EPA/600/X-05/027
July 2005

# Methods/Indicators for Determining when Metals are the Cause of Biological Impairments of Rivers and Streams: Species Sensitivity Distributions and Chronic Exposure-Response Relationships from Laboratory Data

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

## NOTICE

The U.S. Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to the Agency's peer and administrative review and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

## ABSTRACT

This report provides exposure-response information from laboratory toxicity tests for use in the strength-of-evidence step of the Stressor Identification (SI) process (U.S. EPA, 2000). The species sensitivity distribution and exposure-response models in the appendices will assist assessors in analyzing the plausibility of the exposure-response relationship implied by the site exposure data (metals concentrations) and site response data (the biological impairment) by comparing that relationship to the models in this report. That is, the inference that a toxicant metal is the cause of an impairment is supported if the response intensity observed at the site is consistent with the response intensity expected, as indicated by the models from this report, at that metal concentration. If the site-observed response intensity is not consistent with expected intensity at the site-observed metal concentration, other stressors would be implicated. The information in this report will be supplemented by a report analyzing field-derived data for effects of metals and relating the laboratory and field information. The metals exposure-response models in this report will be incorporated into the Causal Analysis Diagnosis/Decision Information System (CADDIS). That Web site was developed and prepared by U.S. EPA's National Center for Environmental Assessment (NCEA) as an information resource and template for the purpose of facilitating the SI process (www.epa.gov/caddis/).

**Preferred citation:**
U.S. EPA. 2005. Methods/Indicators for Determining when Metals are the Cause of Biological Impairments of Rivers and Streams: Species Sensitivity Distributions and Chronic Exposure-Response Relationships from Laboratory Data. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. EPA/600/X-05/027.

## TABLE OF CONTENTS

Page

## TABLE OF CONTENTS cont.

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| BLM | Biotic Ligand Model |
| CHESS | CHemical Equilibria in Soils and Solutions |
| CV | Coefficient of Variation |
| ∆ALAD | Delta Aminolevulinic Acid Dehydratase |
| EC05 | Toxicant concentration at which 5% of organisms are affected |
| EC20 | Toxicant concentration at which 20% of organisms are affected |
| EMAP | Environmental Monitoring and Assessment Program |
| GLM | General Linear Model |
| ICE | Interspecies Correlation Estimations |
| LC01/LC50 | The ratio of the toxicant concentration at which 1% of exposed organisms died to the toxicant concentration at which 50% of exposed organisms died |
| LC50 | In a toxicity test, the toxicant concentration at which 50% of exposed organisms died |
| LOEC | Lowest-Observed-Effect Concentration |
| LT50 | In a toxicity test, the time at which 50% of organisms exposed to a given toxicant concentration die |
| MATC | Maximum Acceptable Toxicant Concentration |
| NOEC | No-Observed-Effect Concentration |
| SAS | Statistical Analysis Software |
| SI | Stressor Identification |
| SSD | Species Sensitivity Distribution |
| TMDL | Total Maximum Daily Load |
| WER | Water Effect Ratio |

## PREFACE

This report provides exposure-response information on the toxicity of metals observed in laboratory tests in the form of species sensitivity distributions (SSDs) and exposure-response relationships. These models are intended for use in the strength-of-evidence step of the Stressor Identification (SI) Process (U.S. EPA, 2000). During the SI process, investigators critically review available information to:

(1) form *causal pathways* that might explain the results of a biological assessment (causal pathways are the cause-and-effect relationships linking stressor sources to biological impairments. Where multiple interacting stressors are involved, the aggregate of these relationships is termed a *causal scenario*),

(2) analyze those pathways using data from the impaired site and elsewhere and

(3) characterize the relative likelihood of each pathway through a strength-of-evidence approach, which considers specificity of response, exposure-response relationships and plausible mechanisms along with other site specific factors.

Ultimately, the process identifies the stressors most likely causing the observed biological impairments. The species sensitivity distribution and exposure-response models in the appendices are intended to assist assessors in analyzing the plausibility of the exposure-response relationship implied by the site exposure data (metals concentrations) and site response data (the biological impairment) by comparing that relationship to the models in this report. That is, the inference that a toxicant metal is the cause of an impairment is supported if the nature and intensity of response observed at the site is consistent with the responses expected at that metal concentration, as indicated by the models from this report. If the nature and intensity of site-observed responses are not consistent with expectations, other stressors would be implicated.

The SSDs and exposure-response models from this report will be incorporated into the Causal Analysis Diagnosis/Decision Information System (CADDIS). This Web site was developed and prepared by U.S. EPA's National Center for Environmental Assessment (NCEA) as an information resource and template for the purpose of facilitating the SI process (www.epa.gov/caddis/). Exposure-response information from

field studies and an analysis of the relationship between responses to exposures in the laboratory and field will supplement these laboratory data-derived models.

## AUTHORS, CONTRIBUTORS AND REVIEWERS

### AUTHORS

Patricia Shaw-Allen
U.S. Environmental Protection Agency
National Center for Environmental Assessment
Cincinnati, OH

Glenn W. Suter II
U.S. Environmental Protection Agency
National Center for Environmental Assessment
Cincinnati, OH

### REVIEWERS

Uwe Borgmann
National Water Research Institute
Environment Canada
Burlington, Ontario, CA

Susan Cormier
U.S. Environmental Protection Agency
National Exposure Research Laboratory
Cincinnati, OH

Charles Delos
U.S. Environmental Protection Agency
Office of Water
Washington, DC

Jerome Diamond
Tetra Tech, Inc.
Owings Mills, MD

Randy Wentsel
U.S. Environmental Protection Agency
Office of Research and Development
Washington, DC

# 1. INTRODUCTION

This report provides species sensitivity distribution and exposure-response models of metal toxicity in freshwater fish, arthropods and non-arthropod invertebrates as tools to assist environmental assessors in determining whether metals have impaired specific biological communities. These models are intended for use in supporting one of the *causal considerations, plausibility of exposure-response,* in the strength-of-evidence step of the Stressor Identification (SI) process (U.S. EPA, 2000). Causal considerations are logical categories of evidence that are consistently applied to support or refute a hypothesized cause. Plausibility of exposure-response is the causal consideration which asks; "Would the effects observed be expected at the level of stressor seen in the environment?" Toxicologic data have not been specifically collected or analyzed with the intent of informing causal analysis in this manner. Point estimates of response (e.g., LOECs and EC20 values) are of limited utility when determining whether the site-observed responses are consistent with the site-observed exposures. Further, traditional approaches for handling toxicity data in criteria development and risk assessment are not appropriate to causal analysis. Causal analysis demands transparency to identify case-specific plausible scenarios rather than general estimates of thresholds for toxic responses in species or taxonomic groups. To address this need, the species sensitivity distributions (SSDs) and exposure-response relationships in this report illustrate the expected intensity of response with respect to intensity of metals exposure. Two important points should be kept in mind when applying these models and examining the data from which they were derived: (1) water chemistry is a critical factor in metals toxicity and (2) laboratory toxicity tests may poorly reflect actual environmental conditions. The causal assessor must therefore carefully choose models that best represent site conditions and explicitly support their model selection in the assessment documentation.

## 1.1. OVERVIEW OF THE STRESSOR IDENTIFICATION PROCESS

During the SI process investigators critically review available information to (1) identify *causal pathways* that might explain the results of a biological assessment (causal pathways are the cause-and-effect relationships linking sources of stressors to biological impairments; where multiple interacting stressors are involved, the aggregate of relationships is termed a *causal scenario*), (2) analyze those pathways using data from the impaired site and elsewhere and (3) characterize the relative likelihood of each

pathway through a strength-of-evidence approach. Ultimately, the process identifies the stressors most likely causing the observed biological impairments.

In the first step, forming causal pathways, investigators use existing information on stressors measured in impaired and reference waters and on stressors associated with point and nonpoint sources of stressors present in the watershed to identify candidate causes of impairment. The outcomes of this step are a list of stressors that potentially contribute to biological impairments and one or more conceptual models illustrating causal pathways.

During the second step, investigators analyze new and existing data to generate evidence that supports, weakens or refutes steps within each causal pathway. Evidence consists of associations of the candidate causes with biological effects. The associations are derived from laboratory tests, field tests or field observational studies.

For the third step, investigators characterize the candidate causes of impairment through a process of elimination, diagnosis and evaluation of the strength of evidence supporting, weakening or refuting each pathway. Elimination of causal scenarios or pathways within a scenario requires site-specific information strongly indicating that an causal pathway is incomplete. While elimination is the strongest method of inference used in the SI process, eliminating candidate causes is seldom possible. For candidate causes that are not eliminated, diagnosis is the next strongest type of inference. Causes are diagnosed when the response of the organisms is clearly indicative of a particular causal agent (Section 4).

Elimination and diagnosis require particular types of high quality site-specific data, while strength-of-evidence analysis organizes all information that supports or weakens the associations illustrated in the conceptual models. Strength-of-evidence analyses are necessary because site-specific data are often insufficient to fully diagnose impairments or confidently eliminate all but one potential cause. Data used for strength-of-evidence fall under one of several categories, termed *causal considerations*. Causal considerations are logical categories of evidence that are consistently applied to support or refute a hypothesized cause. The causal considerations *Specificity* (Section 5) and *Plausibility of Mechanism* (Section 6), are briefly addressed in this report. The models which are the main focus of this report support the consideration *Plausibility of Stressor-Response* (Section 7). The *Stressor Identification Guidance* (U.S. EPA, 2000) gives a complete description of all causal considerations, including Specificity, Plausibility of Mechanism and Plausibility of Stressor Response, potentially used in strength-of-evidence analysis.

## 1.2. ORGANIZATION AND CONTENT

This report contains an introductory chapter and eight major sections. Section 2 places metals contamination in context of other stressors and impairments on the 303(d) list. Section 3 provides valuable information for the development of causal pathways by detailing strategies for characterizing sources of metals. Section 4 discusses potential diagnostic responses to metals while Sections 5-7 cover the causal considerations *Specificity*, *Plausibility of Mechanism* and *Plausibility of Exposure-Response*. Section 7 describes two types of exposure-response relationships, species sensitivity distributions (SSDs) for LC50 values and exposure-response relationships from single species chronic tests. That section also describes how the exposure-response relationships in this report may be used in stressor identification. The methods used to derive the relationships are outlined in Section 8 and the data and models themselves are presented in the appendices. The point of this unusual organization is to ensure that the reader knows how the information will be used before explaining how it was derived. The last section, Section 9 reviews the information contained in this report and its associated uncertainties.

Toxicity data used for generation of SSDs were collected from ECOTOX (ECOTOXicology), the U.S. EPA's public access database that provides single chemical toxicity information for aquatic and terrestrial life. These data, provided in Appendix A, were used to model SSDs for arthropods, non-arthropod invertebrates, invertebrates and vertebrates (Appendices B-E). Exposure response relationships for single species were generated using an internal U.S. EPA database (Appendices F-I).

This work covers the common aquatic metal contaminants, cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn) and the metalloids arsenic (As) and selenium (Se). It does not cover metals in sediment, metals exposure from acid mine drainage or the metals silver (Ag), aluminum (Al) and iron (Fe); Ag, Al and Fe present complexities that are best left to more focused analyses. Information on sediment toxicity for metals and other toxicants can be found in *Predicting toxicity to amphipods from sediment chemistry* (U.S. EPA, 2005).

## 2. IDENTIFYING IMPAIRMENTS: METALS IN THE 303(d) LIST

A Total Maximum Daily Load (TMDL) is a calculation of the maximum amount of a pollutant that a water body can receive and still meet water quality standards, and an allocation of that amount to the pollutant's sources. It specifies the reduction of a pollutant required to meet water quality standards, allocates pollutant load reductions and provides the basis for taking actions needed to restore a water body. In the 2000 water quality inventory, elevated metals were identified as the second most frequent impairment at 11%, of 4800 impaired waters on the TMDL 303d list, and were exceeded only by pathogens, at 14%. Metals are identified far more frequently than pesticides, organics, and other toxicants (Figure 2-1). Impairments identified due to biological criteria account for nearly 4% of the total. While elevated metals may contribute to biological impairments, this is only one group of stressors that may be acting on a system. Their relative prevalence among TMDL listings may reflect the types of chemical data collected by states and tribes and the availability of criteria to which site levels may be compared as well as their importance as aquatic toxicants. While a given site may have been listed due to metals contamination, this does not necessarily mean that a biological impairment exists at the site. Likewise, if a biological impairment is detected at a metals-impaired site, metals are not necessarily the sole or primary cause of the impairment. In the course of stressor identification, the analyst must weigh the evidence for many types of stressors, including those that lack criteria or widely accepted benchmarks indicating levels of concern.

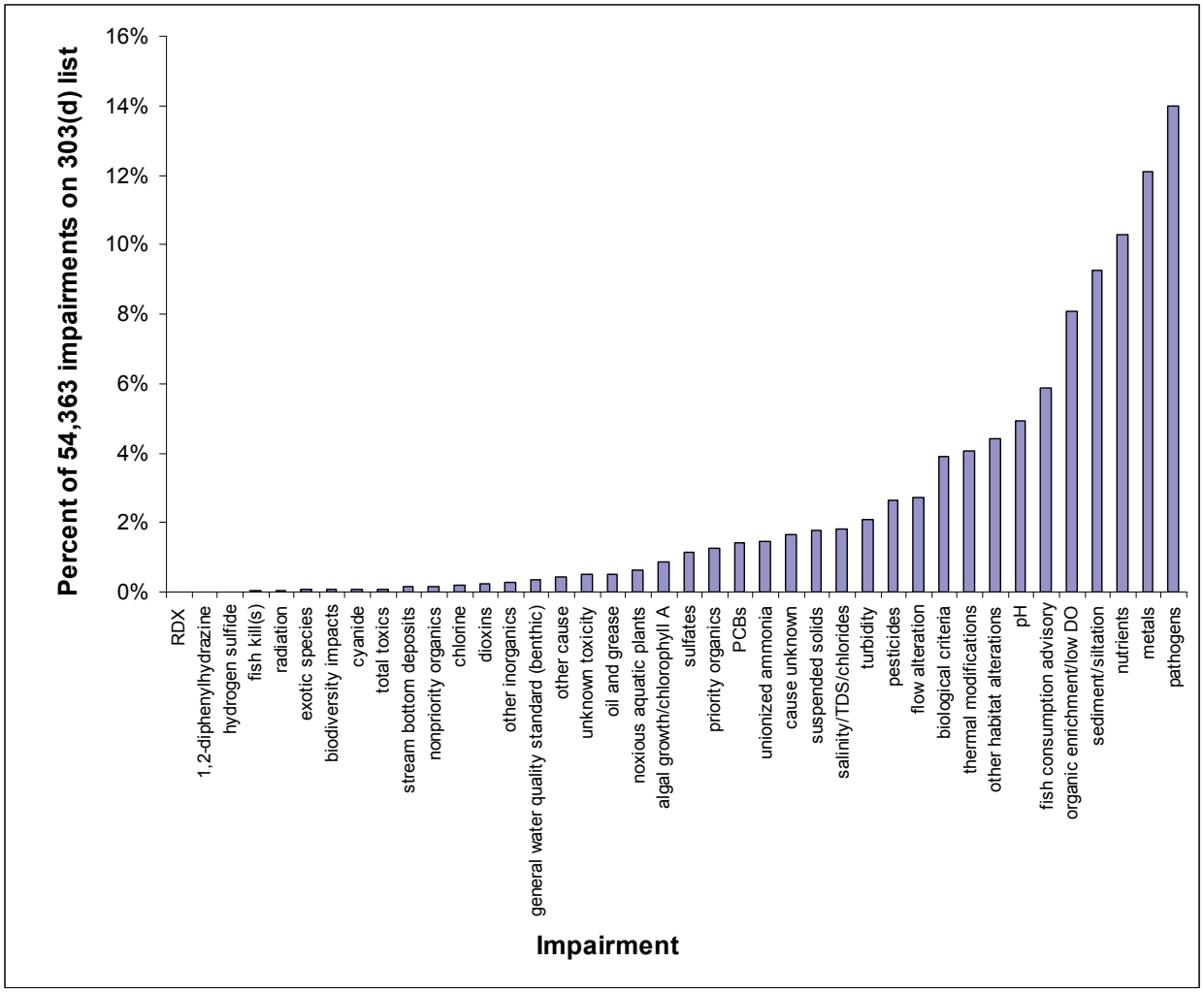



FIGURE 2-1

Frequency of Toxicants Among Biological Impairments Identified in the 2002 Water Quality Inventory

(Data derived from the National 303d list fact sheet available at http://oaspub.epa.gov/waters/national_rept.control).

## 3. LISTING CANDIDATE CAUSES: SOURCES OF METALS

Identification of sources releasing stressors helps the causal analyst to develop a list of candidate causes and supports credible causal pathways for those stressors involved. While lack of a source weakens the causal pathway for a given stressor, it does not eliminate it as a cause since there may be an unknown source. Ideally, adequate site-specific analytical chemistry will be available for both impaired and reference waters. When analytical data are inadequate, alternative data sources may be useful in identifying the potential for metals impairment or in justifying additional analyses. For instance, baseline metal levels and other water quality parameters may be available for the watershed itself or from the region from databases of the U.S. Geological Survey's National Water Quality Assessment Program or the Environmental Monitoring and Assessment Program (EMAP). Metals sources, the relative locations of urbanized areas, recreational areas and regulated facilities releasing toxicants within a watershed can be identified using the U.S. EPA's Enviromapper for water [http://map8.epa.gov/enviromapper/]. While this is no substitute for ground truthing actual source locations and confirming discharge routes, mapping is helpful in conceptualizing spatial associations between potential sources of stressors and an impaired water body. Indirect sources associated with the natural geology of the region, land disposal and stack emissions should be considered in addition to direct releases to surface water. Land disposal may release metals to surface waters during storm events, resulting in episodic exposures to associated stressors. Stack emissions can affect both reference and impaired waters if they are geographically close. Unless explicit air dispersion models or analytical chemistry data indicate otherwise, this circumstance weakens the case for those stressors strictly associated with stack emissions as causes for an impairment.

Table 3-1 identifies the metals released by different industry classes reporting to the 2002 toxics release inventory for those metals reviewed in this report. Certain releases are characteristic of particular industries. For example, the leather industry releases Cr, while other industries such as mining and metals finishing contribute many metals. This information was collected from the Toxicant Release Inventory Explorer [http://www.epa.gov/triexplorer], which is a public access database of industry-reported data that can be queried for identity and relative volume of atmospheric, land and surface water releases for a specific facility, zip code, county, state or one of 25 Standard Industry Class codes. Note that in some cases, location metadata might

identify the company headquarters rather than the actual location of an outfall or stack, so physically confirming point sources is recommended.

Data identifying the chemical releases by specific point sources may also be collected from monitoring and regulatory databases. Actual releases are highly variable due to differences in scale and manufacturing processes of individual companies and shifts in production capacity over time within a given facility. National Pollution Discharge Elimination System permits can help identify sources of metals, but without data on actual discharge content, water flow and hydrology, concentrations in the receiving water cannot be inferred. For these reasons, release data are typically useful only in identifying stressors of potential concern but not in characterizing actual exposure.

Metals from nonpoint sources are more difficult to characterize due to the dispersed and often temporally variable nature of releases. Rainwater and melt water runoff transports contaminants from roads, parking lots, residential areas, recreational areas, landfills, agricultural land, industrial parks and managed forests. Atmospheric emissions from smoke stacks and vehicles enter waterways through wet and dry deposition. In colder climates, snow is removed from roads and placed in snow disposal sites awaiting spring melt. These sites can act as seasonal point sources for toxicants associated with atmospheric deposition, road use and snow management (i.e., salts).

TABLE 3-1

Point Sources Reporting Releases of Metals to Air, Water and Land in the Toxics Release Inventory (TRI, 2002 data)

| Industry | Air | Surface Water | Surface Impoundments | Land Disposal |
|---|---|---|---|---|
| Metal Mining | As Cd Cr Cu Pb Hg Ni Se Zn | As Cd Cr Cu Pb Hg Ni Se Zn | As Cd Cr Cu Pb Hg Ni Se Zn | As Cd Cr Cu Pb Hg Ni Se Zn |
| Coal Mining | As Cr Cu Pb Hg Ni Zn | As Cr Cu Pb Hg Ni Zn | As Cr Cu Pb Hg Ni Se Zn | As Cr Cu Pb Hg Ni Se Zn |
| Food | As Cr Cu Pb Hg Ni Se Zn | Pb Ni Zn | Pb Zn | Cr Cu Pb Hg Ni Zn |
| Tobacco | Pb Hg | Pb Hg | | |
| Textiles and Apparel | Cr Cu Pb Hg Zn | Cr Cu Pb Zn | Cr Cu Pb Zn | Pb Zn |
| Lumber | As Cr Cu Pb Hg Ni Zn | As Cr Cu Pb Zn | Pb | As Cr Cu Pb Hg Zn |
| Furniture | Cr Pb Ni Zn | Cr Pb Ni | | Pb |
| Paper | As Cr Cu Pb Hg Ni Zn | As Cr Cu Pb Hg Ni Zn | Cu Pb Hg Zn | Cr Cu Pb Hg Ni Zn |
| Chemicals and Chemical Wholesalers | As Cd Cr Cu Pb Hg Ni Se Zn | As Cd Cr Cu Pb Hg Ni Se Zn | Cr Cu Pb Hg Ni Zn | As Cd Cr Cu Pb Hg Ni Se Zn |
| Petroleum and Petroleum Bulk Terminals | As Cd Cr Cu Pb Hg Ni Se Zn | As Cd Cr Cu Pb Hg Ni Se Zn | Cr Cu Pb Hg Ni Zn | As Cd Cr Cu Pb Hg Ni Se Zn |
| Plastics | Cd Cr Cu Pb Hg Ni Zn | Cd Cr Cu Pb Hg Zn | Pb Zn | Cr Cu Pb Hg Zn |
| Leather | Cr Pb | Cr | Cr | Cr |
| Stone/Clay/Glass | As Cd Cr Cu Pb Hg Ni Se Zn | As Cd Cr Cu Pb Hg Ni Se Zn | Cr Pb Hg Zn | As Cr Cu Pb Hg Ni Se Zn |
| Primary and Fabricated Metals | As Cd Cr Cu Pb Hg Ni Se Zn | As Cd Cr Cu Pb Hg Ni Se Zn | As Cd Cr Cu Pb Hg Ni Se Zn | As Cd Cr Cu Pb Hg Ni Se Zn |
| Machinery | Cr Cu Pb Ni Zn | Cr Cu Pb Ni Zn | | Cr Cu Pb Ni Zn |
| Electrical Equipment | As Cd Cr Cu Pb Hg Ni Se Zn | Cd Cr Cu Pb Hg Ni Zn | Pb | Cr Cu Pb Hg Ni Zn |
| Transportation Equipment | Cr Cu Pb Hg Ni Zn | Cr Cu Pb Hg Ni Zn | Cu | Cr Cu Pb Ni Zn |
| Printing and Measure/Photography | Cr Cu Pb Hg Ni Se Zn | Cr Cu Pb Hg Ni Se Zn | Cu | Cr Cu Pb Ni Zn |
| Electric Utilities | As Cd Cr Cu Pb Hg Ni Se Zn | As Cr Cu Pb Hg Ni Se Zn | As Cd Cr Cu Pb Hg Ni Se Zn | As Cr Cu Pb Hg Ni Se Zn |

## 4. DIAGNOSIS

Diagnostic evidence is based on the presence or absence of characteristic effects associated with exposure to a particular stressor. This is the second strongest form of causal inference in SI, but there are few useful and truly specific diagnostic indicators. For metals, diagnostic responses include blue stomachs in fish exposed to molybdenum (Meyer and Barclay, 1990) and changes in delta aminolevulinate dehydratase (ΔALAD) with lead exposure. However, internal signs of exposure such as blue stomachs and measures of biochemical responses are not normally included in field investigations for biological assessments. Gross pathologies, such as the presence of black tails in metals-exposed fish caused by metal-induced degeneration of caudal chromophores, are more likely to be reported. This response was observed with exposures to simulated metal effluent (Bengtsson and Larsson, 1986) and to lead concentrations of 120 µg/L over 30 days (Sippel et al., 1983).

## 5. STRENGTH OF EVIDENCE: SPECIFICITY

Specificity is a causal consideration within strength-of-evidence. It differs from diagnosis in that diagnosis is absolute (i.e., if the diagnostic signs all appear the cause is identified), while specificity indicates that a response is typical of one or a few stressors. The induction of the metal binding peptide metallothionein is an example of a specific rather than diagnostic response because, in fish, induction can also result from handling stress (Tort et al., 1996; Ghoshal et al., 1998), changes in temperature (Hermesz et al., 2001) and changes in reproductive status (Van Cleef-Toedt et al., 2000a). However, induction of metallothionein in fish by metals appears to be greater than induction by hormonal or environmental stimuli (Tort et al., 1996; Hermesz et al., 2001; Van Cleef-Toedt et al., 2000b). In invertebrates, exposure to fenhexamid induced metallothionein is a result of oxidative stress (Mosleh et al., 2005). Nevertheless, elevated metallothionein levels are more specific to metals than general responses such as death or diminished growth, and may discriminate metals from other candidate causes.

Again, specific gross pathologies are more likely to be identified in bioassessment efforts than are biochemical responses. The *Manual for Investigation of Fish Kills* (Meyer and Barclay, 1990) describes characteristics of more severe responses indicative of metals exposure. Kills resulting from metal exposure may be characterized by white film on the gills, skin and mouth and sloughing of gill epithelium. Additional information on the types of effects caused by metals can be found in the USGS contaminant hazard reviews [http://www.pwrc.usgs.gov/infobase/eisler/reviews.cfm].

## 6. STRENGTH OF EVIDENCE: PLAUSIBLE MECHANISM

This causal consideration asks, *Given what is known about the biology, physics, and chemistry of the candidate cause, the receiving environment, and the affected organisms, is there a plausible mechanism linking the effect with the candidate cause?* If a mechanism is known and there is evidence that the mechanism is operating in a specific case, the positive evidence is particularly strong. It is important to distinguish a lack of information concerning a mechanism (e.g., the ability of chemical x to induce tumors is unknown) from evidence that a mechanism is implausible (e.g., chemical x is not tumorigenic).

Metals toxicity is a plausible mechanism for most of the negative effects observed in conventional aquatic biological surveys. These include abundance and mass of taxa. Metals toxicity is not a plausible mechanism for most positive effects such as increased abundance and mass. However, it is also important to consider carefully whether some indirect mechanism may be responsible. For example, toxic metals would not directly cause an increase in a fish population but might indirectly cause it by eliminating a more sensitive competitor (U.S. EPA, 2000). Such indirect effects should not be assumed, unless supporting evidence is available such as the absence of a competitor species.

## 7. STRENGTH OF EVIDENCE: PLAUSIBLE EXPOSURE-RESPONSE

This causal consideration asks, *Would the effects observed be expected at the level of stressor seen in the environment?* The comparison of environmental concentrations to laboratory derived exposure-response relationships is a common approach used in chemical risk assessments. For example, if concentrations at the site are as high as levels known to cause relevant effects, then this provides strong evidence of causality. Conversely, if concentrations are below levels known to cause effects in controlled laboratory studies, this weakens the case. Quantitative exposure-response information is very important in strength-of-evidence analysis. This includes threshold data, endpoint (e.g., EC20) data and the shape and slope of the relationship between the stressor intensity and response intensity. It must be noted that in stressor identification, water quality criteria should not be used to determine causation because they indicate safe levels for exposure, not levels causing effects. Criteria are useful in initial screening to determine whether or not potentially unsafe levels are present. Data demonstrating a lack of a response at site exposure levels contribute to the strength-of-evidence analysis by identifying how consistently response may be (or may not be) associated with a given stressor intensity. In this manner, evidence for each candidate cause is weighed, balancing positive and negative support (U.S. EPA, 2000).

The remaining parts of Section 7 detail two types of exposure-response relationships, species sensitivity distributions (SSDs) for LC50 values and exposure-response relationships from single species chronic tests, and explains how they may be used in stressor identification. Response distributions are more useful to SI than single benchmark values because these plots and tables allow the investigator to evaluate whether harmful levels exist at the site, the magnitude of effects reasonably expected to occur at those levels and the certainty with which the evaluation may be reported based on the breadth of the confidence intervals. Those sections are followed by discussions of the treatment of water chemistry and of temporal issues.

### 7.1. SPECIES SENSITIVITY DISTRIBUTIONS

Impairments of aquatic systems are often defined as losses of some proportion of species or changes in the relative abundance of species. Since these effects are a result of differences in the relative sensitivity of the species to metals of other stressors, species sensitivity distributions (SSDs) are potentially helpful models. Species sensitivity distributions are exposure-response relationships that represent the distribution of species sensitivities relative to exposure (e.g., metal concentration).

SSDs are analogous to the distributions of sensitivities of individuals in conventional exposure-response relationships. Because the variance of sensitivities to chemicals among species is often more important to ecological risk assessments than variance among individuals, SSDs have become a common ecological effects model in the U.S., Europe and elsewhere (Posthuma et al., 2002). The SSDs in Appendices B-E of this document assist in determining whether site contamination exists at levels potentially affecting organisms within an impaired community.

Figure 7-1 illustrates how an SSD might be applied to strength-of-evidence analysis. The observed site concentrations are plotted on the SSD to indicate whether site concentrations occur at levels where species reductions are plausible. The assessor then considers whether the magnitude of effect observed at the site is consistent with that suggested by the SSD. For example, if species richness at the impaired sites was observed to be much lower than indicated by the SSD, the assessor would conclude that additional stressors are likely to be contributing to the impairments. On the other hand, if response intensities are generally consistent, the narrative in the stressor identification report might read:

> The LC50s for 20% of species occur at or below 0.0016 mg/L copper. Copper was detected at greater than 0.002 mg/L in baseflow samples from Autoshop station in 1 of 3 observations and at the dry goods store (DG Store) in 2 of 3 storm water samples. Given this information, it is plausible that episodic copper exposure during storm water events at the dry goods store site and sustained copper exposure in baseflow at the auto shop site are contributing to reduced species richness.

**7.1.1. Interpreting Species Sensitivity Distributions.** The SSD generation effort used laboratory LC50 data because these were the most abundant consistent data available and, therefore, provided the greatest number of SSDs. Other effects data for species represented in these plots are included to place the LC50 data in context with other available information on the toxicity of that stressor for that species. However, laboratory LC50 data must be interpreted relative to actual ecological consequences. Since an LC50 is a concentration that kills half of the organisms in a test population, one would expect a fish kill or a temporary reduction in abundance of some species when water concentrations, accounting for bioavailability, equal the LC50 for that species. The SSD permits a prediction of the proportion of species in the community that would be affected in that way. That is, at a concentration equal to 25% fraction affected, one would expect a kill or reduction in abundance of approximately 25% of species at the site. Further, since observable effects could occur at less than

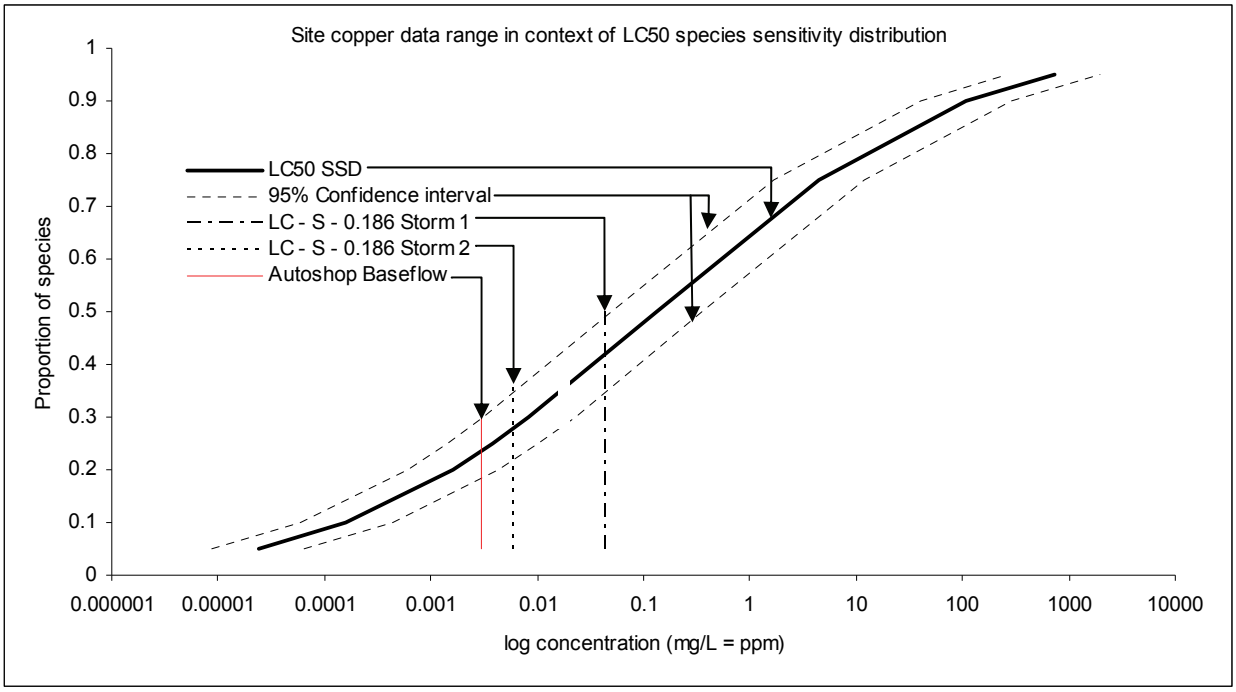



FIGURE 7-1

Example SSD Showing Site Data Plotted Against the SSD to Indicate Plausible Intensity of Response of up to Approximately 30% Species Affected at Autoshop Site and 35% to 50% Affected at the Dry Goods Store Site

50% mortality, we need to consider lower levels. A broad expectation is that if an exposure episode exceeds 0.3 times the LC50 for a given chemical, a kill may be observed or a temporary reduction in abundance may occur. This is based on an analysis that found the LC01/LC50 ratio was below 0.3 in only 9% of tests (U.S. EPA, 1985). Repeated or sustained excursions above the LC50 however, would likely result in local extirpation of species. These interpretations take the models and data at face value.

In comparing SSDs for different species groups, arthropod species are generally more sensitive to metals than non-arthropod invertebrates (see Appendix F), and invertebrates are more sensitive than fish. Among non-arthropod species, bivalves are particularly tolerant of metals. The difference in fish and invertebrate sensitivity may be due to the differing life spans of the two groups. A short (acute) exposure for a relatively long-lived species such as a brook trout is a long exposure (chronic) for invertebrates like caddisflies. Invertebrates are also more likely to adjust or equilibriate to test contaminants over the course of an acute test.

It is important to note that the distributions within species groups do not reflect the tolerant and intolerant species classifications used in bioassessment. Tolerance classifications are based on general sensitivity to polluted environments, particularly to organic loading, not specifically to metals. Note in Figure 7-2 that the intolerant species *Onchorhyncus clarki* has roughly the same LC50 as the tolerant cyprinid *Pytochelius oregoniensis*.

Important factors to consider are the life stages present when exposure was initiated, as well as, the potential for adaptation in tests where multiple generations are exposed. Because acute toxicity tests for fish use post-larval organisms (juveniles or adults), the values available rarely include toxicity to earlier life stages, which often differ in sensitivity. Because of this difference, early life stages of vertebrates are identified separately on the plots. By identifying data for early life stage organisms, the SSDs allow an investigator to consider whether concentrations may be preventing fish recruitment by eliminating a sensitive life stage. In some cases, early life stages are less sensitive than or as sensitive as adults, which might reflect the additional nutritional resources in the yolk of the egg or sac fry. Such effects might also reflect reduced exposure due to the relatively low permeability of the chorion of many fish eggs. Early life stage sensitivities are relevant only if exposure occurs in seasons where they are present.

When comparing species on a given SSD, the potential influence of differing water chemistry or other factors among toxicity tests must be considered (see Section

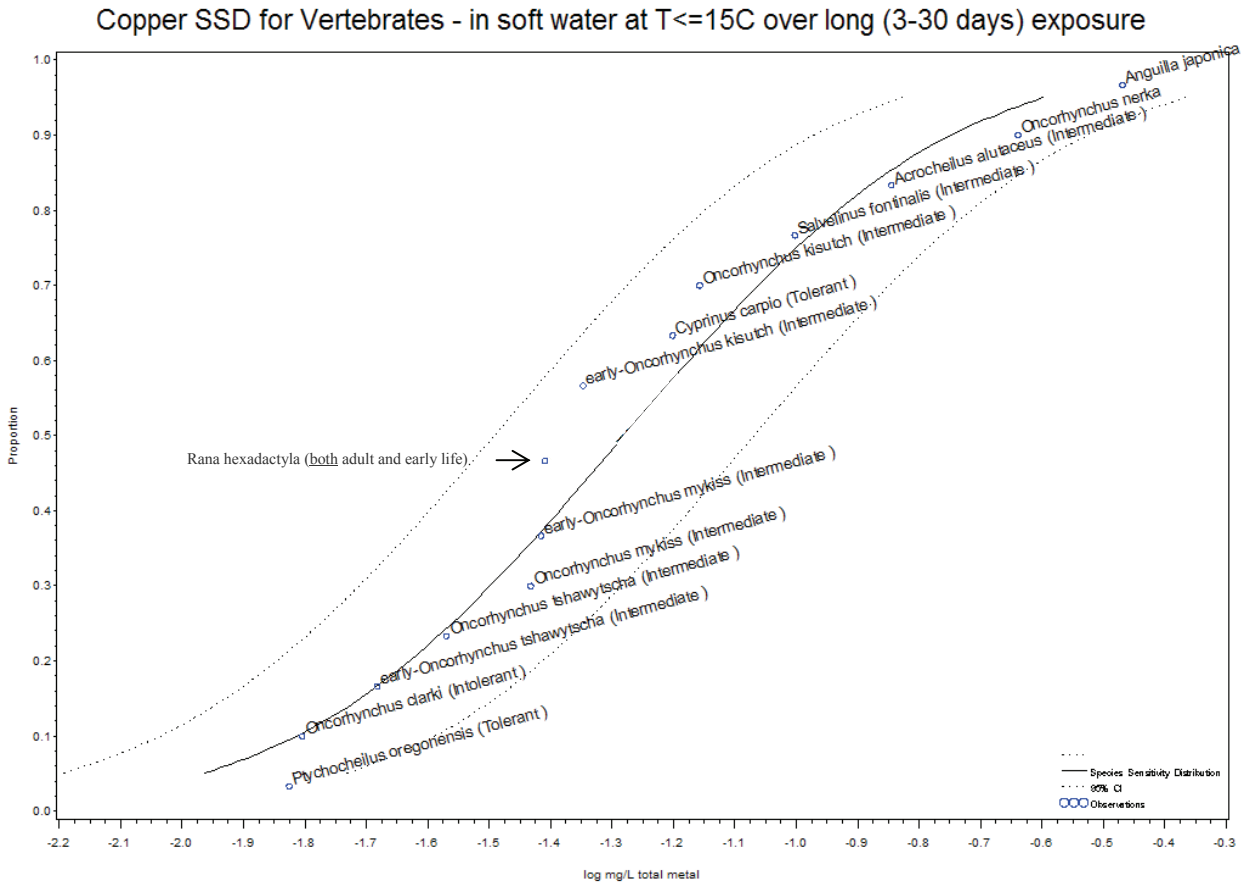



FIGURE 7-2

Example SSD Illustration Showing that Tolerance Classifications (Barbour et al., 1999) Used in Biological Assessment are Not Representative of Tolerance to Metals

7.3). For example, the presence of organic matter, which may complex metal ions, could differ in static, renewal and flow through test designs. However, differences in water chemistry among laboratory water exposures are not expected to be as great as differences found among natural waters.

**7.1.2. Uncertainty in Species Sensitivity Distributions.** The models are uncertain due to uncertainties inherent in the input data and to the sampling error associated with the species that go into the model. The causal analyst must use his or her knowledge of the site at hand to evaluate the utility of these models. Condensed ECOTOX data are presented in Appendix A to allow users to trace plotted values to the citation(s) of origin. If, after examining the actual data, revised SSDs are necessary, the assessor must select and apply controls on the input data that are appropriate to the site at hand. This material is presented to inform users and give them the best available exposure-response analysis for use in SI. That means complete disclosure of the data, as it exists.

Ideally, equivalent endpoints from standard test conditions for a collection of species representative of an ecological assemblage should be used to generate SSDs. ECOTOX data are somewhat diverse, so some sorting and discrimination were necessary.

While the free ion or dissolved metal concentration would be a more appropriate indicator of actual toxic exposure, the analysis presented here focuses on total metals because greater than 90% of the freshwater metals data in ECOTOX and much of the state and tribe metals data are reported as total metals. Since dissolved metals are less than total, this also provides a conservative estimate of exposure. Note that the relative bioavailability of metals in unfiltered lab and natural waters will differ because laboratory water contains little suspended matter.

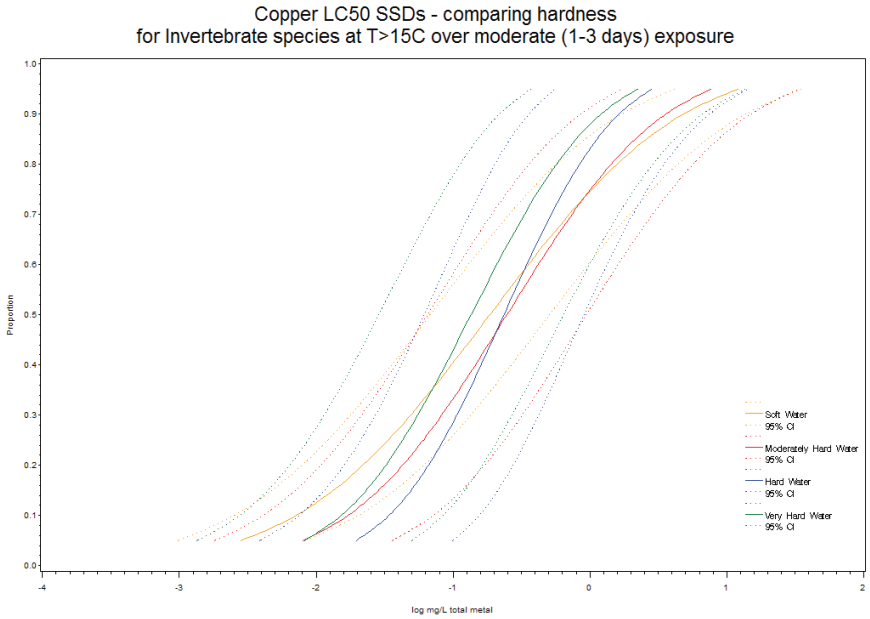
Among the records reporting total metals, about 30% do not report pH, 20% do not report temperature, and 40-50% do not report data for water hardness or alkalinity. Removal of data lines lacking these parameters leaves roughly 10,000 records for the metals of interest in freshwater, about half of which are LC50 values.

The endpoints and effects data other than LC50s under these discrimination parameters include diverse endpoints (EC10, EC50, LT50 etc.) and effect types that cannot be aggregated into large enough sets for SSD generation. Due to these limitations, SSDs were generated using the LC50s for aquatic organisms to total metals at pH 6-8.

The comparability of the plots for each metal was also examined (Appendices G-I). Factors contributing to lack of comparability may be associated with occult differences in laboratory practices and with the influence of water quality parameters other than those used in grouping the data (hardness, temperature and pH) on metal bioavailability. Since water hardness affects metal toxicity (Section 7.3), SSDs generated using data collected under differing water hardness would be expected to illustrate this effect. However, hardness is not the only water quality parameter that may vary among tests and influence toxicity. Figure 7-3a compares the SSDs generated for invertebrate species exposed to copper over a one- to three-day period under differing levels of hardness (other hardness comparisons are provided in Appendix G). The confidence intervals of the SSD relationships overlap considerably, masking any discrimination of a hardness effect on relative toxicity using these models. Further, those curves are not parallel, as would be expected if hardness were the principal factor influencing toxicity. Figure 7-3b, contains SSDs for vertebrates exposed to zinc, and shows the expected parallel curves and clearer discrimination of hardness effects on toxicity. The U.S. EPA Office of Water takes hardness to be a surrogate for Ca and Mg ion content while carbonate is a surrogate for alkalinity and pH. While these parameters potentially influence species sensitivities, relationships which should exist in theory are not easily illustrated with empirical data because toxicity is also influenced by the unmeasured or unreported water quality parameters in the toxicity tests. Such occult factors can include binding of metals to organic material from feces or food or to the exposure apparatus itself (Section 7.3). As with hardness, the expected relationships between temperature and toxicity (Figure 7-4a and b, Appendix H) and between duration and toxicity (Figure 7-5a and b) are apparent in some sets of SSDs but not others, (see also Appendix I).

Lack of comparability among metal SSDs could also be due to differences in the species represented in each SSD, particularly when a small number of species is represented. Plots, species represented and original data should be carefully examined when considering the SSDs. For an extreme example, consider Figure 7-5 for Ni. There is no overlap in species between the SSDs. The disparity between the two SSDs in this case is very great and is clearly driven by the different species groups in each of the models. Data for the more sensitive group are from a single study limited to effects of nickel on protozoans (Madoni, 2000). Nickel sulfate is commonly used to immobilize protozoa for microscopy, because they are particularly susceptible to this metal.

A




B

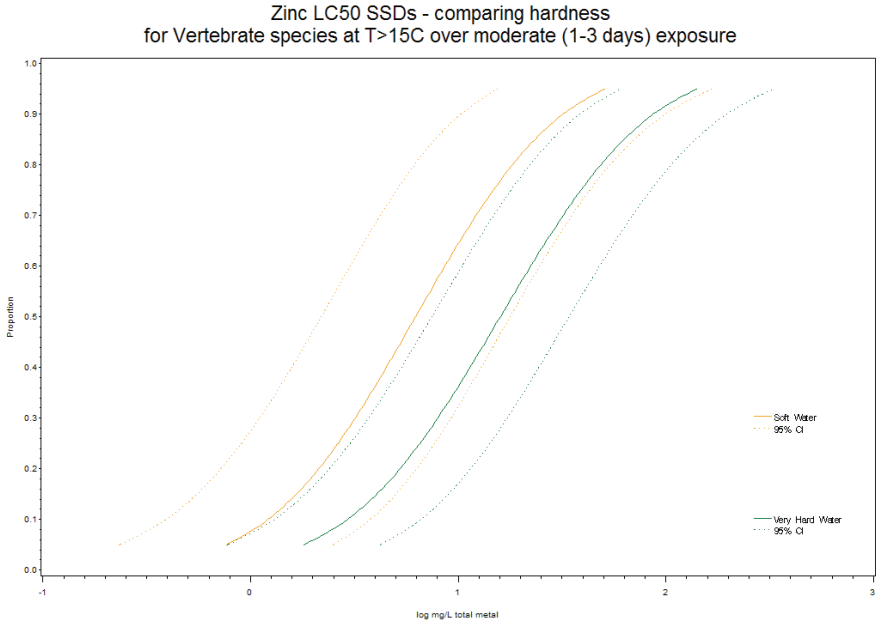
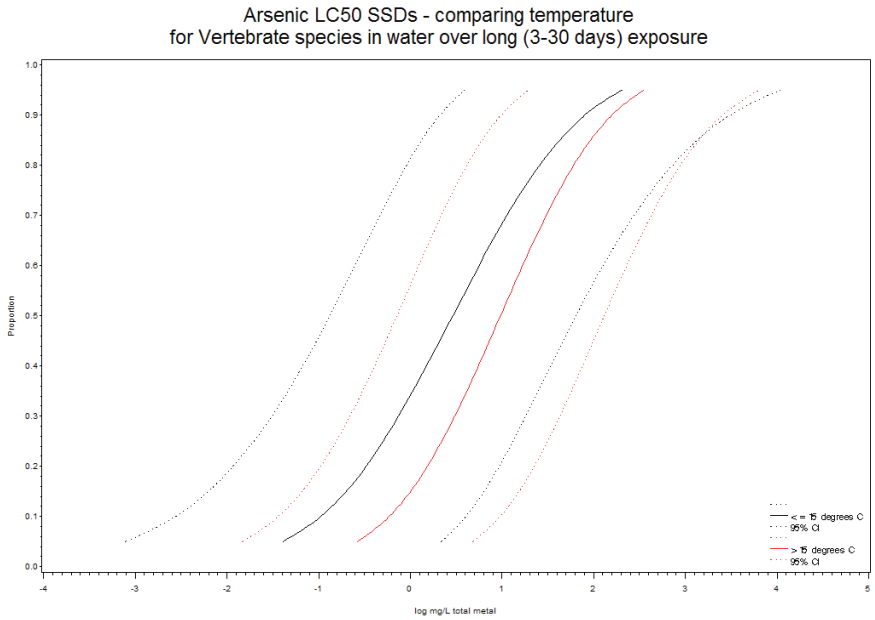



FIGURE 7-3

Species Sensitivity Distributions Illustrating How Data Variability Can Mask the Effect of Hardness on Toxicity (A) in some cases while in other cases (B) the expected relationship where the metal is more toxic in soft water than very hard water and slopes are parallel is apparent.

A




B

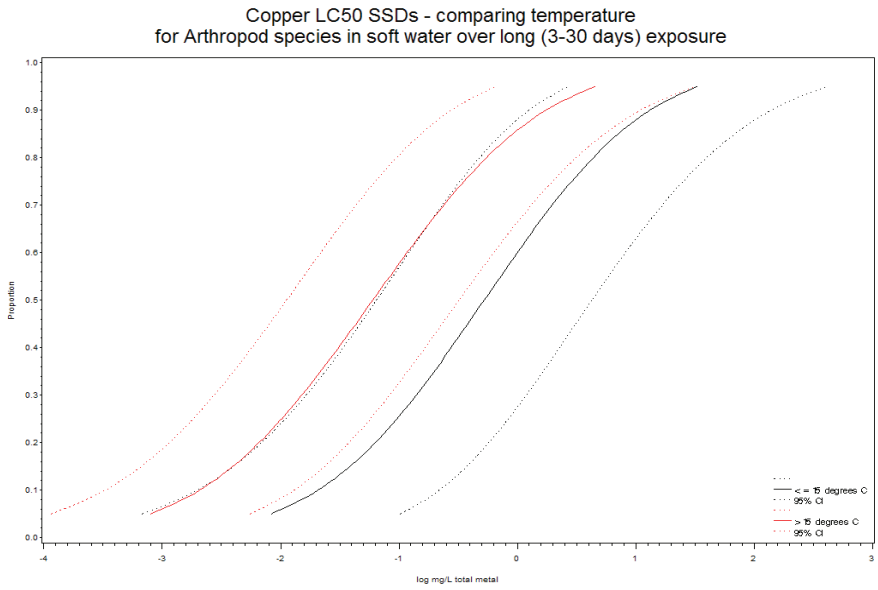
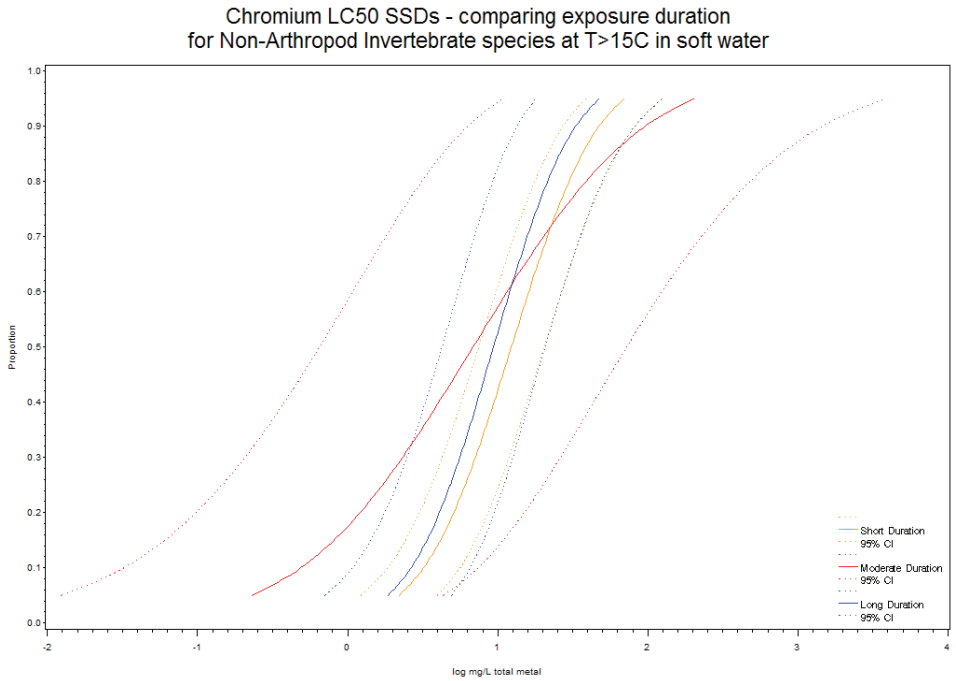



FIGURE 7-4

Species Sensitivity Distributions Illustrating How Data Variability Can Mask the Effect of Temperature on Toxicity (A) in some cases but in other cases (B) the expected relationship where the metal is more toxic in warm water than cold water and slopes are parallel is apparent.

A




B

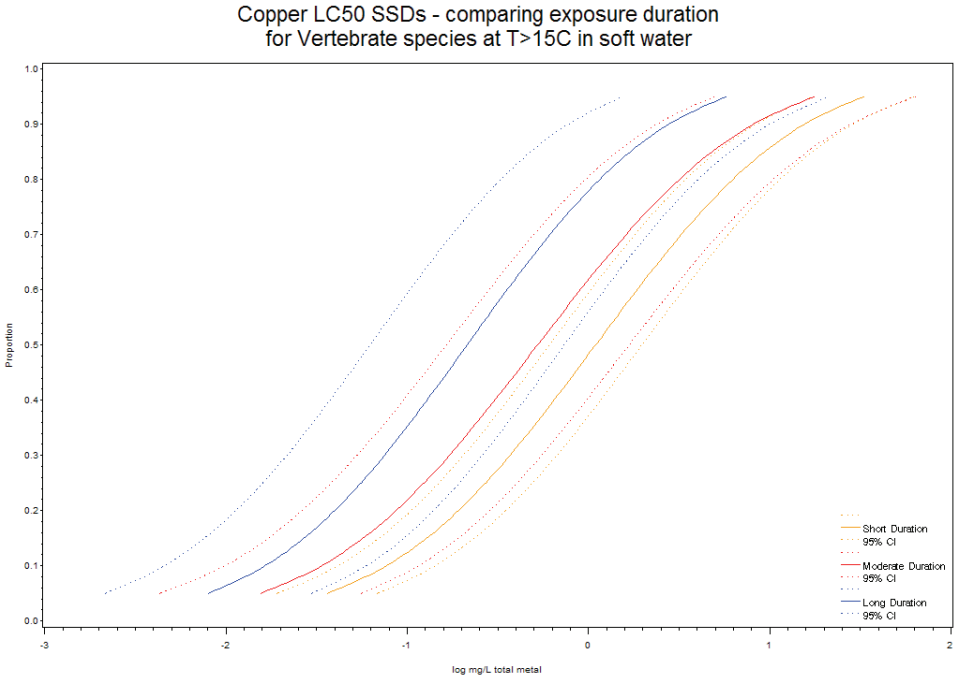



FIGURE 7-5

Species Sensitivity Distributions Illustrating How Data Variability Can Mask the Effect of Duration on Toxicity (A) in some cases but in other cases (B) the expected relationship where the metal is more toxic in under longer exposures and slopes are parallel is apparent.

Uncertainty in the SSDs generated for this report stems from the limited availability of data collected under comparable exposure conditions. ECOTOX data do not include the necessary parameters, such as major ion concentrations, to perform the Biotic Ligand Modeling (BLM) calculations that would allow for more precise estimates (Section 7.3.3). Therefore, these data would have to be estimated for the model.

Theoretically, hardness correction should not affect relative species sensitivities because the use of tested species to represent communities relies on the assumption that regardless of which species are represented, they are an unbiased sample of the community. There is no reason to expect that the available toxicity data is biased because species sensitivities are not known prior to testing. Nevertheless, the importance of species representation and occult differences in test conditions is reflected by the apparent masking of expected relationships among SSDs.

Rather than take all species data available for a given chemical to generate the plots, the more controlled approach identifies a standard suite of species for which standard water conditions data must be available. In theory, the resulting SSDs should be parallel curves showing the influence of modifying environmental conditions as trends of not distinct, non-overlapping response relationships. Species would have to be carefully selected to have reasonable confidence that the models are representative of field community responses. Further, a standard suite of animals appropriate for one type of ecosystem would probably be inappropriate for evaluating a different ecosystem; the most obvious example is cold vs. warm water streams. However, there are currently not sufficient data to optimally characterize realistic exposure conditions that account for durations, temperature, species assemblages. Furthermore, the applicability of such SSDs to SI are still limited because these existing data are not representative of water chemistry conditions (e.g., Ca:Mg ratios) of natural waters.

The differing exposure designs listed in ECOTOX (i.e., static, flow-through, and renewal) were pooled. This was judged to be a relatively insignificant source of uncertainty, because a consistent trend was not observed when comparing LC50s for the same chemical form, species and duration but under differing exposure design (static, flow-through or renewal, Table 7-1). In addition, the “Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods”, found that CVs for LC50s from different laboratories range among species from 20% to 38.5%. This is the best possible precision when using a single, well-defined protocol and well-trained staff (Teresa Norberg-King, personal communication). When applying this data quality expectation for within species tests in the SSD data set, 44% of the CVs among exposure regimes were less than 38.5%. This analysis suggested that,

given equal representation of static, flow-through and renewal test data, only 56% of the data contribute variability in excess of that expected for tests conducted under the same well-defined protocol with well-trained staff. For these reasons, we do not expect that the combining of static, renewal and flow-through exposure designs will be a major contributor to variance in the SSDs beyond the inevitable variance when modeling test data from multiple laboratories.

TABLE 7-1

Difference Between Flow-through, Static and Renewal Tests Given the Same Species, Metals Salt, pH, Hardness Category, Temperature and Exposure Duration
*28% of observations were equal

| LC50 Comparison | % obs < 0 | % obs > 0 |
|---|---|---|
| Flow-through - Static | 46% | 54% |
| Renewal - Static* | 36% | 36% |
| Flow-through - Renewal | 50% | 50% |

The SSDs also pool toxicity data for different inorganic forms of the metals because field data do not report actual metal species. This includes both different species (valence states) and different salts (anions) of a metal. It is generally assumed, as in derivation of water quality criteria, that the specific anion forming the salt of a metal is inconsequential. Examination of the LC50 exposure duration plots for individual species in Appendix A illustrates that in many cases, LC50s for the differing metal salts overlap. However, speciation is potentially important for arsenic, chromium, and selenium. In particular, caution is required when considering the confidence intervals for data on arsenic. Note that where the data are available, the SSDs include both arsenite and arsenate toxicity values for a species of organism. Arsenite, which is favored under low oxygen conditions, is the more toxic form. If the species of arsenic, selenium or chromium is known, an appropriate species-specific SSD should be developed.

**7.1.3. Other Models for Species Sensitivity.** While SSDs are the most common approach for modeling species sensitivities in ecological assessments, other models including linear regression models have been used. In particular, software has been

developed to help quantify the relationship between acute toxicity to a tested species and to a particular untested species. This software, Interspecies Correlation Estimations (ICE) for Acute Toxicity to Aquatic Organisms and Wildlife, would be useful in cases in which the impairment is defined in terms of effects on a particular species and acute toxicity data for chemicals of concern are available for reasonable surrogate species (Asfaw et al., 2004).

## 7.2. EXPOSURE-RESPONSE RELATIONSHIPS FROM CHRONIC TESTS

The chronic exposure-response relationships provided in this report differ from conventional chronic test endpoints (i.e., NOECs, LOECs and MATCs) in that they provide information on the level of specific responses (i.e., survival, growth and fecundity of defined life stages) as functions of concentration. Contrasted with the SSDs which model lethality in relatively short exposures using LC50s, these models allow examination of non-lethal response which may influence ecological communities and, for the survival data, the exposure-lethality relationships for multiple life stages within a species. Interpretation of toxicity endpoints from chronic tests is more complex. For example, exceeding the EC50 for larval growth would have different ecological implications than exceeding the EC50 for reduced fecundity. The species, life stage at exposure initiation, seasonal factors, water quality characteristics, components of an exposure mixture, and duration of exposure all influence an organism's susceptibility to toxic effects.

For each modeled data set, both the confidence intervals for the concentration and response estimates are plotted in red and black, respectively (Figure 7-6). The slope of an exposure-response relationship and the breadth of the confidence intervals around the relationship affect how an assessor will interpret site data. That is, a steep slope implies that the transition from no effect to local extirpation would occur in a narrow concentration range (Figure 7-7). Variability in results associated with the measurement of site stressors and the confidence intervals calculated for the exposure scale of the relationship must be considered. One may be highly confident in the percent response estimates (Figure 7-7a) or less confident (Figures 7-7b, c). The differences between laboratory and natural waters are also important considerations. If the concentration-response slope is steep, and the confidence interval is wide, the variance among individuals becomes less important than the model uncertainty. Other uncertainties in the analysis, particularly uncertainty in the actual exposure intensity, overwhelm partial responses. For all practical purposes, a 50% effect would probably be detected in the field while something less than 20% effect may actually be invisible in

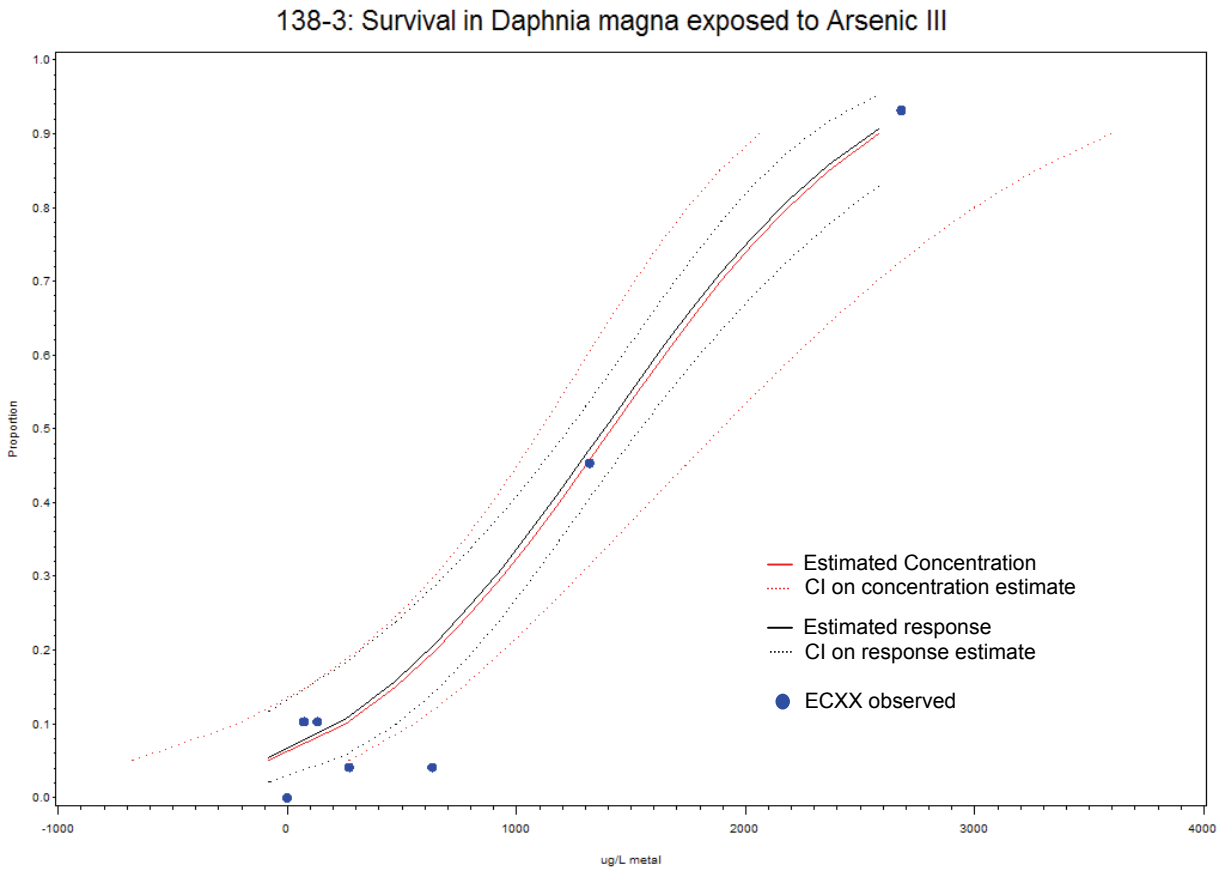
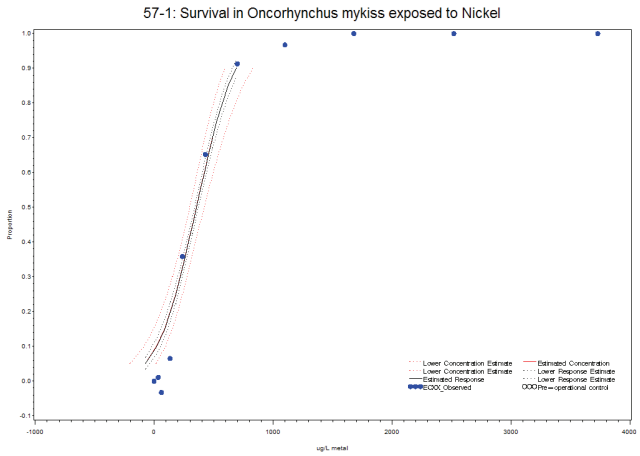


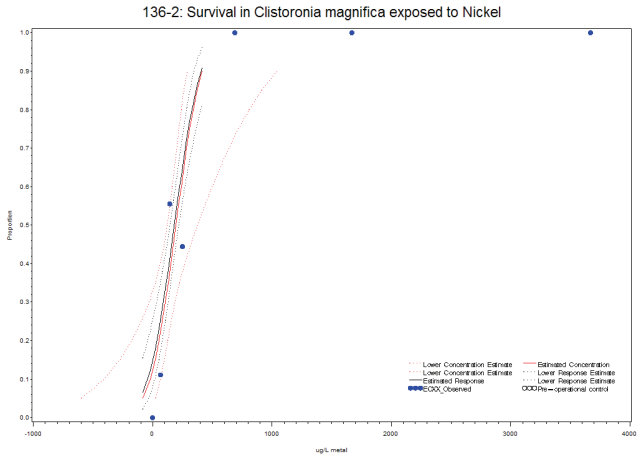

FIGURE 7-6

Exposure Response Plot Illustrating Confidence Intervals for Exposure (red lines) and Proportion Responding (black lines) Along with the Toxicity Test Observations (blue dots) Used to Model the Relationships

**A**




**B**




**C**

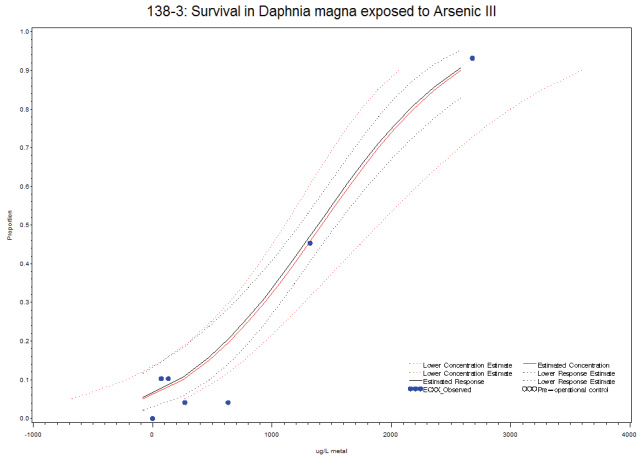



FIGURE 7-7

Exposure Response Plots Contrasting Steep (A, B) and Shallow (C) Slope Exposure-Response Relationships. With a steep slope, the transition from no effect to local extirpation occurs in a narrow concentration range which may (b) or may not (a) be obscured by the exposure confidence interval breadth.

the field. The difference between the EC50 and the EC20 could be negligible compared to other uncertainties in the analysis.

These relationships should be considered in context with the uncertainty associated with the quality and completeness of the data. Exposure-response modeling resulted in few acceptable models (Appendix F). For the remaining data, point to point interpolation between mean responses were used to derive EC05, EC10, EC20 and EC50 endpoint values (Appendix G). The coefficients of variation (CVs) averaged 11% for the non-generational responses (within a single life span) and at 45% for generational responses (tests of reproduction and viability); hence, many of the surrogate replicates for controls were actually equal to or greater than the endpoint values of interest. Further, some reported replicate control responses bracketed the region of interest. Under these circumstances, point-to-point interpolation from the mean responses is the best available approach to arrive at an estimate. Like all estimates, these results should be considered in the context of the variance or expected variance of the data from which they were derived. While point-to-point interpolation does not allow for generation of confidence intervals, the inferred CVs of 11% for non-generational response and 45% for generational responses could be applied to these estimates.

## 7.3. FACTORS MODIFYING METAL BIOAVAILABILITY AND TOXICITY

Analyses of metals as candidate causes of aquatic ecological impairments must take into consideration the influence of water chemistry on bioavailability and toxicity. Surface water chemistry is influential primarily through three mechanisms:

- Organic matter (organic carbon, humic and fulvic acids) and anions (chloride, sulfate) can bind metals to form biologically unavailable complexes (scavenging ligands).
- Nutrient cations (Ca, Mg, Na and K) can compete with pollutant metals for the physiologically active binding sites on organisms (biological ligands),
- Temperature and pH influence the reaction rates and binding capacity of these scavenging and biological ligands.

In regulatory practice, there are potentially four ways to adjust for differences in chemistry between laboratory water and field water, which are discussed in the following subsections:

- empirical adjustments for site-specific bioavailability and toxicity,
- adjustment for hardness,

- Biotic Ligand Model (BLM) and
- use of data with similar water quality.

**7.3.1. Water Effect Ratios.** U.S. EPA (1994) developed the empirical water effect ratio (WER) procedure, which makes site-specific bioavailability adjustments to criteria. This approach relies on comparing results of toxicity tests of a few species in site water with results from standard laboratory water, to derive a WER. The WER is then used to adjust the national criterion to reflect site-specific bioavailability. If a WER has been derived for the impaired water body, it could be applied to the benchmarks of effect derived from the exposure-response models in this report. However, it would not be reasonable to derive a WER for a causal analysis. If toxicity tests are performed in conjunction with a causal analysis, they should be focused on directly identifying the cause rather than adjusting benchmark values.

**7.3.2. Hardness Adjustment.** Adjustment for hardness (i.e., Ca and Mg) using the slope correction factors available in the water quality criteria documents has been an important technique in the development of realistic water quality criteria. The current ambient water quality criteria provide hardness dependant conversion factors for freshwater concentrations of Cd, Cr III, Cu, Pb, Ni, Ag and Zn. However, for reasons discussed in this section, hardness adjustment has not been employed in the development of the models in this report. For the same reasons, the U.S. EPA has been exploring the use of the BLM and other approaches for revising water quality criteria for metals.

The slope (i.e., the relationship between hardness and toxicity) can differ substantially among species and life stages. The composite of toxicity-hardness slopes used to derive the hardness correction factors may mask variation attributable to species sensitivity and result in the loss of information. For example, the Cd acute toxicity-hardness slopes for 12 species ranged from 0.1086 to 2.031 (U.S. EPA, 2001). After data reduction, which included removal of data for fathead minnow fry, the slopes were found to be equivalent and a composite slope value of 1.0116 was calculated for the criterion. In SI, the variability among species and life stages informs the analysis. Hence, species-specific correction factors, rather than the generic factors, are preferred if hardness corrections are to be made when performing site-specific assessments.

Another problem with hardness corrections results from the fact that laboratory test water is created using appropriately equivalent concentrations of Ca and Mg, whereas the Ca:Mg ratio of natural waters can range up to 5:1 on a molar basis. Data

for Cu indicate that the Ca:Mg ratio influences species specific toxicity, with Ca being more protective for some species than others (Naddy et al., 2002). Actual Ca and Mg ion content is therefore an important factor in the influence of hardness on toxicity in natural waters. Further, in natural waters, other ions (Na, $SO_4$, $PO_4$) often co-vary with Ca and Mg and influence bioavailability.

Finally, as discussed in the following section, many water quality characteristics other than hardness are important determinants in metal bioavailability.

**7.3.3. The Biotic Ligand Model.** The effects of water quality parameters and temporal variation in bioavailability and toxicity may be predicted using the BLM (DiToro et al., 2001). This model predicts acute lethality of Ag, Cd, Co, Cu, Ni, and possibly soon Pb in fresh water to aquatic animals on the basis of physical and chemical factors affecting speciation, complexation and competition of metals for interaction at the biotic ligands (i.e., the binding sites on the gills in the case of fish). Software for the model is available for download from [http://www.epa.gov/waterscience/criteria/copper/] (Click on Biotic Ligand Model (BLM), a 3.5MB zip file). At the time of this writing, regulatory use of the BLM is under review.

The BLM incorporates metal speciation through the Windermere Humic Aqueous Model (WHAM) of metal-DOM complexation and CHemical Equilibria in Soils and Solutions (CHESS). Required parameter inputs to the model include temperature, pH, dissolved organic carbon, major cations (Ca, Mg, Na and K), major anions ($SO_4$ and Cl), alkalinity and sulfide. This demand for water chemistry data for both ambient water and test water is the primary impediment to use of the BLM.

While site-specific data for each potentially modifying factor are seldom available, an understanding of how these conditions affect toxicity is helpful in determining whether metals contribute to an impairment. This is particularly important for cases in which metals concentrations are reported as total metals, which includes free ion as well as biologically unavailable metal complexes. Even data for dissolved metal content (that which passes through a 0.40 or 0.45 µm filter) will still include small particulate metal, hydrated metals and small metal complexes in addition to free ionic metal. The following points briefly describe some of the important parameters of the BLM.

- The alkalinity of water reflects its buffering capacity. Buffering capacity is the ability to resist changes in pH. It is determined by net carbonate ($CO_3$), bicarbonate ($HCO_3$) and carbonic acid ($H_2CO_3$) ions. Collectively the $CO_3$, $HCO_3$ and $H_2CO_3$ are dissolved inorganic carbon. Bicarbonate is the dominant species

between pH 6.35 and 10.33. Many metals form carbonate complexes, which have low biological availability.

- Other base cations that may contribute to alkalinity include hydroxide, borates, silicates, phosphates, ammonium, sulfides, and organic ligands. In freshwaters, the anion $SO_4$ may be the dominant anion and may be an important determinant of charge balance and ionic strength.
- Wastewater treatment plant effluents can have elevated sulfide concentrations. Sulfide has a strong affinity for many metals and is therefore an important consideration in determining metal speciation and bioavailability.
- Organic matter, in particular humic and fulvic acids, from decomposing organic materials, also plays an important role in determining metal speciation and bioavailability.
- The chemical speciation of many metals is directly affected by pH because it influences speciation and solubility. Water pH also influences the formation of metal complexes, because it affects the complexation capacity of dissolved organic carbon, and because it is an important determinant in the speciation of inorganic carbon.
- The nutrient cations Ca, Mg, Na and K can compete with a metal at biotic ligand sites, and therefore, these cations affect metal toxicity.

In addition to water chemistry parameters, the BLM incorporates the density of physiologically active biologic ligands of established laboratory species to address species-specific sensitivities. Since the affinities and densities of biological ligands differ among species, cations competing for ligands can influence relative species sensitivities, whereas the formation of biologically unavailable complexes with anions and organic matter will not. Ligand density is seldom known for site species and, when it is known, it is nearly always been estimated empirically from relative sensitivities to estimated free ion concentrations.

If adequate water quality parameter data for the site of interest and ligand density estimates for the site species are available, application of the BLM might be appropriate. Regional baseline conditions can sometimes substitute for site-specific data. For example, the state of Maine Department of Environmental Protection lists a default value for hardness at 20 mg/L $CaCO_3$. Expected regional water hardness data were mapped in 1975 (Briggs and Ficke, 1977). Such baseline values are not expected to change over time or seasons (Figure 7-8). However, parameters like organic carbon content and temperature are site specific and should not be inferred from regional data.

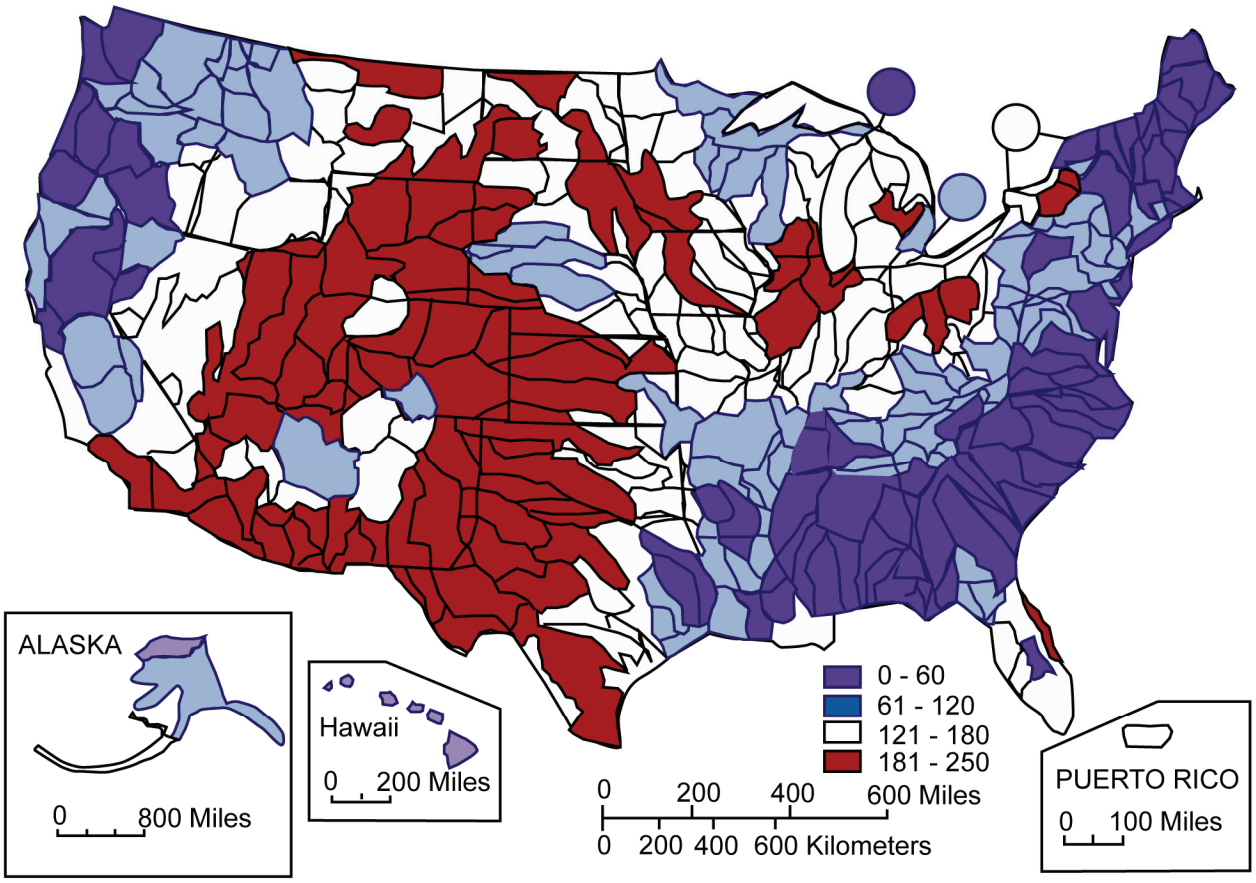



FIGURE 7-8

Mean Hardness for Regions of the United States (Briggs and Ficke, 1977)

The BLM may be particularly useful to account for anticipated periods of altered bioavailability at a site that may not be captured by purely empirical methods. Changes in bioavailability may result from litter fall in autumn, shifts in chloride content of runoff during snowmelt and differences in rainwater runoff characteristics relative to storm frequency and intensity. However, that use requires water chemistry data for the exposure episodes in addition to the baseline water chemistry.

**7.3.4. Matching Water Quality.** The simplest approach to adjusting for water chemistry is to use toxicity data from tests using water that was sufficiently similar to the site water. We adopted that approach in this report, classifying data according to the parameters that were available and avoiding complex modeling by the user of this document. Toxicity data from ECOTOX may include water quality data for hardness, alkalinity, salinity, temperature, pH and organic carbon. Among these parameters, data for hardness, temperature and pH were most commonly available and were used to group data for development of the SSDs provided in this report. Hence, we generated easy to use plots and tables of data classified by hardness, temperature and exposure duration. To make our treatment of the data as transparent as possible we include Appendix A, a compressed table of the ECOTOX data used in the SSDs as well as plots of LC50 x exposure duration for individual species, and Appendix L contains the original data used in the chronic exposure-response plots (see Appendices J and K).

It is recommended that the SSD generated with data most closely resembling site water conditions and exposure regimes be selected for evaluation of the impaired site. The water chemistry for the chronic exposure-response models should be examined to determine whether it is sufficiently similar to the site water.

## 7.4. TEMPORAL CONSIDERATIONS

The effects of metals are determined by the duration of exposure as well as the concentration. The conventional division of toxicity data into acute and chronic does not adequately describe the applicability of test data to exposure events in the field. For example, mortality of larval fish, a chronic effect, may occur more quickly than the 96-hour acute lethality to a juvenile or adult fish. Further, an effect in a small invertebrate is likely to occur more quickly than in a larger invertebrate or a fish simply because of kinetic limitations.

Seasonal events such as snow melt or temporary stormwater inputs are important determinants of the duration of elevated metals exposures. Depending on the intensity of exposures and species involved, responses of organisms to episodic

exposures can range from acute lethality to transient toxic effects or temporary avoidance of a polluted area. Prolonged exposures may extirpate certain species, produce chronic sublethal effects and population decline of other species and eventually lead to dominance of more tolerant species. The possibility for shifts in water quality during episodic releases should also be considered. If the input from a storm event is likely to include metals binding substances, then the bioavailability of the introduced metals is likely to be low and effects would be diminished.

## 7.5. MIXTURES OF METALS

Another limitation of conventional laboratory toxicity data is that they do not address exposure to mixtures of metals, which is usually the case under field conditions. The default approach assumes that metal toxicity is generally concentration additive, and the toxic unit approach is used to evaluate complex exposures (Sprague, 1970). Toxic units are toxicity-normalized concentrations, obtained by dividing the concentration of each chemical in a mixture by a common toxicologic benchmark, such as a *Daphnia* EC50. If the sum of toxic units for a mixture approaches, or is greater than one, that suggests that the mixture could cause toxic effects. However, less than additive, strictly additive and more than additive responses to metal mixture effects were recently reported to occur at 43, 27 and 29% of the time, respectively (Norwood et al., 2003). Hence, the assumption of concentration additivity is reasonable for complex metal mixtures in which more-than and less-than additive effects may approximately balance, but when only two or three metals are involved, the interactions of those specific metals should be considered.

Toxicologic benchmarks for estimation of mixture effects, like those for single metals, should be chosen to approximate the sensitivity of the affected biota at the impaired site. They can be derived from either the SSDs or the single species exposure-response relationships provided in this report. Interspecies Correlation Estimation (ICE) software may also be helpful in estimating benchmarks for a species of concern. Once individual quotients are obtained, the toxic unit approach sums the quotients of stressors with the same mode of action to assess the potential for their additive toxicity. With heavy metals, disturbances in ionic balance is a primary mechanism for acute toxicity at the gill while neurotoxic, nephrotoxic and other mechanisms occur in extended exposures. It is not recommended to incorporate data for As and Se in this approach because their toxic mechanisms differ.

# 8. METHODS

Two sources of data contribute to the analyses presented in this report. Conventional aquatic toxicity data for use in generating SSDs were harvested from the ECOTOX database. A previously unpublished collection of chronic exposure-response datasets originating from David Hansen and Glenn Thursby of the U.S. EPA laboratory in Narragansett, RI was used to generate exposure-response curves for different effects. This dataset was created by extracting exposure-response data from original publications of chronic tests that were judged to be suitable for deriving national ambient water quality criteria. Data clean up, statistical analyses, and graphical plotting for this report were performed using Microsoft® Excel, Microsoft® Word and SAS® statistical software. Tabular and graphical results from these procedures are provided in the appendices for use in stressor assessments. Plots and tables provided in the text are for illustrative purpose.

## 8.1. SPECIES SENSITIVITY DISTRIBUTIONS

Removal of data lines lacking the required water quality parameters of pH, temperature and hardness from ECOTOX data left roughly 10,000 records for the metals of interest in freshwater, about half of which were LC50 values. The test endpoints other than LC50s under these discrimination parameters included EC10, EC50, LT50and others that cannot be aggregated into large enough sets for SSD generation. SSDs were, therefore, generated using the LC50s for aquatic organisms to total metals at pH 6-8. These data were grouped according to exposure durations of one day or less to represent storm event exposures, of one to three days to represent seasonal releases over longer periods such as snowmelt, and three to 30 days to represent more sustained exposures. Data were also grouped by temperatures greater than or less than 15°C and water hardness using the USGS classifications of very soft (<18 mg $CaCO_3$/L), soft (18-60 mg $CaCO_3$/L), moderately hard (60-120 mg $CaCO_3$/L), hard (120-180 mg $CaCO_3$/L) and very hard (>180 mg $CaCO_3$/L). Finally, early life stage data for fish were plotted independently from juvenile and adult data to accommodate potential differences in sensitivities. In summary, SSDs were calculated from studies reporting LC50s, total metal concentrations, pH 6-8, duration, temperature and, except for As and Se, hardness. Data were grouped according to the following characteristics:

- Duration blocks of <1 day, 1-3 days, and 3-30 days
- Temperature greater or less than 15$^{o}$C
- Hardness <18, 18-60, 60-120, 120-180 or >180 mg $CaCO_3$/L.

**8.1.1. ECOTOX Data Selection and Preparation.** Data for the metals of interest for effects in fresh water fish and invertebrates were obtained from the ECOTOX database in July of 2004. Files were converted to a Microsoft® Word document and text was removed from the numeric fields to correct field entries which would interfere with the sorting and identification of usable data. In ECOTOX, the slash code indicates a remark field comment concerning that particular entry. All slash codes were removed and remark fields are reported in the abbreviated records tables (Appendix A). In the endpoint field of ECOTOX, an asterisk (*) indicates that the endpoint value was inferred from the author's data. For example, if an author stated that half of the organisms died at a given exposure, this was coded as an LC50. In water chemistry parameter fields, an asterisk indicates that the value reported was not measured in the actual exposure media, but reflects conditions measured in the culture, holding tank, acclimation, control, dilution water or pretest conditions. Where an author reports approximate values for water quality parameters, the field is coded with a tilde (~). In exposure concentration fields, an asterisk denotes the concentration has been recalculated from the author's original units to the standard µg/L or from the metal compound to the metal concentration. Once extraneous codes were removed, the data tables were converted to SAS® so that they could be merged with taxonomic data from the USDA Integrated Taxonomic Information System database and with tolerance and trophic guild data for fish and invertebrates collected from the *Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers* (Barbour et al., 1999). Trophic values and status were harvested from FishBase (Froese and Pauly, 2004). This augmentation of the ECOTOX data allows sorting, selection and labeling of data according to taxon, trophic guild or tolerance class in future analyses. In the case of vertebrate data, if the organism comment field of a record clearly indicated that early life stage organisms were used, the record was coded as such to allow discrimination of differences in sensitivity. Finally, data fields for test duration, water quality parameters and exposure concentrations were converted to consistent units. Condensed records for the ECOTOX source data for this work, CAS numbers and code definitions are summarized in Appendix A. Data used in this report and full citations are also provided to allow closer examination of individual records and to identify the citation of origin. When information from the plots and tables provided in this report are used in making decisions or recommendations, it is important that the original citations be examined to guard against errors in reporting or interpretation.

**8.1.2. Generation of Species Sensitivity Distributions.** Generation of SSDs followed the approach used in a previous analysis of dioxin toxicity in birds (U.S. EPA, 2003). Most regulatory practitioners of SSD modeling recommend a minimum of five to eight observations, but Dutch standards may be derived with as few as four (Suter et al., 2002). For this work, we required a minimum of five species within each discriminant class for SSD generation. If multiple acceptable values were available for a species, the geometric mean was used as the species value. Effect concentration data for all relevant species were ranked from the lowest to the highest. Ranks were then converted to proportions using the formula, proportion = ($i$ - 0.5)/$n$, where $i$ is the rank and $n$ is the number of species. That value is the empirical proportion of all tested species with an effective concentration less than or equal to that particular species' effective concentration.

Models were then fit to the data (species' ranks expressed as proportions paired with corresponding species' effect concentrations). The proportions were converted to the probit scale and the SAS General Linear Models (GLM) procedure was used to fit the log-probit model, the most commonly used for SSD modeling. The log probit model is:

$$\text{Probit}(p) = a + b(\log 10\ LC50) \qquad \text{(Eq. 1)}$$

where:

Probit($p$) = the probit transformation of the species proportion

$a$ and $b$ = the fitted intercept and slope variables, respectively.

The 95% confidence intervals for these relationships were calculated by taking the antilog of the predictions using the Bonferroni approach described in Neter et al. (1990, pages 173-175, equations 5.33 and 5.33A), specifically, the log of the mean concentration +/- critical value of $t_{\alpha=0.05,\ df}$ times the square root of the SSQ. The SSQ is:

$$\text{SSQ} = \text{Mean Squared Error}/\text{Slope}^2 * [1 + 1/\text{Number of species} + (\text{logCentralTendency} - \text{GrandMean})^2/\text{Corrected sum of squares}] \qquad \text{(Eq. 2)}$$

The resulting models and the data from which they were derived are plotted using the proportion scale against the log concentration. These plots and associated data for arthropods, non-arthropod invertebrates, invertebrates and vertebrates are

provided in Appendices A-D. Following each plot, the fitted parameters or the models are presented along with the species means used to generate the models and any additional effects data for species represented on that plot. These additional effects data are plotted using that species LC50 proportion value and the log concentration at which that particular effect was observed. For example, if the LC50 for species X placed it at the 35$^{th}$ percentile of the SSD, then other effective concentrations for that species would be plotted at 35% on the Y axis of the plot (e.g., behavioral effect for *A. aquaticus* plotted on Figure 8-1). Also note that in some cases, no effect values may occur at higher concentrations than some of the sublethal effects, based on the type of response measured or response magnitude recorded (e.g., EC10 vs. EC100, in Figure 8-1 *Daphnia magna* NR-LETH-MOR at higher concentration than LOEC-MOR). These plots are intended to convey condensed graphical information that can be compared with exposure and response data from the site to determine if the concentration of metals could cause the effects observed.

## 8.2. EXPOSURE-RESPONSE RELATIONSHIPS FROM CHRONIC TESTS

Exposure response relationships (Appendices J-L) were modeled with data for chronic toxicity exposures under standard laboratory conditions. These include binary (e.g., proportion surviving, proportion normal), count (e.g., eggs per female) and continuous (e.g., growth in mm) response data. This database was provided by David Hansen and Glenn Thursby of the U.S. EPA laboratory in Narragansett, RI, checked against original sources by C.L. Tsao at Oak Ridge National Laboratory, and further checked and corrected for this analysis.

Data were first screened to remove those sets having no apparent exposure-response relationship and those having maximum response magnitudes less than 20% different from controls. Many sets also had response intensities at lower concentrations that were less than or equivalent to that of the controls. To avoid any effect of this leading tail on the modeling, the maximum concentration at which a non-response occurred was identified as the operational control for these sets, and data for responses below these concentrations were omitted from the analysis.

Many data in the database were reported with only mean responses and did not include individual data points or standard deviations. To explore the uncertainty associated with incomplete data sets, those data sets providing standard deviations or replicate data were used to infer likely standard deviations for the remaining sets and allow random generation of surrogate replicates and datasets. In examining the coefficients of variation (CVs) on Table 8-1, it became evident that responses

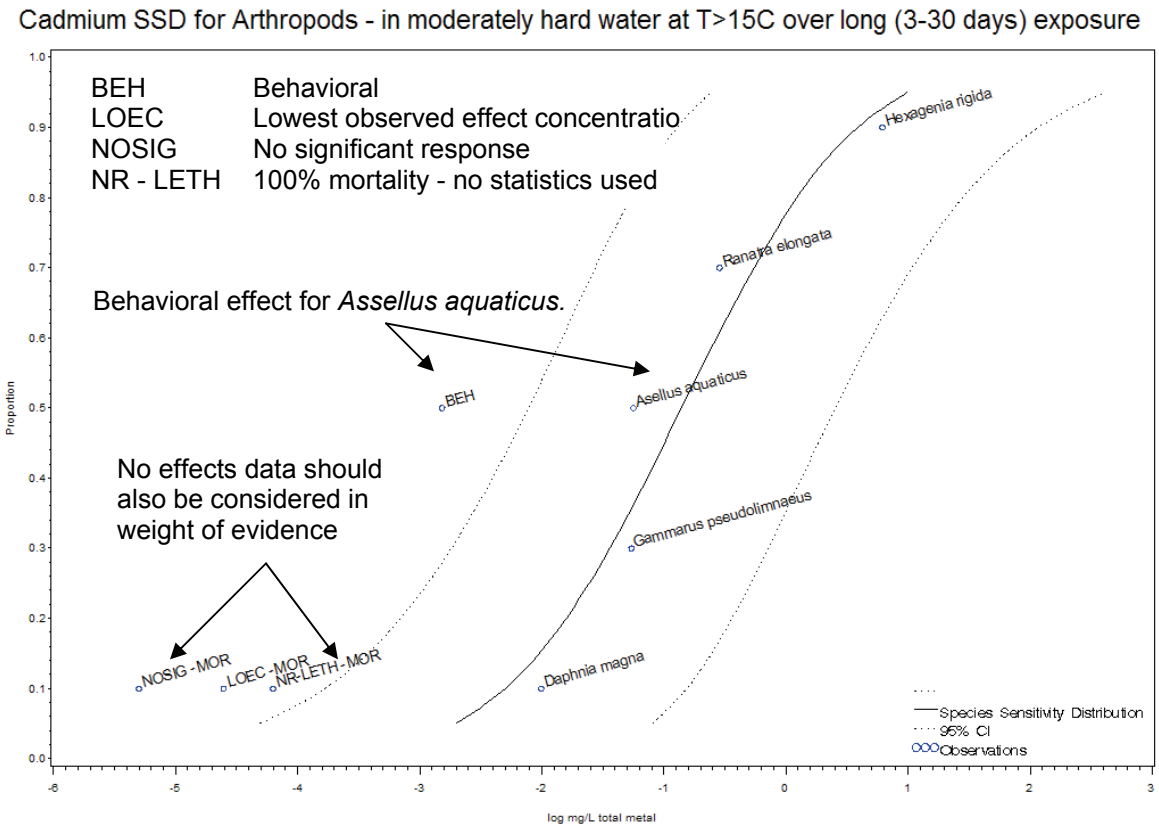



FIGURE 8-1

Example SSD Identifying Non-LC50 Data Which May also Inform a Weight of Evidence Analysis

TABLE 8-1

Summary of the Coefficients of Variation (CVs=standard deviation/mean) Among Replicates

| Parameter | N | Mean CV |
|---|---|---|
| Growth | 27 | 0.03 |
| Growth over generations | 6 | 0.03 |
| Development | 16 | 0.07 |
| Survival | 52 | 0.18 |
| Survival over generations | 11 | 0.21 |
| Reproduction over generations | 6 | 0.25 |
| Fecundity over generations | 2 | 0.28 |
| Reproduction | 41 | 0.46 |

associated with fecundity (e.g., number of eggs per female) or survival over generations (e.g., percent second generation survival) typically had higher variances than responses not dependent on parental exposure. Such responses are termed *generational responses* in the remainder of this report and are discussed below.

**8.2.1. Statistical Treatment of Generational and Non-Generational Data.** Separate surrogate CVs were required for generational and non-generational data. The third quartile of the cumulative CV distributions was selected because it represents a reasonably conservative estimate of expected variation for datasets lacking information on response variability (Figure 8-2). These values were 45% for generational response and 11% non-generational responses. The 45% or 11% values for variance were used to generate minimum and maximum expected observations for incomplete datasets by multiplying the reported mean by the appropriate CV thereby obtaining an estimated standard deviation, then using that value with the mean to obtain an estimated 95% range for the expected responses. Having identified minimum and maximum expected responses, five random values within those ranges were generated to provide five surrogate replicates for each exposure concentration. This process was repeated 100 times to generate 100 datasets having artificially generated replicates which were used to calculate the exposure-response models and 95% confidence intervals needed to determine the concentrations at which the response intensities of primary interest occurred.

Since the response intensities of primary interest are the 10% and 20% change from controls, only those datasets having greater than two exposure points bracketing 10% and 20% response levels were retained for exposure-response modeling. Point-to-point interpolation was used to obtain EC05s, EC10s, EC20s and EC50s for datasets with fewer than two points in this range and those datasets not producing useful exposure response plots after generation of surrogate replicates. The number of datasets remaining was too cumbersome to apply the Benchmark Exposure Software developed by U.S. EPA-NCEA so the procedure was programmed using SAS® statistical software and the general linear models method described by Kerr and Meador (1996). This approach requires that the distribution for the data and a link function describing the relationship between the response intensity (proportion responding) and the linear combination of predictors be specified. In the case of these simple exposure response relationships, this is concentration of the metal eliciting the response. The link function used for binary data was the probit, for count data (Poisson distribution) the log

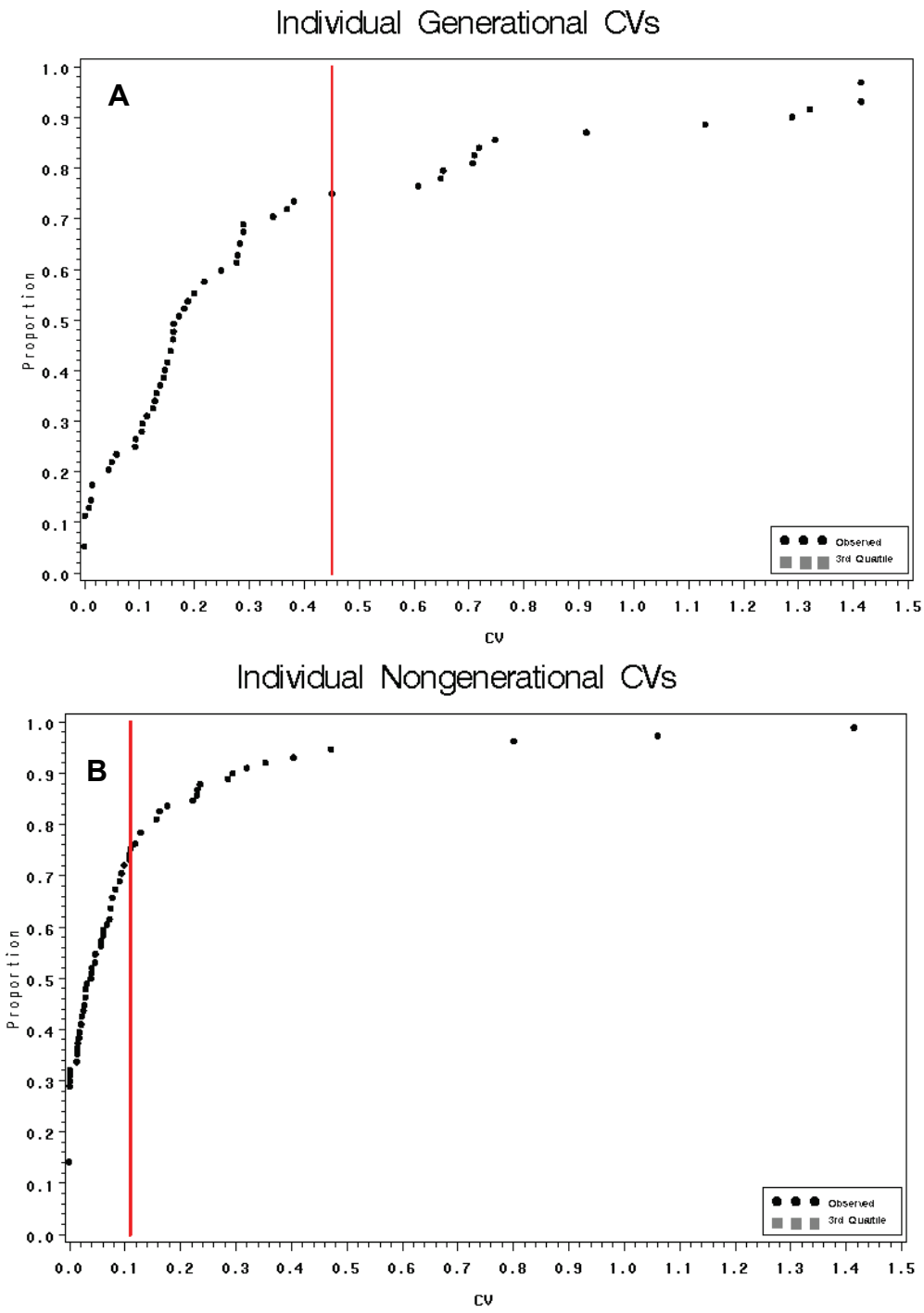



FIGURE 8-2

Cumulative Distribution of the Coefficients of Variation Among Replicate Responses at a Given Concentration in Laboratory Toxicity Test Data. Generational CVs (A) are reproductive effects (number of spawnings) and measured in the offspring of exposed organisms. Non-generational effects (B) include growth in length of mass, survival, and development of organisms exposed post fertilization.

link was used and for continuous data (gamma distribution), the inverse power function was used. Regression coefficients obtained in GLM models are asymptotically normally distributed, making it possible to create a confidence interval for response intensities using link units. A 1 - $\alpha$ interval for link($p$) is:

$$\text{link}(p) \pm Z_{\alpha/2} * \text{square root (variance[link}(p)\text{])} \quad \text{(Eq. 3)}$$

where:

link($p$) = the link-transformed proportion

$Z_{\alpha/2}$ = the Z score for the confidence interval.

The Z score for 95% confidence interval is 1.96.

Confidence intervals for the exposure intensity were estimated using Feiller's methods. Calculations used to arrive at the actual values are described below. See Kerr and Meador (1996) for details on the derivation of these equations.

$$\text{link}(p) = \text{Link transformation(Proportion to be predicted)} \quad \text{(Eq. 4)}$$

$$\text{Part A} = \beta_1^2 - (1.96^2 \times \beta_1\text{Error}^2) \quad \text{(Eq. 5)}$$

$$\text{Part B} = (2\beta_0 \times \beta_1) - (2\beta_1 \times \text{link}(p)) - (2 \times 1.96^2) \times \beta_0\text{Error} \times \beta_1\text{Error} \times \text{Corr} \quad \text{(Eq. 6)}$$

$$\text{Part C} = \beta_0^2 + \text{link}(p)^2 - 2\beta_0 \times \text{link}(p) - (1.96^2 \times \beta_0\text{Error}^2) \quad \text{(Eq. 7)}$$

Finally, the 95% confidence interval is given by:

$$\text{Part B} \pm \text{square root((2PartB - 4PartC) x PartA)/2PartA} \quad \text{(Eq. 8)}$$

where:

link($p$) = the link transformation of the response proportion to be predicted (e.g., 20%, 30% etc.)

Corr = the parameter correlation estimate for the slope and intercept

$\beta_1$ = estimate for the intercept

$\beta_0$ = estimate for the slope

$\beta_1$Error = standard error of this estimate

$\beta_0$Error = standard error of this estimates.

The arithmetic mean of the Corr, $\beta_1$, $\beta_0$, $\beta_1$Error and $\beta_0$Error parameters for the 100 artificially generated replicate datasets were used in the calculation of the central tendency and confidence intervals.

## 9. FUTURE WORK

To make the best possible use of the available data in environmental assessment, it is important to be consistent and thorough in the interpretation of information applied to the assessment. This includes data derived from measurements of site media and biota data collected from other sources for use in evaluating site-specific data. Metal toxicity to communities in the field is influenced by factors not addressed in the laboratory studies used in this analysis. These data offer little information regarding multiple exposures, acclimation, avoidance and the influence of interspecies competition. Additional analyses using data from field observations and field experiments are necessary to provide a more complete understanding of the expected ecological responses to metals contamination in context of other stressors under realistic exposure conditions. Field studies are a separate line of evidence having its own strengths and weaknesses. A database containing information on community responses to complex metals exposures under field conditions is nearing completion. It is planned that these data will be synthesized and related to the laboratory data distilled in this report for the purpose of compiling existing knowledge on metals toxicity to aquatic organisms for application to causal analysis.

## 10. REFERENCES


Asfaw, A., M.R. Ellersieck and F.L. Mayer. 2004. Interspecies Correlation Estimations (ICE) for AcuteToxicity To Aquatic Organisms and Wildlife: II. User Manual and Software. University of Missouri-Columbia and U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory.

Barbour, M.T., J. Gerritsen, B.D. Snyder and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA/841/B-99/002.

Bengtsson, B.E. and A. Larsson. 1986. Vertebral deformities and physiological effects in fourhorn sculpin (*Myxocephalus quadricornis*) after long-term exposure to a simulated heavy metal-containing effluent. Aquat. Toxicol. 9:215-229.

Briggs, J.C. and J.F. Ficke. 1977. Quality of Rivers of the United States, 1975 Water Year—Based on the National Stream Quality Accounting Network (NASQAN). U.S. Geological Survey. Open File Report No. 78-200. p. 436.

DiToro, D.M., H.E. Allen, H.L. Bergman et al. 2001. Biotic ligand models of the acute toxicity of metals: I. Technical basis. Environ. Toxicol. Chem. 20(10):2383-2396.

Froese, R. and D. Pauly, Ed. 2004. FishBase. Last updated August 2004. Available at www.fishbase.org.

Ghoshal, K., Y.J. Wang, J.F. Sheridan and S.T. Jacob. 1998. Metallothionein induction in response to restraint stress - transcriptional control, adaptation to stress, and role of glucocorticoid. J. Biol. Chem. 273:27904-27910.

Hermesz, E., M. Abraham and J. Nemcsok. 2001. Tissue-specific expression of two metallothionein genes in common carp during cadmium exposure and temperature shock. Comp. Biochem. Physiol. 128C:457-465.

Kerr, D.R. and J.P. Meador. 1996. Modeling dose response using generalized linear models. Environ. Toxicol. Chem. 15(3):395-401.

Madoni, P. 2000. The acute toxicity of nickel to freshwater ciliates. Environ. Pollut. 109:53-59.

Meyer, F.P. and L.A. Barclay, Ed. 1990. Field Manual for the Investigation of Fish Kills. U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC. Resource Pub. 177.

Mosleh, Y.Y., S. Paris-Palacios, M. Coudershet, S. Biagianti-Risbourg and G. Vernet. 2005. Metallothionein induction in *Tubifex tubifex* (Oligochaete) exposed to the fungicide, fenhexamid. Environ. Pollut. 135(1):73-83.

Naddy, R.B., W.A. Stubblefield, J.R. May, S.A. Tucker and J.R. Hockett. 2002. The effect of calcium and magnesium ratios on the toxicity of copper to five aquatic species in freshwater. Environ. Toxicol. Chem. 21(2):347-352.

Neter, J., W. Wasserman and M.H. Kutner. 1990. Applied Linear Statistical Models, 3rd ed. Irwin, Boston, MA.

Norwood, W.P., U. Borgmann, D.G. Dixon and A. Wallace. 2003. Effects of Metal Mixtures on Aquatic Biota: A Review of Observations and Methods. Human Ecol. Risk Assess. 9(4):795-811.

Posthuma, L., G.W. Suter II and T.P. Traas. 2002. Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, Boca Raton, FL.

Sippel, A.J.A., J.R. Geraci and P.V. Hodson. 1983. Histopathological and physiological responses of rainbow trout (*Salmo gairdneri* Richardson) to sublethal levels of lead. Water Res. 17(9):1115-1118.

Sprague, J.B. 1970. Measurement of pollutant toxicity to fish. Water Res. 4:3-32.

Suter II, G.W., T.P. Traas and L. Posthuma. 2002. Issues and practices in the derivation and use of species sensitivity distributions. In: Species SensitivityDistributions in Ecotoxicology, L. Posthuma, G.W. Suter II and T.P. Traas, Ed. Lewis Publishers, Boca Raton, FL. p. 437-474.

Tort, L., B. Kargacin, P. Torres, M. Giralt and J. Hidalgo. 1996. The effect of cadmium exposure and stress on plasma cortisol, metallothionein levels and oxidative status in rainbow trout (*Oncorhynchus mykiss*) liver. Comp. Biochem. Physiol. 114C:29-34.

TRI (Toxics Release Inventory). 2002. TRI explorer. Available at http://www.epa.gov/triexplorer/.

U.S. EPA. 1985. Technical Support Document for Water Quality-Based Toxics Control. U.S. Environmental Protection Agency, Office of Water Regulations and Standards. EPA/440/4-85/032.

U.S. EPA. 1994. Appendix L: Interim guidance on determination and use of water effect ratios for metals. In: Water Quality Standards Handbook, 2nd ed. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA/823/B-94/005a.

U.S. EPA. 2000. Stressor Identification Guidance Document. U.S. Environmental Protection Agency, Office of Research and Development, Office of Water, Washington, DC. EPA/822/B-00/025.

U.S. EPA. 2001. Update of Ambient Water Quality Criteria for Cadmium. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA/822/R-01/001.

U.S. EPA. 2003. Analyses of Laboratory and Field Studies of Reproductive Toxicity in Birds Exposed to Dioxin-Like Compounds for Use in Ecological Risk Assessment. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. EPA/600/R-03/114F.

U.S. EPA. 2005. Predicting Toxicity to Amphipods from Sediment Chemistry. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC. EPA/600/R-04/030. Available from: National Technical Information Service, Springfield, VA, and online at http://www.epa.gov/ncea.

Van Cleef-Toedt, K.A., L.A.E. Kaplan and J.F. Crivello. 2000a. The relationship between reproductive status and metallothionein mRNA expression in the common killifish. *Fundulus heteroclitus*. Environ. Biol. Fish. 57:97-105

Van Cleef-Toedt, K.A., L.A.E. Kaplan and J.F. Crivello. 2000b. Metallothionein mRNA expression in spawning and non-spawning *Fundulus heteroclitus* following acute exposure to starvation and waterborne cadmium. Fish Physiol. Biochem. 22:319-327.

## 11. GLOSSARY OF ACRONYMS AND ABBREVIATIONS

BLM – Biotic Ligand Model, a model that incorporates information on biotic ligand sites of organisms and the effects of water chemistry on metal bioavailability to estimate toxicity

CHESS – CHemical Equilibria in Soils and Solutions, a model for calculating chemical equilibria in soils and solutions, used in the BLM

CV – Coefficient of Variation, variation in a set of observations normalized to the mean, calculated by dividing the standard deviation by the mean

∆ALAD – delta aminolevulinic acid dehydratase, enzyme inhibited by lead resulting in impaired heme synthesis

EC05 – Toxicant concentration estimated to affect 5% of organisms

EC20 – Toxicant concentration estimated to affect 20% of organisms

ECOTOX – The ECOTOX (ECOTOXicology) database provides single chemical toxicity information for aquatic and terrestrial life

GLM – General Linear Models

ICE – Interspecies Correlation Estimations – A method and software developed for estimating acute toxicity of chemicals to species, genera, and families when data are lacking

LC01/LC50 Ratio – The ratio of the toxicant concentration estimated to kill 1% of exposed organisms to the toxicant concentration estimated to kill 50% of exposed organisms

LC50 – In a toxicity test, the toxicant concentration estimated to kill half of exposed organisms

LOEC – In a toxicity test, the lowest toxicant concentration at which statistically significant effects were observed

LT50 – In a toxicity test, the time at which 50% of organisms exposed to a given toxicant concentration are estimated to be killed

MATC – Maximum acceptable toxicant concentration (the geometric mean of the LOEC and NOEC)

NOEC – The highest toxicant concentration in a toxicity test at which no statistically significant effects were observed

SAS – Statistical Analysis Software, Cary NC

SI – Stressor Identification

SSD – Species Sensitivity Distribution

TMDL – A Total Maximum Daily Load is a calculation of the maximum amount of a pollutant that a waterbody can receive and still meet water quality standards, and an allocation of that amount to the pollutant's sources.

USDA – United States Department of Agriculture

USGS – United States Geological Survey

WER – Water Effect Ratio, the ratio of the toxicity of a metal in the site water to the toxicity of the same metal in standard lab water