

CONTAMINANT SENSITIVITY OF THREATENED AND ENDANGERED FISHES
COMPARED TO STANDARD SURROGATE SPECIESLINDA C. SAPPINGTON,*† FOSTER L. MAYER,‡ F. JAMES DWYER,§ DENNY R. BUCKLER,† JOHN R. JONES,|| and
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Abstract—Standard environmental assessment procedures are designed to protect terrestrial and aquatic species. However, it is not known if endangered species are adequately protected by these procedures. At present, toxicological data obtained from studies with surrogate test fishes are assumed to be applicable to endangered fish species, but this assumption has not been validated. Static acute toxicity tests were used to compare the sensitivity of rainbow trout, fathead minnows, and sheepshead minnows to several federally listed fishes (Apache trout, Lahontan cutthroat trout, greenback cutthroat trout, bonytail chub, Colorado pikeminnow, razorback sucker, Leon Springs pupfish, and desert pupfish). Chemicals tested included carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin. Results indicated that the surrogates and listed species were of similar sensitivity. In two cases, a listed species had a 96-h LC50 (lethal concentration to 50% of the population) that was less than one half of its corresponding surrogate. In all other cases, differences between listed and surrogate species were less than twofold. A safety factor of two would provide a conservative estimate for listed cold-water, warm-water, and euryhaline fish species.

Keywords—Endangered fishes Acute toxicity *Oncorhynchus mykiss* *Pimephales promelas* *Cyprindodon variegatus*

INTRODUCTION

The U.S. Environmental Protection Agency (U.S. EPA) is the primary federal agency that regulates the registration of chemical substances in the United States. This authority is granted primarily within three statutes: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA; PL80-140), the Toxic Substance Control Act (TSCA; PL94-469), and the Clean Water Act (CWA; Section 101(a)(3)). The FIFRA is used to regulate pesticides that are manufactured specifically for their toxicity and are intended for direct application to the environment. The TSCA regulates the production, use, transportation, and disposal of chemicals of commerce, excluding pesticides. The CWA prohibits the discharge of pollutants in toxic amounts to water bodies of the United States.

The Endangered Species Act (Act), enacted in 1973, affords additional environmental protection. Section 7 of the Act requires Federal agencies to insure that any action authorized, funded, or carried out by them is not likely to jeopardize the continued existence of listed species or modify their critical habitat. Miller et al. [1] reported that, during the last 100 years, 3 genera, 27 species, and 13 subspecies of North American fishes have become extinct. Factors contributing to the decline and extinction of 82% of the fishes are habitat alteration, introduction of exotic species, and hybridization. Chemicals,

habitat alteration, and pollution were also noted as contributing to extinction 38% of the time.

The U.S. EPA and the U.S. Fish and Wildlife Service (U.S. FWS) have been cooperating to determine the effects of pesticides and other chemicals on listed species under Section 7 of the Act. In June 1989, a biological opinion [2] was completed by the U.S. FWS that listed 165 threatened and endangered species (primarily aquatic) in association with 112 chemicals. Additionally in July 1989, the U.S. EPA published its proposed Endangered Species Protection Program in the Federal Register [3].

Protection of endangered species from chemical hazards is primarily based on standardized toxicity tests using standard test organisms as surrogates for other species. Toxicity testing under FIFRA may require four categories of data, including acute toxicity tests with freshwater, estuarine, and marine fish and invertebrates; embryo-larval and life-cycle studies with fish and invertebrates; residue studies; and field testing based on a four-tier testing regime.

The CWA provides an integrated approach to protection of aquatic ecosystems through the development of water quality criteria and the control of toxic discharges (National Pollutant Discharge Elimination System). Water quality criteria are derived to protect aquatic organisms from unacceptable adverse effects. The Water Quality Criteria, promulgated under the CWA, uses a community-based statistical approach based on a minimum multispecies data base [4]. As part of the National Pollutant Discharge Elimination System permit system, protection of freshwater aquatic environments from toxic discharges commonly includes whole effluent toxicity tests with

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This paper has been reviewed in accordance with U.S. Environmental Protection Agency policy. Reference to trade names or manufacturers does not imply government endorsement of commercial products.

Ceriodaphnia dubia, fathead minnows, and the alga (*Selenastrum capricornutum*) [5].

In these programs, there is allowance for some adverse effects, including a small reduction in survival, growth, or reproduction in sensitive species [4]. It is assumed that the test species used for toxicity assessments are generally protective of other species, including those that are threatened or endangered. However, Mayer and Ellersieck [6] compiled an acute toxicity data base for 410 chemicals and 66 species of freshwater animals and reached three conclusions, which were that, for a given chemical, acute toxicity among species ranged over five orders of magnitude; for a given species, acute toxicity among chemicals ranged over nine orders of magnitude; and no single species was always the most sensitive to all chemicals.

Surrogate species are typically organisms that are easily tested using standardized methods. However, these species may or may not represent the sensitivity of populations of threatened and endangered (listed) species. The wide use of pesticides and other commercial chemicals potentially poses a risk to threatened and endangered species because, by definition, the distribution of listed species is limited and further adverse effects on these populations could lead to further extinction. Under current regulations, listed species may not be protected, or conversely, unnecessary regulatory programs may be implemented if their sensitivity to toxic chemicals is not evaluated.

The selection of surrogate species used in aquatic toxicity testing is critical to the regulatory processes because of the need to be predictive of a large number of species, including imperiled species, that may be exposed to chemicals. At present, toxicological data obtained from studies with surrogate test fishes are assumed to be applicable to endangered fish species, but this assumption has not been validated.

The freshwater species rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) and the saltwater species sheepshead minnow (*Cyprindodon variegatus*) are recognized as resident North American species used in toxicity tests [4]. Many toxicity assessments, including the derivation of water quality criterion calculation or pesticide registration, utilize these fish species. Our study objective was to determine the applicability of using rainbow trout, fathead minnows, and sheepshead minnows as surrogate test species for listed fishes. To meet this objective, we conducted 130 static acute toxicity tests over a two-year period using five chemicals with three standard test species, i.e., rainbow trout, fathead minnow, and sheepshead minnow. Listed species tested were Apache trout (*Oncorhynchus apache*), Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*), greenback cutthroat trout (*Oncorhynchus clarki stomias*), bonytail chub (*Gila elegans*), Colorado pikeminnow (*Ptychocheilus lucius*), razorback sucker (*Xyrauchen texanus*), Leon Springs pupfish (*Cyprindodon bovinus*), and desert pupfish (*Cyprindodon macularius*). Static tests were used to prevent listed species from escaping into nonnative habitats.

MATERIALS AND METHODS

Test organisms

Freshwater static acute toxicity tests for cold-water species were conducted with rainbow trout (mean wt \pm standard deviation, 0.71 ± 0.38 g) and three listed species of salmonids, i.e., Apache trout (0.62 ± 0.33 g), Lahontan cutthroat trout (0.46 ± 0.16 g), and greenback cutthroat trout (0.31 ± 0.17

g). Freshwater static acute toxicity tests for warm-water species were conducted with fathead minnows (0.41 ± 0.09 g) and two listed species of cyprinids, i.e., bonytail chub (0.41 ± 0.17 g) and Colorado pikeminnow (0.33 ± 0.01 g) and one catostomid, the razorback sucker (0.32 ± 0.01 g). Saltwater static acute tests were conducted with the euryhaline sheepshead minnow (0.24 ± 0.03 g) and two listed species of pupfish, i.e., Leon Springs pupfish (0.42 ± 0.09 g) and desert pupfish (1.27 ± 0.57 g, adults).

Rainbow trout, Apache trout, Lahontan cutthroat trout, greenback cutthroat trout, bonytail chub, Colorado pikeminnow, razorback sucker, sheepshead minnow, Leon Springs pupfish, and desert pupfish were obtained from various government and commercial sources [7]. Fish for freshwater testing were received during the spring and summer of 1992 and 1993 as eyed eggs at the Columbia Environmental Research Center ([CERC] Columbia, MO, USA). Fathead minnows were obtained from cultures at the CERC or were purchased commercially. Sheepshead minnows were from TRAC Laboratory cultures (Gulf Breeze, FL, USA). Leon Springs pupfish were received during the summer of 1994 as pond-reared juveniles, and desert pupfish were received as adults from the U.S. FWS National Fish Hatchery and Technology Center (Dexter, NM, USA). All listed fishes used were from surplus stock produced in excess of U.S. FWS needs for restocking or reintroduction.

Once received at the CERC, freshwater fish were cultured in flowing well water (alkalinity 258 mg/L as CaCO_3 , hardness 286 mg/L as CaCO_3 , pH 7.8) until testing began. All fish were cultured at 18°C except that salmonids were hatched and cultured until swim-up in well water chilled to 10°C. Pond-reared Leon Springs and desert pupfish were received and held in natural Gulf of Mexico seawater diluted with deionized water to 2‰. Prior to dilution, the seawater was analyzed for all priority pollutants, with arsenic (10 $\mu\text{g/L}$) being the only one equal to or greater than U.S. EPA reporting limits. Desert pupfish were received as adults and were used in only one test with carbaryl due to the small numbers of individuals available.

Before the start of the freshwater toxicity tests, fish were acclimated to the test water over a 4-d period [8,9]. Freshwater fish were incrementally acclimated to the test water and temperature during the first 48 h, moved to clean containers, and held for an additional 48 h at the test temperature in 100% test water. Fish were fed during the first 48 h of acclimation but were not fed during the last 48 h of acclimation or during the test. Saltwater fish were acclimated to 2‰ natural seawater and 20°C test temperature for 6 d prior to test initiation.

Chemicals

Chemicals (carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin) tested were selected in consultation with U.S. EPA Offices of Pesticide Programs and Pollution Prevention and Toxics to represent different chemical classes and a broad range of toxic modes of action (Table 1). Carbaryl, copper, pentachlorophenol, and permethrin were chosen based on the existence of a large database. Nonylphenol was selected because of a lack of toxicity data, its widespread use in the manufacturing of nonylphenol ethoxylate detergents, and its continuing occurrence in the environment. In addition, carbaryl, copper, and permethrin currently are on the U.S. FWS pesticide profile list [2], which was developed based on likelihood of exposure at the request of U.S. EPA in accordance with Section 7 of the Act. The profile evaluated pesticides for

Table 1. Sources and percent active ingredient of chemical used in acute toxicity tests with rainbow trout, fathead minnow, sheepshead minnow, and associated listed species

Chemical	Mode of action	Source	Active ingredient (%)
Carbaryl	Cholinesterase inhibition	Rhône-Poulenc Agricultural Co., Research Triangle Park, NC, USA	99.7
Copper sulfate	Alters cell permeability	Fisher Chemical, St. Louis, MO, USA	25.5
4-Nonylphenol	Up-regulates vitellogenin and is suspected estrogen-mimicking compound	Fluka Chemical, New York, NY, USA	85.0
Pentachlorophenol	Oxidative phosphorylation uncoupler	Aldrich Chemical, Milwaukee, WI, USA	99.0
Permethrin	Neurotoxin	IC America, Richmond, CA, USA	95.2

agricultural, forestry, rangeland, and pastureland use that might jeopardize aquatic species.

Carbaryl is a carbamate insecticide that inhibits cholinesterase activity [10]. Copper occurs from mining, industrial applications, and in fungicide formulations. Copper alters the permeability of cellular membranes such as those associated with gills [11]. Nonylphenol is a monoalkyl phenol that has been shown to up-regulate vitellogenin expression in rainbow trout and is a suspect estrogen-mimicking compound [12]. Pentachlorophenol is a chlorinated phenol used as a wood preservative and molluscicide and is an uncoupler of oxidative phosphorylation. Permethrin is a pyrethroid insecticide and causes neurotoxicity [13]. Organic chemical stocks were prepared by dissolving the chemical in reagent-grade acetone or triethylene glycol, while copper was dissolved in deionized water. Maximum volume of solvent added to any test container was 7.5 ml (0.5 ml/L).

Freshwater tests

Freshwater static acute toxicity tests were conducted in accordance with U.S. EPA [8] and the American Society for Testing and Materials [9] procedures. Fish exposures were conducted in 20-L glass jars containing 15 L of test solution. Salmonids were tested at 12°C and cyprinids and the catostomid at 22°C. Test water was reconstituted hard water (alkalinity 110–120 mg/L as CaCO₃, hardness 160–180 mg/L as CaCO₃). Water quality (alkalinity, hardness, and pH) was measured on each batch of reconstituted water. Dissolved oxygen was measured in the control, low-, medium-, and high-exposure concentrations at 0 h and in the same exposures, if fish survived, at 48 and 96 h of exposure. Additionally, pH was measured in the control, low, medium, and high concentrations at 0 h and in the same treatments, if fish survived, at 96 h of exposure. Tests were conducted under ambient laboratory lighting conditions.

Toxicity tests were conducted in each of two years with each listed species (except greenback cutthroat trout). A test series consisted of six exposure concentrations (three replicates per concentration) with a 60% dilution factor. Both a solvent control and a dilution water control were included for each species (three replicates for each combination). Individual test series were randomly assigned to a waterbath and a location within a waterbath [7].

Due to differences in fish age, size, and availability, all tests could not be conducted concurrently. A surrogate was always tested as a reference with each listed species except during the second year, in which two rainbow trout and two listed trout tests were run separately. This allowed us to de-

termine the repeatability and reproducibility of tests with each fish species and chemical combination.

Fish were counted into groups of five with two groups pooled for each exposure replicate (10 fish/jar or 30 fish/exposure concentration). Mortality was the biological endpoint observed at 12, 24, 48, 72, and 96 h of exposure, and all dead fish were removed at those times. Mortality was defined as a lack of movement for a 5-s observation with the unaided eye. The study design is summarized in U.S. EPA [7].

Saltwater tests

Saltwater static toxicity tests were conducted in accordance with the U.S. EPA [14], American Society for Testing and Materials [8], and TRAC Laboratory's Quality Assurance Plan (Gulf Breeze, FL, USA). Tests were conducted in an environmental chamber in which the temperature was maintained at 20 ± 1.0°C. Test containers were 3.8-L glass jars containing 3 L of spiked or control water. All treatments were duplicated with 10 test animals per replicate, resulting in 20 animals per concentration. Test water was natural seawater diluted with deionized water to 2‰, and lighting was 16 h light:8 h dark. Dissolved oxygen, pH, and temperature were measured in two replicates of all treatments on days 0 through 4. Survival of the test animals was recorded at 24-h intervals.

Statistical analysis

The LC50 (lethal concentration to 50% of the population) and 95% confidence interval at 12, 24, and 96 h of exposure were calculated for each test according to recommendations in Stephan [15]. Probit analysis was normally used. However, when there were no partial responses, LC50s and confidence intervals were calculated using moving average, untrimmed Spearman-Kärber, or a nonlinear interpolative procedure. The data were not corrected for control mortality.

All LC50 calculations were based on nominal concentrations. Except for the saltwater tests, a detailed list of toxicity data (LC50, confidence interval, and slope) for each chemical, species, test (pooled replicates), and time period is given in U.S. EPA [7].

Analysis of variance and least square difference mean tests were used to compare freshwater LC50s at 12, 24, and 96 h of exposure. Differences between surrogate species and listed species were evaluated separately for cold- and warm-water species. Only those tests for which an LC50 could be calculated were used for statistical analysis.

Distribution of LC50s usually cannot be tested for normality due to an insufficient number of LC50 estimates. Thus, a geometric mean (GM) was used to summarize the LC50s

Table 2. Twelve-hour LC50s (lethal concentration for 50% of population) for all chemicals and species tested; toxicity values are the LC50 geometric mean (range; *n*); significant differences from respective surrogates are indicated with asterisks

	Carbaryl (mg/L)	Copper (mg/L)	4-Nonylphenol (mg/L)	Pentachlorophenol (mg/L)	Permethrin (μ g/L) ^a
Cold water					
Rainbow trout	6.8 (5.5–7.5; 4)	0.40 (0.15–1.0; 4)	0.35 (0.27–0.44; 6)	0.2 (0.17–0.29; 6)	5.8 (3.4–8.3; 6)
Apache trout	3.3** (2.6–4.2; 2)	0.18 (0.16–0.21; 2)	0.30 (0.27–0.34; 2)	0.21 (0.19–0.23; 2)	3.9 (3.7–4.1; 2)
Greenback cutthroat	8.5* (NA ^b)	>0.03 (NA)	0.38 (NA)	>0.01 (NA)	>1.0 (NA)
Lahontan trout	4.4** (4.3–4.5; 2)	0.39 (0.28–0.55; 2)	0.29 (0.27–0.3; 2)	0.27 (NA)	3.3 (2.4–4.7; 2)
Warm water					
Fathead minnow	12 (NA)	1.3 (0.9–2.8; 4)	0.38 (0.28–0.45; 6)	0.33 (0.15–0.54; 6)	13.4 (10.3–7.3; 4)
Bonytail chub	7.9 (7.6–8.3; 2)	0.30** (0.25–0.35; 2)	0.56 (0.44–0.72; 2)	0.42 (0.33–0.54; 2)	>25.0 (NA)
Colorado pikeminnow	>10 (NA)	>1.0 (NA)	0.45 (0.44–0.45; 2)	0.23 (0.13–0.40; 2)	>25.0 (NA)
Razorback sucker	8.9 (NA)	>1.0 (NA)	0.29 (0.27–0.31; 2)	0.53 (0.45–0.63; 2)	13.1 (NA)
Euryhaline					
Sheepshead minnow	NA	NA	NA	NA	NA
Leon Springs pupfish	NA	NA	NA	NA	NA
Desert pupfish	NA	No test	No test	No test	No test

^a Note μ g/L for permethrin while all other chemicals are mg/L.

^b NA = not applicable; no LC50 reported or only one observation in test concentration range.

* $p < 0.05$.

** $p < 0.1$.

[16]. Freshwater replicates were pooled within a test and the pooled LC50s were used to calculate a GM for each chemical and species (Tables 2 to 4).

For the saltwater tests, the 95% confidence intervals (CI) were used to determine differences between the surrogate and listed species ($p \leq 0.05$). Differences were considered significant if CIs did not overlap. The CIs were used for comparison because there was only one test per species–chemical combination.

Biological opinions written as a result of consultations held under Section 7 of the Act usually estimate the number of individuals likely to be taken as a result of an action [17]. However, most environmental assessment procedures estimate effects on proportions of populations rather than individual members of a population. Because a greater degree of protection is required for endangered species (i.e., protection of in-

dividuals rather than specified portions of populations), it was necessary to minimize type 2 errors (i.e., acceptance of the null hypothesis that LC50s are the same when they are actually different). For this reason, differences in LC50 values for the freshwater species were considered statistically significant at $p \leq 0.1$. However, the differences at $p \leq 0.05$ were also presented for the freshwater and saltwater tests.

RESULTS

Quality control

Control survival for rainbow trout and listed cold-water species was $\geq 96.7\%$. The fathead minnow and listed warm-water species control survival was 100%. Sheepshead minnow control survival was 100%, Leon Springs Pupfish was $\geq 99\%$ for all tests, and the desert pupfish was $\geq 90\%$ (one test).

Table 3. Twenty-four-hour LC50s (lethal concentration for 50% of population) for all chemicals and species tested; toxicity values are the LC50 geometric mean (range; *n*); significant differences from respective surrogates are indicated with asterisks

	Carbaryl (mg/L)	Copper (mg/L)	4-Nonylphenol (mg/L)	Pentachlorophenol (mg/L)	Permethrin (μ g/L) ^a
Cold water					
Rainbow trout	4.0 (3.4–4.8; 6)	0.12 (0.07–0.23; 4)	0.30 (0.27–0.35; 6)	0.17 (0.13–0.25; 6)	3.8 (3.4–8.3; 6)
Apache trout	2.5** (2.0–3.1; 2)	0.09 (0.08–0.10; 2)	0.24** (0.22–0.25; 2)	0.21 (0.15–0.30; 2)	2.3* (2.0–2.7; 2)
Greenback cutthroat	3.6 (NA ^b)	>0.03 (NA)	0.30 (NA)	>0.01 (NA)	>1.0 (NA)
Lahontan trout	3.6 (3.5–3.6; 2)	0.11 (0.09–0.14; 2)	0.25** (0.23–0.27; 2)	0.23 (0.19–0.28; 2)	1.9** (1.4–2.6; 2)
Warm water					
Fathead minnow	9.8 (9.6–10; 2)	0.73 (0.53–1.7; 4)	0.33 (0.21–0.41; 6)	0.30 (0.14–0.53; 6)	9.7 (9.2–11; 5)
Bonytail chub	6.1 (5.3–7.1; 2)	0.24** (0.22–0.27; 2)	0.49 (0.38–0.62; 2)	0.34 (0.28–0.40; 2)	>25 (NA)
Colorado pikeminnow	6.3 (NA)	0.64 (0.46–0.89; 2)	0.28 (0.28–0.28; 2)	0.16 (0.10–0.27; 2)	>25 (NA)
Razorback sucker	6.7 (5.8–7.6; 2)	0.39 (NA)	0.22 (0.21–0.23; 2)	0.29 (0.27–0.47; 2)	8.9 (NA)
Euryhaline					
Sheepshead minnow	>4.8 (NA)	2.5 (NA)	0.70 (NA)	0.06 (NA)	22 (NA)
Leon Springs pupfish	>8.0 (NA)	>4.8 (NA)	>0.48 (NA)	0.09** (NA)	18 (NA)
Desert pupfish	>8.0 (NA)	NA	NA	NA	NA

^a Note μ g/L for permethrin while all other chemicals are mg/L.

^b NA = not applicable; no LC50 reported or only one observation in test concentration range.

* $p < 0.01$.

** $p < 0.05$.

Table 4. Ninety-six-hour LC50s (lethal concentration for 50% of population) for all chemicals and species tested; toxicity values are the LC50 geometric mean (range; *n*); significant differences from respective surrogates are indicated with asterisks

	Carbaryl (mg/L)	Copper (mg/L)	4-Nonylphenol (mg/L)	Pentachlorophenol (mg/L)	Permethrin ($\mu\text{g/L}$) ^a
Cold water					
Rainbow trout	1.9 (1.2–3.1; 6)	0.08 (0.05–0.11; 4)	0.19 (0.16–0.27; 6)	0.16 (0.12–0.19; 6)	3.3 (1.7–4.8; 6)
Apache trout	1.5 (1.4–1.7; 2)	0.07 (NA) ^b	0.17 (0.16–0.18; 2)	0.11** (0.01–0.11; 2)	1.7* (1.3–2.2; 2)
Greenback cutthroat	1.6 (NA)	>0.03 (NA)	0.15 (NA)	>0.01 (NA)	>1.0 (NA)
Lahontan trout	2.3 (2.0–2.5; 2)	0.07 (0.06–0.08; 2)	0.18 (0.14–0.22; 2)	0.17 (0.16–0.18; 2)	1.6* (1.1–2.2; 2)
Warm water					
Fathead minnow	5.2 (3.9–7.4; 6)	0.47 (0.29–0.81; 6)	0.27 (0.17–0.36; 6)	0.25 (0.14–0.44; 6)	9.4 (6.7–16; 6)
Bonytail chub	3.5** (3.4–3.6; 2)	0.22** (0.2–0.25; 2)	0.29 (0.27–0.31; 2)	0.23 (0.20–0.26; 2)	>25 (NA)
Colorado pikeminnow	3.1** (2.3–4.1; 2)	0.43 (0.38–0.48; 2)	0.26 (0.24–0.27; 2)	0.14 (0.10–0.18; 2)	24 (NA)
Razorback sucker	4.4 (4.3–4.4; 2)	0.27** (0.22–0.34; 2)	0.17 (0.16–0.19; 2)	0.28 (0.27–0.28; 2)	6.0* (4.6–7.7; 2)
Euryhaline					
Sheepshead minnow	4.4	2.5 (NA)	0.46 (NA)	0.05 (NA)	17 (NA)
Leon Springs pupfish	4.5	4.6** (NA)	0.48 (NA)	0.08** (NA)	21** (NA)
Desert pupfish	7.2**	No test	No test	No test	No test

^a Note $\mu\text{g/L}$ for permethrin while all other chemicals are mg/L.

^b NA = not applicable; no LC50 reported or only one observation in test concentration range.

* $p < 0.1$.

** $p < 0.05$.

Water quality for each batch of reconstituted hard water was within acceptable ranges for alkalinity and hardness, but average pH was above 8.0 [7]. Test chemicals added to the test water did not affect the pH. Overall, dissolved oxygen decreases were isolated and interspersed throughout the exposures [7]. For two rainbow trout tests (tests 2 and 4), dissolved oxygen concentrations were below acceptable saturation limits. These two tests had the largest average biomass, which probably contributed to the low dissolved oxygen concentration at 48 and 96 h [7]. However, the LC50s were not significantly different for any other test. Therefore, no tests were eliminated from the statistical analysis because of dissolved oxygen falling below acceptable saturation limits.

Organic and inorganic chemical stocks were analyzed as a confirmation of nominal concentrations. Organic chemical analysis was conducted at either Mississippi State Chemical Laboratory (Mississippi State, MS, USA) or ABC Laboratories (Columbia, MO, USA) using gas chromatography. Copper stocks were confirmed at the CERC by atomic absorption spectrophotometry. The average percent of nominal concentrations were carbaryl 86% ($n = 13$), copper 90% ($n = 13$), 4-nonylphenol 118% ($n = 10$), pentachlorophenol 98% ($n = 12$), and permethrin 111% ($n = 9$). Three individual chemical stocks (copper, pentachlorophenol, and permethrin) had aberrant measured concentrations (percent nominal concentrations of 10, 572, and 308%, respectively). However, biological results from the tests using these stocks were no different than the tests conducted with other stocks for those chemicals. Therefore, the reported values for these samples are suspect and those percent recoveries were not included in calculation of the average percent of nominal concentration.

Toxicity

After 96 h of exposure, fish were most sensitive to permethrin and least sensitive to carbaryl (Tables 2 to 4). The two phenolic compounds (4-nonylphenol and pentachlorophenol) exhibited similar toxicity. For cold-water species, the order of toxicity from most toxic to least toxic was permethrin > copper > pentachlorophenol \geq 4-nonylphenol > carbaryl. For the warm-water and euryhaline species, the order of tox-

icity from most toxic to least toxic was permethrin > pentachlorophenol \geq 4-nonylphenol > copper > carbaryl.

Carbaryl

Fish exposed to higher concentrations of carbaryl were quickly immobilized. Fish dying from carbaryl exposure generally exhibited arched backs, gaping mouths, and flared gills and fins. Apache trout and Lahontan cutthroat trout were significantly more sensitive to carbaryl exposure (3.3 and 4.4 mg/L, respectively) than rainbow trout (6.8 mg/L) at 12 h of exposure (Table 2), while the greenback cutthroat trout were less sensitive (8.5 mg/L). Apache trout were also more sensitive (2.5 mg/L) than rainbow trout (4.0 mg/L) at 24 h of exposure (Table 3). However, by 96 h of exposure (Table 4), there was no significant difference in sensitivity among any cold-water species; LC50s ranged from 1.5 mg/L for Apache trout to 2.3 mg/L for Lahontan cutthroat trout. Warm-water fishes exhibited a different time-dependent relationship than that of cold-water fishes. No significant differences in sensitivity existed at 12 and 24 h of exposure among warm-water species (Tables 2 and 3). But by 96 h of exposure (Table 4), LC50s for bonytail chub (3.5 mg/L) and Colorado pikeminnow (3.1 mg/L) were significantly less than the fathead minnow (5.2 mg/L). While the bonytail chub and Colorado pikeminnow were more sensitive than the fathead minnow, they were less sensitive than rainbow trout. Results from the tests with euryhaline species at 96 h (Table 4) indicated desert pupfish (7.2 mg/L) were significantly less sensitive than the sheepshead minnow (4.2 mg/L) and Leon Springs pupfish (4.5 mg/L). Even though the desert pupfish were five times larger than the sheepshead minnow, the almost twofold difference in desert pupfish and sheepshead minnow 96-h LC50s cannot be attributed to size alone [6]. The sensitivity of euryhaline fishes to carbaryl was similar to that of fathead minnows but 2 to 3.5 times less than that of rainbow trout.

Copper

The sensitivity of listed cold-water species to copper was not significantly different from rainbow trout at 12, 24, or 96 h of exposure (Tables 2 to 4). The LC50s at 96 h ranged from

0.07 mg/L for the Apache and Lahontan trout to 0.08 mg/L for rainbow trout. Bonytail chub were significantly more sensitive to copper than fathead minnows at 12, 24, and 96 h of exposure. At 96 h of exposure, razorback suckers (0.27 mg/L) were more sensitive than fathead minnows (0.47 mg/L). The sheepshead minnows were approximately twice as sensitive to copper as Leon Springs pupfish at 24 and 96 h of exposure. However, as was the case for carbaryl, both surrogate and listed warm-water and euryhaline species were less sensitive to copper than was rainbow trout.

4-Nonylphenol

Fish exposed to higher concentrations of 4-nonylphenol often exhibited increased mucus production and formation of a white particulate material on the fins and gills. No difference in sensitivity existed at 12 h of exposure among cold-water fishes (Table 2). At 24 h, Apache and Lahontan cutthroat trout were significantly more sensitive (0.24 and 0.25 mg/L, respectively) to 4-nonylphenol than rainbow trout (0.3 mg/L, Table 3). However, by 96 h of exposure, all four cold-water species again had similar LC50s (0.15–0.19 mg/L, Table 4). No significant differences existed among the LC50 values for listed warm-water species and fathead minnows at 12, 24, or 96 h of exposure. The 96-h LC50s ranged from 0.17 mg/L for razorback sucker to 0.29 mg/L for bonytail chub. At 96 h of exposure, no significant difference existed between the Leon Springs pupfish and the sheepshead minnow (0.48 and 0.46 mg/L, respectively). Cold- and warm-water species all had similar LC50s, while the euryhaline species appeared to be 1.5 to 2.5 times less sensitive than the rainbow trout and fathead minnow.

Pentachlorophenol

Apache trout were significantly more sensitive to pentachlorophenol (0.11 mg/L) than rainbow trout (0.16 mg/L) at 96 h of exposure (Table 4). No significant differences existed among the LC50s at 12 and 24 h of exposure for any of the cold-water species (Tables 2 and 3). None of the LC50s for the listed warm-water species were significantly different from those for fathead minnows, regardless of time period. The 96-h LC50s for warm-water fish ranged from 0.23 mg/L for bonytail chub to 0.28 mg/L for razorback sucker. Leon Springs pupfish were significantly less sensitive to pentachlorophenol than the sheepshead minnow (96-h LC50s, 0.06 and 0.05 mg/L, respectively). Euryhaline species were three to four times less tolerant than cold- and warm-water species at salinities tested.

Permethrin

Permethrin-exposed fish exhibited hyperactivity (e.g., darting, tremors) prior to death. The LC50s for permethrin with Apache and Lahontan cutthroat trout were significantly less than that for rainbow trout at 24 and 96 h (Tables 2 and 4). After 24 h of exposure, LC50s for Apache and Lahontan cutthroat trout were 2.3 and 1.9 $\mu\text{g/L}$, respectively, compared with 3.8 $\mu\text{g/L}$ for the rainbow trout. At 96-h of exposure, LC50s for Apache (1.7 $\mu\text{g/L}$) and Lahontan cutthroat trout (1.6 $\mu\text{g/L}$) were about twice as sensitive as the rainbow trout LC50 (3.3 $\mu\text{g/L}$). There were no significant differences in 12-h LC50s among the cold-water fishes (Table 2). Razorback suckers were significantly more sensitive to permethrin at 96 h of exposure than fathead minnows (Table 4). Bonytail chubs and Colorado pikeminnows were much less sensitive to per-

methrin than fathead minnows at 12, 24, and 96 h of exposure. The LC50 for Leon Springs pupfish was not different than that of the sheepshead minnow at 24 h. But at 96 h, Leon Springs pupfish were less sensitive. Warm-water fish were 1.8 to 7.5 times less sensitive than the rainbow trout. The 96-h LC50s for sheepshead minnows and Leon Springs pupfish were two to six times higher than those for rainbow trout and fathead minnows.

DISCUSSION

Comparisons with other studies

Macek and McAllister [18] exposed 12 species of fish (five families) to a range of insecticides and determined that salmonids were the most sensitive and the cyprinids and ictalurids the least sensitive. Mayer and Ellersieck [6] compared the sensitivity of four fish families to 65 chemicals and also found that salmonids were the most sensitive and cyprinids the least sensitive. Results from our study are consistent with their findings.

Few studies have evaluated the sensitivity of endangered species to chemical contaminants. Beleau and Bartosz [19] conducted toxicity tests with the Colorado pikeminnow and humpback chub and a closely related cyprinid, the northern pikeminnow (*Pimephales oregonensis*). They exposed these fish to 13 inorganic and 8 organic chemicals. The authors stated that there was a margin of safety associated with the use of channel catfish (*Ictalurus punctatus*) and fathead minnows as surrogates because the listed species were less sensitive to the contaminants they tested. In our study, we found that Colorado pikeminnows, razorback suckers, and bonytail chub were generally either more sensitive than or equally as sensitive as fathead minnows. Permethrin was an exception, where both Colorado pikeminnows and bonytail chub were less sensitive. Beleau and Bartosz [19] made their comparison based on toxicity values reported in the literature for fathead minnows and channel catfish. In addition, their tests were conducted in water qualities that were different than those for fathead minnow and channel catfish study values used for comparison in Johnson and Finley [20]. In our studies, concurrent testing of listed and surrogate species and the use of standardized water quality enhanced our ability to make interspecies sensitivity comparisons.

The current research indicated that bonytail chub and Colorado pikeminnow were more sensitive to carbaryl exposure than were fathead minnows but less sensitive than rainbow trout. This finding is supported by work done by Beyers et al. [10], who conducted 4-d renewal acute toxicity tests with the cholinesterase inhibitors carbaryl, Sevin 4-oil, and malathion. The authors compared their results with acute data summarized by Mayer and Ellersieck [6]. Byers et al. [10] determined that, for carbaryl, the Colorado pikeminnow and bonytail chub were similar in sensitivity to cutthroat trout (*O. clarki*), Atlantic salmon (*Salmo salar*), rainbow trout, and brook trout (*Salvelinus fontinalis*). The listed species were 2 to 10 times more sensitive than fathead minnows, bluegill (*Lepomis macrochirus*), and channel catfish. Conversely, Colorado pikeminnows and bonytail chub exposed to malathion were similar in sensitivity to fathead minnows and channel catfish and were less sensitive than the rainbow trout and bluegill.

Beyers et al. [10] exposed bonytail chub and Colorado pikeminnows to carbaryl in static-renewal tests using 26-d-old Colorado pikeminnows and 6-d-old bonytail chub. The 96-h LC50 values obtained in their tests were 1.3 mg/L for the Colorado

pikeminnow and 2.0 mg/L for the bonytail chub. Their results are similar to our findings under static conditions with larger fish. The LC50s for rainbow trout and fathead minnows from the current studies with carbaryl are similar to reported values calculated from static toxicity tests. Mayer and Ellersieck [6] reported 96-h LC50s for rainbow trout exposed to carbaryl ranging from <0.32 to 3.5 mg/L, with a GM of 1.6 mg/L. Marking et al. [21] reported LC50s ranging between 0.94 and 1.7 mg/L for rainbow trout exposed to carbaryl. Reported 96-h LC50s for fathead minnows exposed to carbaryl range from 7.7 to 14.6 mg/L [5] and 6.7 to 12 mg/L [22]. The LC50s obtained for euryhaline fish were two times higher than values reported in U.S. EPA's ACQUIRE database (<http://www.epa.gov/ecotox/>).

The 96-h LC50s for copper in the current study are similar to previously published values. Mayer and Ellersieck [6] reported a copper LC50 of 0.14 mg/L for rainbow trout (hardness 44 mg/L as CaCO₃) and 0.84 mg/L for fathead minnows (hardness 272 mg/L as CaCO₃). Calamari and Marchetti [23] reported a copper LC50 of 0.89 mg/L for rainbow trout (hardness 290 mg/L as CaCO₃). Fathead minnows tested under static conditions had LC50s ranging from 0.02 to 1.8 mg/L [24] (hardness 20–360 mg/L as CaCO₃). In the ambient water quality criteria for copper [25], the species mean acute value for rainbow trout is 0.043 mg/L and 0.12 mg/L for fathead minnows (hardness of 50 mg/L as CaCO₃).

Buhl and Hamilton [26] obtained 96-h LC50 copper values for Colorado pikeminnow and bonytail and razorback sucker juveniles that are consistent with our results (0.66, 0.23, and 0.33 mg/L, respectively). Also, Hamilton and Buhl [27] obtained 96-h LC50 copper values for Colorado pikeminnow and razorback sucker larvae within a factor of two of our results (0.31 and 0.27 mg/L, respectively). Results from these two studies were conducted with two different site-specific water qualities. The site waters had ionic compositions different than water we used in our assessment; however, the results are similar.

The 96-h LC50s for sheepshead minnows (2.5 mg/L) and Leon Springs pupfish (4.6 mg/L) in our study were 6 to 12 times higher than those reported by Hughes et al. [28] for larval sheepshead minnow (0.37 mg/L). The difference in 96-h LC50s may be partly due to the life stage tested; we tested 0.24-g juveniles and Hughes et al. [28] tested larvae. Little information is available on the acute toxicity of 4-nonylphenol. However, the U.S. EPA Draft Report [29] reported ranges of 96-h LC50s of 0.56 to 0.92 mg/L for rainbow trout and 0.14 mg/L to 0.3 mg/L for fathead minnows. These LC50s for rainbow trout are higher than those obtained in the current study, but the LC50 values for fathead minnows are similar to our results.

In the current study, 96-h LC50s for rainbow trout exposed to pentachlorophenol were about three times lower than those previously reported by other authors, while results with fathead minnows are similar to reported values. Davis and Hoos [30] reported that LC50s for rainbow trout exposed to pentachlorophenol ranged between 0.44 and 0.92 mg/L, with a GM of 0.68 mg/L, for rainbow trout. Mayer and Ellersieck [6] reported LC50s for rainbow trout ranging between 0.03 and 0.12 mg/L, with a GM of 0.06 mg/L. These authors also reported a 96-h LC50 for pentachlorophenol with fathead minnows of 0.21 mg/L. The ambient water quality criteria for pentachlorophenol [31] reports a species mean acute value of 35.34 µg/L for rainbow trout and 63.11 µg/L for fathead minnows.

Borthwick and Schimmel [32] reported 96-h LC50 values (0.22 and 0.39 mg/L) for pentachlorophenol and sheepshead minnow were 2.9 to 7.8 times higher than the values for the sheepshead minnow and Leon Springs pupfish (0.05 and 0.08 mg/L). However, their tests were conducted with sheepshead minnows ranging from 1 d old to 6-week-old fry in 10‰ salinity at 30°C. Our sheepshead minnows weighed 0.24 g and were tested in 2‰ salinity at 20°C. Higher salinity in the former study may have reduced the toxicity of pentachlorophenol. Brecken-Folse et al. [33] found increased toxicity with decreased salinity in cyprinodontids exposed to 4-nitrophenol and 2,4-dinitrophenol. Nordlie [34] suggested that these results were not unexpected because sheepshead minnows have increased metabolic rates at reduced salinities, thus increasing gill exposure to the chemicals.

Mayer and Ellersieck [6] reported 96-h LC50s for rainbow trout exposed to permethrin ranged from 2.9 to 8.2 µg/L, with a GM of 5.1 µg/L. The LC50 for fathead minnows was 5.7 µg/L. These LC50s are similar to those derived in the current study for rainbow trout and fathead minnows.

Management implications

Test variation also needs to be considered when conducting hazard assessments. Interlaboratory tests performed by Schimmel [35], Lemke [36], and DeGraeve et al. [37] found that there was a two- to fivefold difference in LC50s. However intralaboratory test comparisons, by the same researchers, showed that LC50s seldom varied by more than a factor of two. Causes for the variation may include animal size, water quality, method of toxicant preparation, and the data analysis method used [36].

Results from the current studies indicate that the sensitivity of standard test species (rainbow trout, fathead minnow, and sheepshead minnow) were usually not greatly different from that of the corresponding listed species. In only two cases did a listed species have a 96-h LC50 that was less than one half that of its surrogate. However, for about 30% of the possible surrogate/listed species comparisons, the 96-h LC50 for the listed species was 1.5 to 2.1 times lower than that for the respective surrogate species.

Environmental protection procedures usually focus on protection of populations or communities and not on individuals, as may be necessary for listed species. Protection of individuals may require additional margins of safety in certain circumstances. For this reason, caution must be exercised when National Water Quality Criteria are modified by states in setting state water quality standards. Unless specific data are generated for listed species, our data indicates that sensitive species such as rainbow trout should not be eliminated from the database.

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