minnows was similar to variation in IC25s for survival and biomass end points, but variation of IC10s for growth was consistently greater than that for IC25s (Fig. 1). The reliability of IC10 effect levels should also be considered relative to inherent levels of variability in toxicity test responses. Most standard test methods consider 10% mortality to be "acceptable" performance for animals in control groups (ASTM 2003a; USEPA 1994, 1996b), but a 10% loss of a listed species from chemical exposure may be considered a significant "take."

For our tests with 4 fish species and 2 chemicals, effect concentrations for the biomass end point generated by the linear interpolation method were sensitive and had low among-test variation. Reliance on ANOVA-based LOECs resulted in apparent overestimation or underestimation of sensitivity in several tests with fathead minnows, resulting in high among-test variation (>50% RSD) for sublethal end points, notably the growth end point for copper and the biomass end point for PCP (Fig. 1). The expression of ANOVA-based effect concentrations as chronic values decreased but did not eliminate the variability of effect concentrations for growth and biomass. Among-test variation in IC25 values, which ranged from 10% to 40% RSD, was equal to or lower than that for LOECs or IC10s for all end points for both chemicals (Fig. 1). When results of our tests were characterized using IC25s, the biomass end point provided the most sensitive effect concentrations for 15 of 16 tests (Tables 3 and 4).

### *Sensitivities of Listed and Commonly Tested Species*

The two listed species, fountain darters and spotfin chubs, represented the extremes of sensitivity to the toxic effects of copper of the four species tested. Both IC*p*s and chronic values for effects of copper on biomass of fountain darters were less than those for the other three species (Fig. 2). These differences among species were even more pronounced for effects on survival (Table 3). Although chronic values for effects of copper on biomass were similar between the spotfin chub and rainbow trout, IC25s for biomass (Fig. 2) and all other end points (Table 3) were from 30% to > 300% greater for spotfin chubs than for the other species. Previous studies have reported that the fountain darter is highly sensitive to acute toxicity of copper. Dwyer *et al.* (2004) found that fountain darters were the most sensitive of 14 species tested in acute toxicity tests with copper in ASTM hard water (170 mg/L as $CaCO_3$). Median lethal concentrations (LC50) of copper in 96-hour tests were 57 μg/L for the fountain darter and 90 μg/L for the spotfin chub compared with 80 μg/L for rainbow trout and 470

**Table 5.** Comparison of effect concentrations determined by logistic regression (EC10 and EC25) and linear interpolation (IC10 and IC25)[a] Differences between paired values (EC10/IC10, EC25/IC25) are expressed as relative percent difference (RPD = difference/mean, expressed as percent)

| Toxicant | Effect Concentration (μg/L) | | | | | |
|---|---|---|---|---|---|---|
| Species - End point - Test | EC10 | IC10 | RPD (%) | EC25 | IC25 | RPD (%) |
| Copper | | | | | | |
| Minnow - biomass - 2 | 6.0 | 6.9 | 14 | 13 | 13 | 2.3 |
| Minnow - biomass - 3 | 18 | 15 | 13 | 25 | 24 | 2.1 |
| Trout - biomass - 4A | 18 | 15 | 17 | 22 | 21 | 2.4 |
| Trout - biomass - 4B | 18 | 16 | 22 | 27 | 25 | 7.7 |
| Pentachlorophenol | | | | | | |
| Minnow - survival - 3 | 90 | 83 | 7.5 | 131 | 112 | 16 |
| Minnow - biomass - 1 | 107 | 88 | 8.8 | 153 | 143 | 6.1 |
| Minnow - biomass - 3 | 82 | 76 | 7.0 | 109 | 102 | 6.6 |
| Chub - biomass - 4 | 47 | 48 | 17 | 81 | 75 | 6.4 |
| Trout - biomass - 5A | 60 | 40 | 40 | 71 | 65 | 8.1 |
| Mean | | | 16 | | | 6.6 |

[a] Differences between paired values (EC10/IC10, EC25/IC25) are expressed as RPD.
EC = Effect concentration.
IC = Inhibition concentration.
RPD = Relative percent difference expressed as percent.

μg/L for fathead minnow. The high acute copper LC50 for fathead minnows is consistent with the low sensitivity of the survival end point in our chronic tests. Fountain darters are apparently more sensitive to copper toxicity than other darters (Percidae: Etheostominae). Dwyer *et al.* (2004) found that greenthroat darters (*E. lepidum*) were less sensitive to copper than fountain darters with a 96-hour LC50 of 260 μg/L. Previous acute toxicity tests with copper in a water of similar hardness (200 mg/L as $CaCO_3$) found 96-hour LC50s of 320 and 850 μg/L, respectively, for rainbow darters (*E. caeruleum*) and orangethroat darters (*E. spectabile*) and 440 to 490 μg/L for fathead minnows (Geckler *et al.* 1976). One-year chronic exposures of 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 minnow or bluntnose minnows, *Pimephales notatus*) at copper concentrations ranging from 91 to 107 μg/L (Geckler *et al.* 1976).

Fountain darters were significantly affected by chronic exposure to copper concentrations well below the current WQC. Chronic values and IC25s for survival and biomass, and the chronic value for growth were between 8 and 9 μg/L (Table 3), less than the national criterion of 14.7 μg/L at a hardness of 170 mg/L (Figure 2; USEPA 1996a, 1999). Dwyer *et al.* (2004) concluded that the fountain darter would be adequately protected by the national acute WQC for copper. However, the WQC for chronic exposure to copper was derived from the acute copper criterion using an acute–chronic ratio (ACR) of 2.823, an average value derived from testing of several species (USEPA 1996a). The lesser protectiveness of the WQC for chronic exposure of fountain darters reflects the much larger ACR (7.125) derived from our chronic exposures and the acute tests conducted in our laboratory by Dwyer *et al.* (2004).

Fountain darters were also the most sensitive species in chronic tests with PCP. Effect concentrations for fountain darters were lower by a factor of three than those for spotfin chubs or rainbow trout and lower by a factor of six than those for fathead minnows (Fig. 2). IC25s for effects of PCP on biomass (69 μg/L for rainbow trout and 142 μg/L for fathead minnows) were near the low end of the range of published chronic values for rainbow trout (36 to 274 μg/L) and fathead minnows (66 to 393 μg/L) after these values were adjusted to the same pH (USEPA 1986, 1996a; Hickie and Dixon 1987; Hamilton *et al.* 1986; Arthur and Dixon 1994). The current chronic WQC for PCP (24 μg/L at pH 8.3) is adequate to protect one of the two listed species, spotfin chub, and both commonly tested species but may not adequately protect the listed fountain darter. The chronic value and IC25s for biomass of fountain darters were approximately equal to the WQC, but the IC10 for this end point was lower (<15 μg/L), suggesting that biologically significant effects would occur below the WQC (Fig. 2). In contrast, IC10s for the other three species were greater than the WQC by at least a factor of two, suggesting that no adverse effects would be expected. USEPA (1997) has proposed a revised WQC for PCP for application under the California Toxics Rule that would provide slightly greater protection (chronic WQC = 21 μg/L), but it would still be greater than the biomass IC10 for fountain darters. We calculated ACRs for the four species based on chronic values for biomass and 96-hour LC50s for juvenile fish tested in our laboratory: fountain darter 160 μg/L; spotfin chub 320 μg/L; fathead minnow 450 μg/L; and rainbow trout 150 μg/L (N. Wang [CERC], unpublished data, 2000). As was the case for copper, both the ACR for fountain darters and the average ACRs for the four species tested (5.61) were greater than the average ACR used to derive the PCP criterion (3.166; USEPA 1986).

### *Strategies for Protection of Listed Species*

Our results suggest that listed fish species are not consistently more sensitive to chronic toxic effects of waterborne pollutants

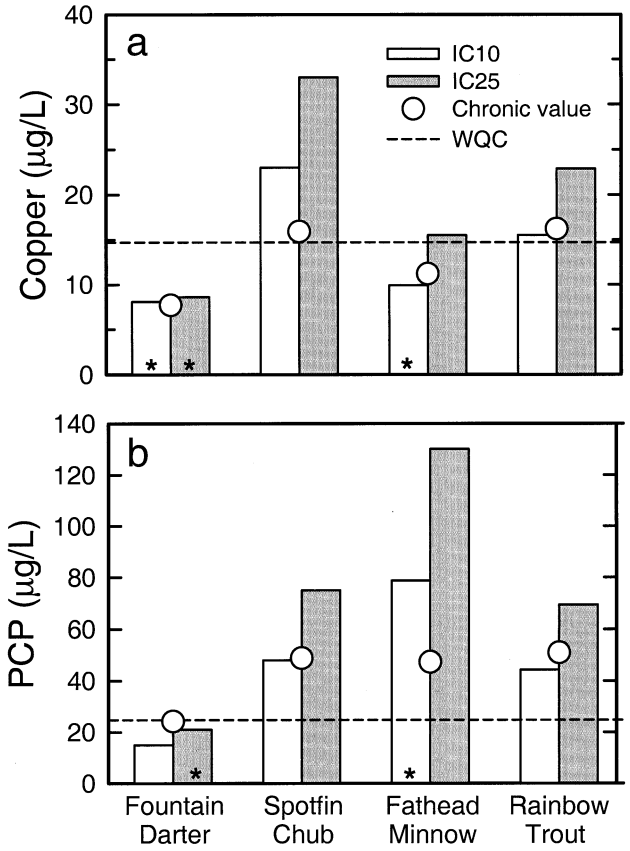


**Fig. 2.** Sensitivity of chronic effect concentrations for biomass (total dry weight per replicate) for four fish species (a) Copper (b) Pentachlorophenol. Values are results of individual tests or geometric means of results from multiple tests. Asterisks indicate means calculated using ≥1 "less than" values (Tables 3 and 4). Horizontal lines indicates chronic WQC (WQC for copper adjusted for hardness of 170 mg/L as $CaCO_3$, and WQC for PCP adjusted for pH 8.3; USEPA 1996). WQC = water-quality criteria

than are two commonly tested species often used to derive national chronic WQCs. However, 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" (i.e., loss of individuals or critical habitat) associated with exposure to toxic chemicals. This is a greater degree of protection than that expected from national WQCs, which are expected to protect 95% of species tested from "unacceptable effects" (USEPA 1985). Although standard toxicity tests and standard methods for analysis of toxicity test data are not amenable to establishing standards to protect individual organisms, efforts to provide greater levels of protection from decreases in survival, growth, and biomass at the population level will correspond to greater levels of protection for individual organisms.

One approach for improving the protectiveness of chronic WQCs may be to increase the sensitivity and consistency of effect concentrations derived from chronic toxicity tests. We found that the percent decrease of toxicity end points associated with chronic values derived by ANOVA, the standard effect concentration recommended for developing WQCs, could vary substantially among repeated tests conducted with the same chemical, test organism, and experimental design. This inconsistency can be decreased by using nonlinear regression or linear interpolation methods to derive effect concentrations based on consistent percent effects (Tables 3 and 4). The $IC_p$ method has the advantage that it can be calculated from less-extensive data sets than are needed for regression, although we recommend that ANOVA should be conducted to establish the overall statistical significance of observed effects before $IC_p$ calculations are used. We found that IC25s derived by linear interpolation had lower among-test variation than ANOVA-based chronic values (Fig. 1). Although IC10s would provide a greater degree of protection than IC25s, IC10 values also showed relatively high among-test variation. A more reliable approach for estimating thresholds for effects at the 10% inhibition level may be to extrapolate from IC25s. Across the chemicals, species, and end points we tested, IC10 values averaged 32% (range 8% to 74%) lower than corresponding IC25s. Applying safety factors of 0.5, 0.33, or 0.25 to IC25 values from our tests would protect the species we tested against 10% inhibition of particular end points in 84%, 95%, and 100% of cases, respectively.

A major limitation in developing chronic WQCs capable of protecting a wide range of fish species is the scarcity of suitable chronic toxicity data. Chronic WQCs for most chemicals, including current criteria for both copper and PCP, are derived from acute-toxicity data using average ACRs for several species. In some cases, average ACRs may be inadequate to extrapolate accurately from acute to chronic values, as is evident from comparisons of our chronic toxicity results with the current chronic WQCs for copper and PCP (Fig. 2). The limitations of the ACR approach were noted in a recent draft for a revised WQC for copper, which derived a proposed new chronic criterion based only on chronic toxicity data (Great Lakes Environmental Center 1998). This approach, when combined with the greater range of chronic toxicity studies conducted since the previous revision (USEPA 1996a), generated a draft chronic criterion that would be adequately protective for the listed and commonly tested species we tested (3.4 µg/L at the hardness of our tests; Great Lakes Environmental Center 1998). Although this approach is desirable, wholesale revision of chronic WQCs is not a short-term solution for protection of listed species from chronic effects of environmental contaminants.

Guidelines for development of WQCs acknowledge that adverse effects may occur in some species at or below these criteria and endorse the modification of WQCs to meet site-specific conditions or to protect "important species" (USEPA 1994). An interim approach for increasing protection of listed species would be to apply "safety factors" that would apply to areas where listed species occur or to designated critical habitats. Dwyer *et al.* (2004), based on toxicity tests with 17 listed species and 5 chemicals (copper, pentachlorophenol, 4-nonylphenol, carbaryl, and permethrin), 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 current chronic WQCs for copper and PCP, would provide an appropriate margin of protection for the 2 listed species we tested (Fig. 2). However, such safety factors are based on data for only a limited number of species and chemicals, and it is uncertain whether this

approach would adequately protect the large number of listed species that have not been evaluated in any toxicity tests (>100 species; personal communication, T. Augspurger, USFWS). Our experience from the current study and other recent studies in our laboratory (Dwyer *et al.* 1999, 2004; Sappington *et al.* 2001) indicates that standard toxicity test methods can be successfully applied to many listed species. Additional chronic testing with listed species would be most appropriate for species that are known or suspected to encounter increased concentrations of particular toxic chemicals in their native habitats.

*Acknowledgments.* We thank Eugene Greer for culturing the test organisms and Doug Hardesty, David Whites, Chris Ivey, James Kunz, and Eric Brunson for their technical assistance during testing. We also thank Tom Brandt of the USFWS National Fish Hatchery and Technology Center (San Marcos, TX) and Pat Rakes and J.R. Shutes of Conservation Fisheries, Inc. (Knoxville, TN) for providing the fountain darters and spotfin chubs, respectively. We thank Tom May, Ray Wiedmeyer, and William Brumbaugh for conducting the copper analyses and Carl Orazio, Kevin Feltz, and Mike Tanner for conducting PCP analyses. We appreciate the thorough reviews of the manuscript by Denny Buckler and Steve Hamilton of CERC; Tom Augspurger of US Fish and Wildlife Service; William Fisher, Larry Goodman, and Mike Lewis of USEPA; and three anonymous reviewers. This research was funded by USEPA, Office of Water (Project No. DW14-937809-01-0) and Office of Research and Development (Project No. DW-14-93900201-0). Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

## References

American Public Health Association, American Water Works Association, Water Environment Foundation (1995) Standard methods for the examination of water and waste water, 19th ed. American Public Health Association, Washington, DC

American Society for Testing and Materials (2003a) Standard guide for conducting early life-stage toxicity tests with fishes (E1241-98). In: Annual book of ASTM standards. Volume 11.05. American Society for testing and Materials, West Conshohocken, Pennsylvania, p. 550

American Society for Testing and Materials (2003b) Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians (E729-96). In: Annual book of ASTM standards. Volume 11.05. American Society for Testing and Materials, West Conshohocken, Pennsylvania, p. 220

Arthur AD, Dixon DG (1994) Effects of rearing density on the growth response of juvenile fathead minnow (Pimephales promelas) under toxicant-induced stress. Can J Fish Aquat Sci 51:365–371

Besser JM, Dwyer FJ, Ingersoll CG, Wang N (2001) Early life-stage toxicity of copper to endangered and surrogate fish species (EPA/600/R-01/051). United States Environmental Protection Agency, Washington, DC

Beyers DW, Keefe TJ, Carlson CA (1994) Toxicity of carbaryl and malathion to two federally endangered fishes as estimated by regression and ANOVA. Environ Toxicol Chem 13:101–107

Beyers DW (1995) Acute toxicity of Rodeo registered herbicide to Rio Grande silvery minnow as estimated by surrogate species: Plains minnow and fathead minnow. Arch Environ Contam Toxicol 29:24–26

Bonner TH, Brandt TM, Fries JN, Whiteside BG (1998) Effects of temperature on egg production and early life stages of the fountain darter. Prog Fish Culturist 127:971–978

Crane M, Newman MC (1999) What level of effect is a no observed effect? Environ Toxicol Chem 19:516–519

Dominguez SE, Chapman GA (1984) Effect of pentachlorophenol on the growth and mortality of embryonic and juvenile steelhead trout. Arch Environ Contam Toxicol 13:739–743

Dwyer FJ, Hardesty DK, Henke CE, Ingersoll CG, Whites DW, Mount DR, et al. (1999) Assessing contaminant sensitivity of endangered and threatened species: Effluent toxicity tests (EPA/600/R-99/099). United States Environmental Protection Agency, Washington, DC

Dwyer FJ, Mayer FL, Sappington, LC, Buckler DR, Bridges CM, Greer IE, Hardesty DK, et al. (2005) Assessing contaminant sensitivity of endangered and threatened aquatic species: Part I. Acute toxicity of five chemicals. Arch Environ Contam Toxicol 48:143–154

Geckler JR, Horning WB, Neiheisel TM, Pickering QH, Robinson EL, Stephan CE (1976) Validity of laboratory tests for predicting copper toxicity in streams (EPA-600/3-76-116). United States Environmental Protection Agency, Duluth, MN

Great Lakes Environmental Center (1998, unpublished) Ambient aquatic life water quality criteria for copper. Report submitted to USEPA Office of Research and Development, Duluth MN. Great Lakes Environmental Center, Traverse City, MI

Hamilton SJ, Cleveland L, Smith LM, Lebo JA, Mayer FL (1986) Toxicity of pure pentachlorophenol and chlorinated phenoxyphenol impurities to fathead minnows. Environ Toxicol Chem 5:543–552

Hickie BE, Dixon DG (1987) The influence of diet and pre-exposure on the tolerance of sodium pentachlorophenate by rainbow trout (Salmo gairdneri). Aquat Toxicol 9:343–353

Marchini S, Tosato M, Norberg-King TJ, Hammermeister DE, Hoglund MD (1992) Lethal and sublethal toxicity of benzene derivatives to the fathead minnow using a short-term test. Environ Toxicol Chem 11:187–195

May TW, Wiedmeyer RH, Brumbaugh WG, Schmitt CJ (1997) The determination of metals in sediment pore waters and in 1N HC 1-extracted sediments by ICP-MS. At Spectrosc 18:133–139

McKim JM, Eaton JG, Holcombe GW (1978) Metal toxicity to embryos and larvae of eight species of freshwater fish. II. Copper. Bull Environ Contam Toxicol 19:608–616

Orazio CE, Kapila S, Manahan SE (1983) High-performance liquid chromatographic determination of phenols as phenolates in a complex mixture. J Chromatog 262:434–440

Sappington LC, Mayer FL, Dwyer FJ, Buckler DR, Jones JR, Ellersieck MR (2001) Contaminant sensitivity of threatened and endangered fishes compared to standard surrogate species. Environ Toxicol Chem 20:2869–2876

Seim WK, Curtis LR, Glenn SW, Chapman GA (1984) Growth and survival of developing steelhead trout (Salmo garigneri) continuously or intermittently exposed to copper. Can J Fish Aquat Sci 41:433–438

Stephan CE, Rogers JW (1985) Advantages of using regression analysis to calculate results of chronic toxicity tests. In Bahner RC, Hansen DJ (eds) Aquatic toxicology and hazard assessment: Eighth symposium (STP 891). American Society for Testing and Materials, Philadelphia, PA, p. 328

United States Environmental Protection Agency (1985) Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses (EPA/833/R-85/100). United States Environmental Protection Agency, Washington, DC

United States Environmental Protection Agency (1986) Ambient water quality criteria for pentachlorophenol—1986 (EPA 440/5-86-009). United States Environmental Protection Agency, Duluth, MN

United States Environmental Protection Agency (1994) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms (EPA/600/4-91/002). United States Environmental Protection Agency, Washington, DC

United States Environmental Protection Agency (1996a) 1995 updates: Water quality criteria documents for the protection of aquatic life in ambient water (EPA-820-B-96-001). United States Environmental Protection Agency, Washington, DC

United States Environmental Protection Agency (1996b) Environmental effects test guidelines (EPA 712-C-96-121). United States Environmental Protection Agency, Washington, DC

United States Environmental Protection Agency (1997) Water quality standard: Establishment of numeric criteria for priority toxic pollutants for the State of California. Federal Register 62 (150): 42159–42208

United States Environmental Protection Agency (1999) National recommended water quality criteria—Correction EPA 822-Z-99-001. United States Environmental Protection Agency, Washington DC

United States Environmental Protection Agency (2000) Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates (EPA/600/R-99/064). United States Environmental Protection Agency, Duluth, MN

Wolf K, Rumsey G (1985) The representative research animal: Why rainbow trout (Salmo gairdneri Rich.)? J Appl Ichthyol 1:131–138

Woltering DM (1984) The growth response in fish chronic and early life stage toxicity tests: A critical review. Aquat Toxicol 5:1–21