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PROTECTIVENESS OF SPECIES SENSITIVITY DISTRIBUTION HAZARD CONCENTRATIONS FOR ACUTE TOXICITY USED IN ENDANGERED SPECIES RISK ASSESSMENT

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Abstract—A primary objective of threatened and endangered species conservation is to ensure that chemical contaminants and other stressors do not adversely affect listed species. Assessments of the ecological risks of chemical exposures to listed species often rely on the use of surrogate species, safety factors, and species sensitivity distributions (SSDs) of chemical toxicity; however, the protectiveness of these approaches can be uncertain. We comprehensively evaluated the protectiveness of SSD first and fifth percentile hazard concentrations (HC1, HC5) relative to the application of safety factors using 68 SSDs generated from 1,482 acute (lethal concentration of 50%, or LC50) toxicity records for 291 species, including 24 endangered species (20 fish, four mussels). The SSD HC5s and HC1s were lower than 97 and 99.5% of all endangered species mean acute LC50s, respectively. The HC5s were significantly less than the concentrations derived from applying safety factors of 5 and 10 to rainbow trout (*Oncorhynchus mykiss*) toxicity data, and the HC1s were generally lower than the concentrations derived from a safety factor of 100 applied to rainbow trout toxicity values. Comparison of relative sensitivity (SSD percentiles) of broad taxonomic groups showed that crustaceans were generally the most sensitive taxa and taxa sensitivity was related to chemical mechanism of action. Comparison of relative sensitivity of narrow fish taxonomic groups showed that standard test fish species were generally less sensitive than salmonids and listed fish. We recommend the use of SSDs as a distribution-based risk assessment approach that is generally protective of listed species.

Keywords—Endangered species Risk assessment Aquatic toxicology Species sensitivity distributions

INTRODUCTION

Chemical habitat degradation is one of the primary threats to imperiled species in the United States [1,2], and exposure to environmental contaminants is a primary conservation concern for endangered species [3]. It is estimated that exposure to environmental contaminants has contributed up to 38% of fish extinctions in the last century [1]. The U.S. Environmental Protection Agency (U.S. EPA) determines the risk posed to listed species by the manufacturing, use, and disposal of chemicals and pesticides under the Toxic Substance Control Act (www.epa.gov/history/topics/tsca/index.htm); the Federal Insecticide, Fungicide and Rodenticide Act (www.epa.gov/oecaagct/lfra.html); and the Clean Water Act (www.epa.gov/waterscience/standards/303.htm). The Federal Insecticide, Fungicide and Rodenticide Act and the Clean Water Act protect aquatic ecosystems through either the required toxicity testing of representative aquatic organisms or a distribution-based approach using multispecies databases [4,5].

Ecological risk assessments of toxicants identify the severity and probability that contaminant exposure will adversely affect organisms and populations. The absence of empirical data makes sensitivity unknown for many species and chemicals and increases risk assessment uncertainty. While endangered species toxicity testing can be done [6–12], it is often not feasible and risk managers must estimate the degree of protection or develop approaches to derive protective measures. Standardized toxicity tests of surrogate species (e.g., rainbow trout, *Oncorhynchus mykiss*) and application of safety factors may provide protection of special-status species [9]; however, the sensitivity of standard test species compared to listed species and their appropriateness as surrogates for special-status species is not well understood [13]. Alternatively, species sensitivity distributions (SSDs) are cumulative probability distributions of species sensitivities (acute toxicity) that are used in endangered species risk assessment to derive hazard concentrations, such as the fifth percentile hazard concentration (HC5) considered protective of 95% of species [5,14].

The use of standard safety factors and SSDs to derive protective concentrations for most listed species relies on the assumptions that listed species are no more sensitive than nonlisted species and SSD HC5s will be protective of listed species. Previous studies compare the acute and chronic toxicity of five chemicals (carbaryl, copper sulfate, 4-nonylphenol, pentachlorophenol, and permethrin) representing diverse modes of action for 17 aquatic listed species and three surrogate test species [9–11,15]. The authors of those studies report that the tested endangered species are not consistently more sensitive than the surrogate species and support earlier conclusions that no species is consistently the most sensitive to chemicals over a wide range of modes of action [16]. While these studies indicate that risk assessors can apply surrogate species toxicity data with safety factors to protect endangered species, they are

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limited in scope in both the range of chemicals and the species evaluated.

The objective of the present study was to comprehensively examine the relative sensitivities of federally listed and nonlisted aquatic species to a broad range of chemicals and determine the protectiveness of SSD hazard concentrations used in endangered species risk assessment. We developed SSDs for 68 chemicals from an expansive acute toxicity database that included toxicity data for 24 listed species. We conducted analyses to assess the relative sensitivity of listed and unlisted taxa over a broad range of chemicals, the frequency of chemicals for which HC5s and HC1s (first percentile) are less than listed species mean acute values, and how SSD hazard concentrations compare to environmental concentrations derived from the application of safety factors. We also propose two potential methods that may reduce uncertainty in SSDs used in risk assessment. In the first method, we identify mechanism of action (MOA)–sensitive taxonomic groups that may guide selection of taxa for SSD development. In the second method, we develop linear models to determine whether the percentile of a surrogate species can predict the percentile of a listed species, which risk assessors may use to identify appropriate hazard concentrations.

MATERIALS AND METHODS

We compiled an extensive acute toxicity database from the U.S. EPA Office of Pesticide Programs ecotoxicity database, ECOTOX/ACQUIRE (cfpub.epa.gov/ecotox, accessed May 2007); Ambient Water Quality Criteria documents [17,18]; the U.S. EPA Gulf Ecology Division Interspecies Correlation Estimation database [19]; and the open literature [12,16,20–48]. All data were required to adhere to acute toxicity test standards for test conditions and methods appropriate for each species [49,50]. Because we combined data from multiple sources, a high level of standardization was necessary to reduce the effects that test conditions, life stage, and chemically active ingredient may have on organism toxicity. This high level of standardization helped to ensure that the modeled relationships were a reflection of species sensitivity and not extraneous factors that contribute to variation in toxicity data. As a result, we excluded some data from previous studies of endangered species toxicity (Dwyer et al. [9]; some data omitted due to active ingredient requirement) that did not meet our standardization requirements described here.

We standardized all data by using 96-h lethal concentration of 50% (LC50) or effective concentration of 50% (EC50) values for fish and most invertebrates (mysids and mussels) and 48-h EC50 values for some invertebrates (daphnids). All toxicity values were expressed as μg/L. We only used toxicity data for juveniles for fish (between 0.2 and 5.0 g, <50 mm, or <3 months), mussels, oysters, shrimp, and blue crabs to reduce variability associated with life stage. While earlier life stages (larvae) may be more sensitive, we used the juvenile life stage because it was a consistent life stage among taxa and the stage for which most data were available. For freshwater species, we adjusted the toxicity values for metals to a hardness of 50 mg/L, pentachlorophenol to pH 6.8, and ammonia to pH 8.0. Duplicate records collected from multiple sources, chemical formulations of less than 90% active ingredient and open-ended toxicity values (>100 μg/L or <100 μg/L) were excluded from the database. We used the geometric mean of multiple toxicity values for a chemical per species as the mean acute value. We removed toxicity records for which the minimum and maximum values exceeded a 10-fold range from the database to limit variability associated with inconsistent toxicity test observations [19,51].

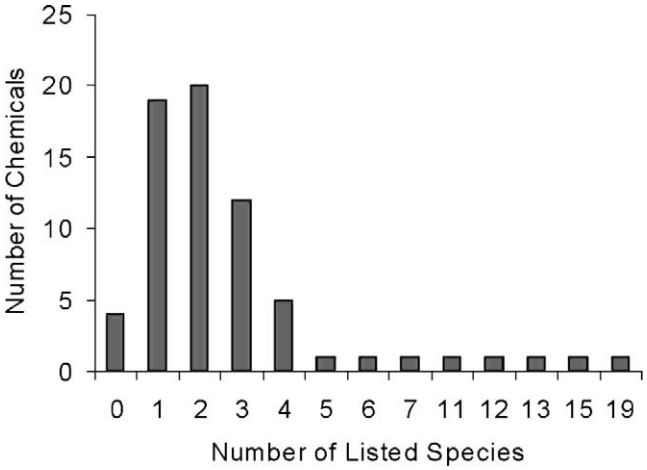

Fig. 1. The number of chemical species sensitivity distributions containing different numbers of listed species.

We identified all chemicals that contained toxicity values for a minimum of 10 species and extracted all toxicity records for each chemical for SSD development. For each chemical, we pooled saltwater and freshwater organisms because preliminary SSDs developed with all species showed no significant difference in the percentiles of freshwater and saltwater species (Mann-Whitney U test, $Z = 1.033$, $p = 0.302$). De Zwart [52] also notes that in a dataset of more than 1,000 chemicals and 500 species, the average toxicity values for freshwater and saltwater species are comparable and combining data from the two media is appropriate for SSD development.

Species sensitivity distributions may be generated using several types of distributions [53], often with little difference in protection levels [54]. We used a log-logistic model for SSD development because it often provides the best overall fit to toxicity datasets [55,56] and more conservative HC5s [57]. For each chemical, a log-logistic model described the distribution of all species sensitivities such that

$$Y = 1/\{1 + \exp[(\alpha - X)/\beta]\}$$

where Y is the cumulative probability; X is log-transformed acute toxicity; α is the intercept parameter determined from the average of the log-transformed sample; and β is the scale parameter estimated from the standard deviation, s, of the log-transformed sample as $\beta = \sqrt{3}/\pi \cdot s$ [52]. For each SSD, the percentile of each species was the cumulative probability corresponding to the log-transformed toxicity value. The HC5 and HC1 of each SSD were the concentrations corresponding to the fifth and first percentile, respectively.

We obtained the most recent list of federally threatened and endangered species from the Threatened and Endangered Species module of Environmental Conservation Online System (ecos.fws.gov/tess_public/StartTESS.do, accessed August 2007) and identified the listed species within each SSD. Of 68 SSDs, 64 contained one or more listed species (Fig. 1). The protectiveness of HC5 and HC1 generated from the 64 SSDs that included listed species was determined from the frequency of listed species occurring above the fifth and first percentiles, respectively.

We compared HC5 and HC1 values to concentrations derived by applying safety factors of 5, 10, and 100 to rainbow

Table 1. Listed species and the number of chemicals of each represented by 64 species sensitivity distributions

Taxa	Family	Common name	Species	No. of chemicals	Median percentile
Fish	Acipenseridae	Shortnose sturgeon	*Acipenser brevirostrum*	4	0.14
	Acipenseridae	Atlantic sturgeon[a]	*Acipenser oxyrinchus*	1	0.17
	Catostomidae	Razorback sucker	*Xyrauchen texanus*	5	0.53
	Cyprinidae	Spotfin chub	*Erimonax monachus*	5	0.27
	Cyprinidae	Bonytail chub	*Gila elegans*	5	0.68
	Cyprinidae	Cape Fear shiner	*Notropis mekistocholas*	5	0.49
	Cyprinidae	Colorado pikeminnow	*Ptychocheilus lucius*	5	0.68
	Cyprinodontidae	Leon Springs pupfish	*Cyprinodon bovinus*	4	0.51
	Cyprinodontidae	Desert pupfish	*Cyprinodon macularius*	1	0.79
	Gasterosteidae	Threespine stickleback	*Gasterosteus aculeatus*	2	0.45
	Percidae	Fountain darter	*Etheostoma fonticola*	5	0.16
	Poeciliidae	Gila topminnow	*Poeciliopsis occidentalis*	4	0.54
	Salmonidae	Cutthroat trout	*Oncorhynchus clarkii*	29	0.58
	Salmonidae	Apache trout	*Oncorhynchus gilae apache*	5	0.35
	Salmonidae	Coho salmon	*Oncorhynchus kisutch*	24	0.48
	Salmonidae	Rainbow trout[b]	*Oncorhynchus mykiss*	59	0.56
	Salmonidae	Sockeye salmon	*Oncorhynchus nerka*	3	0.53
	Salmonidae	Chinook salmon	*Oncorhynchus tshawytscha*	14	0.36
	Salmonidae	Atlantic salmon	*Salmo salar*	13	0.63
	Salmonidae	Bull trout	*Salvelinus confluentus*	3	0.07
Mollusca	Unionidae	Oyster mussel	*Epioblasma capsaeformis*	3	0.12
	Unionidae	Pink mucket	*Lampsilis abrupta*	2	0.13
	Unionidae	Shinyrayed pocketbook	*Lampsilis subangulata*	1	0.90
	Unionidae	Scaleshell	*Leptodea leptodon*	1	0.06

[a] Subspecies Gulf sturgeon.
[b] Subspecies steelhead.

trout toxicity acute values. Rainbow trout was the selected surrogate species because it has been established as a sensitive species that is an appropriate surrogate for endangered species risk assessment [9] and was the most abundant species in the database. We used Wilcoxon signed rank tests of matched pairs to compare HC5s with concentrations derived from safety factors of 5, 10, and 100 and the HC1 with the concentration derived from a safety factor of 100.

We assessed the differences in the relative sensitivity of listed and unlisted species in broad and narrow taxonomic groups. The broad taxonomic groups included annelids, crustaceans, insects, listed mollusks, unlisted mollusks, listed fish, unlisted fish, and amphibians. All listed mollusks were mussels, whereas the unlisted mollusks group was composed of mussels, other bivalves (oysters, clams, and scallops), snails, and abalone. The narrow taxonomic groups focused on fish and were rainbow trout, listed salmonids (species in family Salmonidae other than rainbow trout), unlisted salmonids, other listed fish (nonsalmonids), bluegill (*Lepomis macrochirus*), fathead minnow (*Pimephales promelas*), sheepshead minnow (*Cyprinodon variegates*), and catfish (*Ictalurus punctatus*). We selected the narrow groups to compare listed species with unlisted species within the same family (Salmonidae), as well as to compare listed fish species with standard toxicity test species. The listed salmonid group excluded data for rainbow trout to retain independence in the analyses and consisted of cutthroat trout (*Oncorhynchus clarkii*, $n = 29$), apache trout (*O. gilae apache*, $n = 5$), coho salmon (*O. kisutch*, $n = 24$), sockeye salmon (*O. nerka*, $n = 3$), Chinook salmon (*O. tshawytscha*, $n = 14$), Atlantic salmon (*Salmo salar*, $n = 13$), and bull trout (*Salvelinus confluentus*, $n = 3$). Unlisted salmonids included brown trout (*Salmo trutta*, $n = 16$), brook trout (*Salvelinus fontinalis*, $n = 20$), lake trout (*Salvelinus namaycush*, $n = 21$), and pink salmon (*O. gorbuscha*, $n = 1$). For each analysis, we determined the proportion of observations located within the four quartiles of the SSDs for each taxonomic group. We compared median sensitivities among groups using Kruskal-Wallis test and nonparametric multiple comparison [58].

We qualitatively assessed the effect of chemical mechanism of toxic action on taxa sensitivity using the broad taxonomic groups and well-represented MOAs. The MOAs with sufficient number of chemicals for analysis were organophosphorus insecticides (OPs), neurotoxicants (primarily organochlorine and pyrethroid pesticides), metallic stressors, and nonpolar narcotics (a diversity of chemicals causing toxicity through nonspecific mechanisms) [59]. Organophosphorus insecticides were treated as a separate class of chemicals because of their specific binding to and inhibition of acetylcholinesterase, whereas the general class neurotoxicants encompassed a broader range neurotoxicity mechanisms that did not involve acetylcholinesterase inhibition. The sensitivity of narrow taxonomic groups within MOA categories was not determined due to small sample sizes.

We used a linear model to determine whether relative sensitivity (percentile in the SSD) of a surrogate species could predict the relative sensitivity of a listed species. We used percentile data for two surrogate species (rainbow trout and fathead minnow) and two listed species (cutthroat trout and Cape Fear shiner, *Notropis mekistocholas*). We selected the representative listed and surrogate species based on data availability and taxonomic relatedness of surrogate and listed species (Salmonidae:trout; Cyprinidae:minnow, shiner).

RESULTS

From our database of 713 chemicals, with 5,901 LC50s for 308 species, we developed SSDs for 68 of these chemicals, consisting of 1,482 LC50s and 291 species, including 203 toxicity records for 24 endangered species (20 fish, four mussels; Table 1 and Table S1 [http://dx.doi.org/10.1897/08-157.S1]). Most SSDs contained 1 to 3 listed species, with five chemicals

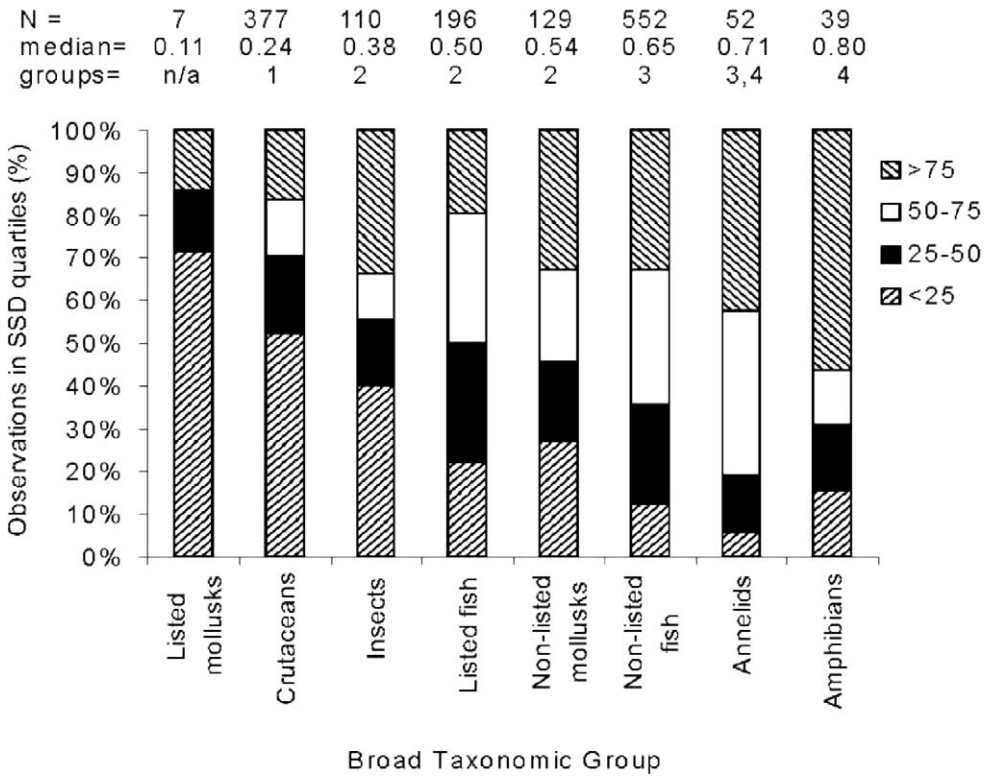

Fig. 2. Proportion of observations in each quartile of the species sensitivity distributions for broad taxonomic groups. Numbers in the Groups row indicate taxonomic groups that are not significantly different from one another.

containing more than 10 listed species (Fig. 1). All SSDs had a minimum of 10 species and eight families with the exception of four that were represented by seven families (potassium permanganate, $n = 12$; coumaphos, $n = 11$; temephos, $n = 11$; and clonitralide, $n = 17$). The four chemicals represented by seven families maintain taxonomic diversity and contained crustaceans, insects, mollusks, and fish. Acute toxicity values in the SSDs ranged from 0.001 μg/L (*Polyodon spathula*; antimycin A) to 1,870,000 μg/L (*Agnetina capitata*; chromium VI), and HC5s ranged from 0.0018 μg/L (antimycin A) to 2,587 μg/L (metolachlor). Of 203 toxicity records for listed species, 97% were greater than the fifth percentile of their respective SSD and 99.5% were greater than the first percentile of the SSD.

Median percentiles of listed species ranged from 0.06 (*Leptodea leptodon*, $n = 1$) to 0.90 (*Lampsilis subangulata*, $n = 1$). Sturgeons (*Acipenser* spp.), spotfin chub (*Erimonax monochus*), and the fountain darter (*Etheostoma fonticola*) were among the more sensitive listed species, with median percentiles equal to or less than 0.27. Most salmonid species median percentiles were approximately 0.5 and ranged from 0.07 (*S. confluentus*, $n = 3$) to 0.63 (*S. salar*, $n = 13$). Listed mollusks were all in the first quartile of their respective SSDs with the exception of *L. subangulata*, which was in the 90th percentile of the malathion SSD (Table 1).

In the comparison of HC5 and concentrations derived from safety factors, the median HC5 (5.1) of all SSDs (10th percentile of all HC5s = 0.15, 90th percentile of all HC5s = 209.4) was significantly lower than the median concentrations derived from a safety factor of 5 (median = 42.0, 10th percentile = 0.91, 90th percentile = 1,451.3; Wilcoxon signed rank tests, $Z = -4.53$, $p < 0.0001$). The HC5s were also significantly lower than environmental concentrations derived from a safety factor of 10 (median = 21.0, 10th percentile = 0.46, 90th percentile = 725.63; Wilcoxon signed rank tests, $Z = -2.41$, $p = 0.016$). Concentrations derived from a safety factor of 100 (median = 2.10, 10th percentile = 0.05, 90th percentile = 72.6), were significantly less than HC5s (Wilcoxon signed rank tests, $Z = 2.84$, $p = 0.004$), but not significantly different from HC1s (median HC1 of all SSDs = 0.64, 10th percentile of all HC1s = 0.007, 90th percentile of all HC1s = 67.4; Wilcoxon signed rank tests, $Z = -0.38$, $p = 0.70$).

The statistical comparison of relative sensitivity among broad taxonomic groups determined that crustaceans were the most sensitive group, followed by insects, listed fish, and unlisted mollusks ($\chi^2 = 171$, degrees of freedom, $df = 6$, $p < 0.0001$; Fig. 2). The sensitivity of listed mollusks ($n = 7$) was not statistically compared to that of the other groups due to the small sample size; however, they were generally among the more sensitive taxa, with a median percentile of 0.11. As a group, listed fish were significantly more sensitive compared to all unlisted fish, amphibians, or annelids. The comparison of relative sensitivity among narrow fish groups indicated that rainbow trout, unlisted salmonids, listed salmonids, and other listed fish all had similar sensitivity. With the exception of bluegill, which had sensitivity similar to rainbow trout, all other standard test species were significantly less sensitive than salmonids and listed fish ($\chi^2 = 44.6$, $df = 7$, $p < 0.0001$; Fig. 3).

Taxa sensitivity, determined as the percentage of observations in the first quartile of the SSD, varied within chemical MOA categories. Crustaceans were consistently sensitive to all MOAs examined, while insects were sensitive to neurotoxicants and OPs but much less sensitive to nonpolar narcotics and metallic stressors. The sensitivity of listed fish was similar

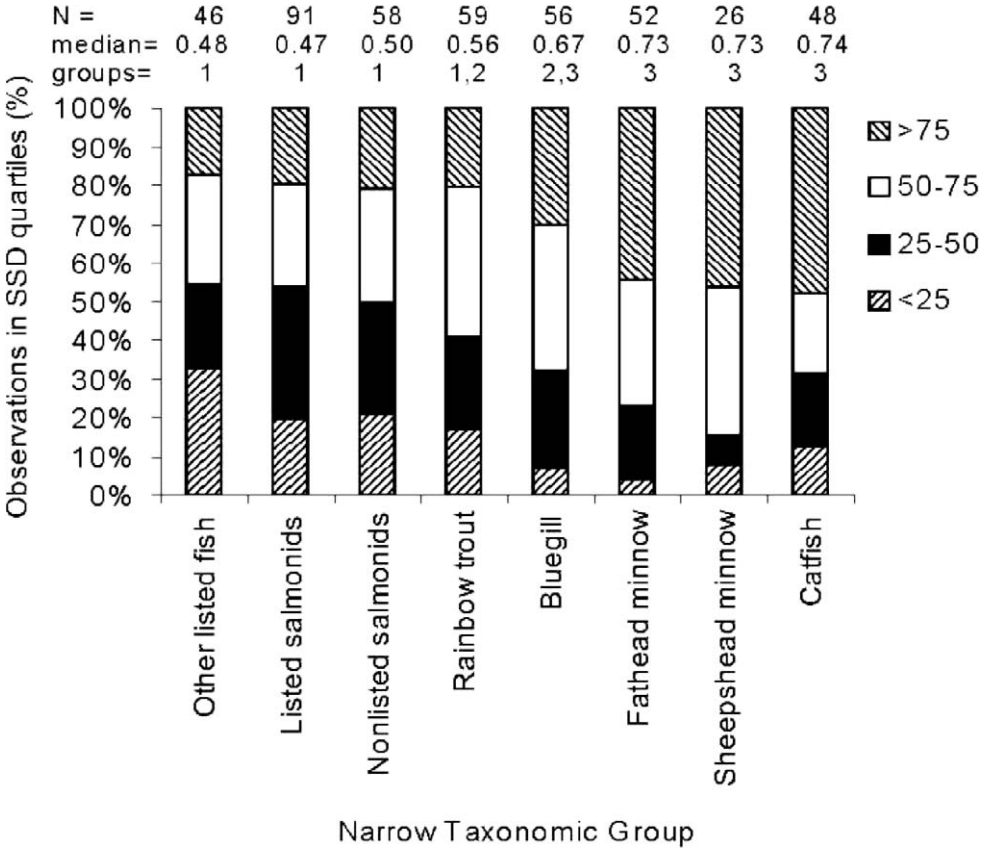

Fig. 3. Proportion of observations in each quartile of the species sensitivity distributions (SSDs) for narrow taxonomic groups. Numbers in the Groups row indicate taxonomic groups that are not significantly different from one another.

to that of unlisted fish to neurotoxicants (16–18%), nonpolar narcotics (16–24%), and OPs (2–3%). Listed fish were generally more sensitive to metallic stressors than unlisted fish (listed −30%, unlisted −3%). There were not enough observations within chemical MOA categories for listed mollusk species to determine trends in relative sensitivity. However, unlisted mollusks had greater sensitivity to nonpolar narcotics and metallic stressors and were relatively less sensitive to neurotoxicants and OPs (Table 2).

Significant relationships existed between the sensitivities of surrogate and the sensitivities of listed fish species (Fig. 4). Linear models developed for cutthroat trout contained 27 and 23 observations for rainbow trout and fathead minnow, respectively. Cape Fear shiner models contained five observations each for rainbow trout and fathead minnow. Based on model R^2, mean square error, and p value, models developed with a rainbow trout surrogate were more robust than models developed with fathead minnow for both cutthroat trout and Cape Fear shiner (Fig. 4).

DISCUSSION

Ecological risk assessments often rely on the SSD approach to determine risks based on single-species toxicity test data [56], and the present study demonstrates the protectiveness of SSD hazard concentrations for 24 endangered aquatic animals. Our results demonstrate that for 64 chemicals, HC5s are lower than 97% of listed species mean acute values and HC1s are less than 99.5% of the listed species values. Our study is the first to compare the relative sensitivity of more than 20 listed species with almost 300 unlisted species for more than 60 chemicals and demonstrates the protectiveness of using distribution-based approaches in endangered species risk assessment.

Of the listed species not protected by the respective HC5, rainbow trout and Chinook salmon are below the HC5 for cadmium (n = 90), the shortnose sturgeon (*Acipenser brevirostrum*) and fountain darter are at or below the HC5 of 4-nonylphenol (n = 17), and the oyster mussel (*Epioblasma capsaeformis*) is at the third percentile of copper (n = 80). In addition, bull trout was less than the HC1 for cadmium. Both cadmium and copper are well-populated SSDs with a diversity of taxa, and the relative sensitivity of these species supports earlier conclusions that salmonids are among the most sensitive species to contaminants [9,15] and that freshwater mussels are particularly sensitive to copper [60]. Sturgeon is also identified as a sensitive fish species [9] with a toxicity value below the HC5 for 4-nonylphenol. The 4-nonylphenol SSD, although composed of 17 species and nine families, contained only vertebrates (fish and amphibians), and the underprotection of the sturgeon in this example may be mitigated by inclusion of additional taxonomic diversity in the SSD.

Stephan [61] recommends that SSD datasets include taxa known to be sensitive to the pollutant to increase the probability that percentiles are adequately protective; however, a comprehensive analysis of taxa sensitivity with relation to chemical classes or MOAs has been lacking. Our analysis of broad taxonomic groups for all chemicals and for chemicals within selected MOAs can guide taxa selection for SSD development to include relatively sensitive species. Our results show that crustaceans occupy the lower quartile of an SSD for

Table 2. Proportions of observations in each species sensitivity distribution quartile for broad taxonomic groups and chemical mechanism of action

Mode of action (No. of chemicals)	Broad taxonomic group	n[a]	Proportion of observations in quartile			
			1	2	3	4
Neurotoxicants (13)	Annelids	2	0.00	0.00	0.00	1.00
	Crustaceans	71	0.38	0.18	0.15	0.28
	Insects	34	0.47	0.15	0.18	0.21
	Nonlisted mollusks	11	0.00	0.18	0.36	0.45
	Nonlisted fish	104	0.18	0.38	0.31	0.13
	Listed fish	31	0.16	0.42	0.32	0.10
	Amphibians	13	0.00	0.00	0.00	1.00
Nonpolar narcosis (14)	Crustaceans	54	0.37	0.19	0.17	0.28
	Insects	17	0.12	0.12	0.18	0.59
	Nonlisted mollusks	25	0.36	0.24	0.24	0.16
	Listed mollusks	2	0.50	0.50	0.00	0.00
	Nonlisted fish	119	0.24	0.19	0.33	0.24
	Listed fish	31	0.16	0.39	0.29	0.16
	Amphibians	7	0.29	0.14	0.29	0.29
Organophosphorus insecticides (18)	Crustaceans	89	0.76	0.17	0.04	0.02
	Insects	32	0.63	0.28	0.06	0.03
	Nonlisted mollusks	15	0.00	0.00	0.33	0.67
	Listed mollusks	1	0.00	0.00	0.00	1.00
	Nonlisted fish	128	0.02	0.20	0.21	0.57
	Listed fish	39	0.03	0.15	0.33	0.49
	Amphibians	7	0.00	0.14	0.43	0.43
Metallic stressors (9)	Annelids	37	0.05	0.16	0.46	0.32
	Crustaceans	106	0.54	0.18	0.13	0.15
	Insects	14	0.00	0.07	0.00	0.93
	Nonlisted mollusks	67	0.37	0.21	0.18	0.24
	Listed mollusks	4	1.00	0.00	0.00	0.00
	Nonlisted fish	88	0.03	0.11	0.39	0.47
	Listed fish	30	0.30	0.40	0.23	0.07
	Amphibians	3	0.33	0.67	0.00	0.00

[a] n = number of observations in taxonomic group.

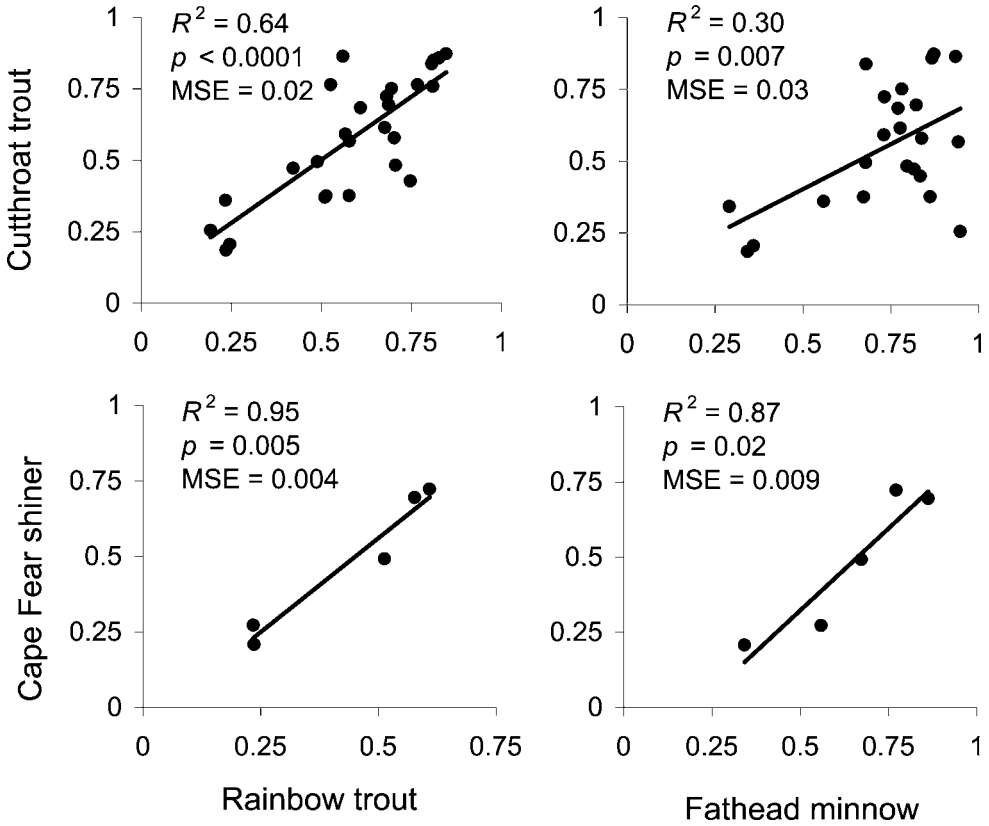

Fig. 4. Relationship of species sensitivity distributions percentiles for surrogate (rainbow trout, fathead minnow) and listed species (Cape Fear shiner, cutthroat trout). MSE = mean square error.

more than 50% of all occurrences and the lower half of the SSD for 70% of all occurrences (Fig. 2). In addition, chemical MOA can be an important determinant of toxicity [59]. Our study of broad taxa sensitivity indicates that, while no single taxon is the most sensitive for all MOAs, relative taxon sensitivity is related to MOA. These results could increase the protectiveness of SSD hazard concentrations by determining a priori which taxa to include in an SSD for specific chemicals or MOAs. In the example of 4-nonylphenol (a nonpolar narcotic) and shortnose sturgeon described earlier, inclusion of crustaceans and mollusks could populate the SSD with taxa sensitive to nonpolar narcotics and facilitate the protection of the sturgeon (Table 2). A quantitative assessment of MOA-sensitive taxa could be the focus of future research efforts to include more MOAs and narrower taxonomic groups.

In addition to taxonomic diversity, SSDs are dependent upon data availability and sample size. Minimum dataset requirements for SSDs have been explored using a diversity of datasets, and approaches and recommendations range from a minimum of 8 up to 55 species [5,62–64]. We required a minimum of 10 species in our SSDs, most of which contained a minimum of eight families, to preserve taxonomic diversity while maximizing the number of SSDs obtainable from our dataset. The SSDs we developed using 10 to 12 species contained between one and three endangered species, which were generally salmonids, and all had toxicity values greater than the fifth percentile. While this is not an exhaustive analysis of SSD sample size requirements for endangered species, it demonstrated that taxonomically diverse SSDs with a minimum of 10 species were protective of listed fish species.

The protection of listed mussels is receiving much attention in endangered species risk assessment [65,66]. Recent research identifies mussels as a consistently sensitive group to specific compounds and highly susceptible to contaminant exposures [12,20]. While we had insufficient data to determine the relative sensitivity of listed species of mussels in our dataset, we determined that unlisted mollusks are more sensitive to metallic stressors and nonpolar narcotics but less sensitive to OPs and neurotoxicants compared to other taxa. Our results support earlier summations that mussels are sensitive to some pollutants while relatively intolerant of others [66] and identify relative mollusk sensitivity as a function of chemical MOA. The data for listed mussels included in the present study support these conclusions: All four toxicity records for listed mussels exposed to metallic stressors were in the first quartile of their respective SSD, toxicity for mussels exposed to nonpolar narcotics were in the first and second quartiles, and toxicity of the listed mussel exposed to an OP was in the upper quartile. Others have proposed the use of daphnids and zooplankton as surrogates for freshwater mussels [13,67]. Our database did not include zooplankton for comparison; however, we demonstrate the relative sensitivity of crustaceans across all MOAs.

A fundamental assumption in endangered species risk assessment is that standard toxicity test species are appropriate surrogates for listed species and that the application of safety factors protects the individuals of endangered species [4,9,13]. In U.S. pesticide risk assessment [4], levels of concern (risk quotients) can be 10-fold lower for listed species than unlisted species. Dwyer et al. [9] suggests applying a safety factor of three to the toxicity data of the standard test species rainbow trout because of general sensitivity to contaminants. In our study, safety factors of 5 and 10 applied to rainbow trout toxicity values are less protective than HC5s and safety factors of 100 are comparable to HC1s, indicating that HC5s are generally more protective than application of safety factors.

Previous studies comparing the sensitivity of listed species with standard test species conclude that listed species are generally not more sensitive and the use of surrogate species is appropriate for endangered species risk assessment [9,15]. Many authors concur that, while no one species is consistently the most sensitive, rainbow trout and other salmonids are generally more sensitive than other species and provide adequate surrogacy for listed species [9,15,16]. Our study shows that the standard test species fathead minnow, sheepshead minnow, catfish, and bluegill are significantly less sensitive than salmonids and other listed fish, suggesting caution when using nonsalmonid surrogates. The discrepancy between our results and the results of earlier work [9,15] is likely to do the limited scope of chemicals tested in previous studies, as well as analytical differences. While the earlier studies compared toxicity values among listed and surrogate species, the present study focused on relative sensitivity as a function of a particular species location within SSDs. Our approach addressed species sensitivity and can be used to protect listed aquatic species for which no toxicity tests have been conducted. Hazard levels from taxonomically diverse SSDs can be used to protect listed species with a greater level of certainty than is obtainable through use of general safety factors.

We created linear models describing the relationship between the relative sensitivity of surrogate species (rainbow trout and fathead minnow) and that of listed species (cutthroat trout and Cape Fear shiner). All models have significant linear relationships and demonstrate that the location of a surrogate species within an SSD may be used to predict the percentile within the SSD of a listed species. Such models can be used to further reduce uncertainty in the protectiveness of SSD hazard concentrations by identifying in which quartile of the SSD a listed species is likely to fall, based on the position in the SSD from a surrogate species. Development of a suite of models for surrogate and listed species for application in risk assessment has large data needs but could be explored in future research.

The SSD is a distribution-based approach that reduces reliance on toxicity testing of only a few standardized surrogate species to protect listed species with unknown sensitivity to a chemical. The relatedness of SSDs to natural communities is a primary source of uncertainty in SSDs [57], and past research addresses this through analysis of taxonomic diversity and the appropriate number of species to include [5,56]. The lack of available data generally limits the number and diversity of species for SSD development and may hinder a priori selection of SSD taxa. However, recent research has shown that interspecies correlation estimation models can be used to populate SSDs by providing toxicity estimates for a diversity of species [14,64,68]. Raimondo, Vivian, and Barron [19] and Raimondo, Mineau, and Barron [51] validated interspecies correlation estimation models for both aquatic and wildlife species, which are available on the U.S. EPA's Center for Exposure Assessment Modeling website [19; www.epa.gov/ceampubl/fchain/webice]. Hazard levels derived from SSDs using toxicity values derived from interspecies correlation estimation have been demonstrated to be similar to hazard levels derived from SSDs of measured data for aquatic organisms and wildlife [14,64,68].

Regardless of the initial cause of decline, endangered species may be at a greater risk of extinction from adverse impacts

of environmental contaminants than unlisted species because of their rarity. Smaller population sizes inherent to endangered species make them more susceptible to extinction risk than healthy populations when additional impacts occur [69]. Although SSDs are used to derive hazard concentrations in the United States and many other countries [5,14,70], the protectiveness of the HC5 for a diversity of endangered species has not been comprehensively assessed until now. While our analysis included a modest number of species relative to the total number of listed species, we demonstrate the protectiveness of using distribution-based approaches in the protection of threatened and endangered species from acute toxicity.

SUPPORTING INFORMATION

Table S1. Species sensitivity distributions for 68 chemicals. Toxicity values represent species mean values.

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