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Hazard/Risk Assessment

ASSESSING ACUTE AND CHRONIC COPPER RISKS TO FRESHWATER AQUATIC LIFE USING SPECIES SENSITIVITY DISTRIBUTIONS FOR DIFFERENT TAXONOMIC GROUPS

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Abstract—Using copper as an example, we present a method for assessing chemical risks to an aquatic community using species sensitivity distributions (SSDs) for different taxonomic groups. This method fits probability models to chemical exposure and effects data to estimate the percentage of aquatic species potentially at risk and expands on existing probabilistic risk assessment methodologies. Due to a paucity of chronic toxicity data for many chemicals, this methodology typically uses an acute–chronic ratio (ACR) to estimate the chronic effects distribution from the acute effects distribution. We expanded on existing methods in two ways. First, copper SSDs were developed for different organism groups (e.g., insects, fish) that share similar sensitivities or ecological functions. Integration of exposure and effects distributions provides an estimate of which organism groups may be at risk. These results were then compared with a site-specific food web, allowing an estimation of whether key food web components are potentially at risk and whether the overall aquatic community may be at risk from the perspective of ecosystem function. Second, chronic SSDs were estimated using the relationship between copper ACRs and acute toxicity (i.e., the less acutely sensitive a species, the larger the ACR). This correction in the ACR removes concerns previously identified with use of the ACR and allows evaluation of a significantly expanded chronic data set with the same approach as that for assessing acute risks.

Keywords—Copper Probabilistic risk assessment Acute–chronic ratio Species sensitivity distributions

INTRODUCTION

Ecological risk assessment methodologies have continuously developed over the last two decades. In its simplest form, risk can be estimated using the hazard quotient (HQ) approach where a point estimate of the exposure concentration is divided by some benchmark, such as a water quality criterion [1,2]. Using this type of approach, an HQ greater than one generally suggests that a stressor is present at levels that may pose risk to receptors. However, the relative risks associated with HQs of 1, 10, and 20, for example, are unclear. An HQ of 10 may imply more or less than 10 times the risk of an HQ of one and will be different depending on the exposure scenario and the dose–response relationship of the stressor of concern. As a result, HQs based on conservative point estimates of exposure and effects are useful as a screening tool (i.e., risks exist or don't exist) but provide little information on the actual magnitude of risks to an ecological community.

Probabilistic risk estimates account for variability in the estimated exposure and effect levels of a stressor and estimate uncertainty [1]. Potential risk can be expressed as a probability distribution rather than a single value. Parkhurst et al. [3] developed a methodology for conducting probabilistic aquatic life risk assessments, and several others have published probabilistic risk assessments using similar methods [4–6].

Using this methodology, probability distributions are calculated for both the exposure and effects data for the stressor being evaluated. The effects data are typically laboratory derived, such as those compiled by the U.S. Environmental Protection Agency (U.S. EPA) in ambient water quality criteria documents (e.g., [7]). The distribution of species sensitivities to a stressor, based on laboratory toxicity data, is assumed to represent the distribution of species sensitivities in a generic and diverse aquatic community [8,9]. Using Monte Carlo sampling, the probability distribution functions (PDFs) for exposure and effects are mathematically integrated, and risk is estimated as the percentage of species expected to be affected in an aquatic community. The greater the degree of overlap between the exposure and effects PDFs, the greater the estimated risk to the aquatic community.

Because of the paucity of chronic toxicity data for most stressors, the chronic species sensitivity distribution (SSD) is often estimated from the acute distribution using a chemical-specific acute–chronic ratio (ACR) [8]. The U.S. EPA uses the geometric mean of available ACRs or a subset of available ACRs for a chemical in calculating chronic ambient water criteria. The ACRs are calculated by dividing a species' acute LC50 by the geometric mean of the chronic no-observed-effect concentration and lowest-observed-effect concentration. The acute and chronic values used to derive an ACR should be calculated based on data from side-by-side experiments or, at a minimum, from experiments using the same dilution water [8]. The Parkhurst et al. [3] methodology recommends using a constant ACR in estimating the chronic SSD, although for some chemicals, the ACR increases as the species mean acute value (SMAV) increases [8].

Generic example of Parkhurst et al. methodology

For this example, assume a freshwater aquatic community is exposed to bioavailable copper concentrations that are log-

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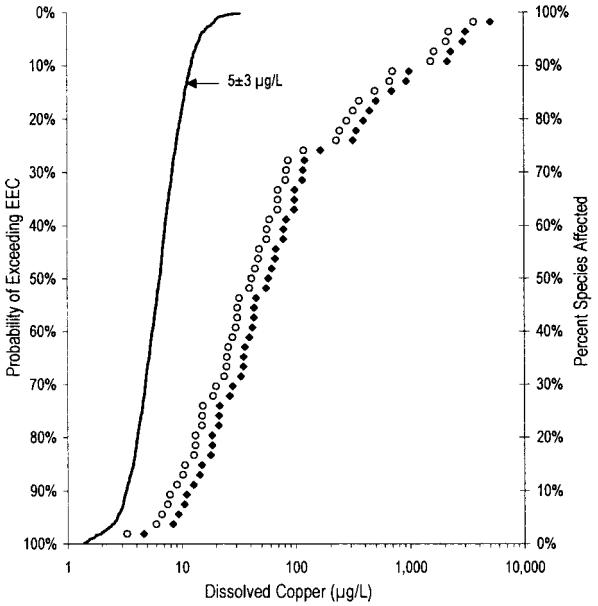


Fig. 1. Example of cumulative exposure and effects distributions for bioavailable copper using the Parkhurst et al. [3] methodology. Copper exposure concentrations (——), acute toxicity data (♦), and estimated chronic toxicity data (○).

normally distributed with a mean of 5 µg/L and a standard deviation of 3 µg/L (shown as a cumulative probability distribution in Fig. 1). Using the copper acute toxicity database compiled by the U.S. EPA [7], the Parkhurst et al. [3] methodology recommends that a logistic regression model be developed to characterize the acute SSD (Fig. 1). The chronic SSD may then be estimated from the acute distribution using the ACR of 2.8 for copper [7] (Fig. 1). The percentage of species acutely or chronically at risk equals the probability of a given expected environmental concentration (EEC) times the percentage of species acutely or chronically affected at that EEC. If the EEC distribution is the same for both acute and chronic exposures, 9 and 12% of the species would be estimated to be acutely and chronically at risk, respectively. This approach clearly provides more information than a simple HQ but leaves key questions unanswered regarding the significance a given reduction in species diversity has on the aquatic community. In this article, we suggest a means to begin to answer this question.

Continuing to use copper as an example, we expand on the Parkhurst et al. [3] methodology for estimating and interpreting risk in the context of a site-specific food web. The methodology considers the species at risk and the functional groups they comprise. Instead of developing one SSD for all aquatic organisms, we developed SSDs for different taxonomic groups [10,11]. The estimated risks to these taxonomic or functional groups can then be compared to a site-specific food web in order to assess potential risks to the aquatic community. In expanding the Parkhurst et al. [3] methodology, we also evaluated the ACR data for copper and developed a revised approach for estimating the chronic SSDs.

METHODS

Development of copper toxicity database

In order to develop SSDs for different taxonomic groups, it was first necessary to expand the U.S. EPA's [7] copper toxicity database. The U.S. EPA acute toxicity database for copper [7] was expanded by querying the AQUIRE database [1] and reviewing the scientific literature. Additional studies were reviewed for test acceptability using U.S. EPA guidelines [8] with the exception that toxicity data for non-North American species were included in the expanded database (Appendix 1). Non-North American species were included to test the hypothesis that species were globally similar in sensitivity to copper. Because copper bioavailability to freshwater aquatic organisms is hardness dependent, all LC50 values were normalized to a hardness of 50 mg/L (as $CaCO_3$) using the U.S. EPA's equation for hardness normalization [7]. When multiple acceptable acute LC50 values were available for a species, the species mean acute value (SMAV) was calculated as the geometric mean of individual LC50 values. We used SMAVs rather than genus mean acute values, as the U.S. EPA uses in criteria development, because the sensitivities of organisms within a genus can be quite variable [12].

Sensitivities of taxonomic groups

There are several mechanistic reasons why groups of organisms would have different sensitivities to copper or any other metal. The three primary reasons are homeostatic regulatory strategies for copper, membrane permeability, and allometric considerations.

Because copper is an essential metal, aquatic organisms have developed strategies for regulating internal copper concentrations. Regulatory strategies range across a continuum from active regulation to storage [13–15]. Active regulators excrete excess copper or limit net uptake [15,16]. This compares with species that store excess copper by sequestering the metal in forms that are either metabolically available (e.g., metallothioneins) or unavailable (e.g., calcium phosphate granules) [13,17–20]. Within this continuum, the majority of species use a combination of active regulation and storage strategies [13].

Membrane permeability also can influence sensitivity to metals, and some organisms adapt to elevated copper levels by reducing their membrane permeability [21,22]. The third factor is allometric considerations such as the amount of permeable membrane relative to absolute body size [23,24].

In order to develop SSDs for different taxonomic groups, copper toxicity data for different groups were plotted as cumulative distributions. All SMAVs were divided by two to estimate an acute safe concentration [8]. Within each of these sensitivity distributions, we evaluated the following potential relationships that have been suggested by other researchers: whether species from the same feeding guild (e.g., herbivores, omnivores, carnivores, and detritivores) had similar sensitivities [25], whether species from the same phylogenetic group (e.g., order, family) had similar sensitivities [26–28], and whether species from the same climatic regime (e.g., cold water, warm water, tropical) had similar sensitivities [29].

The cumulative distributions developed based on the above relationships were visually inspected to identify potentially unique SSDs for different taxonomic categories. The SSDs were also evaluated to determine whether differences between SSDs were statistically significant. For example, differences between means and variances can both be tested. From a statistical perspective, the acute values used in these SSDs do not really constitute random samples from independent populations of acute values. Consequently, testing a null hypothesis that there is no difference between the means that cannot be explained by sampling error is not really appropriate. In

Table 1. Acute–chronic ratios for copper[a]

Species	SMAV (μg/L)	SMCV (μg/L)	ACR	Reference
Campeloma decisum (snail)	1,860.0	11.9	156.2	[34]
Physa integra (snail)	42.7	11.9	3.6	[34]
Daphnia magna (cladoceran)	20.2	8.4	2.4	[7]
Gammarus pseudolimnaeus (amphipod)	21.9	6.6	3.3	[34]
Oncorhynchus tshawytscha (salmon)	61.9	13.9	4.5	[7]
Salvelinus fontinalis (brook trout)	109.4	14.1	7.8	[35]
Pimephales notatus (bluntnose minnow)	72.9	2.8	26.4	[36]
Pimephales promelas (fathead minnow)	124.0	11.1	11.2	[37–41]
Lepomis macrochirus (bluegill sunfish)	1,203.5	31.7	38.0	[42]

[a] SMAV = species mean acute value (normalized to a total hardness of 50 mg/L [as $CaCO_3$] per U.S. Environmental Protection Agency (U.S. EPA [7]); SMCV = species mean chronic value (normalized to a total hardness of 50 mg/L [as $CaCO_3$] per U.S. EPA [7]); ACR = acute–chronic ratio.

addition, a difference in variance provides evidence that populations are not identical, either because the populations are truly different or because the populations have not been sampled comparably. However, from a risk perspective, although the mean acute values or variances of two populations may be the same, differences in parts of the distribution, especially the lower tails, can have significant implications for risk estimates. Thus, a combination of statistics and visual interpretation (from a risk perspective) was used to identify unique SSDs.

Estimation of the chronic SSDs

As mentioned above, Parkhurst et al. [3] used a constant ACR, and this approach has been used in other published risk assessments [6]. However, it has been observed for some chemicals, including copper, that the ACR tends to increase or decrease in relation to the SMAVs [8]. We explored this relationship and how it influences chronic species sensitivity distributions for copper. Copper ACRs meeting data quality criteria [8] were identified for nine freshwater species (Table 1). Each ACR was plotted against its corresponding SMAV (normalized to a hardness of 50 mg/L). The relationship between the copper SMAVs and ACRs was then quantified and used to estimate chronic SSDs for the taxonomic and functional groups identified in development of the acute SSDs.

Risk characterization

Risks based on the EEC distribution previously provided using the Parkhurst et al. [3] methodology were reassessed using our new acute and chronic SSDs. We present the results two ways, first as presented in Parkhurst et al. [3] and then as described in Giesy et al. [30]. The second method graphically presents the results such that the percent of time that a given EEC is exceeded is compared with the percentage of species that are expected to be affected at that EEC [30]. This provides a more complete description of the risk distribution. For the purposes of this example, risks to different taxonomic groups are then discussed in the context of a hypothetical site-specific food web. The potential benefits of using this approach in an ecological risk assessment context are discussed.

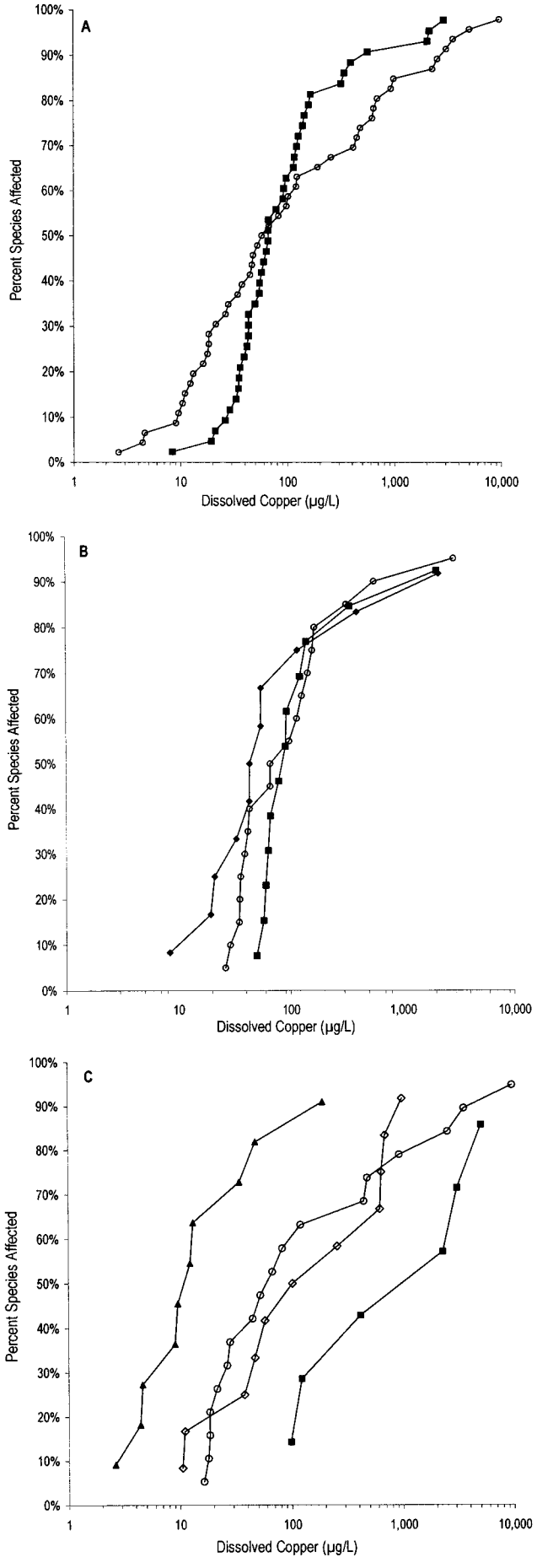
RESULTS

Estimation of acute SSDs

Based on our review, the copper acute toxicity database was expanded from 53 to 87 species (Fig. 2A). Using these data, the SSDs for invertebrates and fish have different variances but similar means, with invertebrates appearing to be more sensitive at concentrations less than 100 μg/L (Fig. 2A). The distribution of toxicity data for each of these general groups was then evaluated further. For fish, we were unable to identify any trends in relative sensitivity with respect to feeding guild or phylogenetic relationship. There was, however, a general trend that temperate cold-water species appear to be more sensitive than temperate warm-water species, which in turn appear to be more sensitive than tropical species (Fig. 2B). We evaluated this trend further to ensure that it was not simply a function of toxicity test design (e.g., less sensitive life stages of tropical or temperate warm-water species were tested) and were unable to identify any causative factors. In the vast majority of tests with temperate cold-water, temperate warm-water, and tropical species, juvenile life stages were tested. The mechanistic basis for this trend is unclear since changes in water temperature have been observed to both increase and decrease the toxicity of compounds [31]. It has been suggested that toxicity may be greater at warm temperatures because absorption of chemicals may be accelerated, but other studies suggest that low water temperatures may increase chemical toxicity by slowing down processes such as excretion and detoxification [31].

Of the invertebrates, cladocerans are clearly the most sensitive taxonomic group and insects the least sensitive (Fig. 2C). Of the six insect species for which there are copper toxicity data, the life stage tested is not reported for three (the stonefly *Acroneuria lycorias*, an unidentified caddisfly, and an unidentified damselfly). The other three species with copper toxicity data are all midges (*Chironomus tentans*, *C. riparius*, and *C. decorus*). The copper SMAVs for these three species are 99, 124, and 417 μg/L. The life stages tested were first instar, second instar, and fourth instar, respectively, thus suggesting that the differences in sensitivity between these three midge species may be at least partially related to the life stage tested rather than to species-specific factors. As a result, the insect sensitivity distribution may be somewhat biased by insensitive life stages. However, sensitive life stages of two midge species were tested (i.e., first and second instars), and the difference between insect LC50s and those for other invertebrates groups (e.g., cladocerans) is large. Therefore, it does appear that insects as a whole may be a relatively insensitive taxonomic group with regard to acute copper toxicity.

The sensitivities of other taxonomic groups, such as amphipods and copepods, did not appear to cluster into separate groups when compared with the entire invertebrate SSD (most



toxicity data for these taxa are based on tests with juveniles or adults). The distribution of toxicity data for crustaceans other than cladocerans is also fairly broad. Overall, though, crustaceans tend to be slightly less sensitive than invertebrates not included in the cladoceran group and more sensitive than insects (Fig. 2C). For many other taxonomic groups (e.g., worms, snails), data were unavailable for a sufficient number of species to derive SSDs and assess their relative sensitivity. Accordingly, the SSD termed other invertebrates represents the sensitivities of taxonomic groups other than cladocerans, crustaceans excluding cladocerans, and insects.

To assess acute risks probabilistically, PDFs were fit to the toxicity data for the different SSDs identified. A variety of authors recommend use of the log-logistic probability function for modeling toxicity data [3,9,32,33], and so this distribution type was used to model the data. While the overall fits of the logistic regression models were quite good, with r^2 values of 0.71 to 0.93 (Table 2), the models tend to overestimate toxicity in the lowest quartile, which is critical for the risk assessment. Figure 3 provides examples of typical model fits to the data for cladocerans, insects, and cold-water fish. Although beyond this article's scope, it is clear that alternative models that better fit the SSDs would further improve risk assessment procedures.

Estimation of chronic SSDs

On a log-log scale, a strong linear relationship ($r^2 = 0.83$) between acute sensitivity to copper and the ACR for a given species was observed (Fig. 4) and indicates that the use of a constant ACR for copper is inappropriate. The linear relationship was used to estimate the chronic sensitivities of species for which there were no acute toxicity data using

$$\text{ACR} = 10^{0.786\times\log \text{ Acute Value}-0.582} \quad (1)$$

$$\text{chronic value} = \frac{\text{acute LC50}}{\text{ACR}} \quad (2)$$

For the most acutely sensitive species, the regression equation results in chronic values greater than the acute values (i.e., chronic toxicity less than acute toxicity). This is because the acute distribution is based on the SMAVs divided by two and the estimated ACRs for the most sensitive species were less than two. For these species, the ACR was set equal to two; in other words, the acute sensitivity (expressed as an estimate of the LC-low) and chronic sensitivity were assumed to be equal. Not setting this somewhat arbitrary bounding limit on the ACR–SMAV relationship would result in predicting chronic values higher than the acute toxicity values expressed as the SMAV/2. Since this is not possible, the bounding limit appears justified. Further, the lowest measured species mean ACR is 2.4 [7], so the limit does not contradict any existing data.

The implications of using a variable ACR are shown in Figure 5. First, the acute toxicity distribution for all species is depicted along with the standard technique of using a constant ACR to estimate the chronic SSD. Also depicted is the estimated chronic SSD using a variable ACR. The resulting estimated chronic SSDs are quite different using the two ap-

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Fig. 2. Acute copper toxicity empirical cumulative distribution functions for (**A**) invertebrates (○) and fish (■); (**B**) cold water (♦), temperate/warm water (○), and tropical (■); and (**C**) cladocerans (▲), crustaceans excluding cladocerans (♦), insects (■), and other invertebrates (○).

Table 2. Slope, intercept, and r^2 values for acute logistic regression analyses

Taxonomic group	Slope	Intercept	r^2
Cladocerans	2.42	−2.76	0.93
Other crustaceans	1.88	−3.93	0.93
Insects	1.65	−4.75	0.91
Other invertebrates	1.58	−3.32	0.88
Tropical fish	2.68	−5.54	0.71
Temperate fish	2.73	−5.38	0.86
Cold-water fish	2.00	−3.60	0.88

proaches. For comparative purposes, the distribution of actual chronic toxicity data (n = 17) are also plotted (Appendix 2). As can be seen, the variable ACR approach provides an improved estimate of the available chronic toxicity data compared with the constant ACR.

Logistic regression models were fit initially to the estimated chronic SSDs for the various species groups, as was done for the acute SSDs. Because the chronic SSDs are a linear function of the acute SSDs, the r^2 values are the same. However, with the exception of cladocerans, logistic regressions tended to provide extremely poor fits to the lower tail of the estimated chronic SSDs. The poor fit in the lower tails is because they are much steeper and lack the sigmoid shape of the acute SSDs. Accordingly, alternative models were considered to describe the chronic SSDs for groups other than cladocerans. Considering the general shape of the distributions, we selected the Pareto model to describe the chronic SSDs, except for cladocerans, for which we used the log logistic (Fig. 6).

Risk characterization

Assuming a copper EEC distribution with a mean of 5 μg/L and standard deviation of 3 μg/L, the following provides a brief example of how the acute and chronic SSDs described above can be used to estimate potential risks to specific components of the food web. Assuming the exposure data represent a temporal distribution of spatially averaged copper concentrations over an appropriate exposure duration, negligible risk from direct exposure to copper is predicted for insects using this example (Fig. 7). Risks to warm-water fish, crustaceans excluding cladocerans, and other invertebrates is low to negligible >85% of the time. The estimated risk to cladocerans is much higher. Up to 50% of the cladoceran species are expected to be affected 20% of the time and 25% of the cladoceran species affected 45% of the time.

Ideally, such results can be applied in the context of a site-specific food web. In this example, assume the assessment endpoint being evaluated is survival, growth, and reproduction of recreationally important fish species. Assume for simplicity

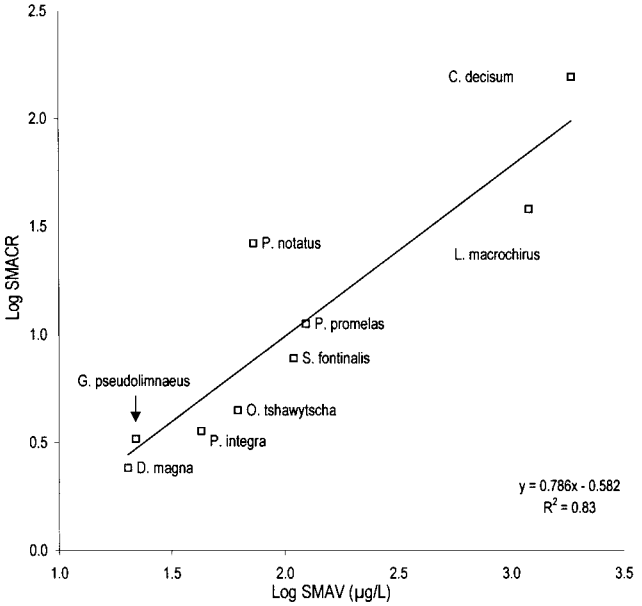

Fig. 4. Relationship between freshwater species mean acute values (SMAV) and acute–chronic ratios for copper.

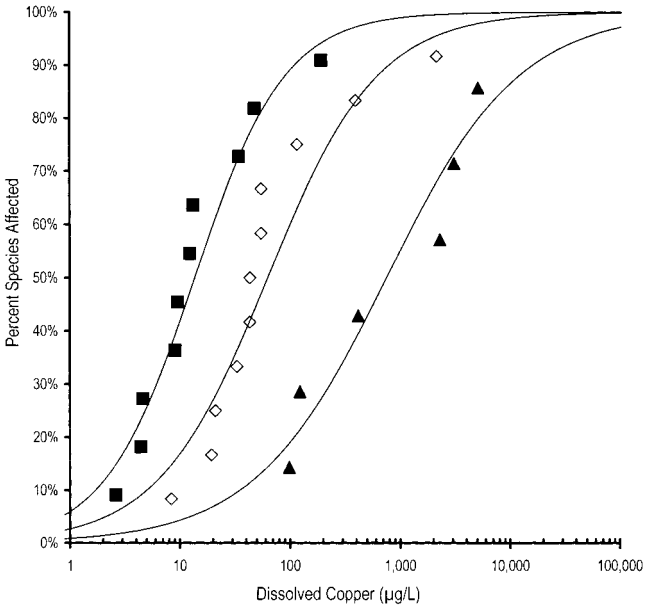

Fig. 3. Logistic regressions fit to acute toxicity data for cladocerans, cold-water fish, and insects. Cladocerans (■), cold-water fish (♦), and insects (▲).

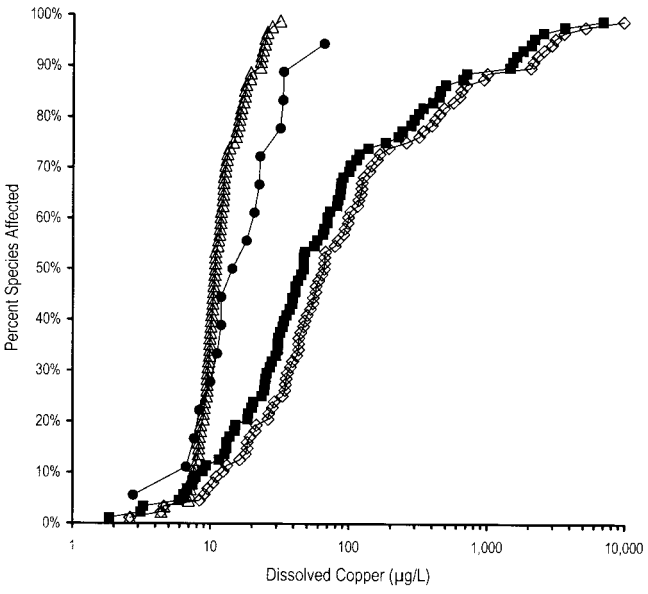

Fig. 5. Empirical cumulative distribution functions based on measured acute and chronic toxicity data compared with estimated chronic cumulative distribution functions using constant and variable ACRs. Acute toxicity (♦), estimated chronic using a constant ACR (■), estimated chronic using adjusted ACR (△), and measured chronic toxicity (●).

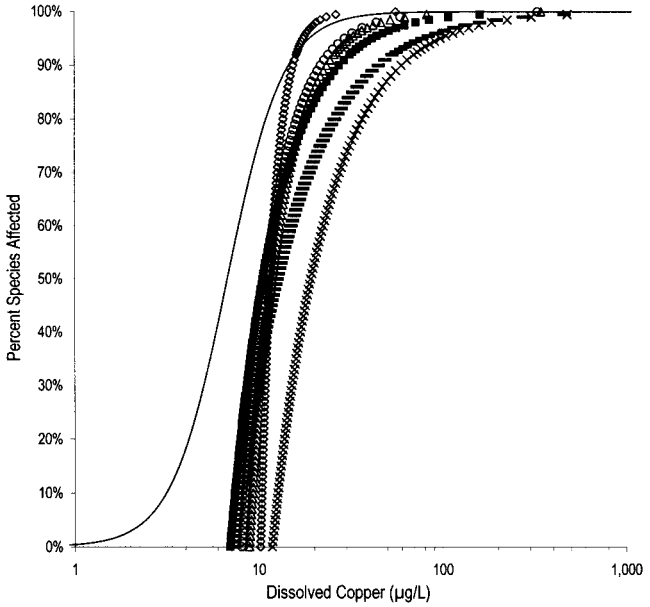

Fig. 6. Pareto distribution function fit to the estimated chronic toxicity data for different taxonomic groups (logistic distribution for cladocerans). Cladocerans (——), cold-water fish (■), temperate/warm-water fish (Δ), tropical fish (◆), crustaceans excluding cladocerans (bold line), other invertebrates (○), and insects (×).

that the food web at this site consists of zooplankton, aquatic insects, planktivorous fish, omnivorous fish, insectivorous fish, piscivorous fish, and detritivores (Fig. 8). The greatest source of carbon for these fish species is planktivorous fish that feed mostly on zooplankton (e.g., cladocerans). Based on the generic analysis above, it appears that copper poses no direct risk to the piscivorous, planktivorous, or insectivorous fish (i.e., fish in general are not at risk). In addition, insects do not appear to be at risk due to copper, so potential risk to insectivorous fish from reduced food sources appears to be negligible. However, it is possible that cladocerans would be sufficiently at risk such that planktivorous fish biomass would be

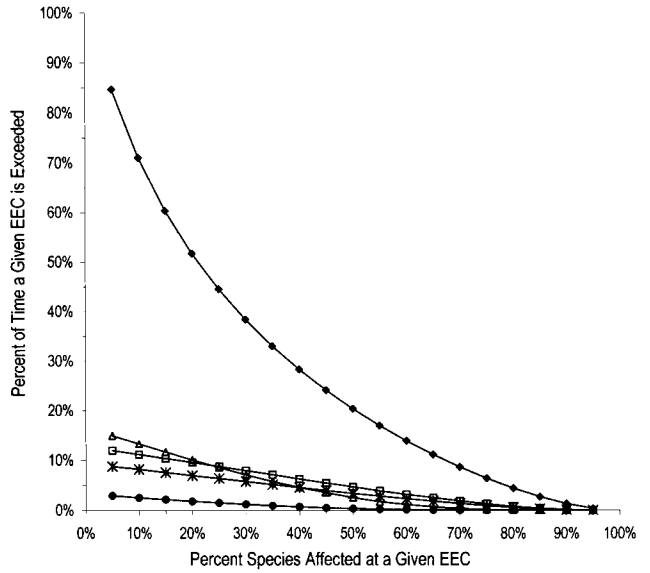

Fig. 7. Example: Estimated copper risks based on expected environmental concentration of 5 ± 3 µg/L bioavailable copper. Cladocerans (◆), other invertebrates (□), crustaceans excluding cladocerans (△), insects (●), and warm-water fish (*).

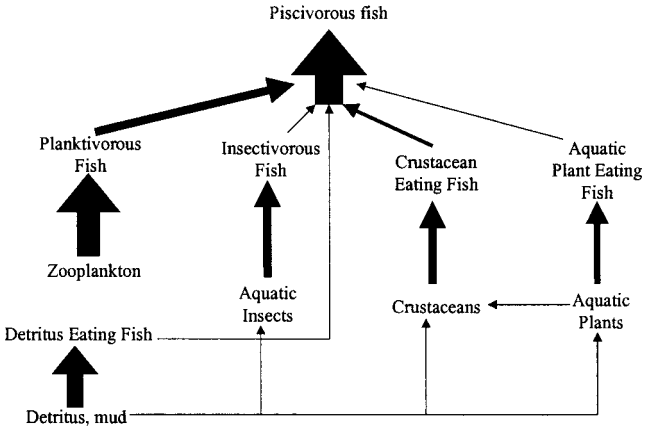

Fig. 8. Example: Foodweb.

reduced enough to put piscivorous fish populations at risk. In this example, given the importance of piscivorous fish as a recreational species, significant risk may be considered unacceptable.

DISCUSSION

Probabilistic risk assessment methodologies have been accepted generally as a more useful approach for conducting aquatic life risk assessments than deterministic approaches [1]. The primary benefits of this approach are that the risk estimates reflect the variability in both exposure concentrations and in the sensitivities of aquatic organisms. In principle, the data required for this approach are the same as those needed for deterministic techniques, with the primary difference being that exposure and effects data are based on probability distributions rather than point estimates. The key underlying assumptions in these two approaches are the same, i.e., laboratory toxicity data are indicative of effects in natural systems, the species for which toxicity data are available are representative of the aquatic system being evaluated, and the exposure data represent bioavailable forms of the stressor.

The first of these assumptions, that laboratory toxicity data are reflective of effects concentrations in natural systems, is a controversial subject that has been extensively studied and debated and that we will not delve into in this article. The second assumption, that the effects data are representative of the aquatic life occurring in a system can be quite important. In the example provided in this article, substantial risk for cladocerans, and consequently to the food web as a whole, was predicted because, in this hypothetical scenario, the zooplankton–planktivorous fish–piscivorous fish food chain was identified as critical to the overall functional integrity of the food web. However, use of the same effects data for a second-order stream might not be appropriate because cladocerans and zooplankton in general are unlikely to be critical components of this systems foodweb. Therefore, site-specific consideration of foodwebs and the applicability of various SSDs to those sites is critical and achievable with this method. Caution should be exercised though in removing phylogenetic groups (particularly sensitive groups) when the sensitivity of other groups not well characterized are identified as important to the site. In these instances, the use of sensitive groups as a conservative surrogate for untested groups can be important [8].

The assumption that the exposure characterization reflects bioavailable copper is critically important when using this methodology. This is particularly true for assessments of

chronic risk where, because of the steepness of the effects PDF, relatively small changes in exposure result in significant changes in risk predictions. Inaccurate characterization of bioavailable copper or use of conservative assumptions (e.g., assuming total or dissolved metal equates to bioavailable metal) will potentially lead to significant overestimations of chronic risk with this method.

If these three assumptions are considered valid or at least conservative, the primary benefit of the probabilistic methodology is that risk managers are provided more information such that decisions can be made depending on the level of risk deemed acceptable for their site. Although risk presented as percent species affected provides more information than an HQ, it still provides limited information on what a given level of species effects has on ecosystem function.

Separating the toxicity data into different taxonomic groups requires data for a large number and wide variety of aquatic species. Consequently, our proposed approach requires sufficient information in order to be able to evaluate the differential sensitivities of various taxonomic groups and to fit PDFs to the toxicity data for the different taxonomic groups identified. We have evaluated the toxicity data for a variety of other metals (both essential and nonessential) and have observed very similar patterns, i.e., that the same taxonomic groups have similar relative sensitivities for many metals. As discussed previously, it is not surprising that these patterns exist because of varying metal regulatory strategies and allometric considerations. It is interesting to note that these patterns are much more evident for the acute toxicity data. When the chronic data are evaluated, the differences in sensitivities between taxonomic groups appear to be much narrower such that a threshold effect is observed for all species groups except cladocerans. The reason cladocerans are an exception to this trend is unclear, although it is interesting that this species group is also the most sensitive to copper.

Estimation of the chronic effects curve using a variable ACR can substantially influence chronic risk estimates. However, this approach appears to be an improved model because it is reasonably consistent with the measured chronic toxicity data for a variety of species (i.e., the measured chronic distribution is much steeper than the acute effects distribution). These results suggest that there may be a chronic threshold for copper related to the ability of organisms to regulate copper, an essential metal.

In conclusion, we believe our approach provides an improvement in the methodology for assessing risks from copper to a freshwater aquatic system. This methodology could be easily applied to other stressors, provided sufficient effects data are available.

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APPENDIX 1
Acute sensitivities of freshwater organisms to copper (μg/L)[a]

Species	*N*	SMAV adjusted to 50 mg/L hardness	Reference
Acrocheilus alutaceus (chiselmouth)	1	133.0	[43]
Acroneuria lycorias (stonefly)	1	10,242.0	[44]
Alona affinis (cladoceran)	1	386.3	[45]
Ambassis sp. (chanda perch)	2	115.4	[27]
Amnicola sp. (snail)	1	900.0	[46]
Anguilla rostrata (American eel)	4	4,305.5	[47–50]
Anodonta imbecilis (mussel)	1	105.3	[51]
Brachydanio rerio (zebrafish)	2	99.8	[29]
Campeloma decisum (snail)	1	1,877.4	[34]
Campostoma anomalum (central stoneroller)	1	78.5	[52]
Carassius auratus (goldfish)	1	289.1	[53]
Ceriodaphnia reticulata (cladoceran)	1	5.2	[54]
Cherax destructor (26-d) (crayfish)	2	1,254.7	[29]
Chironomus decorus (midge)	1	833.6	[55]
Chironomus riparius (midge)	1	247.1	[56]
Chironomus tentans (midge)	1	197.2	[57]
Corbicula malinensis (asiatic clam)	1	7,184.8	[58]
Corbiculina australis (orb-shell mussel)	1	5,115.3	[29]
Crangonyx pseudogracilis (amphipod)	1	1,290.0	[59]
Craterocephalus marjoriae (Marjorie's hardihead)	1	182.2	[29]
Craterocephalus stercusmuscarum (fly-specked hardihead)	2	127.3	[60]
Cyprinus carpio (common carp)	2	317.5	[61]
Cypris subglobosa (ostracod)	2	115.8	[45,62]
Daphnia ambigua (cladoceran)	1	24.8	[63]
Daphnia magna (cladoceran)	12	18.1	[7,54,64–68]
Daphnia parvula (cladoceran)	1	26.4	[63]
Daphnia pulex (cladoceran)	2	8.8	[54,67]
Daphnia pulicaria (cladoceran)	8	9.3	[40]
Daphnia rosea (cladoceran)	1	69.0	[69]
Denariusa bandata (penny fish)	1	120.8	[70]
Echinogammarus berilloni (amphipod)	1	75.7	[71]
Etheostoma caeruleum (darter)	1	86.7	[52]
Etheostoma spectabile (darter)	1	230.2	[52]
Fundulus diaphanus (banded killifish)	2	790.6	[47,48]
Galaxias maculatus (jollytail)	1	70.7	[29]
Gambusia affinis (mosquitofish)	2	196.1	[72]
Gammarus pseudolimnaeus (amphipod)	1	22.1	[34]
Gammarus pulex (amphipod)	7	21.0	[71,73]
Goniobasis livescens (snail)	2	166.2	[74]
Gyraulus circumstriatus (snail)	1	56.2	[75]
Heteropneustes fossilis (sccobranch catfish)	2	4,104.2	[45,76]
Ictalurus nebulosus (brown bullhead)	3	69.8	[52,77]
Ictalurus punctatus (channel catfish)	8	255.3	[78]
Lepomis gibbosus (sunfish)	7	640.9	[79]
Lepomis macrochirus (bluegill)	5	1,134.3	[42,80–83]
Limnodrilus hoffmeisteri (tubificid worm)	1	53.1	[75]
Lophopodella carteri (bryozoan)	1	135.0	[84]
Lumbriculus variegatus (worm)	1	242.7	[85]
Macquaria ambigua (golden perch)	1	158.1	[29]
Macrobrachium sp. (prawn)	1	204.4	[29]
Melanotaenia nigrans (black rainbowfish)	2	245.8	[60]
Melanotaenia splendida inornata (chequered rainbowfish)	5	280.3	[29,60,70]
Melanotaenia splendida splendida (chequered rainbowfish)	1	186.0	[29]
Moina dubia (cladoceran)	1	19.2	[45]
Morone americanus (white perch)	2	5,859.5	[47]
Morone saxatilis (striped bass)	1	52.4	[86]
Nais sp. (worm)	1	90.0	[46]
Notropis chrysocephalus (striped shiner)	2	331.8	[52]
Oncorhynchus clarki (cutthroat trout)	9	66.3	[87]
Oncorhynchus kisutch (salmon)	3	87.0	[7,88]
Oncorhynchus mykiss (rainbow trout)	39	38.9	[7,79,87–92]
Oncorhynchus nerka (sockeye salmon)	5	233.8	[93]
Oncorhynchus tshawytscha (salmon)	10	42.3	[7,94,95]
Oronectes rusticus (crayfish)	1	1,397.3	[96]
Paratya australensis (atyid shrimp)	3	94.0	[29,97]
Pectinatella magnifica (bryozoan)	1	37.0	[84]
Philodina acuticornis (bdelloid rotifer)	2	969.1	[98]
Physa heterostropha (snail)	1	35.9	[75]
Physa integra (snail)	1	43.1	[34]

APPENDIX 1
Continued

Species	*N*	SMAV adjusted to 50 mg/L hardness	Reference
Pimephales notatus (minnow)	8	72.2	[36,52]
Pimephales promelas (fathead minnow)	12	132.7	[37–40,52,99]
Plumatella emarginata (bryozoan)	1	37.0	[84]
Poecilia reticulata (guppy)	3	58.0	[100–102]
Porochilus rendahli (eel-tailed catfish)	1	133.4	[70]
Procambarus clarkii (crayfish)	1	1,989.6	[103]
Ptychocheilus oregonensis (northern squawfish)	1	16.7	[43]
Rhinichthys atratulus (blacknose dace)	1	86.7	[52]
Salmo salar (trout)	2	109.9	[104,105]
Salvelinus fontinalis (brook trout)	1	110.4	[35]
Semotilus atromaculatus (creek chub)	1	84.0	[52]
Simocephalus serrulatus (cladoceran)	3	95.9	[106]
Tilapia mossambica (Mozambique tilapia)	1	684.3	[107]
Tropocyclops prasinus mexicanus (cyclopoid copepod)	3	516.9	[108]
Tubifex tubifex (oligochaete)	2	32.7	[109]
Unidentified caddisfly	2	6,200.0	[46]
Velesunio angasi (freshwater mussel)	1	19,531.1	[29]

[a] LC50 = median lethal concentration; SMAV = species mean acute value (geometric mean of LC50 values for a given species).

APPENDIX 2
Chronic sensitivities of freshwater organisms to copper

	Species mean chronic value normalized to hardness of 50		
Species	*N*	mg/L	Reference
Campeloma decisum (snail)	1	11.9	[34]
Physa integra (snail)	1	11.9	[34]
Daphnia magna (cladoceran)	4	7.7	[7,110]
Gammarus pseudolimnaeus (amphipod)	1	6.7	[34]
Oncorhynchus mykiss (rainbow trout)	1	20.6	[111]
Salmo trutta (brown trout)	1	33.4	[111]
Salvelinus fontinalis (brook trout)	4	8.3	[35,111,112]
Salvelinus namaycush (lake trout)	1	33.1	[111]
Esox lucius (northern pike)	1	65.6	[111]
Pimephales notatus (bluntnose minnow)	1	2.8	[36]
Pimephales promelas (fathead minnow)	5	11.2	[37–41]
Catostomus commersoni (white sucker)	1	22.7	[111]
Ictalurus punctatus (channel catfish)	2	10.0	[112]
Lepomis macrochirus (bluegill)	1	31.7	[42]
Stizostedion vitreum (walleye)	1	22.4	[112]
Clinostornia magnifica (caddisfly)	1	18.2	[57]
Oncorhynchus tshawytscha (chinook salmon)	1	14.4	[7]