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Assessing Contaminant Sensitivity of Endangered and Threatened Species: Toxicant Classes

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by

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Abstract

Under the Federal Insecticide, Fungicide and Rodenticide Act, the Toxic Substances Control Act and the Clean Water Act, the U.S. Environmental Protection Agency (EPA) is charged with determining if the manufacture, use, or disposal of a chemical will present an unreasonable risk of harm to the environment. Typically, management decisions are based on protecting populations of organisms. However, the Endangered Species Act requires that, in some cases, managers must estimate the take of individuals to determine if the loss of individuals might adversely affect a population of an endangered or threatened (listed) species. The most direct assessment would be to determine the sensitivity of a listed species to a particular contaminant or perturbation. However, this direct approach would be time consuming and expensive because it might require development of organism culturing and handling procedures, some species may not be amenable to culture, there might be multiple species to be considered, and would be contaminant specific.

It is not possible to test all listed species that may need protection from environmental contaminants. Therefore, decisions need to be made for listed species using toxicity data obtained from standard test procedures and using surrogate organisms typically tested in laboratory toxicity assessments (e.g., rainbow trout *Oncorhynchus mykiss*, fathead minnow *Pimephales promelas*, and the cladoceran *Ceriodaphnia dubia*). These surrogate species are easily tested using standardized methods; however, there is concern that these species or procedures may not adequately represent populations of listed species. By evaluating the sensitivity for a number of listed species, it is possible to make generalizations regarding the protection afforded listed species through standard regulatory programs. This research project had two objectives: (1) determine the relative sensitivity to contaminants of listed species using standard acute toxicity tests; and (2) determine the degree of protection afforded listed fish species through the use of standard species used in whole effluent toxicity tests.

Previous cooperative research conducted between the EPA and U.S. Geological Survey primarily evaluated the similarity in response to five chemicals with different modes of action (carbaryl, copper, 4-nonylphenol, pentachlorophenol and permethrin) between surrogate (rainbow trout and fathead minnows) and listed species within the same taxonomic family (Salmonidae, Cyprinidae) using standard acute toxicity tests. The present study expands this data base by testing five additional species with these five chemicals. Species were listed either by the U.S. Fish and Wildlife Service (FWS) or state agencies or were species identified as surrogates in FWS Recovery Plans. Organisms included: (1) the Family Percidae fountain darter (*Etheostoma rubrum*, Federally listed), greenthroat darter (*Etheostoma lepidum*, state listed - Texas); (2) the Family Acipenseridae, shovelnose sturgeon (*Scaphirhynchus platorynchus*, identified as surrogate for the Federally listed pallid sturgeon - *Scaphirhynchus albus*); (3) the Family Poeciliidae, Gila topminnow (*Poeciliopsis occidentalis*, Federally listed); and (4) the Family Bufonidae, boreal toad tadpoles (*Bufo boreas*, state listed - Colorado).

The data we have generated indicates that in 96-h acute toxicity tests, if rainbow trout is used as a test species, a species typically used in pesticide registration or water quality criteria derivation, those procedures which protect the rainbow trout would likely be protective of most listed aquatic fish species. If a safety factor is needed to estimate 96-h LC_{50}s for listed fish species, our data indicates that 0.5 would be a conservative estimator. Also, if EPA water quality criteria are recalculated by eliminating certain species from the data set, such as rainbow trout, then listed fish species might not be adequately protected.

Notice

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Contents

Introduction

Under the Federal Insecticide, Fungicide and Rodenticide Act, the Toxic Substances Control Act and the Clean Water Act, the U.S. Environmental Protection Agency (EPA) is charged with determining whether the manufacture, use, or disposal of a chemical will present an unreasonable risk of harm to the environment. Typically, management decisions are based on protecting populations of organisms. However, the Endangered Species Act requires that, in some cases, managers must estimate the take of individuals to determine if the loss of individuals might adversely affect a population of an endangered or threatened (listed) species. The most direct assessment would be to determine the sensitivity of a listed species to a particular contaminant or perturbation. However, this direct approach would be time consuming and expensive because it might require development of organism culturing and handling procedures, some species may not be amenable to culture, there might be multiple species to be considered, and would be contaminant specific. Therefore, it is not possible to test all listed species that might need protection from environmental contaminants.

An indirect approach for determining the sensitivity of listed species would be to use toxicity data obtained from standard test procedures and using surrogate organisms typically tested in laboratory toxicity assessments (e.g., rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), bluegill (*Lepomis macrochirus*). These surrogate species are easily tested using standardized methods (EPA 1975, ASTM 1998); however, there is concern that these species or procedures may not adequately represent population of listed species. By evaluating the sensitivity for a number of listed species, it is possible to make generalizations regarding the protection afforded listed species through standard regulatory programs.

Previous cooperative research conducted by the EPA and U.S. Geological Survey (EPA 1995) primarily evaluated the similarity in sensitivity between surrogate and listed species within the same taxonomic family. Acute toxicity tests were conducted for 96 h with the rainbow trout, fathead minnow, and the following listed species: Apache trout (*Oncorhynchus apache*), Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*), greenback cutthroat trout (*Oncorhynchus clarki stomias*), bonytail chub (*Gila elegans*), Colorado squawfish (*Ptychocheilus lucias*), and razorback sucker (*Xyrauchen texanus*). Endpoints evaluated included mortality at 3, 6, 9, 12, 18, 24, 48, 72, and 96 h of exposure. Chemicals tested included: carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin. These chemicals were selected to represent different chemical classes and toxic modes of action. Results from these studies indicated that the standard test organisms (rainbow trout and fathead minnow) often had a similar sensitivity to toxicant exposure as the listed salmonid and cyprinid species. However, for about 30% of the possible surrogate/listed species comparisons, the listed species was more sensitive than the standard surrogate species of the same family.

The objective of the present study was to expand this acute toxicity data base by conducting tests on the same five chemicals with five additional species. The selection of species tested in the present study was based on availability of listed organisms. Species were listed either by the U.S. Fish and Wildlife Service (FWS) or state agencies, or were species identified as surrogates in the FWS Recovery Plan. Organisms evaluated in the present study included: (1) Percidae - fountain darter (*Etheostoma rubrum*, Federally listed), greenthroat darter (*Etheostoma lepidum*, state listed - Texas); (2) Acipenseridae - shovelnose sturgeon (*Scaphirhynchus platorynchus*, identified as surrogate for the Federally listed pallid sturgeon - *Scaphirhynchus albus*); (3) Poeciliidae - Gila topminnow (*Poeciliopsis occidentalis*, Federally listed); and (4) Bufonidae-boreal toad tadpoles (*Bufo boreas*, state listed-Colorado). Toxicity tests were attempted with the shortnose sucker (*Chasmistes brevirostris*, Family Catostomidae, Federally listed), but this species exhibited excessive mortality during test acclimation and therefore was not tested. Toxicity tests have been successfully conducted elsewhere with shortnose suckers of the same size used in this study (L. Cleveland, USGS, Columbia, MO, personal communication). We believe that this particular lot of fish was inferior because handling and acclimation to test conditions resulted in substantial mortalities.

Materials and Methods

Test organisms

Fountain darters, greenthroat darters, shovelnose sturgeon, gila topminnow, and boreal toads were obtained from various federal and state sources during 1995 and 1996 (Table 1). The fishes were received as fry and the toads were received as tadpoles.

Fishes and tadpoles 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 Columbia Environmental Research Center (CERC, Columbia, MO) until acclimation began. Before the start of a toxicity test, organisms were acclimated for a total of 96 h (EPA 1975, ASTM 1998). For the first 48 h, organisms were acclimated to the test water and temperature. The test organisms were then moved to clean containers and held for an additional 48 h at the test temperature in 100% test water. Organisms were not fed during the 48 h of holding in 100% test water.

Chemicals

The chemicals used in testing were carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin (Table 2). Chemicals were selected to represent different classes of chemical and modes of toxic action. Organic chemical stock solutions were prepared by dissolving the chemical in reagent grade acetone, whereas stock solutions for copper were prepared by dissolving copper in deionized water. The maximum acetone concentration in any test container was 0.05 mL/L.

Organic and inorganic chemical stocks were analyzed to confirm nominal concentrations. Organic chemical analysis was conducted at either Mississippi State Chemical Laboratory (Mississippi State, MS) or ABC Laboratories (Columbia, MO) using gas chromatography. Copper stocks were confirmed at either the CERC or Mississippi State Chemical Laboratory by atomic absorption spectrophotometry. Overall, the mean percent nominal concentration was 110% (n = 9), with a mean range of 63% (copper) to 160% (permethrin). One 4-nonylphenol stock had a percent nominal concentration of 320%. However, biological results from the tests using these stocks were similar to tests conducted with other 4-nonylphenol stocks. Therefore, we believe the reported value for this sample is incorrect and that percent recovery was not included in the average percent of nominal concentration.

Table 1. Source and size of test organisms used in toxicity tests.

Species	Scientific Name	Source	Size
Fountain darter	*Etheostoma rubrum*	San Marcos National Fish Hatchery and Technology Center, San Marcos, TX	62 mg ± 19 20.2 mm ± 2.0
Greenthroat darter	*Etheostoma lepidum*	San Marcos National Fish Hatchery and Technology Center, San Marcos, TX	133 mg ± 19 22.6 mm ± 0.4
Shovelnose sturgeon	*Scaphirhynchus platorynchus*	Blind Pony Missouri State Fish Hatchery, Sweet Springs, MO	719 mg ± 237 60.1 mm ± 0.8
Gila topminnow	*Poeciliopsis occidentalis*	Dexter National Fish Hatchery, Dexter, NM	219 mg ± 65 27.2 mm ± 2.6
Boreal toad	*Bufo boreas*	Colorado Division of Wildlife, collected from the West Fork of Clear Creek, near Georgetown, CO	all tests except carbaryl - 12 mg (mean of 20 weighed as group) 9.6 mm ± 0.7 carbaryl - class 2 (about 200 mg)

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	Donated by Rhone-Pôulenc Agricultural Co., Research Triangle Park, NC	99.7	carbamate insecticide	inhibitor of cholinesterase activity
Copper sulfate	Fisher Chemical, St. Louis, MO	25.5	mining, industrial, fungicide	interferes in osmoregulation
4-nonylphenol	Fluka Chemical, New York, NY	85.0	nonylphenol ethoxylate detergents	narcotic and oxidative stressor
Pentachlorophenol	Aldrich Chemical, Milwaukee, WI	99.0	wood preservative, molluscicide	uncoupler of oxidative phosphorylation
Permethrin	Donated by ICI Americas Inc., Richmond, CA	95.2	pyrethroid insecticide	neurotoxin

Toxicity tests

Static acute toxicity tests were conducted in basic accordance with procedures described in EPA (1975) and ASTM (1998). Exposures were conducted in 19.6-L glass jars containing 15 L of test solution. All tests were conducted at 22°C. Test water was reconstituted hard water (alkalinity 110 to120 mg/L as $CaCO_3$, hardness 160 to180 mg/L as $CaCO_3$ - ASTM 1998). One study with the boreal toad was conducted in CERC well water. Tests were conducted under ambient lighting.

The exposure series consisted of six concentrations with a 60% dilution series tested in duplicate (except for the tests with the boreal toad, which were tested in triplicate). When a solvent was used, both a solvent control and a dilution water control were included for each species. Individual test series were randomly assigned to a waterbath and location within a waterbath (complete block design).

Fishes and tadpoles were counted into two groups (3 to 5 organisms per group depending on availability) and pooled for each exposure replicate (7 to 10 organisms/replicate). Mortality was the endpoint measured at 6, 12, 24, 48, 72, and 96 h of exposure and was defined as lack of movement for a 5-s observation with the unaided eye. Dead animals were removed at each observational time. The study design for each species is summarized in Table3.

Carbaryl concentrations used in the test conducted with the boreal toad tadpoles were not high enough to estimate LC_{50} concentrations. Subsequent testing with boreal toad tadpoles was being performed concurrently with this study. In that testing, exposures were conducted in the well water used for culture and the carbaryl testing had a 70% dilution series. All other conditions were similar.

Water quality

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 suggested ranges, but average pH (8.4) was slightly above the suggested value of 8.0 (Table 4).

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

Test Type:	Static acute
Static Acute	15 L
Test Volume:	15 L
Test Temperature:	22°C
Water Quality:	Reconstituted ASTM hard (alkalinity 110 to 120 mg/L as $CaCO_3$, hardness 160 to 180 mg/L as $CaCO_3$)[1]
Chemicals:	Carbaryl, copper, 4-nonylphenol, pentachlorophenol, permethrin
Dilution Series:	60%
Replicates/number of organisms per replicate:	Fountain darter - 2 replicates/10 fish per replicate Greenthroat darter - 2 replicates/7 fish per replicate Shovelnose sturgeon - 2 replicates/9 fish per replicate Gila topminnow - 2 replicates/10 fish per replicate Boreal toad - 3 replicates/10 tadpoles per replicate
Observations:	Mortality at 6, 12, 24, 48, 72, and 96 h of exposure

[1]Carbaryl exposures with boreal toads were conducted in well water.

Table 4. Average (± standard deviation)

Water Quality Characteristic	Nominal Value	Measured (n = 4)
Alkalinity[1]	110 - 120	115 ± 1
Hardness[1]	160 - 180	167 ± 5
pH	7.8 - 8.0	8.4 ± 0.1

[1]mg/L as $CaCO_3$

The pH was measured on the control, low, medium, and high exposure concentrations at 0 h and in those same treatments if organisms survived to 96 h of exposure. Test chemicals spiked into the test water altered pH , but not in a consistent pattern. Appendix1 lists the pH which represents the lowest and highest pH measured for all exposure concentrations and replicates within a test at the start and end of the exposure.

Dissolved oxygen was measured on the control, low, medium, and high exposure concentrations at 0 h and in those same treatments if organisms survived to 48 and 96 h of exposure. Appendix 2 is a list of the exposure replicates and concentrations for which dissolved oxygen was below 60% saturation at 48 h of exposure or below 40% saturation at 96 h of exposure. Any drop in dissolved oxygen was isolated and interspersed throughout the exposures. However, in toxicity tests with shovelnose sturgeon, jars with acetone added either as a control or as a chemical carrier had low concentrations of dissolved oxygen at 48 h of exposure. The low concentrations of dissolved oxygen in those jars may have been the cause for the mortalities observed in that test at 72 h of exposure. For this reason, data generated from toxicity tests with shovelnose sturgeon using solvent carriers should be interpreted with caution. We did not include any toxicity data for shovelnose sturgeon toxicity tests using acetone as a carrier solvent beyond 48 h of exposure.

Statistical Analysis

The LC_{50} and 95% confidence interval for each test was usually calculated using probit analysis. However, when probit analysis was not appropriate (i.e., less than two partial mortalities), LC_{50}s and confidence intervals were calculated using moving average or a non-linear interpolative procedure (Stephan 1977). The LC_{50}s and confidence intervals were determined using nominal concentrations.

In the previous study conducted by the EPA and USGS (EPA 1995) six different tests (3 replicates per test) for each of the five chemicals were conducted with the rainbow trout and fathead minnow and two different tests for the six listed species. In that study, similar test conditions were used (static acute toxicity tests, reconstituted hard water and 60% dilution series) with coldwater species being tested at 12°C and warmwater species tested at 22°C.

In the previous study, statistical tests to determine differences between species used analysis of variance and least square mean separations on ranked LC_{50}s (EPA 1995). For the present study, we did not have multiple tests for the listed species. Therefore to compare listed species responses to rainbow trout, an overall LC_{50} and confidence interval for each chemical was calculated for rainbow trout by combining all replicates from the six tests. The LC_{50}s for fountain darters, greenthroat darters, shovelnose sturgeon, gila topminnow, and boreal toads calculated in the present study were then compared to the overall LC_{50} for rainbow trout. Differences between LC_{50}s were tested for statistical significance using the procedure described by Sprague and Fogels (1976).

Results and Discussion

Control survival, with and without solvent, was always greater than 90% for all species except the shovelnose sturgeon (Table 5). Appendix 3 is a listing of all LC_{50}s and confidence intervals for each chemical, species, and observation time period.

In the toxicity tests with fountain darters, average control survival without acetone was 97% and with acetone was 93%. However, there was a 5 to 15 % mortality in most low (below observed concentration-effect curve) exposure concentrations, regardless of the chemical tested.

Tables 6 to 10 summarize the 12, 24 and 96 h LC_{50}s for all five chemicals and each species. In general, at 96 h of exposure, permethrin was the most toxic compound and carbaryl was the least toxic compound. These results were similar to those reported in the previous study (EPA 1995). The two phenolic compounds (4-nonylphenol and pentachlorophenol) and copper had LC_{50}s in a similar range of concentrations.

Consistent with previous studies (Macek and McAllister 1970, EPA 1982, Birge and Black 1982, Blank 1984, Mayer and Ellersieck 1986, Reish 1988) no one species was always the most sensitive species to all chemicals. Generally, of the listed species, the fountain darter was more sensitive to contaminant exposure than the greenthroat darter, shovelnose sturgeon, Gila topminnow, or boreal toad. After 96 h of exposure, LC_{50}s for the fountain darter were similar to rainbow trout LC_{50}s for carbaryl (Table 6), pentachlorophenol (Table 9) and permethrin (Table 10). The LC_{50}s for fountain darters were less than those for rainbow trout for copper (Table 7) and 4-nonylphenol (Table 8), however the LC_{50}s were within a factor of 0.6.

The boreal toad was generally more resistant than the rainbow trout with LC_{50}s for carbaryl, copper, pentachlorophenol and permethrin statistically greater (1.5 - 6.5 times) than the LC_{50} for rainbow trout. In contrast, the 4-nonylphenol LC_{50} for boreal toad tadpoles was significantly less than the LC_{50} for rainbow trout. These taxonomic comparisons are consistent with the findings of Mayer and Ellersieck (1986). They determined that amphibians (western chorus frog - *Pseudacris triseriata* and Fowler's toad - *Bufo woodhouseri fowleri*) were

Table 5. Average control survival for listed species. All survival is at 96 h of exposure except where noted.

Species	Control Survival without Solvent (%)	Control Survival with Solvent (%)
Fountain darter	97	93
Greenthroat darter	100	93
Shovelnose sturgeon	48 h - 100 96 h - 100	48 h - 100 96 h - 0
Gila topminnow	100	100
Boreal toad	100	100

Table 6. Acute toxicity of carbaryl (mg/L) at 12, 24, and 96 h of exposure. Also included are the geometric means of LC_{50}s (n=6) for rainbow trout and fathead minnows tested using similar test conditions (EPA 1995). An asterisk (*) indicates the LC_{50} for the listed species is significantly different ($p < 0.05$) than the LC_{50} for rainbow trout. LC_{50}s for fathead minnows are provided for reference purposes.

Species	12-h LC_{50}	24-h LC_{50}	96-h LC_{50}
Fountain darter	>3.0	>3.0	2.02
Greenthroat darter	>3.0	>3.0	2.14
Shovelnose sturgeon	4.90*	3.60	nc[1]
Gila topminnow	>3.0	>3.0	>3.0
Boreal toad[2]	>21	>21	12.31*
Rainbow trout	6.76	4.04	1.88
Fathead minnow	12.00	8.25	5.21

[1]nc = not calculable
[2]toxicity test with boreal toad was conducted in well water

Table 7. Acute toxicity of copper (mg/L) at 12, 24, and 96 h of exposure. Also included are the geometric means of LC_{50}s (n=6) for rainbow trout and fathead minnows tested using similar test conditions (EPA 1995). An asterisk (*) indicates the LC_{50} for the listed species is significantly different ($p \leq 0.05$) than the LC_{50} for rainbow trout. LC_{50}s for fathead minnows are provided for reference purposes.

Species	12-h LC_{50}	24-h LC_{50}	96-h LC_{50}
Fountain darter	0.24*	0.15*	0.06*
Greenthroat darter	>0.3	>0.3	0.26*
Shovelnose sturgeon	>0.6	0.54*	0.16*
Gila topminnow	>0.3	>0.3	0.16*
Boreal toad	0.19*	0.16*	0.12*
Rainbow trout	0.40	0.12	0.08
Fathead minnow	1.30	0.73	0.47

Table 8. Acute toxicity of 4-nonylphenol (mg/L) at 12, 24, and 96 h of exposure. Also included are the geometric means of LC_{50}s (n=6) for rainbow trout and fathead minnows tested using similar test conditions (EPA 1995). An asterisk (*) indicates the LC_{50} for the listed species is significantly different ($p \leq 0.05$) than the LC_{50} for rainbow trout. LC_{50}s for fathead minnows are provided for reference purposes.

Species	12-h LC_{50}	24-h LC_{50}	96-h LC_{50}
Fountain darter	>0.25	0.21*	0.11*
Greenthroat darter	>0.25	0.23*	0.19
Shovelnose sturgeon	0.25	0.20*	<0.13
Gila topminnow	>0.25	>0.25	0.23*
Boreal toad	0.12*	0.12*	0.12*
Rainbow trout	0.35	0.30	0.19
Fathead minnow	0.38	0.33	0.27

Table 9. Acute toxicity of pentachlorophenol (mg/L) at 12, 24, and 96 h of exposure. Also included are the geometric means of LC_{50}s (n=6) for rainbow trout and fathead minnows tested using similar test conditions (EPA 1995). An asterisk (*) indicates the LC_{50} for the listed species is significantly different ($p \leq 0.05$) than the LC_{50} for rainbow trout. LC_{50}s for fathead minnows are provided for reference purposes.

Species	12-h LC_{50}	24-h LC_{50}	96-h LC_{50}
Fountain darter	0.20*	0.17	0.11
Greenthroat darter	0.31*	0.30*	0.18
Shovelnose sturgeon	0.16*	<0.13	nc[1]
Gila topminnow	>0.7	0.64*	0.34*
Boreal toad	>0.7	>0.42	0.37*
Rainbow trout	0.22	0.17	0.16
Fathead minnow	0.33	0.30	0.25

[1]nc = not calculable

Table 10. Acute toxicity of permethrin (ug/L) at 12, 24, and 96 h of exposure. Also included are the geometric means of LC_{50}s (n=6) for rainbow trout and fathead minnows tested using similar test conditions (EPA 1995). An asterisk (*) indicates the LC_{50} for the listed species is significantly different ($p \leq 0.05$) than the LC_{50} for rainbow trout. LC_{50}s for fathead minnows are provided for reference purposes.

Species	12-h LC_{50}	24-h LC_{50}	96-h LC_{50}
Fountain darter	5.60	4.26	3.34
Greenthroat darter	3.10*	2.71*	2.71*
Shovelnose sturgeon	>10.0	nc[1]	nc[1]
Gila topminnow	>10.0	>10.0	>10.0
Boreal toad	>10.0	>10.0	>10.0
Rainbow trout	5.75	3.78	3.31
Fathead minnow	13.43	9.73	9.38

[1]nc = not calculable

generally the more resistant taxonomic group compared to either rainbow trout or fathead minnows. Results from the present study indicate that in acute exposures the boreal toad is generally more resistant than the rainbow trout. The boreal toad LC_{50}s are also greater than the LC_{50}s for fathead minnows exposed to carbaryl, pentachlorophenol, or permethrin but less than the LC_{50}s for the fathead minnow exposed to copper or 4-nonylphenol.

For the following discussion we have included data from the present study and data generated in previous cooperative research conducted between the EPA and USGS (EPA 1995) for the same five chemicals with rainbow trout, fathead minnows and six different listed species - Apache trout, Lahontan cutthroat trout, greenback cutthroat trout, bonytail chub, Colorado squawfish, and razorback sucker. Toxicity data for these tests at 12, 24, and 96 h of exposure are listed in Appendix 4. In order to evaluate species sensitivity, within a chemical, we ranked 96-h LC_{50}s for each species, from 1 (high sensitivity) up to 13 (low sensitivity). Ranks were then averaged across chemicals for each species (Table 11). There were four listed species (Apache trout, greenback cutthroat trout, fountain darter, and Lahontan cutthroat trout) that were overall more sensitive than the rainbow trout, while, overall the fathead minnow was the least sensitive species.

Table 11. Rank of species sensitivity using 96-h LC_{50}s. Within a chemical, the most sensitive species (lowest LC_{50}) was assigned a rank of 1 whereas the least sensitive species was assigned a rank up to 13 (highest LC_{50}). Ranks were then averaged across chemicals for each species.

Species	Carbaryl	Copper	Nonylphenol	PCP[1]	Permethrin	Average Rank
Rainbow trout	3	4	7.5	3	4	4.3
Fathead minnow	10	12	11	7	7	9.4
Fountain darter	4	1	1	1.5	5	2.5
Greenthroat darter	5	9	7.5	5	3	5.9
Shovelnose sturgeon	not ranked	6.5	not ranked	not ranked	not ranked	6.5
Gila topminnow	not ranked	6.5	9	9	not ranked	8.2
Boreal toad	11	5	2	10	not ranked	7
Apache trout	1	2.5	4.5	1.5	2	2.3
Greenback cutthroat trout	2	not ranked	3	not ranked	not ranked	2.5
Lahontan cutthroat trout	6	2.5	6	4	1	3.9
Bonytail chub	8	8	12	5	not ranked	8.3
Colorado squawfish	7	11	10	6	8	8.4
Razorback sucker	9	10	4.5	8	6	7.5

[1] - pentachlorophenol

In addition to relative species sensitivity, the magnitude of difference between LC_{50}s is also important. Using data from the previous study for the six rainbow trout tests with each chemical (EPA 1995), we calculated two factors (lowest 96-h LC_{50}/mean 96-h LC_{50}; mean 96-h LC_{50}/ highest 96-h LC_{50}) which encompassed the range of LC_{50}s for that chemical. For example, for the six toxicity tests conducted with rainbow trout and carbaryl (EPA 1995), the lowest 96-h LC_{50} was 1.22 mg/L, the highest 96-h LC_{50} was 3.11, and the mean 96-h LC_{50} was 1.88 mg/L. Hence, factors calculated for rainbow trout carbaryl exposures were 0.60 and 0.65 with a geometric mean of 0.62. For the five chemicals tested with rainbow trout, the geometric mean factor for all five chemicals was 0.69 with a range of 0.60 (permethrin) to 0.80 (pentachlorophenol). We followed the same procedure for fathead minnows and the five chemicals. Fathead minnows had an geometric mean factor for the five chemicals of 0.65 with a range of 0.57 (pentachlorophenol) to 0.73 (permethrin). If a factor of 0.67 is selected as representative of the normal range in LC_{50} (expected range = LC_{50}X0.67 to LC_{50}/0.67) for a specific chemical and species, then the sensitivities of listed species can be evaluated in terms of how often 96-h LC_{50}s for the listed species differed by more than a factor of 0.67

from the 96-h LC_{50} for either rainbow trout or fathead minnows.

For the 11 listed species tested there were 48 comparisons that could be made to rainbow trout and 46 comparisons to fathead minnows. When a factor of 0.67 is applied to the geometric mean LC_{50} for each of the five chemicals tested with rainbow trout, 24 LC_{50}s for listed species are outside this range but only 4 LC_{50}s are less than the expected range of LC_{50}s for rainbow trout. When the factor of 0.67 is applied to the geometric mean LC_{50} for the five chemicals tested relative to fathead minnows, there are 31 LC_{50}s for listed species outside the range with 28 of the 31 LC_{50}s less than the expected range of LC_{50}s for the fathead minnow. The comparison of rainbow trout and fathead minnows includes species from both Salmonidae and Cyprinidae and five additional families (4 fish and 1 amphibian - Percidae, Acipenseridae, Poeciliidae, Bufonidae, and Catostomidae) and included tests conducted at 12 and 22°C. In only 8% of the comparisons to rainbow trout (4 of 48) was a listed species more sensitive than rainbow trout. This would indicate that for acute environmental assessments, toxicity data for rainbow trout are generally similar to or protective of listed fish species.

Endangered species may require an additional degree of protection since their populations are already in decline and any additional loss of individuals may lead to extinction. The previous discussion has shown that the rainbow trout is generally more sensitive than the fathead minnow and that listed species are frequently more sensitive than fathead minnows. Also, only 4 of 48 tests with a listed species had an LC_{50} less than a factor of 0.67 from that of the rainbow trout. A final evaluation would be to determine the greatest difference between the 96-h LC_{50}s of the rainbow trout and a listed species. Within a chemical, we compared the lowest 96-h LC_{50} for a listed species to the geometric mean 96-h LC_{50} for rainbow trout. For all five chemicals, at least one listed species had a 96-h LC_{50} lower than the 96-h LC_{50} for rainbow trout. There were a total of 16 LC_{50}s for listed species that were less than the comparable LC_{50} for rainbow trout. The average difference was about 0.8 and the maximum difference was about 0.5.

Management Implications

Previous studies have found that no one species is always the most or least sensitive to contaminant exposure (i.e. Macek and McAllister 1970, EPA 1982, Birge and Black 1982, Blank 1984, Mayer and Ellersieck 1986, Reish 1988) and therefore a species is the best surrogate only for itself (Mount 1982). For example, the sea lamprey, generally considered insensitive to contaminant exposure, was found to be the species most sensitive to the lampricide TFM while many desirable fish species, generally considered sensitive to many other chemicals, were much less sensitive to TFM (Cairns 1986).

Mayer and Ellersieck (1986) determined that fish LC_{50}s for a given chemical varied as much as nine orders of magnitude. Blanck (1984) used data from various literature sources and found that chemical sensitivity of algae varied by seven orders of magnitude. Birge and Black (1982) reported LC_{50}s for five or more aquatic species exposed to 50 different organic or inorganic toxicants. They found that LC_{50}s differed by one order of magnitude for 33% of the cases studied, and up to three orders of magnitude for another 33% of the cases. Macek and McAllister (1970) reported the 96-h LC_{50}s for 12 species (five families) varied by up to four orders of magnitude depending on the chemical. In the present study, we did not find the same degree of variability reported in these previous studies. However, the potential variability exhibited in these previous studies emphasizes the difficulties that must be faced when attempting to determine the potential impact of contaminants on a listed species.

The EPA Standard Evaluation Procedure for Ecological Risk Assessment for pesticides and endangered species requires stringent criteria for estimating risk (EPA 1985). A formal consultation is required if the expected environmental concentration is greater than "1/10th the lowest aquatic acute LC10 (when a slope is available) or greater than 1/20th the lowest aquatic LC_{50} (when no slope is available)". While the risk assessment document provides guidance on when a consultation must take place, there is no guidance provided on how contaminant sensitivity should be evaluated. Ultimately, the FWS biologists responsible for the risk assessments will decide if there is substantial risk to the species. However, our data would indicate that if a conservative approach is used for protecting listed fish for which there is no toxicity data, a factor of 0.5 could be applied to the geometric mean LC_{50} of rainbow trout toxicity data and an estimated LC_{50} for a listed species could be determined. Expected environmental concentrations or target environmental concentrations could then be compared to this calculated number and an evaluation of the risk to the species could be made.

Finally, the data we have generated indicates that if rainbow trout is used as a test species, a species typically used in pesticide registration or water quality criteria derivation, those procedures which protect the rainbow trout would likely be protective of most listed aquatic fish species. However, as previously discussed, if a factor is needed to estimate LC_{50}s for listed fish species, our data indicates that 0.5 would be a conservative estimator. Finally, if EPA water quality criteria are recalculated by eliminating certain species from the data set, such as rainbow trout, then listed species might not be adequately protected.

In summary, only direct testing will provide specific information regarding protection of listed species. Our laboratory has evaluated only 11 aquatic vertebrate species (mostly fish) and there are currently over 90 fishes listed by the FWS. The database for fishes should be expanded to include a few additional species from different areas of the United States. Amphibian population declines have been recognized worldwide and the FWS has over 10 listed species; therefore, greater emphasis should also be placed on testing amphibian species. Testing is also needed to evaluate sublethal effects of contaminants on listed species. Finally, other listed species, including freshwater mussels and other invertebrates, should be examined.

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Appendix 1. Exposure water pH for each test (species and chemical) at 0 and 96 h. The pH range represents the lowest and highest pH measured for all exposure concentrations and replicates within a test.

Species	Chemical	0 h	96 h
Fountain darter	carbaryl	7.9-8.3	7.8-8.2
	copper	8.1-8.3	8.1-8.2
	4-nonylphenol	8.3	8.0-8.1
	pentachlorophenol	8.3-8.4	7.9-8.1
	permethrin	8.3	8.0-8.1
Greenthroat darter	carbaryl	7.8-8.0	7.9-8.2
	copper	7.7-7.9	8.2-8.3
	4-nonylphenol	7.7-7.9	8.0-8.2
	pentachlorophenol	7.8-7.9	7.6-8.2
	permethrin	7.8-7.9	7.9-8.3
Shovelnose sturgeon	carbaryl	8.4	nm[1]
	copper	8.2-8.4	6.8-7.4
	4-nonylphenol	8.1-8.4	6.7
	pentachlorophenol	8.3-8.4	nm
	permethrin	8.3-8.5	7.3
Gila topminnow	carbaryl	7.2-8.0	7.9-8.0
	copper	7.9-8.1	8.0-8.1
	4-nonylphenol	7.7-8.1	8.0
	pentachlorophenol	7.6-8.1	8.0-8.1
	permethrin	6.8-7.4	8.0-8.1
Boreal Toad	carbaryl	7.9-8.1	nm[1]
	copper	7.7-8.1	7.7-8.1
	4-nonylphenol	7.5-8.3	7.9-8.0
	pentachlorophenol	7.4-8.1	7.8-8.1
	permethrin	8.0-8.1	7.9-8.1

[1]nm - not measured

Appendix 2. Exposure replicates (rep) and concentrations (conc) for which dissolved oxygen was below 40% saturation (22°C - 5.2 mg/L) at 48-h of exposure or below 60% saturation (22°C - 3.5 mg/L) at 96 h of exposure.

Species	Chemical	rep	conc	48h	96h
Fountain darter	pentachlorophenol	1	low		3.0
Greenthroat darter	pentachlorophenol	2	medium		1.3
Shovelnose sturgeon	carbaryl	1	low	3.5	
		2	low	4.4	
	copper	1	medium		2.9
	4-nonylphenol	1	low	3.3	
	permethrin	1	low	3.1	
			medium	3.3	
		2	low	4.4	
Gila topminnow	carbaryl	1	medium		2.4
			high		1.9
		2	medium		2.8
			high		2.5
	4-nonylphenol	2	low	4.8	
			medium	5.0	
		1	medium		2.5
			high		3.2
	pentachlorophenol	2	medium	4.8	
		1	low		2.1
	permethrin	1	low	5.0	
		1	low		1.8
			high		3.1
Boreal toad	carbaryl	2	low		2.3
		3	low		2.9

Appendix 3. Calculated LC_{50}, 95% confidence interval for each chemical, species and time period. LC_{50}s and confidence intervals were calculated using probit analysis unless otherwise noted.

Carbaryl (mg/L)

Species	Hours					
	6	12	24	48	72	96
Boreal toad	>21	>21	>21	>21	12.31[b] (10.3 - 14.7)	12.31[b] (10.3 - 14.7)
Shovelnose sturgeon	>10	4.90 (4.20 - 5.72)	3.60[c] (2.16 - 6)	1.75 (1.46 - 2.05)	<1.3 Complete mortality	<1.3 Complete mortality
Greenthroat darter	>3.0	>3.0	3.0	2.84 (2.38 - 4.03)	2.44 (1.94 - 3.65)	2.14 (1.73 - 2.88)
Fountain darter	>3	>3[c]	>3[c]	2.77[b] (1.8 - 3.0)	2.07[b] (1.08 - 3.0)	2.02[b] (1.08 - 3.0)
Gila topminnow	>3	>3	>3	>3	>3	>3

Copper (mg/L)

Species	Hours					
	6	12	24	48	72	96
Boreal toad	>0.3	0.19 (0.11 - 0.3)	0.16[b] (0.11 - 0.18)	0.14 (0.13 - 0.15)	0.14 (0.13 - 0.15)	0.12[b] (0.07 - 0.18)
Shovelnose sturgeon	>0.6	>0.6	0.54 (0.44 - 0.78)	0.38 (0.30 - 0.56)	0.21 (0.16 - 0.28)	0.16 (0.12 - 0.21)
Greenthroat darter	>0.3	>0.3	>0.3	0.27 (0.23 - 0.35)	0.26[b] (0.18 - 0.3)	0.26[b] (0.18 - 0.3)
Fountain darter	>0.3	0.24[b] (0.18 -0.3)	0.15[b] (0.11 - 0.18)	0.08 (0.07 - 0.10)	0.06 (0.05 - 0.07)	0.06 (0.05 - 0.07)
Gila topminnow	>0.3	>0.3	>0.3	>0.3	0.23[b] (0.18 - 0.3)	0.16[a] (0.14 - 0.19)

4- nonylphenol (mg/L)

Species	Hours					
	6	12	24	48	72	96
Boreal toad	0.19[b] (0.15 - 0.25)	0.12[b] (0.09 - 0.15)	0.12[b] (0.09 - 0.15)	0.12[b] (0.09 - 0.15)	0.12[b] (0.09 - 0.15)	0.12[b] (0.09 - 0.15)
Shovelnose sturgeon	0.38 (0.34 - 0.44)	0.25[b] (0.13 - 0.36)	0.20 (0.17 - 0.22)	0.20 (0.17 - 0.22)	<0.13	<0.13
Greenthroat darter	>0.25	>0.25	0.23[b] (0.15 - 0.25)	0.22[b] (0.15 - 0.25)	0.20[b] (0.15 - 0.25)	0.19[b] (0.15 - 0.25)
Fountain darter	>0.25[c]	>0.25	0.21[b] (0.15 - 0.25)	0.17[b] (0.15 - 0.25)	0.13[a] (0.11 - 0.15)	0.11[b] (0.09 - 0.15)
Gila topminnow	>0.25	>0.25	>0.25	>0.25	>0.25	0.23[b] (0.15 - 0.25)

[a]--moving average
[b]--nonlinear interpolation
[c]--binomial test

Appendix 3, (Continued).

Pentachlorophenol (mg/L)

Species	Hours 6	12	24	48	72	96
Boreal toad	>0.7	>0.7	>0.42	0.54[b] (0.42 - 0.70)	0.52[b] (0.42 - 0.70)	0.37[b] (0.25 -0.42)
Shovelnose sturgeon	0.27 (0.24 - 0.30)	0.16 (0.14 - 0.19)	<0.13	---	---	---
Greenthroat darter	0.33[b] (0.25 - 0.42)	0.31[b] (0.25 - 0.42)	0.30[b] (0.25 - 0.42)	0.28[b] (0.25 - 0.42)	0.18[b] (0.15 - 0.25)	0.18[b] (0.15 - 0.25)
Fountain darter	0.34 (0.30 - 0.38)	0.20 (0.18 - 0.22)	0.17[a] (0.04 - 0.21)	0.18 (0.17 - 0.20)	0.16[b] (0.09 - 0.25)	0.11[b] (0.09 - 0.15)
Gila topminnow	>0.7	>0.7	0.64[b] (0.42 - 0.70)	0.51[b] (0.42 - 0.70)	0.36[b] (0.25 - 0.42)	0.34[b] (0.25 - 0.42)

Permethrin (ug/L)

Species	Hours 6	12	24	48	72	96
Boreal toad	>10	>10	>10	>10	>10	>10
Shovelnose sturgeon	>10	10	---	---	---	---
Greenthroat darter	4.31 (3.71 - 5.04)	3.10[b] (2.20 - 3.60)	2.71 (2.36 - 3.13)	2.71 (2.36 - 3.13)	2.71 (2.36 - 3.13)	2.71 (2.36 - 3.13)
Fountain darter	>10[c]	5.60 (4.76 - 6.67)	4.26[a] (3.58 - 5.19)	3.34[a] (2.75 - 4.16)	3.34[a] (2.75 - 4.16)	3.34[a] (2.75 - 4.16)
Gila topminnow	>10	>10	>10	>10	>10	>10

[a] – moving average
[b] – nonlinear interpolation
[c] – binomial test

Appendix 4. Acute toxicity of carbaryl (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12-, 24-, and 96-h of exposure. Toxicity values are the geometric mean of the LC_{50}s (number of LC_{50}s in parentheses). Data was abstracted from EPA (1995).

Species	12-h LC_{50} (n)	24-h LC_{50} (n)	96-h LC_{50} (n)
Rainbow trout	6.76 (4)	4.04 (6)	1.88 (6)
Apache trout	3.29 (2)	2.50 (2)	1.54 (2)
Greenback cutthroat	8.50 (1)	3.59 (1)	1.55 (1)
Lahontan cutthroat	4.38 (2)	3.60 (2)	2.25 (2)
Fathead minnow	12.0 (1)	8.25 (3)	5.21 (5)
Bonytail chub	7.93 (2)	6.13 (2)	3.49 (2)
Colorado squawfish	>10.0 (na)[1]	6.31 (1)	3.07 (2)
Razorback sucker	8.88 (1)	6.67 (2)	4.35 (2)

Acute toxicity of copper (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12-, 24-, and 96-h of exposure. Toxicity values are the geometric mean of the LC_{50}s (number of LC_{50}s in parentheses). Data was abstracted from EPA (1995).

Species	12-h LC_{50} (n)	24-h LC_{50} (n)	96-h LC_{50} (n)
Rainbow trout	0.40 (3)	0.12 (4)	0.08 (4)
Apache trout	0.18 (2)	0.09 (2)	0.07 (1)
Greenback cutthroat	>0.03 (na)[1]	>0.03 (na)[1]	>0.03 (na)[1]
Lahontan cutthroat	0.39 (2)	0.11 (2)	0.07 (2)
Fathead minnow	1.30 (4)	0.73 (4)	0.47 (6)
Bonytail chub	0.30 (2)	0.24 (2)	0.22 (2)
Colorado squawfish	>1.00 (na)[1]	0.64 (2)	0.43 (2)
Razorback sucker	>1.00 (na)[1]	0.39 (1)	0.27 (2)

[1] na = not applicable

Appendix 4, (continued).

Acute toxicity of 4-nonylphenol (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12-, 24-, and 96-h of exposure. Toxicity values are the geometric mean of the LC_{50}s (number of LC_{50}s in parentheses). Data was abstracted from EPA (1995).

Species	12-h LC_{50} (n)	24-h LC_{50} (n)	96-h LC_{50} (n)
Rainbow trout	0.35 (6)	0.30 (6)	0.19 (6)
Apache trout	0.30 (2)	0.24 (2)	0.17 (2)
Greenback cutthroat	0.38 (1)	0.30 (1)	0.15 (1)
Lahontan cutthroat	0.29 (2)	0.25 (2)	0.18 (2)
Fathead minnow	0.38 (6)	0.33 (6)	0.27 (6)
Bonytail chub	0.56 (2)	0.49 (2)	0.29 (2)
Colorado squawfish	0.45 (2)	0.28 (2)	0.26 (2)
Razorback sucker	0.29 (2)	0.22 (2)	0.17 (2)

Acute toxicity of pentachlorophenol (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12-, 24-, and 96-h of exposure. Toxicity values are the geometric mean of the LC_{50}s (number of LC_{50}s in parentheses). Data was abstracted from EPA (1995).

Species	12-h LC_{50} (n)	24-h LC_{50} (n)	96-h LC_{50} (n)
Rainbow trout	0.22 (6)	0.17 (6)	0.16 (6)
Apache trout	0.21 (2)	0.21 (2)	0.11 (2)
Greenback cutthroat	>0.01 (na)[1]	>0.01 (na)[1]	>0.01 (na)[1]
Lahontan cutthroat	0.27 (1)	0.23 (2)	0.17 (2)
Fathead minnow	0.33 (6)	0.30 (6)	0.25 (6)
Bonytail chub	0.42 (2)	0.26 (2)	0.23 (2)
Colorado squawfish	0.23 (2)	0.16 (2)	0.24 (2)
Razorback sucker	0.53 (2)	0.29 (2)	0.28 (2)

[1] na = not applicable

Appendix 4, (continued).

Acute toxicity of permethrin (µg/L) to 8 species of fish (2 surrogate and 6 listed) at 12-, 24-, and 96-h of exposure. Toxicity values are the geometric mean of the LC_{50}s (number of LC_{50}s in parentheses). Data was abstracted from EPA (1995).

Species	12-h LC_{50} (n)	24-h LC_{50} (n)	96-h LC_{50} (n)
Rainbow trout	5.75 (6)	3.78 (6)	3.31 (6)
Apache trout	3.88 (2)	2.27 (2)	1.71 (2)
Greenback cutthroat	>1.0 (na)[1]	>1.0 (na)[1]	>1.0 (na)[1]
Lahontan cutthroat	3.33 (2)	1.93 (2)	1.58 (2)
Fathead minnow	13.43 (4)	9.73 (5)	9.38 (6)
Bonytail chub	>25.0 (na)[1]	>25.0 (na)[1]	>25.0 (na)[1]
Colorado squawfish	>25.0 (na)[1]	>25.0 (na)[1]	24.4 (1)
Razorback sucker	13.05 (1)	8.87 (1)	5.95 (2)

[1]na = not applicable

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