Arch. Environ. Contam. Toxicol. 48, 143–154 (2005)
DOI: 10.1007/s00244-003-3038-1

ARCHIVES OF
Environmental
Contamination
and Toxicology

Assessing Contaminant Sensitivity of Endangered and Threatened Aquatic Species: Part I. Acute Toxicity of Five Chemicals

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Received: 28 March 2003 / Accepted: 6 May 2004

Abstract. Assessment of contaminant impacts to federally identified endangered, threatened and candidate, and state-identified endangered species (collectively referred to as "listed" species) requires understanding of a species' sensitivities to particular chemicals. The most direct approach would be to determine the sensitivity of a listed species to a particular contaminant or perturbation. An indirect approach for aquatic species would be application of toxicity data obtained from standard test procedures and species commonly used in laboratory toxicity tests. Common test species (fathead minnow, *Pimephales promelas*; sheepshead minnow, *Cyprinodon variegatus*; and rainbow trout, *Oncorhynchus mykiss*) and 17 listed or closely related species were tested in acute 96-hour water exposures with five chemicals (carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin) representing a broad range of toxic modes of action. No single species was the most sensitive to all chemicals. For the three standard test species evaluated, the rainbow trout was more sensitive than either the fathead minnow or sheepshead minnow and was equal to or more sensitive than listed and related species 81% of the time. To estimate an LC50 for a listed species, a factor of 0.63 can be applied to the geometric mean LC50 of rainbow trout toxicity data, and more conservative factors can be determined using variance estimates (0.46 based on 1 SD of the mean and 0.33 based on 2 SD of the mean). Additionally, a low- or no-acute effect concentration can be estimated by multiplying the respective LC50 by a factor of approximately 0.56, which supports the United States Environmental Protection Agency approach of multiplying the final acute value by 0.5 (division by 2). When captive or locally abundant populations of listed fish are available, consideration should be given to direct testing. When direct toxicity testing cannot be performed, approaches for developing protective measures using common test species toxicity data are available.

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Under the Federal Insecticide, Fungicide and Rodenticide Act, the Toxic Substances Control Act (TSCA) and the Clean Water Act, the United States Environmental Protection Agency (USEPA) is charged with determining whether the manufacture, use, or disposal of a chemical will present an unreasonable risk of harm to the environment. Typically, risk-management decisions are based on data generated for population subsets, and results are intended to represent the sensitivity of a species. However, this approach may allow for effects to occur in a few individuals (e.g., Stephan *et al.* 1985). The Endangered Species Act requires that, in some cases, managers must estimate the potential loss of individuals to determine any adverse effects on populations of federally identified endangered or threatened species. There are currently 612 federally identified threatened or endangered species, 13 federally proposed and 118 federally identified candidate aquatic or aquatic-dependent species. In addition, many states have identified state endangered species (throughout the remainder of this article, federally identified endangered, threatened and candidate, and state-identified endangered species will be collectively referred to as "listed" species). In 1988, the American Chemical Society estimated that there were >63,000 chemicals in use (Ramade 1988), and the TSCA Inventory lists >70,000 chemicals that can be commercially produced and used. Because of the number of listed species and numerous types of chemicals, exposure is likely.

The most direct approach for estimating effects to listed species would be to determine the sensitivity of a listed species to a particular contaminant or perturbation in a full life-cycle

assessment that examines all reasonable routes of exposure. However, this direct approach would be impractical for some species and impossible for others because it might require development of organism culturing and handling procedures, some species may not be amenable to culture, and results might be contaminant specific.

An indirect approach for determining the sensitivity of a listed species would use toxicity data obtained from standard test procedures with common test species (e.g., fathead minnow, *Pimephales promelas*; sheepshead minnow, *Cyprinodon variegatus*; rainbow trout, *Oncorhynchus mykiss*; and bluegill, *Lepomis macrochirus*). These species are easily tested using standardized methods (Committee [Comittee] on Methods for Toxicity Tests with Aquatic Organisms 1975; ASTM 2003); however, there is concern that these species or procedures may not adequately represent the sensitivities of listed species to contaminants in the environment.

During the past several years, acute toxicity exposures (96-hour LC50s) were conducted with common test species (fathead minnows, sheepshead minnows, and rainbow trout) and several listed and related species (Dwyer *et al.* 1995, 1999a, b, c, 2000; Sappington *et al.* 2001). Chemicals tested were carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin. These chemicals were chosen to represent different chemical classes and toxic modes of action. By evaluating the sensitivities of numerous listed and related species using similar testing conditions and chemicals, evaluations regarding the sensitivities of listed species and the protection afforded by standard regulatory approaches can be made.

Toxicity tests were conducted with three common test fish species representing three families (Cyprinidae, Cyprinodontidae, and Salmonidae) and 17 additional species of fish or amphibians from 8 families. These 17 species have been identified as endangered, threatened, or candidates by the United States Fish and Wildlife Service (USFWS) identified as surrogates in a USFWS Recovery Plan, or state identified as endangered (Table 1).

We present a summary of the 96-hour acute toxicity results and compare the sensitivities of listed and related species with common test species tested using similar procedures. These results are for acute water exposures using early life-stage organisms where mortality is the end point and do not include evaluations for other routes of exposure or other toxicologic endpoints. Besser *et al.* (2004) presented results of chronic toxicity tests with pentachlorophenol and copper on early life stages of listed and common test species. Milam *et al.* (2004) presented the results of early life-stage mussel toxicity tests with the same five chemicals used in the present study.

Materials and Methods

A complete description of the study design—including life stages tested, average weight, number of tests conducted, number of replicates per test, number of individuals exposed per replicate, and water quality—are provided in Dwyer *et al.* (1995; 1999a, c; 2000) and Sappington *et al.* (2001). A brief overview of these methods follows.

Test Organisms

All fish were received either as eyed eggs or young-of-year. Boreal toads were received as tadpoles (Table 1). Most test organisms were held in well water (alkalinity 258 mg/L as $CaCO_3$, hardness 286 mg/L as $CaCO_3$, pH 7.8, 18°C) at the United States Geological Survey (USGS), Columbia Environmental Research Center (Columbia, MO) until acclimation before the start of testing. Sheepshead minnows, Leon Springs pupfish, and desert pupfish were held in natural seawater diluted with deionized water to 2‰ (g/L) at the United States Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division (Gulf Breeze, FL).

Before the start of a toxicity test, organisms were acclimated to exposure conditions for 96 hours (Committee 1975; ASTM 2003). For the first 48 hours, organisms were fed and acclimated to the test water and temperature. The test organisms were then moved to clean containers and held for an additional 48 hours at the test temperature in 100% test water without feeding before the start of the exposures.

Chemicals

The chemicals used in testing were technical-grade carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin (Table 2). Chemicals were selected to represent different chemical classes and modes of toxic action. Organic chemical stock solutions were prepared by dissolving the chemical in reagent-grade acetone or triethylene glycol, and stock solutions for copper were prepared with deionized water. Maximum solvent concentration in a test container was 0.5 mL/L (ASTM 2003).

Average percent nominal concentrations for measured stock solutions ranged from 86% for copper ($n = 15$) to 119% for 4-nonylphenol ($n = 11$) and permethrin ($n = 11$) (carbaryl 88%, $n = 15$; pentachlorophenol 100%, $n = 14$). Four individual stock analyses of copper, 4-nonylphenol, permethrin, and pentachlorophenol resulted in concentrations of 10%, 308%, 320%, and 572% of nominal, respectively. Toxicologic results from the tests using these four stock solutions were similar to tests conducted with other stocks for those same chemicals. Thus, we believe that the reported values for those four samples were incorrect, and for that reason those analytic results were not included in calculation of the average percent of nominal concentrations; however toxicologic results from those tests are included in the data analysis. Toxicity values for all tests are based on nominal concentrations.

Toxicity Tests

Static acute-toxicity tests were conducted in basic accordance with procedures described by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and ASTM (2003; Table 3). Freshwater exposures were conducted in 19.6-L glass jars containing 15 L test solution. Saltwater exposures were conducted in 3.8-L glass jars containing 3 L test solution. All tests were conducted at a test temperature appropriate for the species (Table 1).

Reconstituted hard water was used for all freshwater tests (alkalinity 110 to 120 mg/L as $CaCO_3$ and hardness 160 to 180 mg/L as $CaCO_3$; ASTM 2003) rather than soft water to help minimize potential stress to listed species. Saltwater tests were conducted using natural seawater diluted to 2‰ (g/L) with deionized water.

The exposure series consisted of six concentrations with a 60% dilution series. When a solvent was used, both a solvent control and a reconstituted water control were included for each species. Mortality was determined at 24-hour increments or more frequently throughout

Table 1. Source, test temperature, number of individual tests for each of the five chemicals, replicates per test, and number of individuals per replicate for each species used in the acute toxicity exposures[a]

Family	Species	Source	Temperature (°C)	No. of Tests	Replicates per Test	Individuals per Replicate
Cyprinidae	Fathead minnow[b] (*Pimephales promelas*)	USGS–CERC cultures, Columbia, MO Osage Fisheries, Osage Beach, MO	22	6	3	10
Cyprinodontidae	Sheepshead minnow[b] (*Cyprinodon variegatus*)	TRAC Laboratories, Gulf Breeze, FL	20	1	2	10
Salmonidae	Rainbow trout[b] (*Oncorhynchus mykiss*)	Beity's Enterprise, Valley, WA Ennis N F H, Ennis, MT	12	6[c]	3	10
Acipenseridae	Atlantic sturgeon[d] (*Acipenser oxyrhynchus*)	Northeast Fisheries Center, Lamar, PA	17	1	3	9
	Shortnose sturgeon (*Acipenser brevirostrum*)	Bears Bluff National Fish Hatchery, Wadmalaw Island, SC	17	1	3	7
	Shovelnose sturgeon[e] (*Scaphirhynchus platorynchus*)	Blind Pony Missouri State Fish Hatchery, Sweet Springs, MO	22	1	2	9
Bufonidae	Boreal toad[f] (*Bufo boreas boreas*)	Colorado Division of Wildlife, collected from the West Fork of Clear Creek near Georgetown, CO	22	1	3	10
Catostomidae	Razorback sucker (*Xyrauchen texanus*)	NFH and Technology Center, Dexter, NM	22	2	3	10
Cyprindae	Bonytail chub (*Gila elegans*)	NFH and Technology Center, Dexter, NM	22	2	3	10
	Cape Fear Shiner (*Notropis mekistocholas*)	Conservation Fisheries, Knoxville, TN	17	1	3	10
	Colorado pikeminnow (*Ptychocheilus lucius*)	NFH and Technology Center, Dexter, NM	22	2	3	10
	Spotfin chub (*Hybopsis monacha*)	Conservation Fisheries, Knoxville, TN	17	1	2	10
Cyprinodontidae	Desert pupfish (*Cyprinodon macularius*)	NFH and Technology Center, Dexter, NM	20	1	2	10
	Leon Springs pupfish (*Cyprinodon bovinus*)	NFH and Technology Center, Dexter, NM	20	1	2	10
Percidae	Fountain darter (*Etheostoma fonticola*)	San Marcos NFH and Technology Center, San Marcos, TX	22	1	2	10
	Greenthroat darter[g] (*Etheostoma lepidum*)	San Marcos NFH and Technology Center, San Marcos, TX	22	1	2	7
Poeciliidae	Gila topminnow (*Poeciliopsis occidentalis occidentalis*)	NFH and Technology Center, Dexter, NM	22	1	2	10
Salmonidae	Apache trout (*Oncorhynchus apache*)	Williams Creek NFH, White River, AZ	12	2	3	10
	Greenback cutthroat trout (*Oncorhynchus clarki stomias*)	Saratoga NFH, Saratoga, WY	12	1	3	10
	Lahontan Cutthroat trout (*Oncorhynchus clarki henshawi*)	Lahontan NFH, Gardnerville, NV	12	2	3	10

[a] All species are federally listed as endangered or threatened unless otherwise noted.
[b] Common test species.
[c] Only four tests conducted with copper.
[d] State of Connecticut, threatened.
[e] Surrogate species identified in USFWS Recovery Plan for pallid sturgeon (*Scaphirhynchus albus*).
[f] Federal candidate species.
[g] State of New Mexico, threatened.
CERC = Columbia Environmental Research Center.
NFH = National Fish Hatchery.
TRAC = Texas Research Analytical Chemistry.
USFWS = United States Fish and Wildlife Service.
USGS = United States Geological Survey.

Table 2. Source, percent active ingredient, use, and mode of action for chemicals used in toxicity tests

Chemical	Source	Active Ingredient (%)	Use	Mode of Action
Carbaryl	Rhone-Pôulenc Agricultural Co., Research Triangle Park, NC	99.7	Carbamate insecticide	Cholinesterase inhibitor
Copper (from copper sulfate)	Fisher Chemical, St. Louis, MO	25.5	Mining, industrial, fungicide	Osmoregulation interference
4-nonylphenol	Fluka Chemical, New York, NY	85.0	Nonylphenol ethoxylate detergents	Narcotic and oxidative stressor
Pentachlorophenol	Aldrich Chemical, Milwaukee, WI	99.0	Organochlorine, wood preservative, molluscicide	Oxidative phosphorylation uncoupler
Permethrin	ICI Americas Inc., Richmond, CA	95.2	Pyrethroid insecticide	Neurotoxicant

Table 3. General study design for the comparative toxicity of selected chemicals to listed species

Test type	Static acute
Test volume	Freshwater: 15 L Saltwater: 3.0 L
Test temperature	Listed in Table 1
Water quality	Freshwater: Reconstituted ASTM hard (alkalinity 110 to 120 mg/L as $CaCO_3$, hardness 160 to 180 mg/L as $CaCO_3$) Saltwater: Natural seawater diluted with deionized water to 2‰ (g/L)
Chemicals	Carbaryl, copper, 4-nonylphenol, pentachlorophenol, permethrin
Dilution series	60%
Observations	Mortality at 24, 48, 72, and 96 hours of exposure

the exposures and was defined as lack of movement for a 5-second observation with the unaided eye. Dead animals were removed at each observation time.

Carbaryl concentrations used in an initial test with boreal toads were not sufficiently toxic to calculate an LC50. Additional carbaryl testing with boreal toad tadpoles was conducted in well water used for culture and using a 70% dilution series. All other conditions were similar, and toxicity results from the carbaryl exposures conducted in well water were used in this study.

Water Quality

For freshwater exposures, alkalinity, hardness, and pH were measured on each batch of reconstituted water before the start of the exposures. Alkalinity and hardness of reconstituted hard water were within the acceptable ranges outlined by ASTM (2003). In general, average pH was slightly greater than the listed value of 8.0 in ASTM (2003).

For freshwater tests, pH was measured in the control, low-, medium-, and high-exposure concentrations at the start of the exposure and after 96 hours of exposure if organisms survived in those concentrations. Test chemicals occasionally altered pH but not in a consistent pattern. Dissolved oxygen was measured in the control, low-, medium-, and high-exposure concentrations at the start of the exposure and at 48 and 96 hours of exposure if organisms survived in those concentrations. For saltwater tests, dissolved oxygen and pH were measured in two replicates in all treatments daily throughout the test. Chemicals added to saltwater tests did not substantially affect pH. For both freshwater and saltwater tests, any decrease in dissolved oxygen was an isolated event and interspersed throughout the exposures and therefore not considered a chemical-dependent effect.

Data Analysis

The 96-hour LC50 for most tests was calculated using probit analysis. When probit analysis was not appropriate (i.e., less than two partial mortalities), LC50s were calculated using a moving-average procedure or a nonlinear interpolative procedure (Stephan 1977).

For most species, only a single test with $\geq$2 replicate test chambers could be conducted (Table 1). All replicate test chambers within a test were pooled for calculation of LC50s. For species with $>$1 test, individual LC50s from each test were used to calculate a geometric mean LC50 for that species and chemical.

To evaluate relative species sensitivity to a particular chemical, 96-hour LC50s were ranked for each species from 1 (low tolerance = low LC50) to 18 (high tolerance = high LC50). If two LC50s were the same, the two sequential ranks were averaged, and the average rank was assigned to each species. A summary rank was calculated by averaging the individual ranks obtained for each species and chemical and then reranking (Snedecor and Cochran 1989). To adequately evaluate species sensitivity, a species was only included in the summary ranking if there were ranking results for $\geq$4 chemicals.

Ranking provided information regarding relative species sensitivities. Evaluating the range of the specific response (LC50) for multiple exposures of the same species to the same chemical provided additional information regarding the use of data for a commonly tested species as representative of listed species. This evaluation determined how frequently the LC50 of a listed or related species was outside the

Table 4. Acute toxicity of carbaryl to 18 fishes and 1 amphibian including 96-hour LC50s, species sensitivity ranking, comparison of acute value with the range of values for rainbow trout or fathead minnows, and ratio of acute value to that for rainbow trout[a]

Species	LC50 (mg/L)	Rank	RBT Range Comparison	FHM Range Comparison	RBT LC50 Ratio
Fathead minnow	5.21	16	>	–	2.8
Sheepshead minnow	4.36	13	>	=	2.3
Rainbow trout	1.88	5	–	<	–
Atlantic sturgeon	<0.8	1	<	<	NC
Shortnose sturgeon	1.81	4	=	<	1.0
Shovelnose sturgeon	NC	–	–	–	–
Boreal toad	12.3	18	>	>	6.5
Razorback sucker	4.35	12	>	=	2.3
Bonytail chub	3.49	11	>	<	1.9
Cape Fear shiner	4.51	14	>	=	2.4
Colorado pikeminnow	3.07	9	=	<	1.6
Spotfin chub	3.41	10	>	<	1.8
Desert pupfish	7.71	17	>	>	4.1
Leon Springs pupfish	4.54	15	>	=	2.4
Fountain darter	2.02	6	=	<	1.1
Greenthroat darter	2.14	7	=	<	1.1
Gila topminnow	>3.0	NR	NC	NC	NC
Apache trout	1.54	2	=	<	0.8
Greenback cutthroat trout	1.55	3	=	<	0.8
Lahontan cutthroat trout	2.25	8	=	<	1.2

[a] For the range comparison, carbaryl values for rainbow trout (1.22 to 3.11 mg/L, n = 6) or fathead minnows (3.94 to 7.43 mg/L, n = 6) were used (Dwyer *et al.* 1995; Sappington *et al.* 2001). "=" is within the range of 96-hour LC50s for rainbow trout or fathead minnow, ">" is greater than range maximum, and "<" is less than range minimum. The rainbow trout ratio was calculated by dividing the 96-hour LC50 for a species by the geometric mean 96-hour LC50s for rainbow trout.
NC = Not calculated.
NR = Not ranked.
FHM = Fathead minnows.
RBT = Rainbow trout.

LC50 range for either fathead minnows or rainbow trout for a particular chemical.

Using data for six tests with fathead minnows and six tests with rainbow trout for each of the five chemicals (Dwyer *et al.* 1995; Sappington *et al.* 2001), we identified the range of 96-hour LC50s for both fathead minnows and rainbow trout. We then classified the LC50s for listed species as either equal (within the range), greater than (LC50 greater than the range maximum), or less than (LC50 less than the range minimum) the LC50s for rainbow trout or fathead minnows.

We also evaluated the magnitude of the difference between each LC50 for the listed species and the geometric mean of the LC50s for the common test species. The overall "most sensitive" common test species was identified. The relative difference between the sensitive common test species and the listed species was expressed by dividing each listed species' LC50 by the geometric mean LC50 for the selected common test species.

Finally, for each individual test within a species–chemical group, we developed a ratio of the average concentration for replicates that had 0% to 10% mortality to the 96-hour LC50 for that chemical (United States Environmental Protection Agency 1978). This approach provided a factor allowing prediction of no-effect or low-effect concentrations in acute lethality tests. A 10% level of mortality was used in this evaluation because it is considered acceptable control mortality and therefore could not be discerned as an effect of chemical exposure (Committee 1975; ASTM 2003). We selected as the no- or low-effect concentration the highest concentration tested that had ≤10% mortality after 96 hours of exposure. If a species had more than one test conducted, then the individual ratios for each test were averaged. We then calculated the geometric mean of ratios for each species across chemicals and followed the same procedure for ratios across species within a chemical.

Results

Control Survival

Control survival, with and without the addition of a solvent, was always >90% for all species except Atlantic and shovelnose sturgeons. For these two species, control survival was decreased by acetone. With shovelnose sturgeon, survival in water-only exposure (without acetone) was 100%, whereas solvent control survival was 0% after 96 hours. For this reason, we did not use toxicity results for shovelnose sturgeon where acetone was used as the solvent. In the toxicity tests with Atlantic sturgeon, acetone controls had a survival of 70% at 96 hours, whereas water-only control survival was 100%. Mortality in the acetone control was caused by all fish dying in one replicate, and therefore results for Atlantic sturgeon have been included. If a few sturgeon died in either a control or exposure replicate, the water quickly fouled, and most or all of the fish then died in that replicate. These observations indicated that conclusions regarding the chemical sensitivities of sturgeon should be made with caution.

In toxicity tests with fountain darters, average control sur-

Table 5. Acute toxicity of copper to 18 fishes and 1 amphibian including 96-hour LC50s, species sensitivity ranking, comparison of acute value with the range of values for rainbow trout and fathead minnows, and ratio of acute value to that for rainbow trout[a]

Species	LC50 (mg/L)	Rank	RBT Range Comparison	FHM Range Comparison	RBT LC50 Ratio
Fathead minnow	0.47	16	>	–	5.9
Sheepshead minnow	0.63	17	>	=	7.9
Rainbow trout	0.08	5.5	–	<	–
Atlantic sturgeon	0.06	1.5	=	<	0.8
Shortnose sturgeon	0.08	5.5	=	<	1.0
Shovelnose sturgeon	0.16	10.5	>	<	2.0
Boreal toad	0.12	9	>	<	1.5
Razorback sucker	0.27	14	>	<	3.4
Bonytail chub	0.22	12	>	<	2.8
Cape Fear shiner	0.11	8	=	<	1.4
Colorado pikeminnow	0.43	15	>	=	5.4
Spotfin chub	0.09	7	=	<	1.1
Desert pupfish	NT	–	–	–	–
Leon Springs pupfish	1.3	18	>	>	>
Fountain darter	0.06	1.5	=	<	0.8
Greenthroat darter	0.26	13	>	<	3.3
Gila topminnow	0.16	10.5	>	<	2.0
Apache trout	0.07	3.5	=	<	0.9
Greenback cutthroat trout	>0.03	NR	NC	NC	NC
Lahontan cutthroat trout	0.07	3.5	=	<	0.9

[a] For the range comparison, copper values for rainbow trout (0.05 to 0.11 mg/L, n = 4) or fathead minnows (0.29 to 0.81 mg/L, n = 6) were used (Dwyer *et al.* 1995; Sappington *et al.* 2001). "=" is within the range of 96-hour LC50s for rainbow trout or fathead minnow, ">" is greater than range maximum, and "<" is less than range minimum. The rainbow trout ratio was calculated by dividing the 96-hour LC50 for a species by the geometric mean 96-hour LC50s for rainbow trout.
NC = Not calculated.
NR = Not ranked.
NT = Not tested.
FHM: Fathead minnows.
RBT: Rainbow trout.

vival without acetone was 97% and with acetone was 93%. However, a 5% to 15% mortality occurred in most low-exposure concentrations (below observed concentration-effect curve), regardless of the chemical tested, and the results of these tests should also be interpreted with caution.

Toxicity Results and Comparisons

Tables 4 to 8 list the early life-stage 96-hour LC50s for all five chemicals and each species. At 96 hours of exposure, permethrin was generally the most toxic compound, and carbaryl was the least toxic compound. The two phenolic compounds (4-nonylphenol and pentachlorophenol) and copper had similar ranges of 96-hour LC50s. The mean LC50s for rainbow trout were always lower than the mean LC50s for fathead minnows and sheepshead minnow, except for tests with sheepshead minnow and pentachlorophenol.

For fish exposures conducted with carbaryl, 96-hour LC50s ranged from <0.8 (Atlantic sturgeon) to 7.71 mg/L (desert pupfish). The boreal toad LC50 was 12.3 mg/L. Copper LC50s in freshwater ranged from 0.06 (fountain darter and Atlantic sturgeon) to 0.47 mg/L (fathead minnows). Species tested in saltwater had 96-hour copper LC50s ranging from 0.63 (sheepshead minnow) to 1.3 mg/L (Leon Springs pupfish). Toxicity results from freshwater tests with nonylphenol had LC50s ranging from 0.05 (Atlantic sturgeon) to 0.29 mg/L (bonytail chub), whereas saltwater LC50s were 0.46 mg/L for sheepshead minnow and 0.48 mg/L for Leon Springs pupfish. Pentachlorophenol LC50s ranged from 0.05 (sheepshead minnow) to 0.37 mg/L (boreal toad). Permethrin toxicity ranged from <1.2 (Atlantic and shortnose sturgeon) to >25.0 μg/L (bonytail chub).

Rainbow trout had sensitivity ranks of 5 (carbaryl) to 11.5 (4-nonylphenol), with a summary ranking across the five chemicals of 6 (Table 9), and was the "most sensitive" commonly tested species in the study. Ranks for fathead minnows ranged from 11 (permethrin) to 16 (carbaryl and copper). The summary rank for fathead minnows across the five chemicals was 16, and fathead minnow was the most tolerant species tested. Generally, of the listed species, the Atlantic and shortnose sturgeons were two of the most sensitive species (summary ranks of 1 and 2, respectively).

Ranking of data was used to provide information regarding relative species sensitivities. Evaluating the range of the specific response (LC50) for multiple exposures of the same species to the same chemical provided additional information regarding the use of data for a commonly tested species as representative of listed species. The frequency that an LC50 of a listed or related species was outside the LC50 range for either fathead minnows or rainbow trout was also determined (Tables 4 to 8). For the 17 species tested, 70 comparisons could be

Table 6. Acute toxicity of 4-nonylphenol to 18 fishes and 1 amphibian including 96-hour LC50s, species sensitivity ranking, comparison of acute value with the range of values for rainbow trout or fathead minnows, and ratio of acute value to that for rainbow trout[a]

Species	LC50 (mg/L)	Rank	RBT Range Comparison	FHM Range Comparison	RBT LC50 Ratio
Fathead minnow	0.27	15	=	–	1.4
Sheepshead minnow	0.46	17	>	>	2.4
Rainbow trout	0.19	11.5	–	=	–
Atlantic sturgeon	0.05	1	<	<	0.3
Shortnose sturgeon	0.08	2.5	<	<	0.4
Shovelnose sturgeon	<0.13	–	–	–	–
Boreal toad	0.12	5	<	<	0.6
Razorback sucker	0.17	8.5	=	=	0.9
Bonytail chub	0.29	16	>	=	1.5
Cape Fear shiner	0.14	6	=	<	0.7
Colorado pikeminnow	0.26	14	=	=	1.4
Spotfin chub	0.08	2.5	<	<	0.4
Desert pupfish	NT	–	–	–	–
Leon Springs pupfish	0.48	18	>	>	2.5
Fountain darter	0.11	4	<	<	0.6
Greenthroat darter	0.19	11.5	=	=	1.0
Gila topminnow	0.23	13	=	=	1.2
Apache trout	0.17	8.5	=	=	0.9
Greenback cutthroat trout	0.15	7	=	<	0.8
Lahontan cutthroat trout	0.18	10	=	=	0.9

[a] For the range comparison, 4-nonylphenol values for rainbow trout (0.14 to 0.27 mg/L, n = 6) or fathead minnows (0.17 to 0.36 mg/L, n = 6) were used (Dwyer *et al.* 1995; Sappington *et al.* 2001). "=" is within the range of 96-hour LC50s for rainbow trout or fathead minnow, ">" is greater than range maximum, and "<" is less than range minimum. The rainbow trout ratio was calculated by dividing the 96-hour LC50 for a species by the geometric mean 96-hour LC50s for rainbow trout.
NT = Not tested.
FHM = Fathead minnows.
RBT = Rainbow trout.

made to fathead minnows, and 72 comparisons could be made to rainbow trout. There were 44 (63%) listed or related species with LC50s less than the range of LC50s for the fathead minnow. Fourteen listed or related species' LC50s (19%) were less than the range of LC50s for rainbow trout, and 28 LC50s (39%) were above the range of LC50s for rainbow trout. These results indicate that rainbow trout were equal to or more sensitive than that of the listed or related species for 81% (58 of 72) of the tests.

In addition, we calculated a magnitude of difference factor using the 96-hour LC50s of rainbow trout and each listed species. This evaluation provides guidance on estimating factors to apply to LC50s from commonly tested species to estimate LC50s for listed species. Within a chemical, we compared the 96-hour LC50 for a listed species with the geometric mean 96-hour LC50 for rainbow trout (Tables 4 to 8). For all five chemicals, at least one listed species had a 96-hour LC50 lower than the geometric mean 96-hour LC50 for rainbow trout. Of 28 LC50s for listed species that were less than the comparable LC50 for rainbow trout, we were able to calculate factors for only 24 of those LC50s. The geometric mean factor was approximately 0.63, and the lowest factor (Atlantic sturgeon with 4-nonylphenol exposure) was approximately 0.3.

Finally, for a subset of the species tested (species with definitive tests for ≥3 chemicals), a factor was also derived that would allow prediction of no or low-effect concentration from acute lethality data (i.e., [factor]*[96-hour LC50] = 0% to 10% mortality; Table 10; USEPA 1978). A level of mortality ≤10% was used in this evaluation because it is considered to be acceptable control mortality and therefore would not likely be distinguishable from an effect of chemical exposure (Committee 1975; ASTM 2003). For the five chemicals tested with commonly used species, the geometric mean factor was 0.53 (range 0.41 to 0.67) for fathead minnows, 0.48 (range 0.24 to 0.72) for sheepshead minnows, and 0.60 (range 0.50 to 0.69) for rainbow trout. For 13 of the listed and related species, the factors ranged from 0.24 for fountain darter with carbaryl exposure and spotfin chub with copper exposure to 0.83 for greenback cutthroat trout with carbaryl exposure.

Discussion

Previous studies have reported that no one species is always the most or least sensitive to different contaminants (Macek and McAllister 1970; USEPA 1982; Birge and Black 1982; Blanck 1984; Mayer and Ellersieck 1986; Reish 1988). The sea lamprey, generally considered tolerant to contaminant exposure, was the most sensitive species to the lampricide 3-trifluoromethyl-4-nitrophenol (TFM), whereas many fish species generally considered sensitive to other chemicals were much less sensitive to TFM (Cairns 1986). No one species was always the most sensitive in the present study.

Mayer and Ellersieck (1986) determined that fish 96-hour LC50s for a given chemical varied as much as 9 orders of

Table 7. Acute toxicity of pentachlorophenol to 17 fishes and 1 amphibian including 96-hour LC50s, species sensitivity ranking, comparison of acute value with the range of values for rainbow trout or fathead minnows, and ratio of acute value to that for rainbow trout[a]

Species	LC50 (mg/L)	Rank	RBT Range Comparison	FHM Range Comparison	RBT LC50 Ratio
Fathead minnow	0.25	13	>	–	1.6
Sheepshead minnow	0.05	2	<	<	0.3
Rainbow trout	0.16	7	–	=	–
Atlantic sturgeon	<0.04	1	<	<	NC
Shortnose sturgeon	0.07	3	<	<	0.4
Shovelnose sturgeon	NC	–	–	–	–
Boreal toad	0.37	17	>	=	2.3
Razorback sucker	0.28	15	>	=	1.8
Bonytail chub	0.23	11	>	=	1.4
Cape Fear shiner	0.19	10	=	=	1.2
Colorado pikeminnow	0.24	12	>	=	1.5
Spotfin chub	0.26	14	>	=	1.6
Desert pupfish	NT	–	–	–	–
Leon Springs pupfish	0.08	4	<	<	0.5
Fountain darter	0.11	5.5	<	<	0.7
Greenthroat darter	0.18	9	=	=	1.1
Gila topminnow	0.34	16	>	=	2.1
Apache trout	0.11	5.5	<	<	0.7
Greenback cutthroat trout	>0.01	NR	–	–	–
Lahontan cutthroat trout	0.17	8	=	=	1.1

[a] For the range comparison, pentachlorophenol values for rainbow trout (0.12 to 0.19 mg/L, n = 6) or fathead minnows (0.14 to 0.44 mg/L, n = 6) were used (Dwyer *et al.* 1995; Sappington *et al.* 2001). "=" is within the range of 96-hour LC50s for rainbow trout or fathead minnow, ">" is greater than range maximum, and "<" is less than range minimum. The rainbow trout ratio was calculated by dividing the 96-hour LC50 for a species by the geometric mean LC50s for rainbow trout.
NC = Not calculated.
NT = Not tested.
NR = Not ranked.

magnitude. Blanck (1984) used data from various sources and found that the chemical sensitivity of algae varied by 7 orders of magnitude. Birge and Black (1982) reported LC50s for ≥ 5 aquatic species exposed to 50 different organic or inorganic toxicants. They reported that LC50s differed by 1 order of magnitude for 33% of the comparisons and up to 3 orders of magnitude for another 33% of the comparisons. Macek and McAllister (1970) reported the 96-hour LC50s for 12 species (5 families) varied by up to 4 orders of magnitude depending on the chemical. The present study did not find the same degree of reported variability. These studies included only 5 chemicals and were generally conducted under identical test conditions within only 2 laboratories, which likely decreased the variance.

Generally, of the listed species, the Atlantic and shortnose sturgeons were two of the most sensitive species (summary ranks of 1 and 2, respectively). As previously mentioned, because of concerns related to the chemical carrier solvent, any conclusions or applications of these results should be interpreted with caution. Our tests were conducted under static conditions, and additional testing under intermittent-flow or flow-through conditions may be more appropriate. Mayer (1971) found that early life-stage paddlefish, closely related to sturgeons, were much more sensitive to chlordane under static exposure conditions compared with continuously flowing conditions. Subsequent acute copper toxicity tests with shortnose sturgeon indicated similar sensitivities using static, static-renewal, and flow-through conditions (Chris Ivey, USGS, Columbia, MO, unpublished data, March 2003). King and Farrell (2002) conducted static acute toxicity tests with sturgeon exposed to chloramine-T, formalin, and sodium chloride, all therapeutic chemicals used routinely in aquaculture. Their findings indicate that Atlantic sturgeon were generally similar in sensitivity to striped bass but, when compared with rainbow trout, were less sensitive to chloramine-T and salinity but more sensitive to formalin. Their findings and ours indicate that sturgeon could be considered a sensitive species, and the use of contaminant assessments that are protective of sensitive fish species (e.g., rainbow trout) may be appropriate.

The three other most sensitive species included two Salmonidae (Apache trout and Lahontan cutthroat trout) and one Percidae (fountain darter). The Cyprinodontidae species were more sensitive to pentachlorophenol. Previous studies with sheepshead minnows indicated that they are more sensitive to pentachlorophenol at low salinities (Parrish *et al.* 1978; Borthwick and Schimmel 1978). This salinity effect may explain the increased sensitivities (lower LC50s) of sheepshead minnows and Leon Springs pupfish exposed to pentachlorophenol. Although ranking of species provides a general assessment of species sensitivities, the relative difference between LC50s is small in many cases. Therefore, assigning a rank may exaggerate differences between species. However, the results from this analysis are useful for evaluating how the acute sensitivity of a listed species compares with that of common test species.

Rainbow trout was identified as the most sensitive commonly tested species. Using the comparison criteria previously identified, there were 44 instances (61% of the total number of

Table 8. Acute toxicity of permethrin to 17 fishes and 1 amphibian including 96-hour LC50s, species sensitivity ranking, comparison of acute value with the range of values for rainbow trout or fathead minnows, and ratio of acute value to that for rainbow trout[a]

Species	LC50 (μg/L)	Rank	RBT Range Comparison	FHM Range Comparison	RBT LC50 Ratio
Fathead minnow	9.38	11	>	–	2.8
Sheepshead minnow	17.0	12	>	>	5.1
Rainbow trout	3.31	7	–	<	–
Atlantic sturgeon	<1.2	1.5	<	<	NC
Shortnose sturgeon	<1.2	1.5	<	<	NC
Shovelnose sturgeon	NC	–	–	–	–
Boreal toad	>10.0	NR	>	NC	NC
Razorback sucker	5.95	10	>	<	1.8
Bonytail chub	>25.0	15	>	>	NC
Cape Fear shiner	4.16	9	=	<	1.3
Colorado pikeminnow	24.4	14	>	>	7.4
Spotfin chub	1.70	4	=	<	0.5
Desert pupfish	NT	–	–	–	–
Leon Springs pupfish	21.0	13	>	>	6.3
Fountain darter	3.34	8	=	<	1.0
Greenthroat darter	2.71	6	=	<	0.8
Gila topminnow	>10.0	NR	>	NC	NC
Apache trout	1.71	5	=	<	0.5
Greenback cutthroat trout	>1.0	NR	–	–	–
Lahontan cutthroat trout	1.58	3	<	<	0.5

[a] For the range comparison, permethrin values for rainbow trout (1.65 to 4.8 μg/L, n = 6) or fathead minnows (6.68 to 15.7 μg/L, n = 6) were used (Dwyer *et al.* 1995; Sappington *et al.* 2001). "=" is within the range of LC50s for rainbow trout or fathead minnow, ">" is greater than range maximum, and "<" is less than range minimum. The rainbow trout ratio was calculated by dividing the 96-hour LC50s for a species by the geometric mean 96-hour LC50s for rainbow trout.
NC = Not calculated.
NR = Not ranked.
NT = Not tested.

comparisons) where the listed species was equal to or more sensitive than rainbow trout. Of these 44 comparisons, 32 (73%) of those tests included species in the seven families that were tested at a temperature >12°C, the test temperature used for Salmonidae testing. Test results indicate that acute aquatic assessments using rainbow trout data would often be protective of listed fish species. More important, procedures that exclude species because of temperature (such as a state revision to USEPA water-quality criteria) would likely not be protective of sensitive warmwater species. Rainbow trout data likely represent the response of sensitive warmwater species and not merely responses of coldwater species. Mount (1982), in a discussion paper prepared for the Surrogate Species Workshop, stated that "we should not confuse ecological habits or habitat with sensitivity." The representativeness of various species as identified in the present study would also be consistent with Stephan *et al.* (1985) who stated that "results of acute and chronic toxicity tests with representative species of aquatic animals are necessary so that data available for tested species can be considered a useful indication of the sensitivities of appropriate untested species."

It has been shown that intralaboratory tests may range up to a factor of twofold for the same species–chemical test combinations (Schimmel 1981; Lemke 1981; DeGraeve *et al.* 1991). In the present study, only five 96-hour LC50s fell below a factor of 0.5 (intralaboratory variation) and three of those were for sturgeon. These findings indicate that if an aquatic-listed fish species requires protection from acute exposures, a factor

Table 9. Summary rank for species[a]

Family	Species	Summary Rank
Cyprinidae	Fathead minnow	16
Cyprinodontidae	Sheepshead minnow	11
Salmonidae	Rainbow trout	6
Acipenseridae	Atlantic sturgeon	1
Acipenseridae	Shortnose sturgeon	2
Acipenseridae	Shovelnose sturgeon	NR
Bufonidae	Boreal toad	12
Catostomidae	Razorback sucker	10
Cyprinidac	Bonytail chub	14
Cyprinidae	Cape Fear shiner	9
Cyprinidae	Colorado pikeminnow	13
Cyprinidae	Spotfin chub	7
Cyprinodontidae	Desert pupfish	NR
Cyprinodontidae	Leon Springs pupfish	15
Percidae	Fountain darter	4
Percidae	Greenthroat darter	8
Poeciliidae	Gila topminnow	NR
Salmonidae	Apache trout	3
Salmonidae	Greenback cutthroat trout	NR
Salmonidae	Lahontan cutthroat trout	5

[a] The summary rank was calculated by averaging the individual ranks obtained for each species and chemical (Tables 4 to 8) and then reranking. Species were only included if they had rankings for ≥4 chemicals.
NR = Not ranked.

Table 10. Ratios developed within a single species–chemical test[a]

Species	Carbaryl	Copper	4-Nonylphenol	Pentachlorophenol	Permethrin	$\bar{x}$
Fathead minnow	0.55	0.41	0.67	0.58	0.48	0.53
Sheepshead minnow	0.39	0.24	0.63	0.72	0.59	0.48
Rainbow trout	0.50	0.69	0.63	0.60	0.59	0.60
Shortnose sturgeon	0.63	0.63	0.75	0.55	NC	0.64
Boreal toad	NC	0.68	0.74	0.69	NC	NC
Razorback sucker	0.68	0.52	0.67	0.72	0.56	0.63
Bonytail chub	0.50	0.63	0.68	0.65	NC	0.61
Cape Fear shiner	0.39	0.43	0.78	0.68	0.67	0.57
Colorado pikeminnow	0.69	0.39	0.67	0.76	0.54	0.59
Spotfin chub	0.65	0.24	0.75	0.73	NC	0.54
Leon Springs pupfish	0.37	0.57	0.60	0.45	0.48	0.49
Fountain darter	0.24	0.46	0.30	0.72	0.60	0.43
Greenthroat darter	0.44	0.26	0.68	0.44	0.65	0.47
Gila topminnow	NC	0.59	0.65	0.74	NC	NC
Apache trout	0.57	0.43	0.71	0.62	0.57	0.57
Greenback cutthroat trout	0.83	NC	0.62	NC	0.42	NC
Lahontan cutthroat trout	0.54	0.71	0.55	0.53	0.62	0.59
Common test species $\bar{x}$	0.48	0.41	0.64	0.63	0.55	
Listed species $\bar{x}$	0.52	0.48	0.64	0.63	0.56	

[a] This was done by dividing the average concentration for replicates that had a 0% or 10% mortality by the LC50 for that test (USEPA 1978). A 10% level of mortality was used in this evaluation because it is considered acceptable control mortality and therefore could not be discerned from an effect of chemical exposure. Geometric means of ratios were then calculated (overall geometric mean all species = 0.56).
NC = Not calculated.
USEPA = United States Environmental Protection Agency.

of 0.63 can be applied to the geometric mean LC50 of rainbow trout toxicity data. If additional protection is desired, a conservative factor of 0.46 (0.63 − 1 SD) could be applied, which is similar to intralaboratory variation estimates. The greatest single difference found between rainbow trout and a listed species was a factor of 0.3, and it is comparable to a factor of 0.33 (0.63 − 2 SD), the range generally expected to encompass 95% of individual responses within a representative population (Snedecor and Cochran 1989). Regardless of the factor used, these factors are less of an adjustment than using a safety factor of 0.1 (division by a safety factor of 10). The use of factors allows for developing protective measures for untested species by drawing from rainbow trout toxicity data.

For each of the 11 listed and related species that had values for at least 4 chemicals, factors for estimating no- or low-effect concentrations ranged from 0.43 (fountain darter) to 0.64 (shortnose sturgeon). As mentioned previously, the fountain darter had a 5% to 15% mortality in most low-exposure concentrations (below observed concentration-effect curve), regardless of the chemical tested. This mortality would decrease the slope of the dose-response curve and provide the smaller factor (0.43). Therefore, these findings for the fountain darter should be evaluated with caution. Within a chemical, the average factor to calculate a no- or low-effect concentration for listed species ranged from 0.50 (copper) to 0.66 (4-nonylphenol and pentachlorophenol). The overall geometric mean factor for all species to estimate a no- or low-effect concentration from 96-hour LC50 data is 0.56, similar to the 0.5 derived for developing acute water-quality criteria (USEPA 1978) and support the 1985 USEPA guidance for determining a no- or low-acute effect concentration (criterion maximum concentration), which requires dividing the final acute value by 2 (Stephan *et al.* 1985).

Besser *et al.* (2004) compared the chronic toxicity of copper and pentachlorophenol using the commonly tested species fathead minnow and rainbow trout and the federally listed endangered fountain darter and federally listed threatened spotfin chub. The fountain darter was more sensitive than the commonly tested species to both chemicals, but spotfin chub were similar in sensitivity. Augspurger *et al.* (2003) compared the sensitivities of mussels and found that mussels were a sensitive family of species when exposed to ammonia. These results indicated that some species and certain groups of organisms (e.g., Unionidae) may not be adequately protected if appropriate sensitive species (e.g., rainbow) are not included in contaminant assessments. Milam *et al.* (2004) conducted 24-hour acute toxicity tests with early life stages (glochidia) of six freshwater mussel species as well as two commonly tested species, *Ceriodaphnia dubia* and *Daphnia magna*. Chemicals exposures included the five chemicals in the present study and 2,4-D. They found that no mussel species was always the most sensitive, and Daphnidae were generally protective of freshwater mussel glochidia.

The USEPA Standard Evaluation Procedure for Ecological Risk Assessment for pesticides and endangered species defines criteria for estimating risk (USEPA 1986). A formal endangered species consultation (interagency regulatory review) is required if the expected environmental concentration is greater than "1/10th the lowest aquatic acute LC_{10} (when a slope is available) or greater than 1/20th the lowest aquatic LC50 (when no slope is available)" (USEPA 1986). Although the risk assessment document provides guidance on when a consultation must take place, there is no guidance provided on how contaminant sensitivities among species should be evaluated.

Ultimately, resource managers responsible for the risk assessments for listed species will decide if there is substantial

risk to these species. The USFWS has >90 threatened and endangered fish species, making the task to develop specific data for each species and chemical daunting. We directly tested early life stages of 17 species and found that the rainbow trout was equal to or more sensitive than the listed species 81% of the time and, therefore, those procedures that protect rainbow trout would in many cases protect listed fish species.

If an aquatic-listed fish species requires greater protection, a factor of 0.63 can be applied to the geometric mean LC50 of rainbow trout toxicity data, and more conservative estimates can be determined using variance estimates. Regardless of the factor used, these estimates are less of an adjustment than division by a safety factor of 0.1.

Also, a no- or low-effect acute concentration can be estimated by using a factor of approximately 0.56. More conservative estimates of no- or low-effect acute concentrations can be estimated by applying a factor of 0.43. Environmental or target environmental concentrations could then be compared with this calculated number, and an evaluation of the acute mortality risk to the species could be made.

In summary, only direct testing provides specific information regarding protection of listed species. Certain listed fishes are amenable to direct toxicologic assessment using standard methods. When captive or locally abundant populations of listed fish are available, consideration should be given to direct testing (under appropriate state and federal permits). When direct testing cannot be performed, estimates of the degree of protection or approaches for developing protective measures can be made using data from other species that are often available.

Acknowledgments. This project was sponsored in part by the United States Environmental Protection Agency, Offices of Research and Development, Pesticide Programs, Pollution Prevention and Toxics, and Water under Interagency Agreement DW14935155; the New York Department of Environmental Conservation from the Environmental Protection Fund through the Hudson River Estuary Program; and the United States. Fish and Wildlife Service, Ecological Services Field Office in Raleigh, NC. We also thank the federal, state, and private hatcheries and agencies listed in Table 1 that worked with us to provide the species used in testing. The authors thank Jeri Folse and Shannon Holbrook for their technical assistance and Tim Sinnott (New York State Department of Environmental Conservation, Division of Fish, Wildlife, and Marine Resources) for his review. We also thank three anonymous reviewers for providing input on the manuscript. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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