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DEVELOPMENT OF A STANDARDIZED APPROACH FOR ASSESSING POTENTIAL RISKS TO AMPHIBIANS EXPOSED TO SEDIMENT AND HYRDIC SOILS

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The guidance manual presents a standardized two-tiered risk assessment protocol for evaluating potential risks to amphibians. The Tier I Amphibian ERA Protocol comprises a screening level ERA. This approach uses readily available information to identify potential amphibian exposure pathways at a site and determine which exposure pathways are potentially complete. The Tier 1 protocol includes effects-based and background screening steps to determine whether or not potentially complete exposure pathways have the potential to pose a significant environmental risk. Ultimately, the results of the Tier 1 protocol are used to determine whether or not additional amphibian ERA is warranted.

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LIST OF ACRONYMS

ASTM	American Society for Testing and Materials
AWQC	Ambient Water Quality Criteria
BAA	Broad Agency Announcement
BCC	Bioaccumulative Chemicals of Concern
BNS	Binational Toxics Strategy
CBR	Critical Body Residue
CERCLA	Comprehensive Environmental Response Cleanup and Liability Act
DAPTF	Declining Amphibian Populations Task Force
DDD	p,p'-Dichlorodiphenyldichloroethane
DDE	p,p'-Dichlorodiphenyldichloroethylene
DDT	p,p'-Dichlorodiphenyltrichloroethane
DIS	Dissolved Water Samples
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOD	Department of Defense
EC_{50}	Median Effective Concentration
ERED	Environmental Residue Effects Database
ERL	Effects Range-Low
ERM	Effects Range-Median
FETAX	Frog-Embryo Teratogenesis Assay-Xenopus
GLWQI	Great Lakes Water Quality Initiative
HT	Horsetooth Reservoir
IC_{25}	25% Inhibition Concentration
IR	Installation Restoration
LC_{50}	Median Lethal Concentration
LCV	Lowest Chronic Value
LEL	Low Effects Level
LOEC	Low Observed Effect Concentration
NAAMP	North American Amphibian Monitoring Program
NARCAM	North American Reporting Center for Amphibian Malformations
NAS	Naval Air Station (South Weymouth, MA)
NAWQC	National Ambient Water Quality Criteria
NFESC	Naval Facilities Engineering Service Center
nm	Nanometers

NOAA	National Oceanic and Atmospheric Administration
NOEC	No Observed Effect Concentration
NOED	No Observed Effects Dose
NWI	National Wetlands Inventory
OMOE	Ontario Ministry of the Environment
OPPTS	USEPA Office of Prevention, Pesticides and Toxic Substances
PAH	Polycyclic Aromatic Hydrocarbon
PBT	Persistent, Bioaccumulative, and Toxic
PCBs	Polychlorinated Biphenyls
PEC	Probable Effects Concentration
PR	Cache la Poudre River, Colorado
RATL	Database of Reptile and Amphibian Toxicology Literature
RCRA	Resource Conservation and Recovery Act
SAP	Sampling and Analysis Plan
SEL	Severe Effects Level
SETAC	Society of Environmental Toxicology and Chemistry
SMAV	Species Mean Acute Value
SOP	Standard Operating Procedures
SCV	Secondary Chronic Value
TEC	Threshold Effect Concentration
TOC	Total Organic Carbon
TR	Total Recoverable
USACE	United States Army Corps of Engineers
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
UV	Ultraviolet Light
UVB	Ultraviolet Light Radiation at Wavelengths of 290-320 nm

EXECUTIVE SUMMARY

Amphibians are often considered key indicators of possible adverse impacts to wetland ecosystems and considerable research has been dedicated to examining reported teratogenicity and overall declining populations. However, no standardized procedure exists to evaluate the potential toxicity of sediments or hydric soils to amphibians. Therefore, the United States Navy initiated a program to develop a standardized approach for assessing potential risks to amphibians at Navy facilities. The standardized ecological risk assessment (ERA) protocol developed through this program can be used to help the Navy avoid costly and unnecessary wetland alteration based on use of inappropriate ecological endpoints.

This guidance manual presents the framework for a standardized risk assessment protocol for evaluating potential risks to amphibians at sites owned and/or operated by the Navy. This guidance manual serves as the fourth deliverable under the scope of work for the following YO817 project:

> *Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediment and Hydric Soils.*

Previous work for this project included a literature review, developing standardized laboratory testing techniques, validation of the toxicity testing using spiked sediments, and derivation of amphibian screening values. This work has been incorporated into the guidance manual and provided in appendices.

The guidance manual presents a standardized two-tiered risk assessment protocol for evaluating potential risks to amphibians. The Tier I Amphibian ERA Protocol comprises a screening level ERA. This approach uses readily available information to identify potential amphibian exposure pathways at a site and determine which exposure pathways are potentially complete. The Tier I protocol includes effects-based and background screening steps to determine whether or not potentially complete exposure pathways have the potential to pose a significant environmental risk. Ultimately, the results of the Tier I protocol are used to determine whether or not additional amphibian ERA is warranted.

The Tier II Amphibian ERA Protocol comprises a refined ERA or Baseline ERA, and is conducted if recommended at the conclusion of the Tier I assessment. The Tier II protocol approach uses site-specific information to evaluate complete exposure pathways and amphibian ecological resources that are identified through the Tier I screening. This protocol can be used to develop assessment and measurement endpoints for the assessment of potential adverse effects on amphibian receptors. Tier II evaluations may include additional sampling and screening of abiotic media, toxicity or bioaccumulation evaluations, or field surveys. The Tier II evaluation provides quantitative measures and/or risk estimates of potential ecological effects associated with amphibian exposure to chemical stressors.

Use of this ERA approach is designed to allow the Navy and other DOD groups to develop more environmentally relevant risk assessments in a cost-effective manner. Risk managers will be able to use the information provided in the risk assessment, together with other sources, to identify clean-up levels and set remediation goals.

SECTION 1
INTRODUCTION

This guidance manual presents the framework for a standardized risk assessment protocol for evaluating potential risks to amphibians at sites owned and/or operated by the United States Navy. This report has been prepared by ENSR International (ENSR) on behalf of the Naval Facilities Engineering Service Center (NFESC), Port Hueneme, California, under the Navy's YO817 program under Broad Agency Announcement (BAA) Contract No. N47408-01-C-7213. The information contained herein has been developed to address the following Navy Environmental Quality, Research, Development, Testing/Evaluation Requirements:

1.II.02.d - *Regulator Approved Methods and Protocols for Conducting Marine and Terrestrial Risk Assessments*

1.III.01.k - *Improved Field Analytical Sensors, Toxicity Assays, Methods, and Protocols to Supplement Traditional Sampling and Laboratory Analysis*

This guidance manual is intended for risk assessment staff and state/federal regulators involved in the review and approval of risk assessment work plans, reports, and other deliverables.

1.1 Project Scope

This guidance manual serves as the fourth deliverable under the scope of work for the following YO817 project:

Development of a Standardized Approach for Assessing Potential Risks to Amphibians Exposed to Sediment and Hydric Soils.

This project involves the development of a standardized approach for assessing potential ecological risks to amphibians at selected Navy facilities, and is being completed using a phased approach. The phased approach has been adopted to (1) permit technical flexibility: (2) control costs; (3) ensure that the needs of the Navy are incorporated into the laboratory sampling and analysis program; (4) conduct work in an iterative manner so that the latter phases can benefit from knowledge acquired in the earlier phases of work; and (5) ensure that the information acquired for this project will help make informed risk-based management decisions.

The following interim deliverables were provided to the Navy prior to incorporation into this guidance manual:

- An amphibian ecotoxicological literature review;
- Development of laboratory testing techniques for amphibians exposed to sediment;
- Validation of the laboratory testing techniques; and
- Presentation of the program at a national or international scientific meeting.

Amphibians, like this Northern Leopard Frog, are often sensitive indicators of environmental stress.

1.2 Project Background

Since the 1980s, scientists have been researching, and documenting the overall decline in the health and abundance of amphibian populations (Rabb, 1999). Global declines in amphibian populations have been attributed to a number of anthropogenic activities, including habitat destruction, habitat alteration, the introduction of exotic species, exposure to environmental contaminants, climate change, increased acid precipitation, and increased UV flux associated with ozone depletion. Recent studies have illustrated that declines in amphibian population health have also taken place in relatively pristine habitats such as national parks and reserves, where specific environmental stressors are not readily apparent (Declining Amphibians Populations Task Force [DAPTF], 2001).

Possible factors contributing to the decline in amphibian populations include the following:

- Changes in atmospheric conditions contributing to acid rain, increased ultraviolet radiation, ozone layer depletion, and drought.
- Loss or alteration of habitat, specifically freshwater wetlands, vernal pools and other ecosystems necessary to support the complex life history of many amphibians.
- Invasive species that directly or indirectly compete for resources, alter habitats, or act as predators to one or more amphibian life stages.
- Increasing exposure of amphibians to disease and pathogens.
- Chronic and/or acute exposure to environmental contamination.

According to the U. S. Fish and Wildlife Service (USFWS) (USFWS, 2003), there are currently 21 federally listed amphibian species classified as federally threatened or endangered, with an additional nine candidate species. It is unlikely that one specific “smoking gun” will be identified as the causative agent contributing to the overall decline in the health of amphibian populations; however, it is likely that the above-described environmental stressors are contributing to the decline. The decline appears to be, at least in part, due directly or indirectly to human activities.

Recent research has shown that amphibians tend to be sensitive indicators of environmental stress from contaminant exposure as a result of their unique life history and physiology (Meffe and Carrol, 1997; Murphy et al., 2000; McDiarmid, 1994). This research has included evaluation of potential constituents which are no longer commercially available (i.e., aroclor mixtures), as well as controversial studies of commercially available products such as atrazine (i.e., Renner, 2002). Amphibian life-history requirements potentially expose this group of vertebrates to contaminants in surface waters, sediments, and soils at various intensities, depending on developmental stage and the life history unique to each species. Amphibians commonly travel between aquatic and terrestrial habitats, placing them at risk of exposure from the distinct properties associated with each system (Linder, 2000). Although amphibians often inhabit the transition zone between upland and lowland habitats, their home range is generally limited, resulting in constant exposure from egg to adult if contaminants are present (Henry, 2000). Compounding the effects of contaminant exposure, wetland habitats generally serve as a sink for many chemical compounds. Thus, exposure to environmental contaminants in wetland systems may be higher than potential exposure in surrounding upland areas, especially during the critical early life egg and larval stages of development commonly spent in wetland habitats.

In addition to their unique life history, the physiological properties of amphibians heighten their exposure to contaminants in the environment. Amphibians are exposed to contaminants through the direct uptake from water and substrate as well as the ingestion of sediments, soils, and food items (Linder, 2000; McDiarmid, 1994). The skin of amphibians is thin and highly permeable serving as part of the respiratory system (Murphy et al., 2000; United States Geological Survey [USGS], 2000). This permeability maintains the organisms balance in nature, but also creates a route for the potential for uptake and intensifies the risk of contaminant exposure to amphibians by permitting chemical transport across membranes (Henry, 2000).

Although there are a number of laboratory and field studies investigating effects associated with amphibian exposure to environmental contaminants (e.g., United States Environmental Protection Agency (USEPA), 1998; Beyer, 1988 in Henry, 2000), amphibian toxicity is generally under-represented in the literature. Until relatively recently, most available amphibian ecotoxicity information has been limited to contaminant body burden data based on surface water exposures or field collected organisms. Much of the body burden data reported in the literature have no corresponding ecotoxicity data, making it difficult or impossible to interpret these data in the context of an amphibian ecological risk assessment. It has been postulated that amphibian ecotoxicity has not been extensively studied due to the fact that amphibians are of relatively little economic importance in comparison to fish and other wildlife (Sparling et al., 2000b).

In an effort to protect freshwater and saltwater aquatic life, the USEPA has developed chemical specific numeric water quality criteria recommendations (USEPA, 2002). These criteria are currently applied directly to a broad range of surface waters by state standards, including lakes, impoundments, ephemeral and perennial rivers and streams, estuaries, the oceans, and in some instances, wetlands (USEPA, 1990). The numeric aquatic life criteria, although not designed specifically for wetlands, were designed to be protective of aquatic life and according to USEPA are generally applicable to most wetland types. However due to the general paucity of peer-reviewed amphibian ecotoxicological literature, amphibian toxicity data are either not included in the development of numeric criteria for the protection of aquatic life or are grossly underrepresented in comparison to other vertebrate organisms, including fish (Sparling et al., 2000b).

In addition to the potential exposure to contaminants in surface water, amphibians potentially have a greater risk of exposure to contaminants in sediments. Sediment is defined as all the detrital and inorganic matter situated on the bottom of lakes, ponds, streams, rivers, the ocean, or other surface water bodies (USEPA, 1996b). A hydric soil is a soil that formed under conditions of saturation, flooding, or ponding long enough during the growing season to develop anaerobic conditions in the upper part (USDA, 1991). In this manual these terms are used interchangeably to refer to sediments of palustrine emergent wetland complexes. In freshwater ecosystems, contaminant concentrations are typically higher in the sediments than in the overlying surface waters due to the strong affinity of many chemicals to bind to sediments and organic matter and settle out of the water column. The development of sediment quality screening values is an evolving discipline and no single standard has been adopted by regulatory agencies or is necessarily applicable to the sediment types found in freshwater wetland habitat (Wenning and Ingersoll, 2002). Furthermore, the majority of existing sediment quality benchmarks have been developed based primarily on the potential or observed effects associated with contaminant exposure to benthic organisms. These sediment quality benchmarks were developed using a variety of methods, and generally do not include amphibian toxicity endpoints.

There are also emerging methods to evaluate the influence of soil exposures to amphibians and recent literature has shown that dermal exposures can be important to amphibians (e.g., Hall and Swineford, 1979; Johnson et al., 2000 and 1999; Johnson and McAtee, 2000; Johnson, 2003). Some amphibians (i.e. *Plethodontid* and *Ambystomid* salamanders) spend a significant portion of their lives in soil and have been used in soil toxicity experiments.

1.3 Problem Statement

The relevance of available surface water and sediment quality benchmarks in palustrine wetlands where amphibians may represent a dominant vertebrate taxon is uncertain. Although acute exposure toxicity data exist for several inorganic and organic chemicals, a reliable, realistic amphibian model for evaluating chronic exposure to native North American species does not exist. Since chronic effects can often be induced at lower concentrations than those that cause acute mortality, using acute data to define environmental cleanup goals may be under-protective of amphibian populations. Conversely, using toxicity data from sensitive species that may not be present in a wetland, or play a minor ecological role, may result in over-protective (or under-protective) cleanup levels.

Use of an amphibian model is not exclusionary of invertebrate, fish, bird, or mammal models but rather represents a relatively new tool for the risk assessment practitioner that may be appropriate for use in an integrated risk assessment approach or independently, based on site-specific circumstances. Consideration of other species with standardized toxicity tests (e.g., amphipods) may also be appropriate for some wetlands.

Wetland habitats may often form a significant amount of open space in the vicinity of CERCLA sites at Naval facilities. This phenomenon is illustrated at the Naval Air Station (NAS) South Weymouth in Massachusetts, where palustrine wetlands comprise approximately 40 percent of the 1,400 acre facility and are present at 6 of the 7 CERCLA sites currently under investigation (ENSR, 2001). Wetlands at Navy facilities are prime habitat for various amphibian species.

Amphibians play a key ecological role in palustrine wetlands, serving as an important food source for higher trophic level receptors, and as a major consumer of prey items. However, because of the limited availability of chronic exposure amphibian ecotoxicity data, environmentally acceptable endpoints for current CERCLA and other environmental investigations are often based on data from aquatic species that may not be typical of the wetland in question. Sensitive non-wetland species such as fathead minnow and daphnids are often inappropriately used to make key ecological risk-based management decisions at Navy sites as these species may not be representative of the site conditions.

Wetlands comprise approximately 40% of the South Weymouth Naval Air Station site.

As a result of using aquatic species (e.g., fathead minnow *(Pimephales promelas)*) inappropriate to site conditions to make costly risk management decisions, the Navy runs the risk of remediating wetlands when no remediation is required. Not only is this a costly endeavor that potentially could be avoided, it also results in potentially avoidable wetland alterations. Conversely, at some sites the opposite result may occur: there is a potential to conclude that no unacceptable risks exist at a site based on the use of aquatic endpoints, when early life stage amphibians may be at risk.

Evaluation and remediation of contaminated Navy sites involves a determination of remedial cleanup goals, including identification of contaminant concentrations that are protective of ecological resources.

Pursuant to Department of Defense (DOD) guidance, ecological risk-based cleanup goals are typically developed using methodologies that have technical and social foundations. Development of risk-based cleanup goals involves complex risk management decision making. Perhaps the most complex decisions entail balancing the trade-off between destructive and costly remediation and leaving residual contamination in place. This tradeoff is important in wetland environments, which often serve as a "sink" for environmental contamination. Considerable attention has been paid in recent years to wetland losses in our nation; however, remediation of wetlands is environmentally destructive and costly. Remediation of certain wetlands often involves destruction of wetland habitat, and may only provide minimal risk reduction relative to the loss of functional habitat.

1.4 Tiered Framework for Amphibian Risk Evaluation

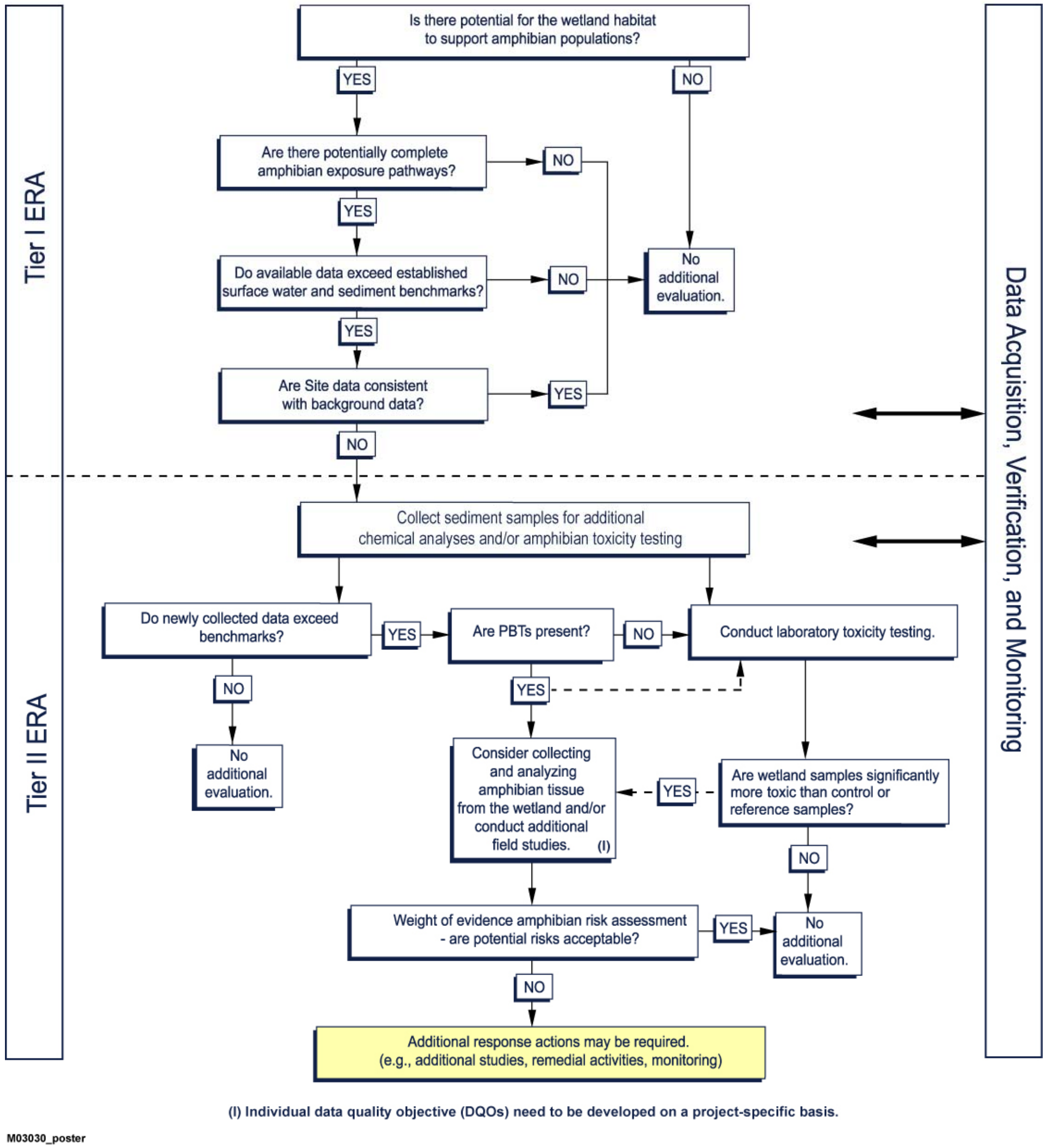
The objective of this guidance manual is to present a standardized risk assessment protocol for evaluating potential risks to amphibians at Navy sites. This protocol may help the Navy avoid costly and unnecessary wetland alteration based on use of inappropriate ecological endpoints**.** This protocol generally focuses on amphibians that fall into the 'pond-breeding' category, which includes amphibians that occupy palustrine wetland complexes often found on Navy sites. Terrestrial exposures are not completely evaluated within the scope of this protocol evaluation and, as such, taxon-specific risk evaluations for appropriate representative species and life stages may require modification of the proposed methodologies.

As presented in Figure 1-1, a tiered approach has been recommended for this standardized risk protocol. This approach is consistent with a tiered approach to ecological risk assessment appropriate for RCRA and CERCLA sites. The Navy also endorses a tiered approach in the Navy Policy for Conducting Ecological Risk Assessments (US Navy, 1999).

Conducting ecological risk assessments (ERAs) in a tiered, step-wise manner allows the risk assessor and risk manager to maximize the use of available site information and sampling data, while providing the opportunity to reduce the uncertainties inherent in the ecological risk assessment process through the use of focused supplemental data collection to fill key data gaps identified in the previous tier of the assessment, if necessary.

- The Tier I Amphibian ERA Protocol comprises a *screening level ecological risk assessment*. This approach uses readily available information to identify potential amphibian exposure pathways at a site; determine which exposure pathways are complete; and conduct effects-based screening using available benchmarks to determine whether or not the complete exposure pathways have the potential to pose a significant environmental risk. In addition, a chemical of ecological potential concern (COPEC) refinement step incorporates amphibian-specific screening values and an ambient conditions evaluation (i.e., background screen) to further refine the list of chemicals requiring evaluation. Although the background screen is recommended in the Tier I Amphibian Screening Level ERA Protocol, under Navy ERA policy (http://web.ead.anl.gov/ecorisk/) background evaluations typically occur during the Baseline Ecological Risk Assessment (BERA) (i.e., *Step 3a - Refinement of Conservative Exposure Assumption),* which is part of the Navy's Tier 2 ERA guidance. Therefore, the Tier I Amphibian ERA Protocol includes elements of both the Navy's Tier 1 and Tier 2 ERA protocol. Ultimately, the results of the Tier I Amphibian ERA protocol are used to determine whether or not additional amphibian ecological risk assessment is warranted.

Figure 1-1
Amphibian Ecological Risk Assessment Decision Matrix



- The Tier II Amphibian Ecological Risk Assessment Protocol comprises a *refined ecological risk assessment*, and will be conducted if recommended at the conclusion of the Tier I assessment. The Tier II protocol approach uses site-specific information to evaluate complete exposure pathways and amphibian ecological resources which are identified through the Tier I screening. This protocol can be used to develop assessment and measurement endpoints for the assessment of potential adverse effects on amphibian receptors, and provides quantitative measures and/or risk estimates of potential ecological effects associated with amphibian exposure to chemical stressors.

Where the results of the Tier I evaluation indicate sufficient potential ecological risk, further ecological risk assessment may be warranted. Tier II evaluations may include additional abiotic sampling and screening, toxicity or bioaccumulation evaluations, or field surveys to more accurately assess potential impacts to amphibians within the wetland study area. The activities outlined within the tiered approach presented in this manual would typically be integrated as a part of the Navy's Tier 1 and Tier 2 ERAs.

This guidance manual follows the general approach and methodology provided described by the USEPA in a number of documents. The risk assessor is encouraged to consult these additional sources for guidance on conducting ecological risk assessments:

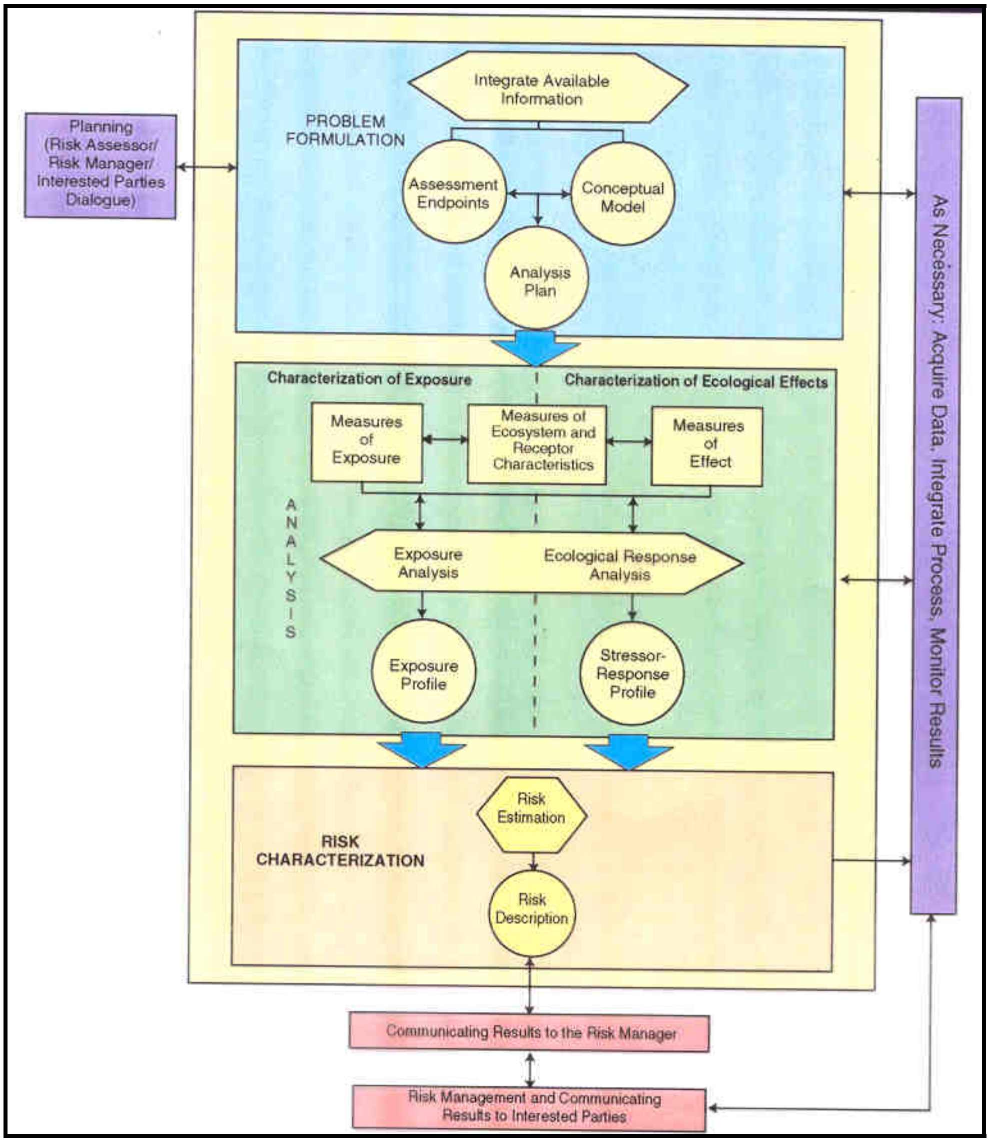
- Framework for Ecological Risk Assessment (USEPA, 1992);
- Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessment, Interim Final. (USEPA, 1997);
- Guidelines for Ecological Risk Assessment (USEPA, 1998); and
- The Role of Screening-Level Risk Assessments and Refining Contaminants of Concern in Baseline Ecological Risk Assessments (USEPA, 2001c).

The Navy Policy for Ecological Risk Assessment (US Navy, 1999) also provides guidance on the manner in which ecological risk assessments are to be conducted for the Navy Installation Restoration (IR) Program. This policy was developed to be consistent with the requirements of the USEPA ecological risk assessment guidance and also uses a phased or tiered approach.

1.5 Document Organization

The remainder of this guidance manual is organized in the following manner:

- Section 2 provides a general description of the life history and ecology of amphibians, with particular emphasis on amphibians as sentinel organisms;
- Section 3 presents the *Tier I Amphibian Ecological Risk Assessment Protocol*;
- Section 4 presents the *Tier II Amphibian Ecological Risk Assessment Protocol*;
- Section 5 includes a summary and recommendations; and
- Section 6 includes a list of references cited in this manual.



The USEPA framework for ecological risk assessment provides a general approach for ecological risk investigations (based on Figure 1-1 in USEPA, 1998).

SECTION 2
AMPHIBIANS AS ECOLOGICAL INDICATORS

Amphibians have been appropriately coined a keystone species as well as an indicator/sentinel member of their ecological community (Murphy et al., 2000). As keystone species, amphibians may play a disproportionately large role in wetland community structure, and may not be readily replaceable in the event of a sudden decline or loss in population size. Their absence within an ecosystem has the potential to lead to a disruption in the balance of the local interdependent community.

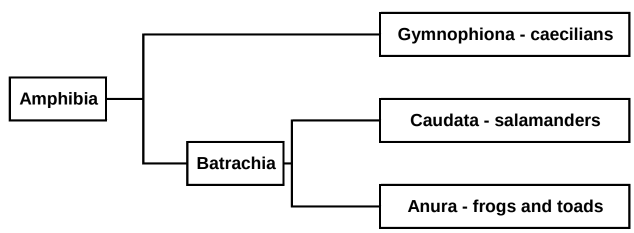
Amphibians are often a significant biomass component in North American ecological systems. For example, Merchant (1972) demonstrated that *Plethodontid* salamanders may occur in densities of several thousand per hectare, and that their total biomass in certain areas may exceed that of resident mammals and birds. While some species of amphibians are wide-ranging, others are habitat specialists and may be especially sensitive to environmental perturbation.

Although many communities exhibit a response to environmental stressors, certain aspects of amphibian physiology (e.g., the relative ease with which chemicals move across their skin) and life history (e.g., complex, bi-phasic life cycle), enable them to serve as excellent indicators of ecosystem health. The highly permeable amphibian integument, which allows gaseous exchange through the skin and via passive exposure, can render these organisms susceptible to changes in the environment (Linder et al., 2003). Amphibians can even be incorporated into a bioassessment and biocriteria program using an approach similar to that used to evaluate invertebrate communities in streams (i.e., Rapid Bioassessment Protocol).

The remainder of this section discusses aspects of amphibian life history which enable them to serve as ecological sentinel species at Navy facilities in North America.

2.1 Amphibian Classification

Two of the three major amphibian groups occur in North America and represent over 190 species (Behler and King, 1995). Salamanders are a group of amphibians that range in length from 6 inches to over 3 feet and superficially resemble lizards. While a few species are terrestrial, most salamanders are strictly aquatic or semi-aquatic. Their life-history traits require that they live in or near water or other moist habitats. Frogs and toads comprise the other North American group of amphibians. Frogs and toads, as adults, are four-legged tail-less amphibians that are found in moist or aquatic habitats for at least a portion of their life history.



Simplified Amphibian Phylogenic Tree

2.2 Amphibian Physiology

All amphibians are poikilotherms, meaning they have a substantially lower metabolic rate than other higher level classes. Poiklothermy presents certain advantages over the homeothermic requirements of other vertebrates such as mammals or avians (Dimmitt and Ruibal, 1980 as cited in Murphy et al., 2000). Their lower metabolic rate enables amphibians to utilize habitat types that have the potential to encounter harsher conditions. Through aestivation or over-wintering, amphibians are able to tolerate adverse conditions such as an intermittent food supply, dry weather, or severe cold where potential competitors do not have the physiological adaptations to survive.

As poikilotherms, amphibians must regulate their activity type and duration in order to regulate their body temperature (Murphy et al., 2000). Amphibians modify their body temperature through physiological attributes and behavioral traits, which enables them to maximize seasonal and daily climate variations. Amphibians cannot physiologically elevate their body temperature; however, they can behaviorally regulate their body temperature via basking in or avoidance of the sun. Amphibians are also able to physiologically lower their body temperature when necessary through evaporative cooling (Lillywhite, 1970 as cited in Murphy et al., 2000). As a result, amphibians may be both diurnally and nocturnally active as they modify their temporal behavior in order to maximize optimal body temperatures.

Amphibians primarily conduct gaseous exchange through the skin; the extent of this exchange varies across species type, developmental stage, and environmental conditions (Henry, 2000). The skin of amphibians is thin, highly permeable and in part breathes for the organism, thereby facilitating chemical transport across membranes (Murphy et al., 2000; USGS, 2000). Some amphibians retain their gills throughout their life span, while other species develop lungs and transform into air breathing adults. These differences and other physiological traits such as glandular/mucus excretions vary the amount of liquid and gaseous exchange that takes place transdermally (Murphy et al., 2000). The amount of gaseous and liquid exchange may also vary within a single species type depending on patchy environmental conditions, such as dissolved oxygen/carbon dioxide concentrations or depending on the developmental stage of the individual organism. This permeability maintains the organisms balance in nature, but also creates the potential for contaminant uptake and intensifies the risk of contaminant exposure to amphibians (Henry, 2000).

2.3 Amphibian Breeding Ecology

An understanding of amphibian breeding behavior is critical to understanding their role as sentinel organisms. Most species of amphibians have a complex, biphasic life cycle (McDiarmid, 1994). Environmental cues such as rain events prompt terrestrial adults to move to permanent or ephemeral aquatic habitats. While in these aquatic habitats, many amphibians engage in courtship behavior. Adults of oviparous species typically release eggs into the water (or near the water). Following hatching, amphibian larvae may serve as a major consumer in the aquatic environment. Following a period of growth (which may range from days to years, depending on the species), amphibian larvae undergo metamorphosis and typically migrate back into terrestrial or wetland habitats where they continue to forage and grow. Eventually, when mature, most amphibians return to the aquatic environment to breed and complete their life cycle.

Amphibian life-history varies with species type, although generally most migrate in and out of aquatic systems on an annual basis to breed (Murphy et al., 2000). The onset of amphibian migration and breeding varies with species type and latitude, but primarily depends on air temperature, precipitation, humidity, and for some species, soil temperature. Since amphibian breeding is regulated by environmental and seasonal conditions, breeding within sub-populations is generally synchronized in onset and duration. As a result, entire amphibian populations are potentially at risk from contaminant exposure if contamination is present in breeding areas.

For most species, reproduction generally occurs via external fertilization (Murphy et al., 2000). Eggs are generally deposited at or near the surface and depending on species may be laid in mass, chains, small clumps or singly attached to aquatic vegetation. Depositing the eggs in the surface microlayer is likely designed to warm the eggs by solar radiation for early spring breeders and expose them to maximal oxygen concentrations for mid-summer breeders, when eutrophication is most likely to occur. Conversely, predation, disease and other natural stochastic events are possibly enhanced in the surface layer where eggs are more accessible to a wide range of predators and parasites. High fecundity may counteract the vulnerability of early life-stages to these circumstances.

Amphibian Egg Mass

Anthropogenic activities also increase the risk for developing offspring in the surface waters before any physiological defenses are likely to develop through an increased risk of direct exposure to dangerous UV-B radiation, aquatic contaminants partitioned into the surface microlayer, parasites, and pathogens. Furthermore, anthropogenic activities may also indirectly affect the development of amphibian eggs and larvae via altering the natural flora surrounding or within water bodies in turn increasing exposure to UV-B radiation, altering pH or dissolved oxygen levels or varying food availability. If these events are remote or stochastic such as predation, or parasitic infection, then the effect on the health of the amphibian community is usually short-lived. Unfortunately many environmental contaminants such as PCBs and pesticides, as well as atmospheric changes such as acid rain and UV-B exposure, are very persistent, and may jeopardize the long-term health of widespread amphibian populations.

2.3.1 Egg and Larval Development

The development and subsequent hatching of amphibian eggs into larvae varies with species and generally ranges in duration between a few days to a month (Murphy et al., 2000). Since the viability of the developing embryo is highly vulnerable, rapid larval progression to metamorphosis is often advantageous. Larval stage amphibians are equally exposed to the same environmental threats as the embryonic stage with additional hazards associated with their dietary intake. Depending on species, the larval stage and the transformation to adult form may occur within a single growing season or the larval phase may extend over several winters. The latter is exemplified by the green frog (*Rana clamitans)*and American bullfrog *(Rana catesbeiana)*which continue aquatic feeding to ensure a successful metamorphosis. This behavior may prolong contaminant exposure during this critical development period.

The larval stage of many amphibian taxa has developed some physiological defensive mechanisms to reduce contaminant exposure through cellular defenses and a liver that metabolizes compounds using non-specific esterases, reductases, and mixed function oxidases. Behavioral defenses include limited mobility that affords larval

Overview of amphibian metamorphosis (approximately Stages 25 through 46)

amphibians the opportunity to remove themselves from adverse conditions (e.g., pond evaporation) as long as an alternative favorable one exists within their range. However, there are relatively few studies in the literature that document the effectiveness of these defenses to significantly limit contaminant exposure or the extent that contaminants affect the larval stage in the natural setting.

2.3.2 Metamorphosis

Not all amphibians undergo metamorphosis or the extent of the transformation may be limited (Henry, 2000). Some amphibians develop directly from the embryonic stage into adult form while others remain primarily aquatic for the duration of their life. Metamorphosis in amphibians represents a critical stage in complex, biphasic amphibian life cycles, and is accompanied by numerous complex physiological and anatomical changes. [The completion of metamorphosis in larval amphibians is often characterized by the re-absorption of the tail; following tail re-absorption, the juvenile physically resembles the adult form (Murphy et al., 2000).] Metamorphosis is a combination of structural, physiological, biochemical, and behavioral changes that vary between species (Duellman and Trueb, 1994 as cited in Murphy et al. 2000). For example, spadefoot toads (*Scaphiopus* spp.), an ephemeral pool genus, may complete metamorphosis in less than 2 weeks, whereas bullfrogs (*Rana catesbiana*), may overwinter as larvae for one or more years (Linder et al., 2003).

In addition to the natural dangers associated with the stress and vulnerability of metamorphosis, the transformation process is also highly sensitive to chemical and physiological changes in the environment that may impair the successful metamorphosis to adult form (Murphy et al., 2000). For example, perchlorate is a known endocrine disruptor which has become widely distributed in surface water and ground water due to its persistence and stability. As perchlorate affects thyroid function, exposure of a developing amphibian to perchlorate can result in abnormal or reduced growth during metamorphosis (Dumont, 2001). In addition, metamorphosis also has the potential to mobilize stored energy reserves that have accumulated persistent contaminants. Although the effect of these toxins on the metamorphic process is relatively unknown, it has been theorized that it may contribute to the sensitivity anurans have to xenobiotics (Murphy et al., 2000). Recently, several studies have suggested that the presence of anthropogenic endocrine disrupting compounds has the potential to adversely affect metamorphosis.

2.3.3 Sexual Development

The age of sexual maturity in amphibians varies among species, but rarely occurs within the first year. Juvenile amphibians are often essentially miniature versions of the adult form; with their use of habitat, diet and behavior consistent with that of adults. For some species, a major differentiator between juveniles and adults is related to the probability of attracting a mate and successful mating. Environmental stressors (e.g., UV radiation, chemical contaminants) in the environment have the potential for acute mortality in juvenile amphibians, but may also result in chronic effects which may threaten the long-term survival of the community. Contaminants introduced into the ecosystem have the potential to alter food supply, act as endocrine disrupters, and affect energy metabolism pathways in effect delaying the onset of sexual maturity (Linder et al., 2003). Endocrine disruptors generally mimic a natural hormone, fooling the body into over-responding to a stimulus or responding at inappropriate times. Other endocrine disruptors may block the effects of a hormone from certain receptors or directly stimulate or inhibit the endocrine system and cause overproduction or underproduction of hormones.

2.4 Habitat Use

Amphibians employ a variety of habitats throughout their complex life-history, each with its own unique pathway of potential direct and indirect exposure to contaminants. Most amphibians begin their early life stages in a submerged aquatic environment where the critical early stages of development may be exposed to contaminants present in wetlands or shallow ponds. Freshwater wetlands serve as an important transition zone between terrestrial uplands and freshwater bodies and generally serve as a sink for many chemical compounds in relation to upland areas. As amphibians are generally intolerant of saline conditions, with some exceptions (see Ultsch et al., 1999) estuarine or brackish wetlands are not typically considered suitable amphibian habitat. Following the embryonic and larval development, some amphibian species gradually metamorphose into air breathing adults while some species remain in the submerged aquatic environment. Adult amphibian habitat type range from terrestrial to aquatic ecosystems, where they may be exposed to contaminants present in the atmosphere, sediments, soils, surface water, and diet depending on species type. On an annual basis most juvenile and adult amphibian species are exposed to a wide-range of habitats during dispersion, migration between breeding ponds or over-wintering habitats, each presenting the potential for exposure to anthropogenic contaminants.

Effects of contaminants may be heightened during aestivation or over-wintering because it is a potentially vulnerable stage for adult amphibians that generally occurs during unfavorable conditions or harsh seasons and there may be direct contact with contaminated matrices (James and Little, 2002). Dermal exposure during this period could potentially contribute to sublethal effects in amphibians (Johnson, 2003). In addition, the synchronicity of breeding grounds and timing presents the risk of exposure to the entire exposed community. Consequently, amphibians are especially sensitive to environmental stressors since all stages of development are exposed to the environment as embryos, gilled larvae and submerged or air-breathing adults in a range of habitat types within a relatively consolidated home-range. The likelihood of exposure is compounded by the susceptibility amphibians have to the uptake of contaminants due to the unique physiology.

2.5 Amphibian Trophic Status

The class Amphibia is extremely diverse, with an enormous array of species-specific habitat preferences, life history patterns, and reproductive strategies (Linder et al., 2003). Amphibians serve as predator and prey to a variety of organisms. Larval stages and tadpoles are large consumers of algae and periphyton (Murphy et al., 2000). Plankton blooms initiating annually in early spring with the increase in light and temperatures coincides with the lifecycle of amphibians, and may provide an abundant source of food and energy for the larvae. Early life-stage amphibians may aid in suppressing large algae blooms through grazing, thereby transforming primary production into body mass for secondary consumption by tertiary aquatic and terrestrial consumers. Not all amphibians are primary consumers. Larvae from some species may be carnivorous, and include inter- and intraspecific prey in their diet. For example, predatory salamander larvae aid in the transfer of zooplankton and other micro aquatic invertebrates into energy for higher level trophic organisms. Juvenile and adult amphibians are carnivorous and primarily feed on insects, worms, terrestrial and aquatic invertebrates. Some larger amphibian species may also include small rodents, birds, snakes and other amphibians in their diet.

Amphibians of all life-stages are a major component of the diet for many predatory vertebrates (Murphy et al., 2000). Adult invertebrates such as arthropods and crayfish that form a large portion of amphibian diet in turn consume the eggs and larvae stages of many amphibian species. The major vertebrate predators of amphibians include mammals such as raccoons, and opossums, birds such as herons and raptors, fish and some snake species. Some voracious fish are so adept as predators they have essentially eradicated amphibians from certain water bodies. However, for most amphibians where successful residency is not as dependent on constant overlying water as it is for most fish species, intermittent water bodies provide a safe refuge for the success of egg and larvae development. The early development and metamorphic stages that need constant overlying water are fairly rapid allowing amphibians to inhabit temporary submerged habitats such as wetlands submerged during the spring-time, depressions made from tire tracks and vernal pools. In many of the intermittent water bodies that cannot sustain fish populations, amphibians serve as the major predator. In the role of top predator, amphibians aid in the maintenance of biodiversity by reducing the densities of single-species that may otherwise dominant the system. In addition, the lack of fish predators in ephemeral pools may also have influenced the selection of these areas as breeding grounds for many species.

The introduction of contaminants into the environment has the potential to disrupt the trophic balance by interfering with the health of prey or predator populations. Inadvertently, contaminants may be incorporated into the food chain both through direct exposure or indirectly through the consumption of lower level organisms or incidental ingestion of inorganic matter. As an intermediary link in the food web, amphibians may concentrate contaminants and transfer them up the food chain to their predators where the concentrations and usually the effects are magnified. Only a limited number of chemicals (generally those classified as persistent, bioaccumulative and toxic (PBT), as described at www.epa.gov/opptintr/pbt/index.htm) have been shown to significantly bioaccumulate through the food chain, and even fewer have been shown to biomagnify.

The risk to top predators including amphibians in certain systems not only threatens the health of the individual population, but also poses a risk to community diversity. In addition to the threat of poisoning top predator population, contaminants concentrated in amphibian tissues may be passed onto their offspring reducing the likelihood of proper development. As a species, regardless of which endpoint is effected, contaminants to varying degrees may directly or indirectly effect the viability of offspring to survive and successfully reproduce. The success of sexual reproduction within a population is the ultimate measure of the health and fitness of an amphibian community or population. As demonstrated in several laboratory studies discussed in Section 3 of this report, species health is likely reduced through contaminant exposure**.** It has recently been postulated (see Linder et al., 2003) that exposure to chemical stressors may play a significant role in the global decline of certain amphibian taxa.

2.6 Other Stressors

Contaminants in the environment are not the only threat to the viability and health of amphibian populations. Several potential anthropogenic factors have been identified as possibly contributing to the increase in the number of malformations detected in amphibians and the decrease in the biomass and diversity of global amphibian distribution. Loss or alteration of habitat, specifically freshwater wetlands, vernal pools and other ecosystems necessary to support the complex life history of many amphibians is rarely disputed as the prime threat to all ecological communities. Historically wetlands were considered wastelands (Mullarkey and Bishop, 1995), and it was not until relatively recently that society has discovered some of the many human-valued and intrinsic functions that wetlands possess that work to sustain overall ecosystem health (Wilen, 2001; Hunt, 1996). In an effort to modify wetlands into more productive areas, conversion of wetlands to agriculture and timber harvesting was encouraged and even supported through legislation (i.e., the Swamplands Acts). It has been estimated that over half of the original 220 million acres of the nations wetlands in the lower 48 states had been drained and converted to other uses by the mid-1980's (Dahl, 1990).

Recent amphibian decline research has focused on changes in atmospheric conditions as a result of anthropogenic emissions. The byproducts of human activity released into the atmosphere contribute to the acidification of freshwater systems, the increase in harmful ultra-violet (UV) radiation and drought. Basking individuals, egg masses and tadpoles in shallow exposed water bodies are at risk to synergistic acute and chronic effects associated with UV-B exposure both directly and indirectly. Murphy et al. (2000) discusses the potential risks posed by the current trends in canopy removal and the thinning ozone layer that may be increasing the exposure of hazardous UV radiation to amphibians. UV-B radiation has been linked to an increased occurrence of immunosuppresion. The acidification of freshwater systems is linked to the decline in several amphibian populations around the world (Corn, 2000). The effects of low pH on amphibians are numerous and highly codependent on other environmental variables and include both acute and chronic toxic effects on all life-stages (Rowe and Freda, 2000). Low pH levels contribute to the toxicity of many inorganic compounds as discussed in the Section 3. There also is speculation over the increased prevalence of drought and changing weather patterns and its' link to the health and biomass of amphibian community (Corn, 2000).

Invasive or exotic species directly or indirectly compete with indigenous populations for resources. Invasive species may pose a risk to amphibian communities by altering the natural habitat or landscape, replacing common prey items in food chains, competing directly with amphibians for resources or space, or introducing disease. Invasive species may also exist as a predator to one or more amphibian life stages and have the potential to extirpate local populations if no natural defense mechanism exists.

Another potential risk to amphibian communities is through disease and parasites. Amphibian malformations and die-offs have been limited to several biological stressors, including fungus injections and iridoviruses at a number of sites. The prevalence of diseased amphibians has apparently increased over the past few decades, and it is possible that susceptibility to disease may result from reduced immunity from other environmental stressors, including environmental contaminants (Corn, 2000).

Although several studies target a specific environmental stressor as the underlying threat to the community under observation, it is unlikely that any one factor is going to be targeted as the predominant risk to global amphibian declines with the exception of humans. Many of proposed factors risking amphibian viability have been the target of research efforts in the laboratory. However, the synergistic effects multiple stressors and the relevance to natural amphibian communities still has evaded any of the current literature.

2.7 State of the Science

During the past 25 years, the extent of ecotoxicological literature has expanded and level of the research has become increasingly more complex and informative (Sparling et al., 2000b). Although vertebrates in general have been the topic of a good portion of the research, recent inquiries into the available literature indicate that little attention was applied to the amphibian class. Sparling et al. (2000b) recently investigated the extent of amphibian ecotoxicology data over a 25-year period and discovered that amphibians represented only 2.7% of the vertebrate data contained within the Wildlife Review and Sports Fisheries Abstracts database representing vertebrate eco-toxicological data between 1972 and 1998. Over 95% of the abstract topics focused on fish, birds and mammal ecotoxicology.

The reason for the lack of literature on amphibian eco-toxicology is poorly understood. Their ecological significance represented by their role in the trophic system and occupancy of unique habitats is well documented and generally accepted by the scientific community. Furthermore the unique life history and physiology of amphibians cannot be represented by a surrogate group of organisms within the literature. Some have speculated that the relatively minor economic role amphibians serve may account at least in part for the disparity in the literature (Murphy et al., 2000). In addition, much of the ecotoxicological work conducted during the past two decades was represented by species that were relatively easily to breed in captivity which did not previously include amphibians (Murphy et al. 2000). The recent discovery and attention drawn to amphibian declines and malformations have boosted the research and attention on amphibian ecotoxicology and ecological significance.

In the available amphibian eco-toxicological literature, the focus of the research is primarily in metal residue and toxicity, acidification and non-chlorinated pesticides (Sparling et al. 2000b). Much of the available metals and acidification literature was focused on the toxic interactions under varying levels. Other stressors represented in the literature but to a much lesser extent include PAHs, PCBs/dioxins/furans, nitrogenous compounds, radioactivity, and UV-radiation. Several of these chemical stressors were investigated further in the following sections and appendices of this document.

The scant information available on general amphibian ecotoxicology does little to further the understanding the effects contaminants have on the local and global distribution of amphibians. In the natural setting, multiple factors contribute to the extent contaminants alter local community structure. Under natural conditions amphibians, as well as many other groups of organisms, may often recover from stochastic events that pose a temporary set back to the population However, the degree to which amphibians are able to respond and overcome natural stresses may be impaired by presence of anthropogenic stressors. The interactions between chemical and environmental variables create multiple conditions that both intensify and counteract the environmental stressors within the system. Amphibians aside, even within vertebrate classes that have a robust eco-toxicology literature base, the applicability of these studies to natural populations under natural conditions is poorly understood and highly speculative.

SECTION 3
TIER I INITIAL EVALUATION

This section presents the *Tier I Amphibian Ecological Risk Assessment Protocol*, which comprises the first tier of the standardized approach for assessing potential risks to amphibians at sites owned and/or operated by the United States Navy. The Tier I protocol serves as a screening level evaluation of potential risks to amphibian receptors associated with exposure to chemical stressors in abiotic media, and includes the following steps:

- Initial evaluation of habitat quality. The purpose of the initial habitat evaluation is to determine whether there is any reason to believe that amphibian receptors and potentially complete exposure pathways are present or potentially present within the wetland study area.
- Effects based screening. The purpose of this ecotoxicological-screening step is to evaluate whether or not site abiotic data (e.g., water quality) are consistent with the available literature values for the protection of aquatic life, including amphibians and other taxa as appropriate.
- Ambient conditions evaluation. The purpose of this step is to evaluate whether or not site abiotic data are consistent with site-specific, local, or regional background data for these media.

3.1 Initial Evaluation of Habitat Quality

This sub-section provides a generic summary of habitat evaluation techniques, and includes references to numerous literature sources relative to evaluation of amphibian habitat quality. Sample habitat evaluation checklists presented in Appendix A may be a useful mechanism to standardize this habitat evaluation procedure**.** It is recommended that regionally appropriate habitat evaluation checklists be identified on a site-specific basis.

In North America, north of Mexico, there are nine amphibian families within the order Anura (i.e., frogs and toads) and nine families within the order Caudata (i.e., salamanders). A complete species list with identification characteristics and range maps can be accessed at the North American Reporting Center for Amphibian Malformation (NARCAM) website (http://www.npwrc.usgs.gov/narcam/idguide/). The amphibian species within these families utilize a wide variety of habitats for overwintering, breeding, and foraging.

Pickerel weed in permanently flooded pond.

Amphibians can be placed into generalized groups relative to their breeding habits. There are amphibians that breed in streams and rivers (e.g., *Desmognathus*, *Eurycea*, *Dicamptodon*), terrestrial-breeding amphibians (e.g., *Plethodon*), and pond-breeding amphibians (e.g., *Ambystoma*, *Rana*, *Pseudacris*, *Hyla*, *Bufo*, *Notophthalmus*). This protocol generally focuses on amphibians that fall into the 'pond-breeding' category, which includes amphibians that occupy palustrine wetland complexes. Breeding amphibians within this group are typically associated with small depressions within uplands, larger wetland ecosystems, or oxbow ponds on river floodplains. However, amphibians also occur within man-made habitats including stream impoundments, farm ponds, quarries, and ditches. Amphibians breeding in ponds will fall into two primary categories; (1) those that typically breed in temporarily flooded ponds (e.g., vernal pools) and (2) those that typically use permanently flooded ponds. For those amphibian species that breed in temporary or ephemeral systems, there may be several months out of each year in which there are no larvae or adults within a given wetland complex. During these periods, a qualitative evaluation of habitat characteristics within the potential breeding pond and the adjacent landscape may provide enough information to assess whether a pond has the potential to support amphibian breeding. Although many of these same characteristics apply to those species breeding in permanently flooded habitats, often times larval tadpoles (e.g., green frog (*Rana clamitans),* American bullfrog (*Rana catesbiana)*) or aquatic adults (e.g., mole salamander (*Ambystoma talpoideum)*,Eastern newt *(Notophthalmus viridescens)*) are present throughout the year in these systems.

3.1.1 Natural History Investigation

The following sub-sections describe relevant sources for amphibian taxonomic identification, outline habitat characteristics important to pond- or wetland-breeding amphibians, remote methods for identifying potential breeding habitat (e.g., use of aerial photographs), and temporal considerations relative to obtaining definitive evidence of amphibian breeding. As in any ecological habitat evaluation program, it is important to appreciate the level of diversity and variation between species, regions, and even species within a region. Therefore the characteristics of amphibian habitat and methods for sampling those habitats are presented as generic, referenced guidance, and more detailed knowledge of the life-history requirements for species within a given region may be critical for accurate evaluations.

State or regional natural resources staff (e.g., Natural Heritage and Endangered Species Inventory Programs) may also provide useful information regarding valuable natural areas or occurrence of amphibians within the study area.

3.1.1.1 Taxonomic Identification

Most adult and juvenile amphibians exhibit diagnostic features that allow for easy identification to species. In addition, characteristics of amphibian habitat, or knowledge of a species life-history requirements (e.g., timing of observations made in the field) may be useful in separating species. Table 3-1 presents a number of national and regional reference sources which provide detailed information on identification, habitat use, and natural history of adult amphibians. The USGS maintains an internet site dedicated to the identification of North American amphibians north of Mexico (http://www.npwrc.usgs.gov/narcam/idguide/), and publications such as Moriarty and Bauer (2000) serve as useful lists of state and regional publications regarding this taxon.

Identification of amphibians during larval stages is typically more difficult than identification of adults. Taxonomic keys may require many hours of practice, looking at teeth rows on tadpoles or gill slits in salamander larvae under a dissecting scope to achieve a certain level of confidence in your identification. Available literature that will assist in the identification of amphibian larvae includes Altig and Ireland (1984) Petranka (1998), and McDiarmid and Altig (1999).

3.1.1.2 Temporal Considerations

The best time to identify amphibian breeding habitat is during the breeding season, which for most pond-breeding amphibians is in the spring (i.e., March through May). Southeastern amphibians may breed earlier, with some species such as American toad (*Bufo americanus)* breeding as early as January or February (Martof et al., 1980)

Table 3-1

National and Regional Amphibian Natural History and Taxonomic References

General Amphibian References

- Adler, K, 1992. Herpetology: Current Research on Amphibians and Reptiles. SSAR, St. Louis, MO.
- Bartlet, P. and R.D. Bartlet, 2003. Reptiles & Amphibians for Dummies. John Wiley & Sons.
- Cogger, H.G. and R.G. Zweifel (eds). 1998. Encyclopedia of Reptiles & Amphibians. 2nd edition. Academic Press, San Diego, CA.
- Collins, J.T. 1997. Standard Common and Current Scientific Names for North American Amphibians & Reptiles. 3rd Edition. SSAR, St. Louis, MO.
- Cope, E.D. 1979. Papers on the Higher Classification of Frogs. SSAR, Oxford, OH.
- Duellman, W.E. and L. Trueb, 1994. Biology of Amphibians. Reprint edition. McGraw Hill, New York, NY.
- Duellman, W.E. 1999. Patterns of Distribution of Amphibians: A Global Perspective. Johns Hopkins Univ Press, Baltimore, MD.
- Elliott, L. 1994. The Calls of Frogs and Toads. NatureSound Studio, NorthWood Press, Inc., Minocqua, Wisconsin. Audio CD Recording.
- Frost, D. R. (editor). 1985. Amphibian Species of the World. A Taxonomic and Geographical Reference. Allen Press, Inc. and The Association of Systematics Collections. Lawrence, Kansas.
- Halliday, T. and K. Adler, 2002. Firefly Encyclopedia or Reptiles and Amphibians. Firefly Books.
- Heatwole, H. and E.M. Dawley (eds). 1998. Amphibian Biology (Volume 3): Sensory Perception. Surrey Beatty & Sons, Chipping Norton, NSW.
- Heatwole, H. and B. K. Sullivan (eds). 1995. Amphibian Biology (Volume 2): Social Behavior. Surrey Beatty & Sons, Chipping Norton, NSW.

General Amphibian References

- Heatwole, H. and G. T. Barthalmus (eds). 1994. Amphibian Biology (Volume 1): The Integument. Surrey. Beatty & Sons, Chipping Norton, NSW.
- Heatwole, H. and R.L. Carroll (eds). 2000. Amphibian Biology (Volume 4): Paleontology: The Evolutionary History. Surrey Beatty & Sons, Chipping Norton, NSW.
- Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster (eds). 1994. Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution Press, Washington, DC.
- Library of Natural Sounds. 1996. Voices of the Night. The Calls of the Frogs and Toads of Eastern North America. Cornell Laboratory of Ornithology, Ithaca. Audio CD Recording.
- Murphy, J., K. Adler, and J. Collins, 1994. Captive Management and Conservation of Amphibians and Reptiles. SSAR, St. Louis, MO.
- O'Shea, M. and T. Halliday, 2001. Reptiles and Amphibians (Dorling Kindersley Handbooks). Dk Pub Merchandise.
- Shaw, G. 1999. General Zoology Volume III. Amphibians and Reptiles.
- Stebbins, R.C. and N.W. Cohen, 1997. Natural History of Amphibians. Reprint edition. Princeton Univ Press, Princeton, NJ.

Identification and Field Guide References

- Bartlett, P., R.D. Bartlett, B. Griswold, 2001. Reptiles, Amphibians and Invertebrates: An Identification and Care Guide. Barrons Educational Series.
- Behler, J.L. and F.W. King, 1979. National Audubon Society Field Guide to North American Reptiles and Amphibians. Knopf, New York, NY.

Identification and Field Guide References

- Behler, J.L., 1988. Familiar Reptiles and Amphibians: North America (Audubon Pocket Guides). Knopf, New York, NY.
- Bishop, S.C. 1943. Handbook of Salamanders, the salamanders of the United States, of Canada, and of Lower California. Comstock Publishing Associates, Ithaca, NY.
- Capula, M. and J.L. Behler (eds), 1990. Simon & Schusters Guide to Reptiles and Amphibians of the World. Fireside.
- Conant, R. 1998. A Field Guide to Reptiles & Amphibians of Eastern & Central North America (Peterson Field Guide Series). 3rd Edition expanded. Houghton Mifflin Co., Boston, MA.
- Corkran, C.C. and C. Thoms. 1996. Amphibians of Oregon, Washington and British Columbia. Lone Pine, Redmond, WA.
- Frost, D.R. W.E. Duellman (eds), 1985. Amphibian Species of the World: A Taxonomic and Geographical Reference. Allen Press Inc., Lawrence, KS.
- Hanson, J. and R.B. Hanson. 1997. 50 Common Reptiles and Amphibians of the Southwest. Southwest Parks & Monuments Association.
- Powell, R., J.T. Collins, and E.D. Hooper, 1998. A Key to Amphibians & Reptiles of the Continental United States and Canada. Univ Press of Kansas, Lawrence, KS.
- Stebbins, R.C. 2003. A Field Guide to Western Reptiles and Amphibians. 3rd edition. Houghton Mifflin Co., Boston, MA.
- Tyning, T.F. 1990. A Guide to Amphibians and Reptiles. Stokes Nature Guide. Little, Brown and Company.
- Wright, Albert, R. McDiarmid and A.A Wright., 1995. Handbook of Frogs and Toads of the United States and Canada. Comstock Pub Assoc; 3rd edition.

Table 3-1 (continued)

National and Regional Amphibian Natural History and Taxonomic References

Tadpole and Larval Salamander Identification Keys

- Altig, R. 1970. A key to the tadpoles of the continental United States and Canada. Herpetologica, 26(2):180-207.
- Altig and Ireland. 1984. A key to larvae and larviform adults of the United States and Canada. Herpetologica, 40(2): 212-218.
- Orton, G. L. 1952. Key to the genera of tadpoles in the United States and Canada. The American Midland Naturalist, 47(2): 382-395.
- Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, DC.
- McDiarmid, R.W. and R. Altig. 1999. Tadpoles: The Biology of Anuran Larvae. Univ. of Chicago Press, Chicago, IL..

Vernal Pools

- Calhoun, A. J. K. and M. W. Klemens. 2002. Best development practices: Conserving pool-breeding amphibians in residential and commercial developments in the northeastern United States. MCA Technical Paper No. 5, Metropolitan Conservation Alliance, Wildlife Conservation Society, Bronx, New York.
- Calhoun, A.J.K. 1997. Maine citizen's guide to locating and describing vernal pools. Maine Audubon Society, Falmouth, ME.
- Colburn, E.A. (ed.) 1997. Certified: a citizen's step-by-step guide to protecting vernal pools. Massachusetts Audubon Society, Lincoln, MA.
- Kenney, L.P. and M. R. Burne. 2000. A Field Guide to the Animals of Vernal Pools. Massachusetts Division of Fisheries & Wildlife's Natural Heritage Program, Westborough, MA.
- Kenny, L..P. 1995. Wicked big puddles: a guide to the study and certification of vernal pools. Vernal Pool Association, Reading, MA.
- Tappan, A. (ed.). 1997. Identification and documentation of vernal pools in New Hampshire. New Hampshire Fish and Game Department, Concord, NH.

Other

- Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek and M.S. Foster (eds). 1994. Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution Press, Washington, DC.
- Hunter, M.L., A..J.K. Calhoun, and M. McCollough, 1999. Maine Amphibians and Reptiles. University of Maine Press, Orono, ME.
- http://www.naturesound.com/frogs/frogs.html, also available as a compact disk entitled The Calls of Frogs and Toads, by Lang Elliott, NatureSound Studio.

State-Specific Identification References

- Bartlett, R. D., and P. P. Bartlett. 1999. A Field Guide to Florida Reptiles and Amphibians. Gulf Publishing Company, Houston.
- Carpenter, C. and J. Krupa, 1989. Oklahoma Herpetology: An Annotated Bibliography. Oklahoma Museum of Natural History Publication. Univ. of Oklahoma Press, Norman, OK.
- Collins, J.T. and S.L. Collins, 1993. Amphibians and Reptiles in Kansas. 3rd edition. Univ. Press of Kansas, Lawrence, KS.
- Degenhardt, W., C. Painter, and A. Price, 1996. Amphibians & Reptiles of New Mexico. University of New Mexico Press, Albuquerque, NM.
- Dundee, H. A., and D. A. Rossman. 1989. The Amphibians and Reptiles of Louisiana. Louisiana State University Press, Baton Rouge and London.
- Grismer, L. L. and H.W. Greene, 2002. Amphibians and Reptiles of Baja California, Its Pacific Islands, and the Islands in the Sea of Cortes. University of California Press, Berkeley, CA.
- Hammerson, G.A. 1999. Amphibians and Reptiles in Colorado. 2nd edition. University Press of Colorado, Niwot, CO.
- Harding, J. and J.A. Holman, 1999. Michigan Frogs, Toads and Salamanders: A Field Guide and Pocket Reference. Michigan State Univ. Bulletin Office.

State-Specific Identification References

- Johnson, T.R, 2000. Amphibians and Reptiles of Missouri (2nd Edition). Missouri Dept Conservation, Jefferson City, MO.
- Karns, D.R., 1974. Illustrated Guide to Amphibians and Reptiles in Kansas. Univ. of Kansas Museum of Natural History, Lawrence, KS.
- Klemens, M.W., 2000. Amphibians and Reptiles in Connecticut: A Checklist With Notes on Status, Identification, and Distribution. Dep Bulletin, No. 32. National Resources Center.
- Klemens, M.W., 1993. Amphibians & Reptiles of Connecticut & Adjacent Regions. State Geology & Natural History Survey of CT. Bulletin Series No. 112.
- Martof, B. S., W. M. Palmer, J. R. Bailey, and J. R. Harrison III. 1980. Amphibians and Reptiles of the Carolinas and Virginia. The University of North Carolina Press, Chapel Hill.
- McKeown, S. 1996. Field Guide to Reptiles and Amphibians in the Hawaiian Islands, Diamond Head Publishing, Inc., Osos, CA.
- McPeak, R.H., 2000. Amphibians and Reptiles of Baja California. Sea Challengers, Monterey, CA.
- Minton, S.A. Jr., 2001. Amphibians & Reptiles of Indiana. 2nd Edition. Indiana Academy of Science, Indianapolis, IN..
- Mitchell, J.D. 1994. The Reptiles of Virginia. Smithsonian Institution Press, Washington, DC.
- Mount, R. H. 1975. The Reptiles and Amphibians of Alabama. Auburn University Agricultural Experiment Station, Auburn, AL.
- Oldfield, B., J.J. Moriarty, and W.J. Breckenridge, 1994. Amphibians & Reptiles Native to Minnesota. Univ of Minnesota Press, Minneapolis, MN.
- Schwartz, V. and D.M. Golden, 2002. Field Guide to Reptiles and Amphibians of New Jersey.

When amphibians migrate to their breeding ponds, readily observable behaviors include spring migrations, courtship and mating processes, chorusing, and depositing of eggs masses. Evaluating amphibian occurrence can often be accomplished in one or two visits. Evidence of breeding may be acquired during other times of the year but will require entering the pond and sampling for aquatic larvae, which are sometimes difficult to catch and can be particularly difficult to identify. Environmental conditions such as temperature, hydroperiod, and rain events may also influence amphibian migration; a regional understanding of these factors is required prior to initiating amphibian surveys.

3.1.1.3 Hydroperiod and Fish Predators

One of the key characteristics of habitat use by pond-breeding amphibians is pond hydroperiod (i.e., the length of time a pond contains standing water within a year). Many pond-breeding amphibians use ponds that typically hold water throughout the spring and summer, eventually drying in the fall during most years. The ephemeral nature of these habitats precludes permanent fish populations from establishing and provides ideal breeding habitat for ephemeral-pool breeding amphibians. Resident fish populations have been shown to negatively impact amphibian species richness (Lehtinen et al. 1999) and breeding population size (Egan and Paton, 2004). However, there are a number of amphibian species that have developed physiological (e.g., unpalatable taste) or behavioral (e.g., hiding in vegetation or leaf litter) adaptations that allow them to successfully utilize permanently flooded habitats with established fish populations. There are also some species whose aquatic lifecycles dictate the use of permanently flooded habitats (e.g., green frog (*Rana clamitans)* takes one full year to complete tadpole stage to metamorphosis). At the opposite end of the hydrologic spectrum are ponds that dry too soon and do not permit larvae to undergo metamorphosis (Paton and Crouch, 2002) creating a sink habitat rather than a source habitat for juvenile recruitment.

In absence of several months, or even years, of hydrologic monitoring within a particular breeding pond, it can be very difficult to determine its hydroperiod. Fortunately, there are some physical and biological characteristics related to pond hydroperiod that may be used to assist in determining habitat suitability for amphibians in general, and for pond-breeding, or ephemeral pond-breeding species. For example:

1) The larger and deeper a pond is, the longer the pond will remain flooded. Amphibian breeding ponds in Rhode Island > 3 feet deep were usually permanent (Egan, 2001);
2) Ponds that are not hydrologically isolated (i.e., exhibit a surface water inlet or outlet) are more likely to be permanently flooded and contain fish;
3) Palustrine forested wetlands that are temporarily to seasonally flooded, often dry too soon to support successful amphibian breeding. However, deeper depressions within forested wetlands where the tree canopy is open and woody shrubs or persistent emergent vegetation predominate, or in closed tree canopy situations where the trees are atop large hummocks, will often have extended hydroperiods and support successful breeding by amphibians.
4) Similarly, isolated ponds that are small, shallow, surrounded by upland habitat, and have a closed tree canopy, will typically dry too soon to support successful amphibian breeding.

In a recent study (Skidds and Golet, 2002), basin depth and tree canopy cover were among the best determinants for habitat suitability for wood frogs (*Rana sylvatica*) and spotted salamanders (*Ambystoma maculatum*).

3.1.1.4 Vegetation Characteristics

Vegetation cover within ponds can also be used to assess habitat suitability for amphibian breeding. The availability of complex microhabitats (i.e., vegetation cover) is suspected to be important in providing refugia for developing larvae (Formanowicz and Bobka, 1989) and as egg attachment sites for some amphibian species (Egan and Paton, 2004; Paton and Crouch 2002). Potential breeding ponds should therefore have woody shrubs (e.g., *Cephalanthus occidentalis*, *Spirea latifolia*, *Ilex verticillata*), sphagnum, and persistent non-woody vegetation (e.g., *Carex spp*., *Scirpus spp*., *Glyceria spp*.) growing throughout the pond or within zones along the edges of the pond. The presence of woody debris (e.g., fallen tree branches) within in the pond may also be important in providing additional egg attachment sites.

Vernal pool at a forested site in the northeastern United States.

3.1.1.5 Chemical and Physical Characteristics

Tolerance to saline habitats varies widely in amphibians, as some species occur in brackish habitats such as salt marshes and areas affected by evaporation, tide or salt spray (Ultsch et al., 1999). Typically, North American amphibians are salt-intolerant and inhabit freshwater systems (Henry, 2000). Little data is available in the literature regarding salinity tolerances of larvae, however, chronic exposure to low pH water can result in growth reduction and other sublethal effects (Rowe and Freda, 2003).

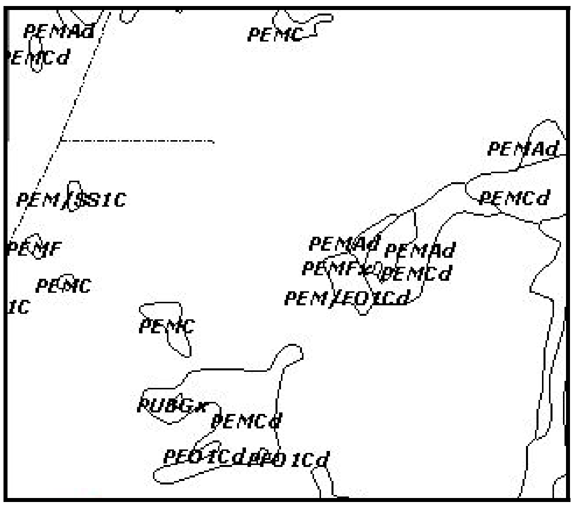
3.1.1.6 Landscape Setting

The adults of many pond-breeding amphibian species spend less than one month out of each year in the breeding pond, with the remainder of their annual cycle in forested upland and wetland habitats adjacent to the pond (Semlitsch, 2000). Therefore, when assessing the suitability of a particular pond as amphibian breeding habitat, it is beneficial to consider the landscape setting of the pond in question. According to Calhoun and Klemens (2002), the landscape adjacent to breeding ponds can be broken into two primary zones, the pool envelope (area within 100 feet of the pool's edge) and the critical terrestrial habitat (area within 100-750 feet of the pool's edge). The pool envelope provides habitat for the high densities of amphibians that congregate at a pond during the breeding season, and provides a buffer for water quality protection of the pool itself. The outer critical zone provides habitat for foraging and hibernating during the non-breeding season. Ideally the habitat within these zones is partially shaded by forest canopy with uncompacted litter and abundant coarse woody debris for amphibian cover. Any development within the pool envelope and > 25% development in the critical terrestrial habitat, can severely impact amphibian populations (Calhoun and Klemens, 2002).

3.1.1.7 Remote Detection (Map Sources)

Potential breeding ponds ≥ 12 m in diameter (≈ 0.045 ha) are reliably identified using large-scale aerial photographs and a stereoscope (Burne, 2001). Ponds smaller than this may be obscured by shadows or coniferous tree cover on the aerial photograph, and will go unnoticed. In certain parts of the country, a large proportion of potential breeding ponds are < 12 m in diameter and identifying them in the landscape is most effectively accomplished through ground surveys. For example, in a Massachusetts study (Stone, 1992) 73.6% (*n* =

78) of all potential breeding ponds were smaller than 0.04 ha. Similarly in a Rhode Island study (Egan and Paton, 2004), 33.1% (n = 41) of all potential breeding pools were smaller than 0.04 ha. For more information on using aerial photographs and other map sources (e.g., NWI maps, USGS maps) to identify potential amphibian breeding habitat, see Calhoun (1997). For a list of sources for aerial photography, see Calhoun and Klemens (2002).



Sample of a National Wetlands Inventory map.

3.1.1.8 Recommendations

The most efficient and deterministic approach for identifying amphibian-breeding habitat is to time field visits based on the breeding phenology of the species in your area. By arriving at a potential breeding pond during the breeding season, the presence of amphibians can be easily assessed through dip-net sampling, nighttime flashlight surveys, calling surveys and identification of egg masses. The use of these surveys is discussed in more detail in Section 4.3 (also see sources such as Heyer et al., (1994) for details on these sampling methods). In the event that a survey must be conducted during the non-breeding season (i.e., when larval or adult amphibians are not present), conducting a qualitative evaluation of a site's potential to provide the amphibian breeding habitat characteristics outlined above is recommended.

3.2 Effects Based Screening

Based on the evaluation of the available habitat and the presence of a historic source/release, if potentially complete exposure pathways exist at the site, then additional media screening is recommended. The complete exposure pathway requires that the contaminant and the habitat overlap in both time and space. If no complete exposure pathways are identified, then the site is unlikely to present significant risks to ecological receptors and no additional ecological evaluations are recommended. The evaluation of media against screening values assumes that abiotic analytical chemistry data are available from previous sampling activities within the study area. Although potential adverse ecological effects for wetland amphibian receptors can be evaluated based on comparisons of site data relative to literature derived screening values, end users should exercise caution interpreting the results of these comparisons. As discussed in Section 3.2.1, the majority of available literature screening values do not include amphibians in the database(s) used for benchmark derivation.

There may considerable differences in sensitivity to contaminants between fish and amphibians, particularly for metals (Birge et al., 2000). A comparison to other effects-based benchmarks may not be sufficiently protective of amphibians. A discussion with the relevant agencies is recommended prior to the elimination of chemicals based on these benchmarks.

3.2.1 Generic Literature Values

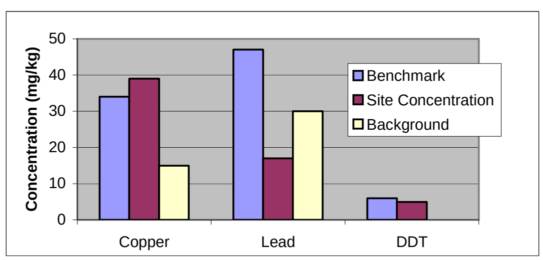
As part of the initial evaluation of the analytes, available surface water and sediment analyte concentrations can be compared to medium-specific screening benchmarks. It is recognized that the majority of these screening values were not derived with explicit consideration of amphibians; however, given the general regulatory acceptance of their use as screening level ERA benchmarks in a variety of federally and state-led programs, and given the conservative nature of the majority of these benchmark screening values, they are recommended for consideration in screening level amphibian ERAs. In cases where these screening values are used to help refine a list of chemical stressors at a site, an assumption must be made that these generic screening values, which primarily were derived to be protective of finfish and benthic organisms, are also protective of early life stage amphibians. This assumption may not be valid on all sites; for instance, if an endangered amphibian is a potential site receptor, this assumption may warrant further evaluation.

Potential sources of the screening benchmarks for this evaluation are described below:

- Surface water – A number of sources are available as potential screening benchmarks for surface water. These include federal Ambient Water Quality Criteria (AWQC) (USEPA, 2002) and state Water Quality Standards (WQS), USEPA No Observed Effect Concentrations (NOECs) and Lowest Observed Effects Levels (LOELs). If none of these values are available, sources such as Oak Ridge National Laboratories (ORNL) documents (Suter and Tsao, 1996) can be reviewed for secondary chronic values (SCV) calculated using Great Lakes Water Quality Initiative (GLWQI) guidance (USEPA, 1993b) or lowest chronic values (LCV). If none of these values are available, the primary ecotoxicology literature can be reviewed for relevant benchmarks or studies.
- Sediment – A number of sources are available as potential screening benchmarks in sediment. It is unknown how relevant these screening values are for the hydric soil matrix typically considered in amphibian ecological risk assessment. Sediment screening values include consensus-based Threshold Effect Concentrations (TECs) and Probable Effect Concentration (PECs) (MacDonald et al., 2000), Low Effect Levels (LELs) and Severe Effect Levels (SELs) from the Ontario Ministry of the Environment (OMOE) (Persaud et al., 1996), and Effects Range-Low (ER-L) and Effects Range-Median (ER-M) values from the National Ocean and Atmospheric Administration (NOAA; Long and Morgan, 1990). If benchmarks are not identified in these sources, sediment screening values may be derived using USEPA (1993a) equilibrium partitioning theory and freshwater chronic surface water screening values.

Additional sources of screening values may also be evaluated if sufficient benchmarks are not readily available**.** Benchmarks can also be developed using surrogate screening values or other risk-based tools (e.g., site-specific toxicity testing). The ECOTOX database (http://www.epa.gov/ecotox/) maintained by the USEPA provides single chemical toxicity information from peer-reviewed literature for aquatic and terrestrial life. Government and private web-sites, peer-reviewed studies and previous risk assessments on other sites may also be investigated. Information obtained from these reviews may then be used to develop screening values. In addition, the Navy conducted a literature review of available benchmarks for a selected number of potentially relevant constituents (Tables 3-2 and 3-3). This review is described further in Section 3.3.1, and details of the review are presented in Appendix B.

In the event that surface water or sediment benchmarks are not identified for certain analytes, these analytes are typically not further evaluated in screening level risk assessments, but should be discussed in the uncertainty section of the risk assessment.



***Sample of an Effects Based Screening*.**

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Table 3-2

Sediment Screening Benchmarks

Analyte	Low Effect Levels			Severe Effect Levels		
	Minimum	**Maximum**	**Source**	**Minimum**	**Maximum**	**Source**
Inorganics (ppm)			**Minimum/Maximum**			**Minimum/Maximum**
Cadmium	0.6	1.2	LEL (OMOE)/ERL (NOAA)	4.98	9.6	Consensus PEC/ERM (NOAA)
Chromium, Total	26	81	LEL (OMOE)/ERL (NOAA)	110	370	SEL (OMOE) at 1% TOC/ERM (NOAA)
Copper	16	34	LEL (OMOE)/ERL (NOAA)	110	270	SEL (OMOE) at 1% TOC/ERM (NOAA)
Lead	31	46.7	LEL (OMOE)/ERL (NOAA)	128	218	Consensus PEC/ERM (NOAA)
Mercury	0.15	0.2	ERL (NOAA)/LEL (OMOE)	0.71	1.06	ERM (NOAA)/Consensus PEC
Nickel	16	22.7	LEL (OMOE)/Consensus TEC	48.6	51.6	Consensus PEC/ERM (NOAA)
Zinc	120	150	LEL (OMOE)/ERL (NOAA)	410	459	ERM (NOAA)/Consensus PEC
Organics (ppb)						
OE Compounds						
2,4,6-Trinitrotoluene	92	--	Talmage et al. (1999) SQB at 1% TOC	--	--	
1,3,5-Trinitrobenzene	2.4	--	Talmage et al. (1999) SQB at 1% TOC	--	--	
1,3-Dinitrobenzene	6.7	--	Talmage et al. (1999) SQB at 1% TOC	--	--	
3,5-Dinitroaniline	--	--		--	--	
2-Amino-4,6-Dinitrotoluene	--	--		--	--	
Hexahydro-1,3,5-Trinitro-1,3,5-Triazine	13	--	Talmage et al. (1999) SQB at 1% TOC	--	--	
Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine	4.7	--	Talmage et al. (1999) SQB at 1% TOC	--	--	
N-Methyl-*N*,2,4,6-Tetranitroaniline	--	--		--	--	
PCBs, Total	22.70	70	ERL (NOAA)/LEL (OMOE)	180	5,300	ERM (NOAA)/SEL (OMOE) at 1% TOC
DDT	4.16	4.16	Consensus TEC	62.9	62.9	Consensus PEC
DDE	2.20	5	ERL (NOAA)/LEL (OMOE)	31.3	31.3	Consensus PEC
DDD	4.88	8	Consensus TEC/LEL (OMOE)	28	28	Consensus PEC
PAHs, total	1,610	4,022	Consensus TEC/ERL (NOAA)	22,800	100,000	Consensus PEC/SEL (OMOE) at 1% TOC
High molecular weight PAHs, total	1,700	1,700	ERL (NOAA)	9,600	9,600	ERM (NOAA)
Low molecular weight PAHs, total	552	552	ERL (NOAA)	3,160	3,160	ERM (NOAA)

Preference was given to the selection of freshwater sediment screening values.
Consensus PEC - Probable effect concentration (MacDonald et al., 2000)
Consensus TEC - Threshold effect concentration (MacDonald et al., 2000)
ERL - Effects range low, NOAA (Long and Morgan, 1990)
ERM - Effects range median, NOAA (Long and Morgan, 1990)
LEL - Low effect level, OMOE (Persaud et al., 1996)
NOAA - National Oceanographic and Atmospheric Administration
OE - Ordnance and explosives
OMOE - Ontario Ministry of the Environment
PAH - Polycyclic aromatic hydrocarbon
PCB - Polychlorinated biphenyl
SEL - Severe effect level, OMOE (Persaud et al., 1996)
SQB - Sediment quality benchmark (Talmage et al., 1999)
TOC - Total Organic Carbon
ppb - parts per billion
ppm - parts per million

Table 3-3

Surface Water Screening Benchmarks

Analyte (ppb)	Chronic Values[1]			Acute Values		
	Value	**Source**	**Notes**	**Value**	**Source**	**Notes**
Inorganics						
Cadmium	0.25	USEPA, 2002		2	USEPA, 2002	
Chromium III	74	USEPA, 2002	NAWQC; All metal water quality criteria are based on the dissolved fraction of metal in the water column; hardness of 100 mg/L as $CaCO_3$	570	USEPA, 2002	
Chromium VI	11	USEPA, 2002		16	USEPA, 2002	NAWQC; All metal water quality criteria are based on the dissolved fraction of metal in the water column; hardness of 100 mg/L as $CaCO_3$
Copper	9	USEPA, 2002		13	USEPA, 2002	
Lead	2.5	USEPA, 2002		65	USEPA, 2002	
Mercury	0.77	USEPA, 2002		1.4	USEPA, 2002	
Nickel	52	USEPA, 2002		470	USEPA, 2002	
Zinc	120	USEPA, 2002		120	USEPA, 2002	
Organics						
OE Compounds						
2,4,6-Trinitrotoluene	90	Talmage et al. (1999)	Tier I	570	Talmage et al. (1999)	Tier I
1,3,5-Trinitrobenzene	11	Talmage et al. (1999)	Secondary Chronic Value	60	Talmage et al. (1999)	Secondary Acute Value
1,3-Dinitrobenzene	20	Talmage et al. (1999)	Secondary Chronic Value	220	Talmage et al. (1999)	Secondary Acute Value
3,5-Dinitroaniline	60	Talmage et al. (1999)	Secondary Chronic Value	460	Talmage et al. (1999)	Secondary Acute Value
2-Amino-4,6-Dinitrotoluene	20	Talmage et al. (1999)	Secondary Chronic Value	350	Talmage et al. (1999)	Secondary Acute Value
Hexahydro-1,3,5-Trinitro-1,3,5-Triazine	190	Talmage et al. (1999)	Secondary Chronic Value	1,400	Talmage et al. (1999)	Secondary Acute Value
Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine	330	Talmage et al. (1999)	Secondary Chronic Value	3,800	Talmage et al. (1999)	Secondary Acute Value
N-Methyl-*N*,2,4,6-Tetranitroaniline	--			--		
PCBs, Total	0.14	Suter and Tsao, 1996	Secondary Chronic Value	--		
Aroclor 1221	0.28	Suter and Tsao, 1996	Secondary Chronic Value	5	Suter and Tsao, 1996	Secondary Acute Value
Aroclor 1232	0.58	Suter and Tsao, 1996	Secondary Chronic Value	1.00	Suter and Tsao, 1996	Secondary Acute Value
Aroclor 1242	0.053	Suter and Tsao, 1996	Secondary Chronic Value	1.2	Suter and Tsao, 1996	Secondary Acute Value
Aroclor 1248	0.081	Suter and Tsao, 1996	Secondary Chronic Value	1.4	Suter and Tsao, 1996	Secondary Acute Value
Aroclor 1254	0.033	Suter and Tsao, 1996	Secondary Chronic Value	0.6	Suter and Tsao, 1996	Secondary Acute Value
Aroclor 1260	94	Suter and Tsao, 1996	Secondary Chronic Value	1700	Suter and Tsao, 1996	Secondary Acute Value
DDT	0.001	USEPA, 2002	NAWQC	1.1	USEPA, 2002	NAWQC
DDE	--			--		
DDD	0.011	Suter and Tsao, 1996	Secondary Chronic Value	0.19	Suter and Tsao, 1996	Secondary Acute Value
PAHs, total	--			--		
High molecular weight PAHs, total	--			--		
Low molecular weight PAHs, total	--			--		

NAWQC - National ambient water quality criteria (USEPA, 2002)
OE - Ordnance and explosives
PAH - Polycyclic aromatic hydrocarbon
PCB - Polychlorinated biphenyl
ppb - parts per billion
[1] Selected chronic values were based upon the Final Chronic Values (FCV)

3.3 Refinement of Chemicals of Potential Ecological Concern

The list of COPECs may be further refined following the initial comparison against effects based screening values. This is consistent with USEPA (1997) and Navy guidance (1999) regarding the refinement of COPECs prior to the baseline, or Tier II, ecological risk assessment. COPECs may be compared against amphibian screening values identified during the Navy literature review and the amphibian toxicological testing. An evaluation of ambient (e.g., background) concentrations of chemicals is also recommended to further refine the list of COPECs.

3.3.1 Navy Y0817 Amphibian Screening Values

Under earlier phases of the Y0817 program evaluation, in an effort to evaluate whether or not sufficient data were available to develop screening values specific to amphibians, the Navy developed preliminary amphibian screening values using both laboratory testing and literature review approaches, which are described in the following sub-sections.

The screening values presented herein are not intended to be used as absolute screening values or to replace more established screening values and criteria, such as those described in Section 3.2.1. However, depending upon site-specific conditions and regulatory contexts, these amphibian screening values may prove to be useful tools to help evaluate site data relative to potential risks to early life stage amphibians in the Tier I amphibian ecological risk assessment protocol.

3.3.1.1 Literature Review Screening Values

The amphibian literature review focused on the following eleven constituents/classes of constituents of potential concern:

- Cadmium
- Chromium
- Copper
- Lead
- Mercury
- Nickel
- Zinc
- Polychlorinated Biphenyls
- 4,4 DDT, 4,4-DDE, 4,4-DDD
- Polycyclic Aromatic Hydrocarbons (PAHs)
- Ordnance and explosives

These constituents were selected because they are commonly identified at CERCLA, RCRA, and other sites being investigated by the Navy under the Installation Restoration (IR) and other environmental programs.

Appendix B provides a brief profile for each constituent describing the sources, uses, and fate and transport characteristics in terms of its relevance to amphibian toxicity. Following the profile, each constituent-specific sub-section includes a summary of the available amphibian toxicity information.

The ecotoxicological literature review presented in Appendix B focused on acute and chronic immersion laboratory studies with amphibians. Aquatic immersion studies were reviewed (rather than injection studies) since the immersion exposure pathway most closely approximates *in situ* exposure pathways in the natural environment. Contaminant tissue residue studies were not reviewed for the subject constituents, since the majority of these studies simply indicate the body or tissue burden of a constituent, without any indication of effects or ecotoxicological endpoints. FETAX (frog embryo teratogenesis assay *Xenopus*) studies were included in the review. However, it is recognized that there are some uncertainties associated with using this bioassay in a traditional risk assessment context, since it uses a species non-native to North America, there are limited comparative sensitivity data available between native North American species and *Xenopus*, it involves relatively finite evaluation of limited life stages (often 96-hour studies), and the FETAX bioassay includes endpoints (e.g., teratogenesis) that are not always considered by risk managers when making ecological risk management decisions.. When possible, solid phase exposure (e.g., sediment) ecotoxicity data were reviewed independently from aqueous phase studies.

Ecotoxicological effects data were divided into the following effects categories:

Mortality - These studies included lethal effects studies associated with the death of the target species. Studies review included median lethal concentration (LC_{50}) studies for tests of various durations.

Developmental - Contaminant exposure in these studies was typically associated with disruptions or alterations to various development processes. Endpoints included delayed metamorphosis and polydactyly.

Growth - Growth endpoints included sub-lethal effects on target organisms length and weight.

Behavior - Contaminant exposure in these studies was associated with behavioral observations, including swimming behavior, predator avoidance behavior, and lethargy.

Reproduction - Reproductive endpoints included altered reproductive activity, such as delayed hatching of eggs, and reductions in adult fertility.

Teratogenesis – Teratogenic endpoints included developmental effects and subsequent fitness reduction as a result of damage to embryonic cells.

Biochemical/cellular/physiological - A broad array of sub-lethal physiological endpoints were grouped under this category, including enzyme induction, ion balance, ocular responses, and hormone level responses.

Much of the material presented in this chapter was obtained from the following two recently published compilations of amphibian ecotoxicity data:

- Ecotoxicology of Amphibians and Reptiles (Sparling et al., 2000a). This resource, published by the Society of Environmental Toxicology and Chemistry (SETAC), provides summaries of several studies that have been conducted with amphibians exposed to a variety of contaminants.
- RATL: A Database of Reptile and Amphibian Toxicology Literature (Pauli, et al., 2000). This resource, published by the Canadian Wildlife Service as a Technical Report, contains numerous data extracted from primary literature for reptiles and amphibians.

When appropriate, focused searches of primary literature were also conducted, and databases such as ECOTOX were searched. Much of the data summarized in this chapter are presented in the context of available sediment and surface water quality criteria (e.g., AWQC) and guidance values, which are summarized in Table 3-2 (sediment) and Table 3-3 (surface water). Table 3-4 presents a summary of the available amphibian aquatic toxicity data, with ranges of effects concentrations on constituent-by-constituent and endpoint-specific basis. These data are further interpreted in Appendix B.

Five constituents (cadmium, copper, mercury, zinc, and DDT) were selected for further evaluation of lethal effects data: the lethal effects data for these five analytes represent the more robust of the amphibian data sets available. In order to establish preliminary effects concentrations for these chemicals in water, the 10^{th} centile and 50^{th} centile of the toxicity distribution were calculated using methods described by Solomon et al. (2001). Observed lethal effects endpoints (LC_{50} values) from all species and measured effects were incorporated into the dataset for the 10^{th} and 50^{th} centile calculations. No adjustment was made to account for the hardness of the water, which, as described in Appendix B, may affect the sensitivity of aquatic organisms to some metals.

Table 3-4

Summary of Surface Water Toxicity Studies

	BEHAVIORAL			BIOCEMICAL/ CELLULAR/ PHYSIOLOGICAL			DEVELOPMENTAL			GROWTH			MORTALITY			OTHER DATA			REPRODUCTIVE		
Chemical	**n**	**Minimum**	**Maximum**	**n**	**Minimum**	**Maximum**	**n**	**Minimum**	**Maximum**	**n**	**Minimum**	**Maximum**	**n**	**Minimum**	**Maximum**	**n**	**Minimum**	**Maximum**	**n**	**Minimum**	**Maximum**
Cadmium	2	1	1.3	5	1.1	4,000	12	<2 - 505	NA	3	30	106	48	9,920	11,648	27	1 - 76.5	77	1	1.34	1.34
Chromium		NA	NA	2	0 - 10,000	125,000	2	2,000	3,200	1	3,200	3,200	8	10,000	57,970	3	0 - 2,500	2,000		NA	NA
Copper		NA	NA		NA	NA	1	20 - 3,700	20 - 3,700	1	100,000 - 500,000	100,000 - 500,000	37	110	843	40	1	9	1	1 - 25	1 - 25
Lead	4	750	0 - 1,000	2	500	1000	7	70	1 - 10,000		NA	NA	13	470 - 900	105,000	12	10 - 4	8,000 - 16,000		NA	NA
Mercury		NA	NA	1	NA	NA	15	800	0 - 5,000	1	50 - 250	50 - 250	76	1	88	9	880	1,000	1	0.49	0.49
Nickel		NA	NA	1	10 - 4	10 - 4		NA	NA		NA	NA	11	11,030	53,210		NA	NA		NA	NA
Zinc		NA	NA	1	0 - 10,000	0 - 10,000	4	3,600	100 - 100,000		NA	NA	29	10	71,870	8	899	11,780,000		NA	NA
DDT	5	1	500	2	0.1 - 0.3	35	4	5	100		NA	NA	30	100	900	7	1	NA	1	25	25
PAH	3	10.97	37.97	11	0 - 12.5	4 - 200	2	247	276	2	17.6 - 602.8	17.2 - 906.1	15	90	12.5 - 500	8	10	900		NA	NA
PCBs		NA	NA	1	0.1	0.1		NA	NA		NA	NA	20	1,030	9,970	1	0.025 - 0.5	0.025 - 0.5		NA	NA

Notes:

n - Number of studies in database.

NA - Not Available

PAH - Polycyclic aromatic hydrocarbon

PCB - Polychlorinated biphenyl

All studies included, regardless of duration or effect

All data presented in parts per billion (ug/L)

No toxicity studies identified for ordnance and explosive compounds.

A lethal effect concentration was estimated for each species in each of the chemical data sets. To maintain the most robust data sets possible, studies of various durations and lifestages were included. Tests for any single species may include several test durations and lifestages of amphibians; no attempt was made to estimate the most sensitive lifestage. The geometric mean of all available LC_{50} values for each species was calculated and used to estimate the species mean acute value (SMAV).

Data were ranked from low to high, and the percentile for each concentration calculated as [100 * *i*/(n+1)], where *i* is the rank of the datum and n is the number of data points in the set. Log-normalized concentration data and the calculated concentration percentile were plotted, and linear regressions were performed. Appendix B presents all SMAVs and the regression analyses performed for the five chemicals.

As described in Appendix B, with the exception of the chronic/10th centile values for zinc, all thresholds calculated using the available amphibian mortality data are higher than their respective acute and chronic AWQC (Table 3-5).

Although there are considerable uncertainties associated with this approach (e.g., differences in test species, duration, exposure conditions, and general test methods can produce highly variable lethal (or sub-lethal) thresholds for any single chemical), evaluation of these thresholds indicates that amphibians may be sensitive to mercury and zinc contamination, and relatively insensitive to cadmium contamination. Amphibian thresholds were generally much higher than the AWQC; however, it is important to recognize that this evaluation considered only lethal effects data, and that the resulting values are not directly comparable to acute and chronic AWQC values. For instance, acute AWQC are based upon the 5th percentile of the SMAV or GMAV (not the 50th percentile), and chronic AWQCs are typically based upon the acute AWQC and an acute-to-chronic ratio. Additional detail regarding derivation of AWQC is presented in a variety of USEPA documents, including the *Guidelines for Deriving Numerical National Water Quality Criteria for Protection of Aquatic Organisms and Their Uses* (EPA 822/R-85-100) While the available data may not allow an amphibian-specific acute-to-chronic ratio to be derived, a default value (from the AWQC methodology) could potentially be used or an uncertainty factor could be applied depending upon site-specific circumstances.

It is possible that the results would differ markedly for sub-lethal effects data, or if exposure duration and life stage data were explicitly considered.

Due to the level of uncertainty inherent in the development of these screening level, literature-derived amphibian benchmarks, regulatory agencies may not accept these values in place of the AWQC or other promulgated standards.

3.3.1.2 Effects Levels Obtained from Y0817 Amphibian Toxicological Testing

Under an earlier phase of the Y0817 program evaluation, the Navy evaluated the toxicity of four metals (cadmium, copper, lead, and zinc) to larval amphibians exposed to sediment/ hydric soil in the laboratory (Appendix C). These activities resulted in a set of no observed effect concentrations and low observed effect concentrations (NOECs and LOECs) for both lethal and sub-lethal endpoints relative to analyte concentrations in amphibian tissue, sediment, and overlying water (total recoverable and dissolved fractions).

These NOECs and LOECs can be incorporated into the initial screening of the available site data. Sediment, water, or tissue concentrations above the laboratory–derived NOECs are likely to require additional investigation. Concentrations below the NOECs are unlikely to cause harm to the local amphibian population. Tables 3-6 and 3-7

Table 3-5

Comparison of Surface Water Screening Benchmarks to Calculated Centiles

Analyte (ppb)	Chronic Values		Acute Values	
	Chronic AWQC	Calculated 10th Centile	Acute AWQC	Calculated 50th Centile
Inorganics				
Cadmium	0.25	444	2	5,962
Copper	9	11.8	13	243
Mercury	0.77	1.52	1.4	54
Zinc	120	94	120	6,050
Organics				
DDT	0.001	107	1.1	1,594

Table 3-6
Summary of NOECs and LOECs – Lethal Endpoints

			Survival [1]	
Compound	**Matrix**	**Taxa** [2]	**NOEC**	**LOEC**
Cadmium	Sediment (mg/Kg)	*Rana*	580 – 760	>580 - 2600
		Bufo	580	>580
	Diss. Metal (mg/L)	*Rana*	1.1 - 1.1	>1.1 - 4.3
		Bufo	1.1	>1.1
	Total Metal (mg/L)	*Rana*	1.8 - 2.6	>1.8 - 7.2
		Bufo	1.8	>1.8
Copper	Sediment (mg/Kg)	*Rana*	64 - 200	>64 - >200
		Bufo	200	>200
	Diss. Metal (mg/L)	*Rana*	0.28 - 0.9	>0.28 - >0.9
		Bufo	0.9	>0.9
	Total Metal (mg/L)	*Rana*	0.39 - 1.2	>0.39 - >1.2
		Bufo	1.2	>1.2
Lead	Sediment (mg/Kg)	*Rana*	2000 - 2400	>2400 - 6100
		Bufo	2600	>2600
	Diss. Metal (mg/L)	*Rana*	0.27 - 0.48	>0.48 - 0.7
		Bufo	0.48	>0.48
	Total Metal (mg/L)	*Rana*	5.1 - 6.2	>6.2 - 17
		Bufo	6.2	>6.2
Zinc	Sediment (mg/Kg)	*Rana*	900 - 1200	>1200 - 1400
		Bufo	1200	2700
	Diss. Metal (mg/L)	*Rana*	3.0 - 5.2	>3.0 - 17
		Bufo	17	64
	Total Metal (mg/L)	*Rana*	3.9 - 6.3	>3.9 - 18
		Bufo	18	64

NOEC - No Observed Effect Concentration
LOEC - Low Observed Effect Concentration
[1] – Values obtained from SOP validation testing (presented in Appendix D).
Bufo tests were performed once for each compound. *Rana* tests were performed twice.
[2] - *Bufo* = American toad (*Bufo americanus*) and *Rana* = leopard frog (*Rana pipiens*)

Table 3-7
Summary of NOECs and LOECs - Sublethal Endpoints

			Growth [1]	
Compound	**Matrix**	**Taxa[2]**	**NOEC**	**LOEC**
Cadmium	Sediment (mg/Kg)	*Rana*	0.46 [3] - 580	510 - >580
		Bufo	0.32[a]	110
	Diss. Metal (mg/L)	*Rana*	0.011 [3] - 1.1	1.1 - >1.1
		Bufo	0.0025 [3]	0.16
	Total Metal (mg/L)	*Rana*	0.006 [3] - 1.8	2.6 - >1.8
		Bufo	0.0025 [3]	0.27
Copper	Sediment (mg/Kg)	*Rana*	64 - 200	>64 - >200
		Bufo	200	>200
	Diss. Metal (mg/L)	*Rana*	0.28 - 0.9	>0.28 - >0.9
		Bufo	0.9	>0.9
	Total Metal (mg/L)	*Rana*	0.39 - 1.2	>0.39 - >1.2
		Bufo	1.2	>1.2
Lead	Sediment (mg/Kg)	*Rana*	2000 - 2400	>2400 - 6100
		Bufo	2600	>2600
	Diss. Metal (mg/L)	*Rana*	0.27 -0.48	0.7 - >0.48
		Bufo	0.48	>0.48
	Total Metal (mg/L)	*Rana*	5.1 - 6.2	>6.2 - 17
		Bufo	6.2	>6.2
Zinc	Sediment (mg/Kg)	*Rana*	900 - 1200	>1200 - 1400
		Bufo	1200	2700
	Diss. Metal (mg/L)	*Rana*	3.0 - 5.2	>3.0 - 17
		Bufo	17	64
	Total Metal (mg/L)	*Rana*	3.9 - 6.3	>3.9 - 18
		Bufo	18	64

NOEC - No Observed Effect Concentration
LOEC – Low Observed Effect Concentration
[1] – Values obtained from SOP validation testing (presented in Appendix D).
Bufo tests were performed once for each compound. *Rana* tests were performed twice.
[2] - *Bufo* = American toad (*Bufo americanus*) and *Rana* = leopard frog (*Rana pipiens*)
[3] – NOEC concentrations for this test and endpoint are from the control treatment; LOEC concentrations are the lowest treatment containing added test material; some NOEC concentrations may be calculated using ½ the detection limit as a conservative measure.

present the NOECs and LOECs for the lethal and sublethal endpoints for both leopard frog (*Rana* (likely *pipiens*)) and American toad (*Bufo americanus)*. These values are based on a limited number of tests performed for each analyte/amphibian pair; site specific factors (e.g., total organic carbon) used in these tests were variable and may impact effects levels.

The effects levels summarized in Tables 3-6 and 3-7 tend to be elevated relative to literature-derived screening values (see Table 3-5**,** Appendix B). Use of these laboratory-derived benchmarks as absolute screening values is not advisable, since the testing protocol used to develop them may not be appropriate for all site-specific conditions and regulatory contexts**.** Site-specific toxicity tests should be considered when potential amphibian exposure pathways are identified.

3.3.2 Ambient Conditions Screening

Navy risk assessment policy (US Navy, 1999) requires consideration of background concentrations of both naturally occurring and anthropogenic chemicals. Under Navy ERA policy, (http://web.ead.anl.gov/ecorisk/), this background evaluation typically occurs during the Baseline Ecological Risk Assessment (BERA) (i.e., *Step 3a - Refinement of Conservative Exposure Assumption),* which is part of the Navy's Tier 2 ERA guidance. However, for the purposes of this amphibian risk assessment protocol, the background evaluation occurs after the initial effects-based screening, in order to refine the chemicals considered in the Tier II evaluation. Therefore, the Tier I Amphibian ERA Protocol includes elements of both the Navy's Tier 1 and Tier 2 ERA protocol.

The ambient conditions screen serves to further refine the list of chemicals requiring additional consideration. Chemicals present at levels below background are generally eliminated from the risk assessment process. It is recommended that consideration of background levels of constituents be discussed with state or federal agencies prior to sampling in order to reach consensus regarding appropriate comparisons.

Generally, if concentrations of constituents in sediments or surface water are consistent with background concentrations, no additional evaluation is necessary. If detected concentrations within the wetland are elevated above these values, additional Tier II evaluation may be recommended to further evaluate the potential impacts to wetland receptors.

Specific US Navy guidance for background screening can be found in the *Guidance for Environmental Background Analysis Volume I: Soil* (Naval Facilities Engineering Command, 2002) and *Guidance for Environmental Background Analysis Volume II: Sediment* (Naval Facilities Engineering Command, 2003). The Navy's final background policy is presented in *Navy Policy on the Use of Background Chemical Levels* (Naval Facilities Engineering Command, 2004). The Tier I protocol review of ambient conditions should consider both naturally occurring background levels of constituents, as well as anthropogenically-influenced "background" conditions.

3.4 Recommendations

The presence of potential habitat within the study area will dictate whether an evaluation of the available analytical data is necessary. If potential amphibian habitat does exist and ecological exposure pathways are potentially complete, available sediment or surface water data should be screened against appropriate ecological screening values. As part of the refinement step of the Tier I evaluation, additional comparisons to site-specific background data or available amphibian-specific benchmarks should be considered. Suggested literature-derived screening values were investigated and are presented in Tables 3-2 through 3-5 and Appendix B. In addition, the validation phase of this project resulted in a range of potential screening values based on laboratory toxicity testing performed with spiked sediments (Tables 3-6 and 3-7, Appendix D). Additional sources of Tier I screening values may be incorporated, as they become available.

There are a number of limitations associated with interpretation of the amphibian ecotoxicological literature summarized in Appendix B and the testing results summarized in Appendix D. Few data are available in the literature for many compounds, and there are no standard test organisms, duration, or study designs. In addition, the majority of the amphibian ecotoxicological literature summarized in Appendix B used surface water as the exposure medium. Therefore, use of these benchmarks as absolute screening values is not advisable, since the protocol used to develop them may not be appropriate for all site-specific conditions and regulatory contexts. None-the-less, the effects level developed for this Y0817 program may be useful as an additional information source to consider in the Tier I amphibian ecological risk assessment protocol

SECTION 4
TIER II REFINED EVALUATION

When the Tier I evaluation of habitat suitability and the initial media screening indicate potential risk of harm to the amphibian receptors, additional site-specific sampling and evaluation may be recommended. The need for additional sampling to evaluate potential risks to amphibians must be reviewed in terms of project-specific objectives. Additional data needs may include sampling and analysis of additional hydric soil or surface water samples from within the study area or appropriate background locations. Site-specific analytical requirements may include evaluation of chemistry in abiotic (i.e., hydric soil, sediment, and surface water) and biotic (i.e., amphibian tissue) matrices. Depending upon site-specific circumstances, collection of amphibian tissue for evaluating bioaccumulation, and collection of hydric soil or sediment for laboratory toxicity testing may also be required. It is possible to evaluate several different exposure scenarios (e.g., direct contact and food chain exposures) contemporaneously, so as to avoid duplication of efforts or project schedule delays.

4.1 Abiotic Media Sampling and Screening

Collection of additional abiotic media (e.g., hydric soil, sediment, and/or surface water) samples will permit the evaluation of recent data collected for specific use in an ecological risk assessment. Often, available historic data may not be collected from the most relevant portions of the Site, may have been analyzed with elevated detection limits or methodologies that introduce a level of uncertainty in the ecological risk assessment, and/or may not be temporally representative of current site conditions. Newly collected samples can be collected from the relevant surface soil/sediment stratum (generally no more than 0-15 centimeters, but region- and state-specific guidance should be consulted), with current analytical methodologies and detection limits low enough to achieve the objectives of the risk assessment. It is recommended that a Tier II Sampling and Analysis Plan (SAP) be prepared prior to sample collection to address specific sampling and analytical methods and concerns. The U.S. Army Corps of Engineers (USACE) Engineering Manual 200-1-3 (USACE, 2001) is one source for guidance on the preparation of DOD SAPs.

Recently collected abiotic analytical results can be compared against the sediment and surface water benchmarks presented in Sections 3.2 and 3.3. These data may also be evaluated in the ambient conditions screen, as described in Section 3.3.2. In addition, it may be possible to adjust some screening values to be more site-specific through the application of site-specific hardness values (for surface water) and total organic carbon (TOC) (for sediments). It is recommended that state and USEPA guidance and agencies be consulted during the development of the SAP to assure that consensus is reached prior to sampling.

It is also important to recognize that some bioaccumulative compounds may be of concern to higher trophic level organisms (i.e., consumers of amphibians), even when these constituents are present at low levels in abiotic media. Constituents included in the Binational Toxics Strategy (BNS) list of persistent, bioaccumulative, and toxic chemicals (PBTs) (USEPA GLNPO, 1999**),** and lists of bioaccumulative chemicals of concern (BCCs) (USEPA GLNPO, 1999; USEPA, 2000b) should be reviewed relative to site data and food chain concerns. Potential PBTs include, but are not limited to, dioxins, PCBs, DDE, DDD, DDT, organochlorine pesticides, selected inorganics (e.g., cadmium, and mercury), and chlorinated dibenzofurans.

Detected concentrations of these compounds should be discussed in the text of the risk assessment report, and retained for additional consideration if present at concentrations above screening values (or if no screening values are available).

If abiotic media concentrations are below screening benchmarks or consistent with background concentrations, no additional Tier II amphibian ecological risk assessment is necessary. However, if concentrations are detected at levels above screening values (or if no screening values are available), additional Tier II evaluation is required.

4.2 Amphibian Toxicity Testing

If a Tier II evaluation is required, laboratory toxicity tests may be recommended to evaluate site-specific bioavailability of chemical stressors within wetland portions on Navy sites. These samples can be collected concurrently with the abiotic samples collected for additional screening (Section 4.1). Additional analyses (e.g. simultaneously extracted metals and acid volatile sulfides (SEM/AVS)), may also be recommended at this time to assess the bioavailability of certain analytes (e.g. divalent metals). In cases where SEM/AVS analysis is conducted, it is important to assess whether or not the basic assumptions inherent in equilibrium partitioning theory are valid at a site. A dynamic equilibrium between pore water, sediment, and biota should not be assumed to exist in all seasonally inundated or saturated palustrine wetlands. Consideration of other factors which may affect hydric soil bioavailability should be considered at this stage in the ERA process. These factors may include the grain size of soil or sediment particles, the texture and composition of the matrix, total organic carbon, dissolved organic carbon, and various other binding phases. It is recommended that toxicity testing and SEM/AVS (or other bioavailability) samples be co-located with a sub-set of samples collected for the Tier II abiotic benchmark screening. Specific procedures for collection and analysis should be presented in the Tier II SAP.

Nearly all of the methods developed for conducting environmental toxicity tests are for water exposure, including effluent testing and testing the toxicity of specific chemicals. The importance of sediments and surface soils as potential contributors to environmental contamination has triggered the development of test procedures for evaluating soil and sediment toxicity, however, relatively few have been issued as standardized SOPs by ASTM or USEPA. The most recent USEPA and ASTM sediment test procedures were published in 2000 and 2001 (USEPA, 2000a; ASTM, 2001a). These methods are for an amphipod (*Hyalella azteca*), dipteran midge (*Chironomus tentans*), and oligochaete (*Lumbriculus variegatus*) and are not necessarily appropriate for evaluating wetland sites. Currently, USEPA and ASTM do not present standardized sediment test methods for amphibians.

However, some standardized amphibian toxicity test methods do exist. ASTM provides two methods that can use amphibians, one for ambient water samples and effluents (1192-97) and one for test materials (729-96) (ASTM, 2001b; and ASTM, 2001c). These methods are both intended for evaluating the exposure of amphibians in a liquid matrix. ASTM also publishes the guide for conducting the Frog-Embryo Teratogenesis Assay-Xenopus (FETAX) (ASTM, 2001d). This study procedure includes the exposure of African clawed frog (*Xenopus laevis)* embryos to a test solution to which some test material has been added. This method was developed as a water-only exposure, however many toxicological labs run this test with a sediment component. In addition, the USACHPPM, Health Effects Research Program and other researchers have recently developed several protocols for surface soil toxicity testing of terrestrial amphibians such as Plethodontid salamanders (Hall and Swineford, 1979; Johnson, 2003; Johnson and McAtee, 2000, Johnson et al., 1999 and 2000).

The USEPA Office of Prevention, Pesticides, and Toxic Substances (OPPTS) also presents guidance for conducting sediment tests with tadpoles (method OPPTS 850.1800; USEPA, 1996c). However, the guidance is intended for use when a sediment or slurry has been spiked with a chemical.

In response to the lack of available amphibian-hydric soil laboratory toxicity testing methods, the Navy has developed an amphibian test method that is applicable to the evaluation of environmental samples. This method is cost-effective enough that a large number of samples can be tested, if needed, and is consistent with already-existing procedures for sediment tests.

In addition, USFWS and others are developing amphibian toxicity testing methods that may become available in the near future. Field techniques for in situ testing with eggs, tadpoles or larvae have also been developed (Bishop and Martinovic, 2000) and may see more widespread use in the future.

4.2.1 SOP Development

Appendix C describes two experimental phases of Standard Operating Procedure (SOP) development, which are 1) Test Development and 2) Test Refinement. The goal of these experimental phases of this Y0817 project was to collect data necessary for the completion of a SOP for conducting sediment toxicity tests with amphibians. To achieve this goal, several factors were investigated, including:

- Organism handling and maintenance, including:
    - Holding conditions
    - Water type
    - Food
    - Temperature
- Acceptable control sediment
- Tolerance limits for ammonia
- Effects of various toxicants on tadpoles
- Most sensitive sublethal endpoint
- Most sensitive organism age
- Appropriate test length

These factors were investigated using two different anuran taxa in a series of studies conducted over several months. The test method developed by the Navy uses an early life stage of a native North American species, and lethal and sub-lethal toxicity endpoints that are relevant to typical ecological risk assessment endpoints. Attachment C-1 presents the SOP developed for the evaluation of sediment toxicity with early life stage amphibians.

Laboratory flow through system for amphibian toxicity testing.

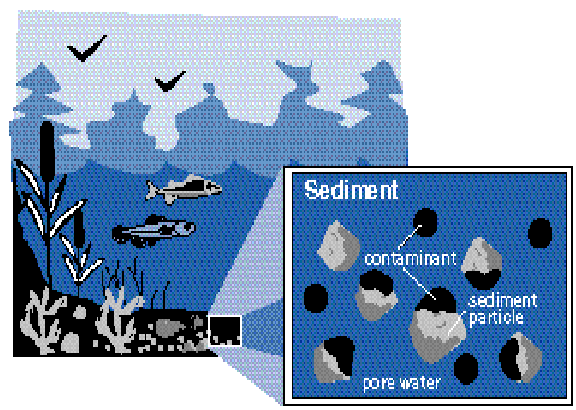
The SOP was validated by conducting the protocol with a number of spiked-sediments (Appendix D). In the validation phase, tadpoles of two North American anurans, *Rana* (likely *pipiens*) and *Bufo americanus* were used to assess the toxicity of copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn) in sediments. Natural sediment was amended with compost and then spiked with solutions containing salts of the four divalent metals of interest. As described in the SOP (Attachment C-1) the tests were conducted under flow-through conditions for 10 days and the biological endpoints measured at test termination were survival, body width, and body length.

The results of the validation phase indicated that the protocol performed well for evaluating potential impacts to early life stage amphibians. In general, the results of this phase of the YO817 study confirmed the results of the Phase I Literature Review (Section 3.2 and Appendix B), which suggested that relative to the toxicity testing endpoints evaluated herein, amphibian test thresholds were generally substantially higher than AWQC and other literature-derived benchmarks. However, as described in Section 3 of this document, direct comparisons of the derived screening values to AWQC is not appropriate, because the screening values are not directly comparable to acute and chronic AWQC values. The values derived from the toxicity testing are useful in screening for short-term effects to amphibians. If additional aquatic species (e.g. fish, aquatic invertebrates) are of concern, AWQC may be more appropriate.

In addition, it was observed that copper and zinc toxicity was strongly associated with the amount of organic carbon in the test. High levels of sediment organic carbon bind these metals, retaining them in the sediment and decreasing concentrations in the water column. This indicates that the level of organic carbon in the sediments at Navy sites may have a significant impact on the bioavailability and toxicity of constituents in the wetlands.

Depending on the size of the study area, it is recommended that the Tier II toxicity testing samples be co-located with a sub-set (or all) of the samples collected for the Tier II abiotic screen. Collection of SEM/AVS data at this time may also be recommended to assess the potential bioavailability of divalent metals in the wetland. If possible, it is useful for the toxicity testing samples to represent a range of constituent concentrations (i.e., a concentration gradient) to allow the development of No Observed Effects Concentrations (NOECs) based on testing results. These site-specific NOECs can be derived for analytes with no literature reported amphibian benchmarks and can be incorporated into the abiotic screening of historic or current data.

In addition to chemical analyses conducted for the abiotic screening, it is recommended that physical parameters (e.g. TOC, grain size) be analyzed for toxicity testing samples, and that at least one or more reference sample be collected and tested for toxicity also. Reference samples should represent locations un-impacted by site-related constituents, but with similar physical and geochemical characteristics. Selection of appropriate reference locations may involve consultation with state and/or USEPA agencies to assure that consensus is reached prior to sampling and testing. The results of study area samples may be statistically compared against the reference samples or laboratory control samples.



Equilibrium theory predicts that the concentration of the contaminant dissolved in the pore water is the concentration that is bioavailable and to which organisms may be exposed. Contaminants may bind to organic carbon in the sediment and be unavailable to potential receptors.

If wetland study area samples are significantly more toxic than reference samples and/or control samples, additional Tier II evaluation may be appropriate. If no toxicity is observed, constituents detected in the sediment may not be bioavailable and may not be impacting amphibian wetland receptors, indicating no need for further evaluation. If lethal or sub-lethal toxicity is observed, additional field surveys and/or bioaccumulation evaluations may be warranted.

A risk assessor or remedial project manager may want to consider collecting multiple lines of evidence as part of the ecological investigation rather than just collecting abiotic (i.e., chemical and physical data) and toxicity information. It may be useful and potentially cost-effective to conduct field surveys at the same time as the sediment collection to limit mobilization/demobilization costs and time needed to complete a baseline ecological risk assessment. Coordination of sampling efforts is best conducted during the amphibian breeding season, when most field surveys would be completed. Additionally, if the presence of potentially bioaccumulative contaminants has been observed and the size of the wetland is significant enough to provide substantial foraging habitat, then collection of amphibian tissue at the same time may be warranted.

4.3 Field Surveys

If Tier II abiotic screens and amphibian toxicity testing indicate potential risk, additional site-specific amphibian field studies may be warranted. These studies may include determining what amphibian species occur, the relative abundance of those species, and collecting and analyzing amphibian tissue. Amphibian field survey results may be compared relative to reference sites to determine if measured concentrations of chemicals in abiotic media are related or correlated with field observations.

The following text provides an outline of the standard techniques used during these inventories. In addition to the options presented here, other sources for bioassessment protocols may also be consulted and can be modified to address amphibians.

Field surveys may be incorporated into a Tier II evaluation.

4.3.1 Chorusing Surveys

During the breeding season, male anurans (i.e., frogs and toads) vocalize to attract potential mates. Therefore, under the right environmental conditions and within the correct timing, conducting calling surveys easily assesses their presence. According to the North American Amphibian Monitoring Program (NAAMP; http://www.mp2-pwrc.usgs.gov/naamp/) sampling during "good frog weather" for a particular region is critical. Environmental condition should be moist and humid, following a rain event, or during a light rain (heavy rain may interfere with hearing ability), and it should not be too windy. In addition, calling surveys should be conducted above minimum temperatures determined by the calling phenology of species in a given region (e.g., above 42°F to 55°F, depending on the time of year). According to a study in Massachusetts (Paton et al. 2001, *unpubl. data*) anurans native to that area exhibited highest calling frequencies within 4 hours after sunset. Therefore, surveys are most efficiently conducted during the evening.

Another efficient means of conducting calling surveys is to use a portable, automated recording device (i.e., frog-loggers). Dr. Michael Dorcas of the Savannah River Ecology Laboratory originally designed the frog-logger to monitor populations of western chorus frogs, southwestern toads, and Pacific chorus frogs in Utah and Idaho. The subsequent use of this device by other researchers has resulted in the detection of species otherwise thought to have been absent. For more information on this see http://www.uga.edu/srel/logger.htm, or Heyer et al. (1994).

The results of the chorusing survey can be used to evaluate the presence or absence of a reproductive population of anurans and can be used to evaluate the study area relative to reference locations.

4.3.2 Quantitative Sampling Techniques

There are a number of standardized techniques that have been developed to estimate relative abundance, species richness, or total breeding population size for amphibians. These include egg mass counts, dip netting, seining, trapping, and enclosure surveys.

Several species, for example wood frogs (*Rana sylvatica*) and spotted salamanders (*Ambystoma maculatum*), deposit large globular egg masses that are easy to identify and are relatively persistent in the environment (Klemens, 1993). Female wood frogs deposit one egg mass (Crouch and Paton, 2000), and female spotted salamanders deposit from one to four egg masses (Petranka, 1998), thus their egg masses provide an index to population size and annual breeding effort. Egg mass counts are easily conducted from within a breeding pond wearing chest waders.

Dip netting, seining, trapping, and enclosure surveys are useful methods for assessing densities of tadpoles and salamander larvae. Dip netting and enclosure surveys are most useful in shallow habitats exhibiting dense vegetation cover. To achieve quantitative results, researchers should standardize the number of dip net sweeps (e.g., based on pond size) or the duration of sampling. Captured tadpoles or salamander larvae will need to be temporarily removed from the pond or marked in some manner (see Heyer et al., 1994 for marking techniques). In addition, it is important to sample the various microhabitats within a pond because different species will utilize different niches within the pond. Seining is effective for habitats that are large, deep and have little vegetation cover. Total numbers of larvae may then be counted and densities calculated. Trapping techniques may be used in ponds with varying degrees of vegetation cover and depths. Use of a drift fence with pitfall traps in upland areas is often recommended for quantitative sampling of adults migrating to/from breeding habitat. Again for this method, the number of traps or the duration for which traps are deployed must be standardized and should be presented in a SAP. Results of these evaluations can be compared against local reference locations or other relevant databases.

These sampling methods can also be used to obtain sufficient amphibian tissue for bioaccumulation evaluations, if necessary. For detailed information on these and other methods, sources such as *Measuring and Monitoring Biological Diversity, Standard Methods for Amphibians* (Heyer et al., 1994) should be conducted. Table 3-1 also presents a list of additional data sources related to amphibians and their habitats.

4.4 Bioaccumulation Evaluations

Although the focus of this manual is on direct toxic impacts to amphibians, at certain sites it may be important to consider potential impacts to higher trophic level receptors that prey on amphibians. It is possible that bioaccumulative chemicals may impact higher trophic level organisms at levels that do not cause toxicity to amphibians. If the Tier II abiotic screen and toxicity testing indicate the potential for risk of harm to wetland receptors, the Tier II sample collection may also include the evaluation of site-specific tissue to evaluate bioaccumulation and potential impacts from exposure to constituents. Tissue collection procedures would be specified in the SAP, but may include sampling for tadpoles or adult amphibians**.** Although no standardized protocols currently exist, long term laboratory bioaccumulation tests can be designed to produce tissues to be analyzed for site-related constituents.

Results of the tissue analyses could potentially be compared relative to critical body residues (CBRs) obtained from the scientific literature. CBRs relate tissue concentrations with potential adverse impacts from exposure to chemicals.

No Observable Effects Dose (NOED) values are recommended as the primary CBR values. NOEDs indicate a body residue concentration at which no adverse effects were observed. The U.S. Army Corps of Engineers' (USACE) Environmental Residue Effects Database (ERED) (http://www.wes.army.mil/el/ered/) is recommended as the primary source of CBRs. The USEPA ECOTOX database **(**www.epa.gov/ecotox**)** is also a valuable source of aquatic toxicological results for many individual chemicals. This database provides chemical toxicity information from numerous peer-reviewed studies for toxicity testing to aquatic species. Additional CBR information can also be obtained from Niimi (1996) and Jarvinen and Ankley (1999), as well as other sources.

Unfortunately, considerable uncertainty is associated with amphibian CBR analysis, since CBRs may not be readily available for many amphibian species. A review of the ERED database in January 2004 indicated only four amphibian species (one salamander, two *Rana* species, and the African clawed frog (*Xenopus*)) listed with a maximum of fourteen chemicals evaluated for a single species. This review indicates that CBR data in the current literature is generally not sufficient to warrant comparisons at this time.

Values for fish may be extrapolated to amphibians, but this adds uncertainty to the risk assessment and should be done with caution due to the broad range of sensitivities between fish and amphibians (Birge, et al., 2000). The results of the SOP validation portion of this Y0817 project resulted in a range of tissue concentrations for both *Rana* and *Bufo* species correlated with no and low observed effects for survival and growth. These values (presented in Table 4-1 and Appendix D) may be used as CBRs for cadmium, copper, lead, and zinc.

Tissue concentrations in excess of available CBRs indicate potentially adverse impacts to amphibian receptors in the wetland. Additional field evaluations or response actions may be warranted for sites where this condition is observed.

Table 4-1
Critical Body Residues Developed During SOP Validation

		Survival [1]	
Compound	**Taxa**	**NOEC**	**LOEC**
LETHAL ENDPOINT - Survival			
Cadmium	*Rana*	47 - 110	>47 - 260
	Bufo	200	>200
Copper	*Rana*	16 - 79	>16 - >79
	Bufo	93	>93
Lead	*Rana*	700 - 870	>870 -1600
	Bufo	620	>620
Zinc	*Rana*	240 - 300	>240 - 310
	Bufo	250[b]	170[b]
		Growth [1]	
Compound	**Taxa**	**NOEC**	**LOEC**
SUBLETHAL ENDPOINTS - Length & Width			
Cadmium	*Bufo*	0.25[a]	28
	Rana	0.8[a] - 47	>47 - 110
Copper	*Bufo*	93	>93
	Rana	16 - 79[d]	>16 - >79[d]
Lead	*Bufo*	620	>620
	Rana	700 - 870	>870 - 1600
Zinc	*Bufo*	250[b]	170[b]
	Rana	240[c] - 300	>240[c] - 310

All tissue concentrations presented in mg/kg on a wet weight basis.
NOEC - No Observed Effect Concentration
LOEC - Low Observed Effect Concentration
1 - Values obtained from SOP validation testing (presented in Appendix D).
Bufo tests were performed once for each compound. *Rana* tests were performed twice.
a - NOEC concentrations for this test and endpoint are from the control treatment; LOEC concentrations are the lowest treatment containing added test material; some NOEC concentrations may be calculated using ½ the detection limit.
b - Measured tissue concentrations of zinc actually decreased with increasing exposure concentrations, therefore, the tissue LOEC is actually less than the NOEC.
c - Measured tissue concentration in the high treatment was 240 mg/Kg Zn. However, the highest body burden was in the second highest test concentration at 270 mg/Kg Zn.
d - Measured tissue concentration in the high treatment was 79 mg/Kg Cu.
However, the highest body burden was in the second highest test concentration at 80 mg/Kg Cu.

SECTION 5
SUMMARY

Wetland habitats may often form a significant amount of open space in the vicinity of CERCLA sites at Naval facilities. Wetlands at Navy facilities are also prime habitat for various amphibian species. Amphibians play a key ecological role in palustrine wetlands, serving as an important food source for higher trophic level receptors, and as a major consumer of prey items. However, because of the limited availability of chronic exposure amphibian ecotoxicity data, environmentally acceptable endpoints for current CERCLA and other environmental investigations are often based on data from aquatic species that may not be typical of the wetland in question. Species such as fathead minnow and daphnids are often inappropriately used to make key ecological risk-based management decisions at Navy sites**,** as these species may not be representative of site conditions.

The ecological risk assessment process described in this guidance manual attempts to address the need to more accurately represent exposure of amphibians to constituents within the wetlands. While initial, conservative Tier I evaluations against existing benchmarks may eliminate some constituents, it is likely that some amphibian risk evaluations will proceed to the Tier II protocol evaluation described in Section 4.0. Tier II evaluations can include the collection and evaluation of new abiotic media, and/or evaluation of site-specific toxicity testing, tissue analysis, and field survey data to more accurately evaluate the impacts to the amphibian population from potential exposure to contaminants in the wetland. If no impacts are identified through the Tier II protocol evaluation, then no additional ecological evaluation is necessary. Additional evaluation or remediation may be necessary if amphibian populations appear to be adversely impacted by site-related constituents in the wetland.

Evaluation and remediation of contaminated Navy sites involves a determination of remedial cleanup goals, including identification of contaminant concentrations that are protective of ecological resources. Pursuant to Department of Defense (DOD) guidance, ecological risk-based cleanup goals are typically developed using methodologies that have technical and social foundations. Development of risk-based cleanup goals involves complex risk management decision making. Perhaps the most complex decisions entail balancing the trade-off between destructive and costly remediation and leaving residual contamination in place. This trade-off is important in wetland environments, which often serve as a "sink" for environmental contamination. Considerable attention has been paid in recent years to wetland losses in our nation; however, remediation of wetlands is environmentally destructive and costly. It has even been demonstrated that remediation of certain wetlands involves destruction of wetland habitat, while only providing minimal risk reduction. Use of the protocols described in this manual will help the Navy and other interested parties make informed risk management decisions with regard to protecting native amphibians in wetland habitats.

SECTION 6
LITERATURE CITED

Altig, R. and P. H. Ireland, 1984. A key to salamander and larviform adults of the United States and Canada. Herpetologica. 40(2):212-218.

American Society for Testing and Materials (ASTM). 2001a. Test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates. E 1707-00. pp. 1109-1225 *in* Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides.

American Society for Testing and Materials (ASTM). 2001b. Standard guide for conducting acute toxicity tests on aqueous ambient samples and effluents with fishes, macroinvertebrates, and amphibians. E 1192-97. pp. 405-417 *in* Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides.

American Society for Testing and Materials (ASTM). 2001c. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians . E 729-96. pp. 175-196 *in* Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides.

American Society for Testing and Materials (ASTM). 2001d. Standard guide for conducting the frog embryo teratogenesis assay-Xenopus (FETAX). E 1439-98. pp. 776-791 *in* Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides.

Behler J.L. and F.W. King, 1995. National Audubon Society Field Guide to North American Reptiles and Amphibians. Chanticleer Press, Inc. New York.

Birge, W.J., A.G. Westerman, and J.A. Spromberg 2000. Comparative Toxicology and Risk Assessment of Amphibians. *In:* Sparling, D.W., G. Linder and C.A. Bishop, editors. 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Bishop, C.A. and B. Martinovic 2000. Guidelines and Procedures for Toxicological Field Investigations Using Amphibians and Reptiles. *In:* Sparling, D.W., G. Linder and C.A. Bishop, editors. 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Burne, M. R. 2001. Massachusetts aerial photo survey of potential vernal pools. Massachusetts Natural Heritage and Endangered Species Program. Westborough, Massachusetts, USA.

Calhoun, A. J. K. 1997. Maine citizen's guide to locating and describing vernal pools. Maine Audubon Society, Falmouth, ME.

Calhoun, A. J. K. and M. W. Klemens. 2002. Best development practices: Conserving pool-breeding amphibians in residential and commercial developments in the northeastern United States. MCA Technical Paper No. 5, Metropolitan Conservation Alliance, Wildlife Conservation Society, Bronx, New York.

Corn, P.S. 2000. Amphibian declines: Review of some current hypothesese. *In:* Sparling, D.W., G. Linder and C.A. Bishop, editors. 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Dahl, T. E. 1990. Wetlands: Losses in the United States -1780's to 1980's. U.S. Fish and Wildlife Service, National Wetlands Inventory. St. Petersburg, Florida http://www.nwi.fws.gov/bha/

DAPTF, 2001. Declining Amphibians Populations Task Force http://www.open.ac.uk/daptf/

Dumont, James. PhD. 2001. The effects of ammonium perchlorate on reproduction and development of amphibians. SERDP Bulletin, Cleanup CU-1236.

Egan, R. S. 2001. Within-pond and landscape-level factors influencing the breeding effort of *Rana sylvatica* and *Ambystoma maculatum.* Thesis, University of Rhode Island, Kingston, RI, USA.

Egan, R. S. and P. W. C. Paton. 2004. Within-pond parameters affecting oviposition by wood frogs and spotted salamanders. Wetlands 24:1-13.

Eisler, R. 1985. Cadmium hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service, Biological Science Report 85(1.2).

ENSR, 2001. Calendar Year 2000 Report: Turtle Investigation Program. Naval Air Station South Weymouth, Weymouth, Massachusetts. April 2001.

Formanowicz, D. R. and M. S. Bobka. 1989. Predation risk and microhabitat preference: an experimental study of the behavioral responses of prey and predator. American Midland Naturalist 121:379-386.

Hall, B., N.S. Bloom, J. Munthe, 1995. An experimental study of tow potential methylation agents of mercury in the atmosphere: CH3I and DMS, Water, Air, and Soil Pollution, 80, 337-341, 1995.

Hall, R.J. and D. Swineford. 1979. Uptake of methoxychlor from food and water by the American Toad (*Bufo americanus*). Bull. Environ. Contam. Toxicol. 23:335-337.

Hem, J.D. 1985. Study and Interpretation of the Chemical Characteristics of Natural Waters. Third Edition. U.S. Geological Survey Water Supply Paper 2254.

Henry, P.F.P. 2000. Aspects of Amphibian Anatomy and Physiology. ***In:*** Sparling, D.W., G. Linder and C.A. Bishop, editors. 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster, editors, 1994. Measuring and monitoring biological diversity: standard methods for amphibians.

Hunt, R. J. 1996. Do Created Wetlands Replace the Wetlands that are Destroyed? Fact Sheet. U.S. Geological Survey. Madison, WI. http://wi.water.usgs.gov/pubs/FS-246-96/

James, S.M. and E.E. Little. 2002. An Assessment of Multiple Routes of Cadmium Exposure using the American Toad (Bufo americanus). In: Symposium on Multiple Stressor Effects in Relation to Declining Amphibian Populations. Sponsored by ASTM Committee E47 on Biological Effects and Environmental Fate. April 16-17, 2002. Pittsburgh, PA.

Jarvinen, Alfred W. and Gerald T. Ankley. 1999. Linkage of Effects to Tissue Residues: Development of a Comprehensive Database for Aquatic Organisms Exposed to Inorganic and Organic Chemicals. SETAC Press.

Johnson, M.S. 2003. Evaluation of 2,4-DNT and RDX exposures to the red-backed salamander (*Plethodon cinereus*). Presented at the TSERAWG Meeting, September 9, 2003. San Antonio, TX.

Johnson, M.S. and M.J. McAtee. 2000. Wildlife Toxicity Assessment for 2,4,6-Trinitrotoluene. U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM). Project Number 39-EJ-1138-00, Aberdeen Proving Ground, Maryland. October.

Johnson, M.S., L.S. Franke, R.B. Lee, and S.D. Holladay. 1999. Bioaccumulation of 2,4,6-trinitrotoluene and polychlorinated biphenyls through two routes of exposure in a terrestrial amphibian: is the dermal route significant? Environ. Toxicol. Chem. 18(5):873-878.

Johnson, M.S., S.D. Holladay, K.S. Lippenholz, J.L. Jenkins, and W.C. McCain. 2000. Effects of 2,4,6-trinitrotoluene in a holistic environmental exposure regime to a terrestrial salamander: *Ambystoma tigrinum*. Toxicological Pathol. 28(2):334-341.

Klemens, M. W. 1993. Amphibians and reptiles of Connecticut and adjacent regions. Bulletin Number 112. State Geological and Natural History Survey of Connecticut, New Haven, CT, USA.

Lehtinen, R. M., S. M. Galatowitsch, and J. R. Tester. 1999. Consequences of habitat loss and fragmentation for wetland amphibian assemblages. Wetlands. 19(1):1-12.

Linder, G. 2000. Ecotoxicology of Metals in Amphibians. *In:* Sparling, D.W., G. Linder and C.A. Bishop, editors. 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Linder, G.L., S.K. Krest and D.W. Sparling. 2003. Amphibian Decline: An integrated analysis of multiple stressor effects. Pensacola, FL, USA: Society of Environmental Toxicology and chemistry (SETAC).

Long, E.R. and L.G. Morgan. 1990. The Potential for Biological Effects of Sediment-Sorbed Contaminants Tested in the National Trends and Status Program. NOAA Technical Memorandum No. 57. NOAA/TM/NOS/ OMA 52. Seattle, WA.

MacDonald, D.D., Ingersoll, C.G. and T.A. Berger. 2000. Development and Evaluation of Consensus-Based Sediment Quality Guidelines for Freshwater Ecosystems. Arch. Environ. Contam. Toxicol. 39:20-31.

Martof, B. S., W. M. Palmer and J. R. Harrison. 1980. Amphibians and reptiles of the Carolinas and Virginia. The University of North Carolina Press, Chapel Hill, NC.

McDiarmid, R. W. 1994. Reptiles and Amphibians. *In:* Our Living Resources National Biological Service A Report to the Nation on the Distribution, Abundance, and Health of U.S. Plants, Animals, and Ecosystems. U.S. Department of the Interior, U.S. Fish and Wildlife Service. http://biology.usgs.gov/s+t/noframe/d906.htm

McDiarmid, R. W. and R. Altig, editors. 1999. Tadpoles: the biology of anuran larvae. The University of Chicago Press, Chicago 60637.

Meffe, G.K. and C.R. Carrol, 1997. Principles of Conservation Biology, 2nd Edition. Sinauer Associates, Inc. Sunderland, MA. USA.

Merchant, H., 1972. Estimated population size and home range of the salamanders *Plethodon jordani* and *Plethodon glutinosus.* Journal of the Washington Academy of Sciences, 62:248-257.

Moriarty J. and Bauer A.M. 2000. State and Provincial Amphibian and Reptile Publications for the United States and Canada. SSAR Herpetological Circular 28. 56 pp.

Mullarkey, D. and R.J. Bishop, 1995. Towards Assessing the Validity of Contingent Valuation of Wetlands. *In:* C. Colgan (ed) Sustaining Coastal Resources. Univ. of S. Maine. 1995

Murphy, J.E., C.A. Phillips, V.R. Beasley, 2000. Aspects of Amphibian Ecology. *In:* Sparling, D.W., G. Linder and C.A. Bishop, editors. 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Naval Facilities Engineering Command, 2002. Guidance for Environmental Background Analysis Volume I: Soil. NFESC User's Guide, UG-2049-ENV. April 2002.

Naval Facilities Engineering Command, 2003. Guidance for Environmental Background Analysis Volume II: Sediment. NFESC User's Guide, UG-2054-ENV. April 2003.

Naval Facilities Engineering Command, 2004. Navy Policy on the Use of Background Chemical Levels. January 30, 2004.

Niimi, A.J. 1996. PCBs in Aquatic Organisms. Environmental Contaminants in Wildlife, Interpreting Tissue Concentrations. Eds. W. Nelson Beyer, Gary H. Heinz, Any W. Redmon-Norwood. SETAC Special Publications Series. Lewis Publishers.

Paton, P. W. C. and W. B. Crouch III. 2002. Using phenology of pond-breeding amphibians to develop conservation strategies. Conservation Biology 18:194-20

Paton et al., unpubl. data. 2001. Dr. Peter Paton, Department of Natural Resources Science, University of Rhode Island, USA.

Pauli, B.D., J.A. Perrault, and S.L. Money, 2000. RATL: A Database of Reptile and Amphibian Toxicology Literature. National Wildlife Research Centre 2000, Canadian Wildlife Service, Environmental Conservation Branch. Technical Report Series Number 357.

Persaud, D., R. Jaagumagi, and A. Hayton. 1996. Guidelines for the Protection and Management of Aquatic Sediment Quality in Ontario, Ontario Ministry of the Environment, Queen's Printer for Ontario; 23 pp.

Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington and London.

Rabb, G. B. 1999. The Amphibian Decline Phenomenon. The Amphibian Crisis. Chicago Zoological Society. Brookfield, IL. http://elib.cs.berkeley.edu/aw/declines/rabb.html

Renner, R. 2002. Amphibian Declines: Conflict Brewing Over Herbicide's Link to Frog Deformities." Science. 938-939.

Rowe, C.L. and J. Freda, 2000. Effects of Acidification on Amphibians at Multiple Levels of Biological Organization. *In:* Sparling, D.W., G. Linder and C.A. Bishop, editors. 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Semlitsch, R. D. 2000. Principles for management of aquatic-breeding amphibians. Journal of Wildlife Management 64:615-631.

Skidds and Golet, 2002. Rapid assessment of hydroperiod in seasonal ponds of the pawcatuck river watershed. Final report for Rhode Island field office, The Nature Conservancy.

Solomon, K.R., J.M. Giddings, and S.J. Maund, 2001. Probabilistic Risk Assessment of Cotton Pyrethroids: I. Distributional Analyses of Laboratory Aquatic Toxicity Data, Envir. Tox. and Chem., Vol 20, N0. 3, pp. 652-659.

Sparling, D.W., 2000. Ecotoxicolgy of Organic Contaminants to Amphibians. *In:* Sparling, D.W., G. Linder and C.A. Bishop, editors. 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Sparling, D.W., G. Linder and C.A. Bishop, 2000a. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Sparling, D.W., C.A. Bishop, and G. Linder, 2000b. The Current Status of Amphibian and Reptile Ecotoxocological Research. *In:* Sparling, D.W., G. Linder and C.A. Bishop, editors. 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Stone, J. 1992. Vernal pools in Massachusetts: aerial photographic identification, biological and physiographic characteristics, and state certification. Master's Thesis, University of Massachusetts, Amherst.

Suter, G.W. and C.L. Tsao, 1996. Toxicological Benchmarks for Screening Potential Contaminants of Concern for Effects on Aquatic Biota: 1996 Revision. Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. ES/ER/TM-96/R2.

Talmage, S.S., D.M. Opresko, C.J. Maxwell, C.J.E. Welsh, F.M. Cretella, P.H. Reno, and F.B. Daniel, 1999. Nitroaromatic Munition Compounds: Environmental Effects and Screening Values. Rev. Environ. Contam. Toxicol. 161:1-156.

Ultsch, G.R., D.F. Bradford and J. Freda. 1999. Physiology: Coping with the Environment. R.W. McDiarmid and R. Altig, editors. 1999. Tadpoles: The Biology of Anuran Larvae. The University of Chicago Press. Chicago, Illinois.

U.S. Army Corps of Engineers (USACE), 2001. Requirements for the Preparation of Sampling and Analysis Plans. Engineering Manual. EM 200-1-3. February.

U.S. Department of Agriculture (USDA), Soil Conservation Service. 1991. Hydric Soils of the United States. Third Edition. In cooperation with the National Technical Committee for Hydric Soils. USDA-SCS, Washington, DC.

USEPA, 1992. Framework for Ecological Risk Assessment. Risk Assessment Forum. EPA/630/R-92/001.

USEPA, 1993a. Technical Basis for Deriving Sediment Quality Criteria for Nonionic Organic Contaminants for the Protection of Benthic Organisms by Using Equilibrium Partitioning. Office of Water, Washington, D.C. EPA/822-R-93-011.

USEPA, 1993b. Water quality guidance for the Great Lakes System and correction; Proposed rules. Federal Register. 58(72):20802-21047.

USEPA, 1996a. 1995 Updates: Water Quality Criteria Documents for the Protection of Aquatic Life in Ambient Water. Office of Water. EPA 820-B-96-001. September 1996.

USEPA, 1996b. Calculation and Evaluation of Sediment Effect Concentrations for the Amphipod *Hyalella azteca* and the Midge *Chironomus riparius*. Great Lakes National Program Office, Region V. Chicago, Illinois. EPA 905/R96/008 April 1996.

USEPA, 1996c. Ecological EffectsTest Guidelines. OPPTS 850.1800. Tadpole/sediment subchronic toxicity test. EPA 712-C-96-132. Office of Prevention, Pesticides, and Toxic Substances.

USEPA, 1997. Ecological Risk Assessment Guidance for Superfund, Process for Designing and Conducting Ecological Risk Assessments (Interim Final). U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Office of Emergency and Remedial Response. EPA 540/R-97/006.

USEPA, 1998. Guidelines for Ecological Risk Assessment. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. EPA/630/R-95/002F. April, 1998.

USEPA, 2000a. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. Second Edition. EPA/600/R-99/064. Office of Water.

USEPA, 2000b. Bioaccumulation Testing and Interpretation for the Purpose of Sediment Quality Assessment: Status and Needs EPA/823/R-00-001 and Appendix EPA/823/R-00-002. Office of Water, Washington, D.C.

USEPA, 2001. The Role of Screening-Level Risk Assessments and Refining Contaminants of Concern in Baseline Ecological Risk Assessments. ECO UPDATE. Interim Bulletin Number 12. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response.

USEPA, 2002. National Recommended Water Quality Criteria : 2002. Office of Water. EPA 822-R-02-047. November 2002.

USEPA GLNPO, 1999. Draft Report on Level 2 Substances in The Binational Toxics Strategy: A U.S. Perspective. USEPA Great Lakes National Program Office December 1, 1999.

USFWS, 2003. Threatened and Endangered Species System. U.S. Listed Vertebrate Animal Species Report by Taxonomic Group. http://ecos.fws.gov/servlet/TESSWebpage Updated April 15, 2003.

USGS, 2000. Where Have All the Frogs Gone? *Research May Solve the Puzzle.* U.S. Department of the Interior, U.S. Geological Survey, Reston, VA, USA URL http://www.usgs.gov/amphibian_faq.html

US Navy, 1999. Navy Policy for Conducting Ecological Risk Assessments. Issued by the Office of the Chief of Naval Operations April 5, 1999. Available at http://web.ead.anl.gov/ecorisk/policy/index.cfm

Wenning RJ, Ingersoll CG. 2002. Summary of the SETAC Pellston Workshop on Use of Sediment Quality Guidelines and Related Tools for the Assessment of Contaminated Sediments; 17-22 August 2002; Fairmont, Montana, USA. Society of Environmental Toxicology and Chemistry (SETAC). Pensacola FL, USA.

Wilen, B.O. 2001. The Nation's Wetlands. *In:* Our Living Resources National Biological Service A Report to the Nation on the Distribution, Abundance, and Health of U.S. Plants, Animals, and Ecosystems. U.S. Department of the Interior, U.S. Fish and Wildlife Service. http://biology.usgs.gov/s+t/noframe/m3128.htm

APPENDIX A

EXAMPLE FIELD EVALUATION FORMS

The field evaluation forms included in this appendix are representative regional forms and checklists. These and other similar field evaluation forms provide a mechanism to document:

- rare species observation(s);
- wetland community types;
- vernal pool presence/absence;
- ecological community surveys;
- aquatic special animal surveys; and
- native species surveys.

For more information or to obtain state-specific forms, contact the project locus state or regional Natural Heritage Program. Program information and forms can be found at the NatureServe Local Program website: http://www.natureserve.org/visitLocal/usa.jsp.

REPRESENTATIVE FORMS FOR THE NORTHEAST REGION

NatureServe

Member Program

New Jersey Natural Heritage Rare Species Reporting Form

This form is used to report a personal field sighting of a rare species tracked by the Natural Heritage Database. It may also be used to summarize locational information from a published or unpublished report. Species tracked include those appearing on the Special Plants of New Jersey List and the Special Animals of New Jersey List. The Office of Natural Lands Management can provide copies of the lists upon request. Note: For anadromous fish species, only reports of spawning areas are requested. For most bird species, only breeding reports are requested. Consult the Endangered and Nongame Species Program to determine if a non-breeding report of a bird species is desired.

In order for this form to be processed, the sections preceded by an asterisk (*) must be completed.

Send completed form to : DEP - Division of Parks and Forestry, Office of Natural Lands Management, Natural Heritage Program, PO Box 404, Trenton, NJ 08625-0404. Forms for endangered and nongame wildlife will be forwarded to the Endangered and Nongame Species Program for review.

Common Name____

***Scientific Name**____

Today's Date____

Location:

***Location Map:** A mapped location of the occurrence must accompany this form. The ideal format is to locate the site on a photocopied section of a USGS 7.5 minute topo map, and to also sketch a second map showing finer details. Be sure to provide the name of the USGS map.

***Directions to Site:** Describe how to get to the site from a readily relocated permanent landmark such as a road intersection.

____

____

____

____

Biology/Habitat:

*Date and Approximate Time of the Observation:

____

Weather Conditions (animal reports):

clear ____overcast ____calm ____windy____

Describe temperature, precipitation, and other significant weather factors:

____

____

____

Identification: How was the species identification made? Was it based on a sighting, tracks, call, or road kill? Name the identification manuals used or the experts consulted. Were there identification problems?

____

____

____

***Number of Individuals Observed:**

1-10 ____ 11-50 ____ 51-100 ____ 101-1000 ____ 1001-10,000 ____ >10,000____

If possible, provide the exact number of individuals. For rhizomatous plants such as grasses and sedges, what was counted as an individual - separate culms or entire clumps or patches?

____

____

____

Life Stages Present: Check off life stages observed or provide an estimate of the numbers of individuals for each life stage.

For plants:

vegetative ____ in bud ____ flower ____ fruit____

seed dispersing ____ seedling ____ dormant ____

For animals:

eggs ____ larvae ____ immature _____ adult female ____

adult male ______ adult, sex unknown ____

Associated Species: List any associated species such as predators, prey, food plants, parasites, host species, and additional rare species observed at the site.

____

____

____

____

***Additional Biological Data:** What else was observed? Provide information on the general condition or vigor of the individuals and viability of the population, and animal behavior such as mating or nesting behavior.

____

____

____

____

____

Habitat Data: Describe the general area where the occurrence is located. List natural community types, dominant vegetation, and information on the physical environment such as substrate type, hydrology, moisture regime, slope, and aspect. Also, if possible, provide information on the surrounding land use.

____

____

____

____

____

Conservation: Are there natural or man made threats to this occurrence? Please describe.

____

____

Ownership: If known, please provide landowner name, address, phone #.

Information Source:*Name and Address and Phone # (of person filing report):

*Does this information come directly from a field visit ____, or a published or unpublished report?____

Citation: For information taken from a published or unpublished report, please provide a copy of the cover page and the pertinent portions of the report. If a copy can not be provided, list below the author, date, title, publisher, and page numbers.

Voucher: Was the observation vouchered with a photograph?____ a specimen?____ If possible, attach a copy of the photograph. If specimen voucher, please provide the name of the repository:

Confirmation: Would you accompany a biologist to the site if needed? ____yes ____no.

Additional Comments: (use extra sheets if needed)

Revised 9/98

Table 4 — HABITAT ASSESSMENT FOR *HIGH* GRADIENT STREAMS

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	40-70% mix of stable habitat; well suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Velocity/Depth Regimes	All 4 velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (slow is <0.3 m/s, deep is >0.5 m)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity / depth regime (usually slow-deep).
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yrs.) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. In stream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank) Note: determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Bank Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, under story shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

HABITAT SCORES	VALUE
OPTIMAL	160 - 200
SUB-OPTIMAL	110 - 159
MARGINAL	60 - 109
POOR	< 60

Table 4 (cont.) — HABITAT ASSESSMENT FOR *LOW* GRADIENT STREAMS

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/Available Cover	Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	30-50% mix of stable habitat; well suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Pool Substrate Characterization	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Pool Variability	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% <20% for low-gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and beads; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yrs.) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. In stream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Channel Sinuosity	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 2 to 1 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank)	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable: 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Bank Vegetative Protection (score each bank) Note: determine left or right side by facing downstream.	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, under story shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

HABITAT SCORES	VALUE
OPTIMAL	160 - 200
SUB-OPTIMAL	110 - 159
MARGINAL	60 - 109
POOR	< 60

Source: Habitat Assessment for Low Gradient Streams, New Jersy DEP, April 2003, http://www.state.nj.us/dep/wmm/bfbm/appendix/habitat.html

APPENDIX E: BLANK VERNAL POOL IDENTIFICATION FORM

PART A. VERNAL POOL IDENTIFICATION

I. GENERAL INFORMATION

Observer: ________________ Date: ________________

Time of day: ______________ Weather: ______________

Photos: Yes_____ No_____ Visit #: ______________

POOL NUMBER: ____________ **LOCATION:** ______________________

MAP: NWI/USGS quad____________ Assessor's Map & Lot # _______________

II. POOL CHARACTERISTICS

Pool type: ☐ temporary ☐ permanent but fishless

☐ artificial ☐ natural

Size: average width_____ average length_____

Water level: ☐ full ☐ half full ☐ one quarter full ☐ less ____ (%)

Water depth: max when observed ______ estimated spring max ______

Hydroperiod (if known): full (date) _______ dry (date) ________

Optional (if known): **Water Temperature** _______° (indicate F° or C°) **pH** ________

Cover: Plant material in the pool

(Put a check mark or write in rough % of area covered by each type.)

_____ Branches, twigs (in pool or touching pool surface)

☐ < 5% ☐ 5-25% ☐ 26-50% ☐ 51-75% ☐ 76-100%

_____ Shrubs

☐ < 5% ☐ 5-25% ☐ 26-50% ☐ 51-75% ☐ 76-100%

_____ Emergent vegetation (e.g., grasses, cattails)

☐ < 5% ☐ 5-25% ☐ 26-50% ☐ 51-75% ☐ 76-100%

_____ Floating vegetation (e.g., water lilies, duckweed)

☐ < 5% ☐ 5-25% ☐ 26-50% ☐ 51-75% ☐ 76-100%

_____ Submergent vegetation

☐ < 5% ☐ 5-25% ☐ 26-50% ☐ 51-75% ☐ 76-100%

_____ Other

☐ < 5% ☐ 5-25% ☐ 26-50% ☐ 51-75% ☐ 76-100%

Dominant wetland plants (if known):

Source: (Calhoun, 1997)

III. INDICATOR SPECIES STATUS

Record the estimated number of each or place a check mark in box where present.

INDICATOR SPECIES	adult	vocalization	amplexus	spermatophores	egg masses	tad/larvae	juvenile
wood frog							
spotted salamander							
blue-spotted salamander							
fairy shrimp							

IV. INDICATOR SPECIES VERIFICATION – Check all boxes that apply.

	Heard or seen	Identified in hand	Photographed
wood frog			
spotted salamander			
blue-spotted salamander			
fairy shrimp			

V. FACULTATIVE SPECIES STATUS

Record the estimated number of each or place a check mark in box where present.

FACULTATIVE SPECIES	adult	vocalization	amplexus	spermatophores	egg masses	tad/larvae	juvenile
eastern newt							
four-toed salamanders							
spring peeper							
gray tree frog							
green frog							
American toad							
painted turtle							
spotted turtle							
wood turtles							
Blanding's turtle							
snapping turtle							
fingernail clams							
amphibious snails							
caddisflies							

VI. COMMENTS/OBSERVATIONS OF OTHER WILDLIFE SPECIES

Please attach an additional sheet with your comments.

PART B. VERNAL POOL SETTING

I. SITE TYPE (Check one)

- ❑ upland-isolated (pool not part of larger wetland)
- ❑ bottomland-isolated (pool in a floodplain; not part of a larger wetland)
- ❑ wetland complex (pool associated w/ larger wetland habitat)

II. HABITAT AROUND THE POOL (within 200' buffer)
Estimate % of each and note compass direction.

______ woodland ❑ hardwoods (> 75% deciduous) ❑ softwood (> 75% evergreen) ❑ mixed (all others)
______ agriculture or open fields
______ gravel pit
______ residential
______ roadside
______ other

III. BORDERING OVERSTORY VEGETATION (Check one)

- ❑ heavy overstory, > 50% trees/shrubs > 5' tall
- ❑ moderate overstory, < 50% trees/shrubs > 5' tall
- ❑ open site (grasses, forbs, scattered shrubs) < 5' tall

IV. LEVEL OF DISTURBANCE (Check one)

A. Pool: ❑ high ❑ medium ❑ low
Describe the nature and extent of disturbance.

B. Surrounding habitat within 200' buffer:
❑ high ❑ medium ❑ low
Describe the nature and extent of disturbance.

V. WRITTEN DIRECTIONS TO THE POOL.

VERNAL POOL DATA FORM
CODE SHEET

This sheet includes descriptions of all the information you need to include on ***Part A*** of the identification form.

I. General Information

Pool Number: Assign a unique number to each pool.
Location: Include name of town, county, road, or other specific information.
Map: Record name of NWI/USGS quad and/or assessor's map number.
Observer: Write in your full name
Date: Record month, day, year
Time of Day: Be sure to include a.m. or p.m.
Weather: Estimate temperature, % cloud cover, and wind speed
Visit #: Record 1st, 2nd, 3rd etc.

II. Pool Characteristics

Pool Type: Check temporary or permanent (but fishless), and artificial or natural.
Size: Determine size by pacing or estimating the average width and length at each visit. Note which method used.
Water status: Note whether the pool is at full, half full, quarter full, less or if it is dry.
Depth: Estimate depth of pool at deepest part in inches or feet if measured.
Cover: Note all emergent, floating, and submergent vegetation present in pool. Put a check mark or write in rough % of area covered by each type of vegetation.
Dominant wetland plants: Fill in the names of dominant wetland plants if known.

III. Indicator and Facultative Species Status

For each indicator and facultative species, put a check mark if present or number if counted in each box.

IV. Indicator Species Verification - Note which method used to verify presence of species.

V. Comments

Record any additional pertinent information here, including observations of other wildlife species or unusual plants.

Commonwealth of Massachusetts

Division of Fisheries & Wildlife

Wayne F. MacCallum, *Director*

Spring 2000

CERTIFICATION CRITERIA

Please read and understand the DOCUMENTATION REQUIREMENTS in the next section before submitting vernal pool certification applications.

Documentation of the biological and physical criteria described in this section is necessary to obtain official certification of any vernal pool.

DOCUMENTATION OF ANY ONE OF THE FOLLOWING (1-3) WILL CONFIRM THE EXISTENCE OF VERNAL POOL HABITAT AND IS SUFFICIENT FOR OFFICIAL CERTIFICATION

1) The Obligate Species Method
2) The Facultative Species Method
3) The Dry Pool Method

1) The Obligate Species Method

Evidence of a confined basin depression with no permanently flowing outlet **AND** one or more of the following:

1A Breeding* Obligate Amphibian
- Wood frog (*Rana sylvatica*)
- Spotted salamander (*Ambystoma maculatum*)
- Blue-spotted salamander (*Ambystoma laterale*)**
- Jefferson salamander (*Ambystoma jeffersonianum*)**
- Marbled salamander (*Ambystoma opacum*)**
- Eastern spadefoot toad (*Scaphiopus holbrooki*)**

OR

1B Adult Obligate Invertebrate
- Fairy shrimp (ANOSTRACA: *Eubranchipus*)

* Acceptable Breeding Evidence

Documentation of **any one** of the following proves that an area functions as vernal pool habitat. For the purposes of official certification, if amphibian evidence is submitted it must show evidence of breeding.

1. Breeding Adults
   - Frogs and toads: breeding chorus and/or mated pairs
   - Salamanders: courting individuals (congressing) and/or spermatophores
2. Egg Masses (**two or more are required**)
3. Larvae (tadpoles or salamander larvae)
4. Transforming Juveniles
   - Frogs and toads: tail remnants evident
   - Salamanders: gill remnants evident

**** State-listed Species**

State-listed Endangered (E), Threatened (T) and Special Concern (SC) species are protected under the Massachusetts Endangered Species Act (321 CMR 10.60); fill out a Rare Animal Observation Form and submit along with Certification Form.

CERTIFICATION CRITERIA

2) The Facultative Species Method

Evidence of a confined basin depression with no permanently flowing outlet **AND** evidence that there is no established, reproducing fish population

AND photographs of two or more of the following:

AMPHIBIANS

Breeding* Spring peeper (*Pseudacris crucifer*)
Breeding* Gray treefrog (*Hyla versicolor*)
Breeding* American toad (*Bufo americanus*)
Breeding* Fowler's toad (*Bufo woodhousii*)
Breeding* Green frog (*Rana clamitans melanota*)
Breeding* Pickerel frog (*Rana palustris*)
Breeding* Leopard frog (*Rana pipiens*)
Breeding* Four-toed salamander (*Hemidactylium scutatum*)**
Adult or Breeding* Red-spotted Newt (*Notophthalmus v. viridescens*)

REPTILES

Spotted turtle (*Clemmys guttata*)**
Blanding's turtle (*Emydoidea blandingii*)**
Wood turtle (*Clemmys insculpta*)**
Painted turtle (*Chrysemys p. pictata*)
Snapping turtle (*Chelydra serpentina*)

INVERTEBRATES

Predaceous diving beetle larvae (*Dytiscidae*)
Water scorpion (*Nepidae*)
Dragonfly larvae (*Odonata: Anisoptera*)
Damselfly larvae (*Odonata: Zygoptera*)
Dobsonfly larvae (*Corydalidae*)
Whirligig beetle larvae (*Gyrinidae*)
Caddisfly larvae (*Trichoptera*)
Leeches (*Hirundinea*)
Freshwater (fingernail) clams (*Pisidiidae*)
Amphibious, air-breathing snails (*Basommatophora*)

3) The Dry Pool Method

Evidence of a confined basin depression containing no standing water (dry pool)

AND one or more of the following:

Cases of caddisfly larvae (*Trichoptera*)
Adults, juveniles or shells of either of the following:
- Freshwater clams (*Pisidiidae*)
- Amphibious, air-breathing snails (*Basommatophora*)

Shed skins (exuvia) of dragonfly or damselfly larvae on vegetation along the edge of pool

DOCUMENTATION REQUIREMENTS

Documentation of the biological and physical characteristics listed in the CERTIFICATION CRITERIA must be submitted for official certification of a vernal pool. Photographic prints or slides are the preferred method of documentation, but video tapes of evidence or audio recordings of calling frogs are acceptable. Field notes are encouraged, but are not accepted as evidence; they must be submitted along with photographic or taped documentation.

Label all photographs as follows:

Location of pool
(or tracking number)
Date of photograph
Observer's name

The following field observations must be adequately documented

1. Biological criteria:
   1A Clear photographs or video of obligate amphibian breeding evidence
   OR
   1B Clear photographs or video of facultative invertebrate or vertebrate species (**AND** 2B or 2C)
   OR
   1C Audio tape of frog breeding chorus

2. Fishlessness:
   2A Evidence of obligate species per CERTIFICATION CRITERIA (1A above)
   OR
   2B Photograph of dry vernal pool
   OR
   2C Scientific evidence (e.g. seining) that documents the absence of fish

3. Physical criteria:
   Clear photographs or video of the vernal pool demonstrating the lack of permanently flowing connections to larger wetlands

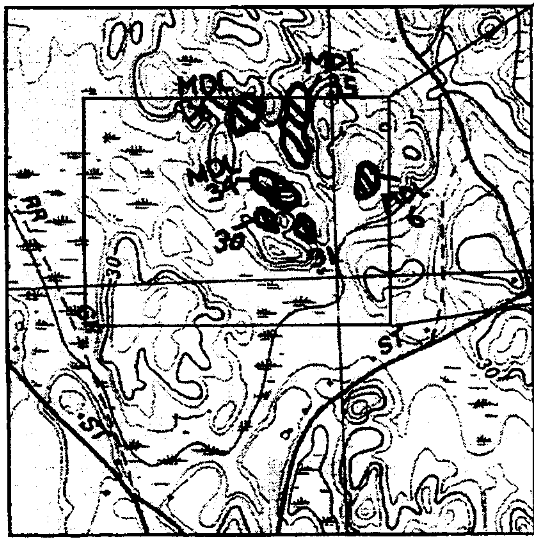
MAPPING REQUIREMENTS

It is critical to provide maps that are accurate and clear when submitting information for state vernal pool certification. A 1:24,000 or 1:25,000 scale **U.S. Geological Survey topographic map is required,** and additional maps that clarify the position of the vernal pool must be submitted. Many maps are acceptable fro this purpose. Large scale street maps generally are not acceptable as supporting maps.

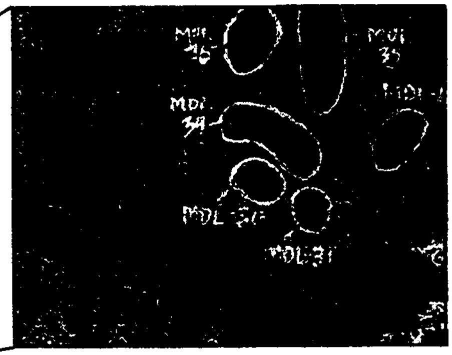
At least one from each of the following groups must be submitted:

GROUP 1	GROUP 2
USGS topographic: The location of the vernal pool must be clearly and accurately marked with an 'X' or dot	**Aerial photograph** Large scale (1:12,000 or better) with pool clearly visible **Compass directions and distances** Magnetic compass direction and distances from two permanent landmarks within 1000 feet of the pool. Landmarks should be readily identifiable in the field and clearly described on the submitted map **Professional survey** Large scale topographic maps or project plans where the depression is evident

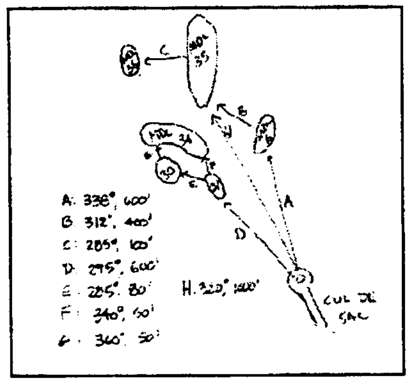
Some examples of required maps



USGS Topographic map section with pools clearly marked



B&W copy of **color infra-red aerial photograph** (1:12,000 scale) with pools also marked



Sketch map with compass directions and distance in feet

Field Observation Form

Application for certification of vernal pool habitat should be made using the standard field observation form (revised in 1999). All requested information should be filled out to the fullest extent possible. Additional directions are provided on the field form.

Please give particular attention to the following items:

Section 1: Written directions to the pool <u>must</u> be provided, noting field markers to help navigation.
Section 2: Please indicate the datesw on which evidence was collected, including the year.
Section 3: Indicate the evidence of obligate and facultative species collected at each pool. If egg masses were found, indicate the number of masses discovered.
Section 4 and 5: Check the boxes corresponding to evidence submitted for each pool (in photographs or tape)
Optional Information: Information provided in this section gives the Natural Heritage & Endangered Species Program a better sense of the type of vernal pools that are being identified through the certification program, and aides in-field identification of the pools should anyone need to visit it. This section is optional, but provides very helpful information.
Section 6: Field forms <u>must be signed</u> at the bottom of page 2.

Incomplete submissions will be returned in full with a letter indicating any missing information. When the requested information has been collected, the application may be resubmitted.

Submit completed applications to:

Vernal Pool Certification
Natural Heritage & Endangered Species Program
Route 135
Westborough, MA 01581

Natural Heritage & Endangered Species Program
Massachusetts Division of Fisheries and Wildlife

Vernal Pool Field Observation Form

(7/99) (For use with *Guidelines for Certification of Vernal Pool Habitat*)

For office use only.

1. Pool location

Town ____________________ County ____________________

USGS Quadrangle name ____________________ ☐ SERIES 7.5' X 7.5' ☐ SERIES 7.5' X 15'

WRITTEN DIRECTIONS TO POOL: ____________________

THIS INFORMATION MUST BE SUBMITTED

(USE ADDITIONAL PAGES, IF NECESSARY.)

2. Observation dates

First date pool/species observed ________

Last date pool observed ________ Last date species observed ________

3 A. Evidence: obligate amphibians

Indicate date of observation.

* = RARE SPECIES	COURTING ADULTS	SPERMATOPHORES	EGG MASSES (2+)	SALAMANDER LARVAE	TRANSFORMING JUVENILES
SPOTTED SALAMANDER					
* BLUE-SPOTTED SALAMANDER					
* JEFFERSON SALAMANDER					
* MARBLED SALAMANDER					
UNIDENTIFIED MOLE SALAMANDER					

	BREEDING CHORUS	MATED PAIRS	EGG MASSES (2+)	FROG TADPOLES	TRANSFORMING JUVENILES
WOOD FROG					
* SPADEFOOT TOAD					

Instructions

For complete information about certification, refer to *Guidelines for Certification of Vernal Pool Habitat.*

Provide all of the information requested in boxes 1-6. If more space is required, attach additional pages. Include all required photographs and documentation. Sign the form in the area provided on the reverse side. **Incomplete or unsigned submissions will be returned.**

The following instructions refer to each of the numbered boxes.

1. The 7.5 X 7.5 series has the legend "7.5 minute series" in the upper right hand corner along with the quadrangle name. The 7.5 X 15 minute series is so labeled in the upper right hand corner and has the quadrangle name in the lower right corner.
Written directions **must** be included.

2 Indicate the first and last dates that the pool or its biological components were observed.

3. Part A and B are for certification by obligate species. Part C is either for additional information (appreciated) or for certification by the facultative species. If certifying by obligate species, provide a photograph of the pool holding water and at least one photograph (or audio tape for chorusing) of breeding activity.
For certification by facultative species, provide photographs of the pool holding water and photographs (or tapes) of the facultative species as required. Additionally, provide a photograph of the pool when dry or otherwise prove that it has no fish.

3 B. Evidence: fairy shrimp

DATE OBSERVED ____________________

3 C. Evidence: facultative organisms

Two or more must be documented. Indicate date of observation.

* = RARE SPECIES	DATE OBSERVED	ACTIVITY OBSERVED
BREEDING SPRING PEEPERS		
BREEDING GRAY TREEFROGS		
BREEDING GREEN FROGS		
BREEDING LEOPARD FROGS		
BREEDING PICKEREL FROGS		
BREEDING AMERICAN TOADS		
BREEDING FOWLER'S TOADS		
* BREEDING FOUR-TOED SALAMANDERS		
RED-SPOTTED NEWT (ADULTS)		
* SPOTTED TURTLES		
* WOOD TURTLES		
* BLANDINGS TURTLES		

	DATE OBSERVED	ACTIVITY OBSERVED
PAINTED TURTLES		
SNAPPING TURTLES		
PREDACEOUS DIVING BEETLE LARVAE		
WATER SCORPIONS		
DRAGONFLY NYMPHS		
DAMSELFLY NYMPHS		
DOBSONFLY LARVAE		
WHIRLIGIG BEETLE LARVAE		
CADDISFLY LARVAE		
LEECHES		
FINGERNAIL (FRESHWATER) CLAMS		
AMPHIBIOUS AIR-BREATHING SNAILS		

Instructions (continued)

4. Indicate the photographs being submitted. Label, date, and sign all photos.
5. Mark the pool clearly on all maps. The pool must be clearly distinguished from other wetlands and be relocateable by others. Provide any maps that would help someone unfamiliar with the area locate the vernal pool in the field.
6. The form **must** be signed. Unsigned submissions will be returned without further action.

Optional information:

Property owner. Provide information about property owner(s), if known. It is recommended that you seek property owner permission prior to certification activities.

Rare species. A photograph is necessary for documentation of rare species habitat.

Description. Provide any information that will distinguish the pool from other wetlands (boulders, debris, tree species, etc.).

4. Photographs

Must be labeled, dated, and signed.

- ☐ Pool holding water
- ☐ Obligate +/or facultative species
- ☐ Dry pool (**required** for evidence 3C)

5. Maps submitted

- ☐ USGS topographic map (**required**)

And one or more of the following:

- ☐ Aerial photograph
- ☐ Distances/compass directions
- ☐ Professional survey
- ☐ Large scale topo
- ☐ Other___________

Optional extra information

- ☐ Sketch map of area
- ☐ Assessor's map
- ☐ GPS longitude/latitude coordinates

____

Optional information

Although the following information is not required for certification, it is useful to NHESP to possibly better protect the vernal pool, its habitat and species.

Property owner

It is strongly recommended that landowner permission be obtained prior to collecting certification documentation.

Name ______________________

Address ______________________

____

Town ______________ State____ ZIP______

Rare wetland species

Y N Were any rare state-listed species observed using this pool?

Y N Is a photograph of the rare species included with this filing?

Description of pool and surroundings

Dimensions: Approximate length Approximate width

Approximate depth

Describe distinctive features (roads, structures, boulders, etc.) which are visible from or near the pool.

Are there other distinctive features about this pool (vegetation types, abandoned vehicles, foot trails, etc.) that would help someone recognize it?

6. Observer information & signature

Name ______________________

Address ______________________

____

Town ______________ State____ ZIP______

Telephone ______________________

e-mail ______________________

I hereby certify under the pains and penalties of perjury that the information contained in this report is true and complete to the best of my knowledge.

Signature ______________________ Date ________

Send completed form and supporting documentation to:

NH&ESP
Vernal Pool Certification
MA Division of Fisheries & Wildlife
Route 135
Westborough, MA 01581

All submissions and supporting documents will be retained by the Natural Heritage & Endangered Species Program. Information submitted on this form and other documents is part of the public record and is available to interested parties under the State Documents Request Law.

Natural Community
FIELD FORM INSTRUCTIONS

Modified for **Massachusetts**
by Patricia Swain, MNHESP
May 10, 2001

from a 1991 draft
Lesley Sneddon, Regional Ecologist
(The Nature Conservancy
Eastern Heritage Task Force
201 Devonshire Street
Boston, Massachusetts)
now
NatureServe
Boston Office
11 Avenue de Lafayette
Boston, MA 02111

Massachusetts Natural Heritage & Endangered Species Program
Division of Fisheries and Wildlife
Rt. 135
Westborough, MA 01581

Field forms were designed to standardize data collection. We have divided the community data into categories, and designed separate forms with different purposes:

COMMUNITY FORM 1: TRANSECT, SITE SURVEY SUMMARY: use this form for reconnaissance, for a new site that is essentially unknown from community description perspective. Use this form to try to "make sense" of the landform: where are the communities in relation to changes in topography? What are the communities? What are the boundaries? For sites that are degraded (obvious C and D ranked community occurrences for which no further activity is planned), this may be the only community form that you will complete. It will serve as a record of the visit and provide some community data, but probably will not be mapped or entered into the database of Priority and Exemplary Communities. Iinformation on low quality community occurrences may be entered into a secondary community database to be tracked for a record of the sites. Form 1 is useful for recording general information along transects, with notes taken when communities change.

FORM 2: NATURAL COMMUNITY SUMMARY AND RANKING form: use to record information on the community location and rank. The natural community will be a part of a property or site: a bog, a hemlock ravine, an isolated stretch of floodplain forest are all communities. Single Form2s may have several plot forms with them. Form 2 is used to assign a rank (element occurrence rank); generally for A or B-ranked occurrences, or best known occurrences (C- or D- ranked common communities for which no pristine examples occur). Explain the basis of your ranking: range wide, state wide, or locally. These ranks are meant to apply state wide: if you are only familiar with the community in part of the state, give it a relative rank, but give your area of comparison. If you are giving it a global rank say so clearly. The assumption is that some protection activity is planned for this occurrence, so contains ownership information and other miscellaneous information that will assist in initiating protection activity. This form will also contain basic information regarding management needs of the community element: burning, exclosures, etc. This form can also be used as a record of subsequent visits, as an update form.

FORM 3, BASIC VEGETATION AND HABITAT INFORMATION: This form is to report plots, usually done in the best occurrences of community types. There can be several Forms 3 for any given community occurrence. This form contains all the basic information fields needed for minimum documentation of community occurrences. The sampling method is the relevé, which appears to be a reasonable compromise between the community "species list" and the more detailed plot techniques (e.g. macro-plots). Relevés are circular, square, or rectangular plots placed in the most representative portion of the community occurrence (but placement within this area should be random). Plots in most cases are not permanently marked (but semi-permanent markers may be used if a return visit is anticipated). Plots may be measured with a tape, but if you are familiar with your pace length, you may simply pace the distance and flag the corners. Identify what size and shape plot were used.

A given community occurrence may have several plots. All the information on Form 3 pertains to the plot. If more than one plot is taken (large community occurrences may require more than one plot), use a new sheet for each plot. Each should be labeled carefully to associate it with other form 3s and with its form 2. Make sure each plot can be identified if the pieces of paper get separated. Each set of forms needs a map associated with it to locate the plots and the community.

Filling out Form 3. Follow these instructions as much as possible. There is a lot of information requested, and you may not be able to supply it all. Soil information is helpful, but requires equipment you may not have with you. Do what you can, balancing information acquisition with time available. General descriptions are very useful.

All forms submitted to NHESP will be photocopied. Interns may transcribe them. You need to be neat and clear. Pencil doesn't photocopy well. Your data is valuable – help us make is useful by being legible!

Form 1 Reconnaissance

A. Identifiers:

1) **Site Name** - "Official" name. Leave blank if you don't know it.

2) **Survey Site Name** - provisional name assigned by field worker; should represent an identifiable feature on topographic map.

3) **Quad name(s)** - USGS quadrangle map name and scale. Note if these are the double or single map(s).

4) **Quad code(s)** - number assigned by MNHESP. Leave blank if you don't know it.

5) **County** - appropriate name from topographic map.

6) **County Code** - assigned by MNHESP, leave blank.

7) **Town** - appropriate name from topographic map.

8) **Directions** - from an easily identified road or other location. Include parking information if useful. these should be precise directions in words; attach a map if appropriate

9) **Source Code** -appropriate code, assigned by MNHESP. Put it and your name on copies of the form before photocopying. The pattern is eight characters with F (for field) 01 (for year), first three letters of your last name then 0X (tie breaker, we assign it). All the records for one year for any one person have the same source code. For example, all Pat Swain's field records for 2001 are F01SWA01. (NOT the same directions as in the NY State instructions).

10) **Survey Date** - year, month, day. Date of survey

11) **State**: - use postal codes for the state

12) **Surveyors** - names and addresses, as appropriate. Each group of surveyors will be assigned different codes

B. Topography:

13) **Transect** - a sequence number for identifying location.

14) **Reconnaissance Diagram** - diagrammatic cross section or toposequence showing changes in elevation and corresponding changes in vegetation and soils. Mark each observation point and releve location on the diagram. (Corresponding brief descriptions for each point are given in part C). Use arrow to show compass direction and indicate approximate elevation changes and distance covered in meters. Indicate scale using ruler or stick figure.

C. Vegetation/Habitat Observations:

16) **Community name** - state or regional vegetation name, if known; provisional name may also be assigned.

17) **Additional data** - state whether site and/or Form 3 were completed for this observation point.

18) - **General Description** - briefly describe the community or feature with the physiognomy and three dominant species of each stratum. If form 3 was filled out, omit, and write "see form 3".

Form 2: Natural Community Summary and Ranking:

Always include a copy of the appropriate USGS topographic map with this form, with the community and any transects shown.

1) **Community Name** - name of the community from the draft classification.

2) **TNC/NVCS Association Name** – an optional field for those working with the National Classification.

3) **Survey Date** - Date the field work was done.

4) **Today's Date** - Date the form is filled out.

5) **Survey site name** - Provisional name of the site, usually named after a geographic feature.

6) **Surveyors name(s)** - give the main surveyors name first. Add addresses if appropriate.

7) **Best Source** – themost complete survey. Leave blank if unknown.

8) **Transcriber** – leave blank, NHESP use only.

9) **USGS Topo Quad Name** – name of quad used, say if old single or more recent double map.

10) **Town** - official town the site is in, not local village

11) **Directions to the site** - from an easily identified road or other location. Include parking information if useful. Give precise directions in words; attach map if appropriate. Use clear sentences that will be understandable to someone who is unfamiliar with the area and has only your directions to follow. Give distances as closely as possible and use compass directions. Give additional directions to the plot within the site.

12) **GPS point(s)** – yes or no, and supply if taken.

13) **Vegetation Description** - formal description of the site with list of key species and community structure.

14) **Physical Description** - Give a word picture of the area, including a general description of the vegetation and the landscape. Describe the setting for the site, including whether there is surrounding conservation land, highways, or development.

15) **Is community within a managed conservatin area:** name if possible, also if private, public, and owner.

16) **Disturbances/Threats/Management** – as described on the form. Generally, threats and evidences of disturbances are from observations while in the field or from information gained from knowledgeable sources. These may lead to management recommendations as appropriate

17) **Protection comments** - to be filled out if the information is known..

18) **General Comments** – notes on sampling techniques, other forms filled out, and other information gathered or needed. Note if photographs were taken and are available.

19) **Owner information** - leave blank if not known

Community Element Occurrence Ranking

These fields are very important, fill out the parts you are comfortable with. Use the comment fields. In the comments field state what the comparisons are to: is this a property, region, state, or range wide assessment? Comment on size, exotics, management possibilities, position in the landscape, ownership or other useful criteria. MNHESP does have draft technical criterea for ranks which will be made available with the 2001 interim draft of the Classification of natural communities.

Form 3 Habitat/Vegetation Description

A. Identifiers:

1) **SName** - State name of the community type. Provisional name assigned by field worker

2) **Gname** - Formal name of community type.

3) **Site Name** - "Official" name. Leave blank if you don't know it.

4) **Survey Site Name** - provisional name assigned by field worker; should represent an identifiable feature on topographic map.

5) **Quad name(s)** - USGS quadrangle map name and scale. Note if these are the double or single map(s).

6) **Quad code(s)** - number assigned by MNHESP. Leave blank if you don't know it.

7) **County** - appropriate name from topographic map.

8) **County Code** - assigned by MNHESP, leave blank.

9) **Town** - appropriate name from topographic map.

10) **Lat.** - latitude in degrees, minutes, and seconds. Do not estimate, NHESP will do unless a GPS is used.

11) **Long.** - longitude as above in 10).

12) **Directions** - from an easily identified road or other location. Include parking information if useful. Give precise directions in words; attach map if appropriate. Use clear sentences that will be understandable to someone who is unfamiliar with the area and has only your directions to follow. Give distances as closely as possible and use compass directions. Give additional directions to the plot within the site.

13) **Source Code** -appropriate code, assigned by MNHESP. Put it and your name on copies of the form before photocopying. The pattern is eight characters with F (for field) 98 (for year), first three letters of your last name then 01 (tie breaker, we assign it). All the records for one year for any one person have the same source code. For example, all Pat Swain's field records from 1998 will be/are F98SWA01. NOT the same directions as in the NY State instructions.

14) **Survey Date** - year, month, day. Date of survey.

15) **Last obs** - May be the same as the survey date, but could be an update without data collection.

16) **First obs** - the first time the site was visited. May be years before, may only be known to the year.

17) **State** - State where community occurrence is located.

18) **Surveyors** - names and addresses, as appropriate. List principle surveyor first.

B. Environmental Description (Topography):

19) **Reconnaissance ID** - observation point number, if indicated on Form 1.

20) **Image annotation #** - patch identifier if noted on aerial photographs.

21) **Elevation** - elevation of the plot, in feet or meters, label which.

22) **Topographic position** - topographic position of the community in the landscape, check off.

23) **Topographic sketch.** - make a topographical sketch and indicate position of plot. Use arrow to show compass direction and indicate approximate elevation changes in meters.

24) **Slope degrees** - measure slope using a clinometer or describe: flat, gentle, moderate, somewhat steep, steep, very steep, abrupt, overhanging.

25) **Slope Aspect** - use a compass and be sure to correct for the magnetic declination. Or describe: flat, variable, N, NE, E, SE, S, SW, W, or NW.

26) **Parent Material/Bedrock** - note the geologic substrate influencing the plant community (bedrock or surficial materials.)

Igneous Rocks

Granitic (Granite, Schyolite, Syenite, Trachyte)
Dioritic (Diorite, Dacite, Andesite)
Gabbroic (Gabbro, Basalt, Pyroxenite, Peridotite)

26) Parent Material/Bedrock - continued

Sedimentary Rocks
- Conglomerates and Breccias
- Sandstone
- Siltstone
- Shale
- Limestone and Dolomite
- Marl
- Gypsum

Metamorphic Rocks
- Gneiss
- Schist
- Slate and Phyllite
- Marble
- Serpentine

Glacial deposits:
- undifferentiated glacial deposit
- till
- moraine
- bedrock and till
- Glacio-fluvial deposits (outwash plains, ice-contacted GF deposits, eskers, kames, pro-glacial deltas, etc.)
- Deltaic deposits (alluvial cones, deltaic complexes)
- Lacustrine and fluvial deposits (glacio-fluvial, fluvio-lacustrine, freshwater sandy beaches, stony/gravelly shore)
- Marine deposits (bars, spits, sandy beaches, old shorelines, old beach ridges, old marine clays, etc.)
- Organic deposits:
  - Peat (with clear fibric structure)
  - Muck
  - Marsh, regularly flooded by lake or river (high mineral content)
- Slope and modified deposits:
- talus and scree slopes
- colluvial
- solifluction, landslide

Aeolian deposits:
- dunes
- aeolian sand flats
- loess deposits
- cover sands

27) Soil Profile Description - Using a shovel with a long narrow blade or a soil auger, dig a pit 2-3 feet deep and note depth, texture, and color (Munsell color chart) of each horizon. Sketch the soil profile representative of the plot. In the sketch indicate depth scale (cm) on left side of profile, horizon designation on right side, boundary characteristics in drawing, and additional information on texture, structure, color, etc. as appropriate.

Simplified Key to Texture (Brewer & McCann, 1982)

A1 Soil does not remain in a ball when squeezed. . . . . . . **sand**
A2 Soil remains in a ball when squeezed. . . . . .B
B Squeeze the ball between your thumb and forefinger, attempting to make a ribbon that you push up over your finger. B1 Soil makes no ribbon. . . . . **loamy sand**
B2 Soil makes a ribbon; may be very short. C
C1 Ribbon extends less than 1 inch before breaking . . . . . . .D
C2 Ribbon extends 1 inch or more before breaking . . . . .E
D1 Add excess water to small amount of soil; soil feels at least slightly gritty . . . . **loam or sandy loam**
D2 Soil feels smooth . . . .**silt loam**
E1 Soil makes a ribbon that breaks when 1-2 inches long; cracks if bent into a ring . . . . F
E2 Soil makes a ribbon 2+ inches long; doesn't crack when bent into a ring . . . . .G
F1 Add excess water to small amount of soil; soil feels at least slightly gritty. . . . .**sandy clay loam or clay loam**
F2 Soil feels smooth . . . . **silty clay loam or silt**
G1 Add excess water to a small amount of soil; soil feels at least slightly gritty . . . . **sandy clay or clay**
G2 Soil feels smooth. . . . . **silty clay**

VON POST SCALE OF PEAT DECOMPOSITION

H1: Completely undecomposed peat; only clear water can be squeezed out.
H2: Almost undecomposed and mud-free peat; water that is squeezed out is almost clear and colorless.
H3: Very little decomposed and very slightly muddy peat; when squeezed water is obviously muddy but no peat passes through fingers. Residue retains structure of peat.
H4: Poorly decomposed and somewhat muddy peat; when squeezed, water is muddy. Residue muddy but it clearly shows growth structure of peat.
H5: Somewhat decomposed, rather muddy peat; growth structure visible but somewhat indistinct; when squeezed some peat passes through fingers but mostly very muddy water. Press residue muddy.

H6: Somewhat decomposed, rather muddy peat; growth structure indistinct; less than 1/2 of peat passes through fingers when squeezed. Residue very muddy, but growth structure more obvious than in unpressed peat.
H7: Rather well-decomposed, very muddy peat; growth structure visible, about 1/2 of peat squeezed through fingers. If water is squeezed out, it is porridge-like.
H8: Well-decomposed peat; growth structure very indistinct; about 2/3 of peat passes through fingers when pressed, and sometimes a somewhat porridge-like liquid. Residue consist mainly of roots and resistant fibers.
H9: Almost completely decomposed and mud-like peat; almost no growth structure visible. Almost all peat passes through fingers as a homogeneous porridge if pressed.
H10: Completely decomposed and muddy peat; no growth structure visible; entire peat mass can be squeezed through fingers.

28) Organic horizon depth - Indicate depth to contact with mineral soil or mixture of organic and mineral soil (O horizon)

29) Organic horizon type -

MOR - acid reaction. lacking in microbial activity except fungi, and composed of several layers of organic matter in varying degrees of decomposition.

MULL - chemically neutral or alkaline reaction; well aerated, and provides generally favorable conditions for decomposition of organic matter. Well decomposed and intimately mixed with mineral matter.

30) - Average pH of mineral soil - measure pH of mineral soil.

31) Moisture Regime - while soil drainage is based on soil morphology only, moisture regime is based on the amount of water available to plants. It is evaluated on the basis of soil drainage, soil structure and texture, and climate. Thus, a well-drained till is much more moist than a well-drained coarse textured glacio-fluvial deposit within the same area, or a well-drained sandy loam in a humid climate is moister than the same soil in a climatically dry region.

EXTREMELY DRY: steep eroding sands, rock piles, gravel.

VERY DRY: medium and coarse sands: shallow soils, not influenced by ground water.

DRY: deep silty sands and loamy sands, not influenced by ground water.

WELL-DRAINED: deep sandy loams and loams, not influenced by ground water.

SOMEWHAT MOIST: loams and sandy loams with some rust mottling in lower part of B or C horizon. Moist variants or zonal soil types.

MOIST: soil surface above the maximum water level; normal soil profile development hampered because of imperfect drainage. Upper 1-2 feet of soil well-aerated during vegetative season. On mineral soils a severely mottled to homogeneous brown horizon (color B) is present. Occurs also on heavy textured soils with perched water table and on dry deep peat.

SOMEWHAT WET: maximum water level at or close to the soil surface. Anaerobic soils; on mineral soils reduced, grey soil matrix with rust mottling. Gleysols, some peat soils.

WET: water level at soil surface for most of vegetative season. Reduced gley layer up to mineral soil surface on mineral soils; mottling usually absent or insignificant. Organic soil, gleysol

VERY WET: water level above soil surface for most part of vegetative season. Minimum water level approximately at soil surface. Organic soil.

PERMANENTLY INUNDATED: (hydric) minimum water level above soil surface, soils permanently inundated.

PERIODICALLY INUNDATED: (hydric) known to be periodically inundated due to flood/drought cycles or other variable moisture regimes.

32) Stoniness - average stoniness of deposit up to 1 m in depth, check off..

33) Soil Drainage - The soil drainage classes are defined in terms of (1) actual moisture content (in excess of field moisture capacity), and (2) the extent of the period during which excess water is present in the plant-root zone.

It is recognized that permeability, level of groundwater, and seepage are factors affecting moisture status. However, because these are not easily observed or measured in the field, they cannot be used generally as criteria of moisture status. It is further recognized that soil profile morphology, for example mottling, normally, but not always, reflects soil moisture status. Although soil morphology may be a valuable field indication of moisture status, it should not be the overriding criterion. Soil drainage classes cannot be based solely on the presence or absence of mottling. Topographic position and vegetation as well as soil morphology are useful field criteria for assessing soil moisture status.

RAPIDLY DRAINED - The soil moisture content seldom exceeds field capacity in any horizon except immediately after water addition. Soils are free from any evidence of gleying throughout the profile. Rapidly drained soils are commonly coarse textured or soils on steep slopes.

WELL DRAINED - The soil moisture content does not normally exceed field capacity in any horizon (except possibly the C) for a significant part of the year. Soils are usually free from mottling in the upper 3 feet, but may be mottled below this depth. B horizons, if present, are reddish, brownish, or yellowish.

MODERATELY WELL DRAINED - The soil moisture in excess of field capacity remains for a small but significant period of the year. are commonly mottled in the lower B and C horizons or below a depth of 2 feet. The Ae horizon, if present, may be faintly mottled in fine-textured soils and in medium-textured soils that have a slowly permeable layer below the solum. In grassland soils the B and C horizons may be only faintly mottled and the A horizon may be relatively thick and dark. excess of field capacity remains in subsurface horizons for moderately long periods during the year. are commonly mottled in the B and C horizons; the Ae horizon, if present, may be mottled. The matrix generally has a lower chroma than in the well-drained soil on similar parent material.

SOMEWHAT POORLY DRAINED - The soil moisture in excess of field capacity remains in subsurface horizons for moderately long periods during the year. Soils are commonly mottled in the B and C horizons; the Ae horizon, if present, may be mottled. The matrix generally has a lower chroma than in the well-drained soil on similar parent material.

POORLY DRAINED - The soil moisture in excess of field capacity remains in all horizons for a large part of the year. The soils are usually very strongly gleyed. Except in high-chroma parent materials the B, if present, and upper C horizons usually have matrix colors of low chroma. Faint mottling may occur throughout.

VERY POORLY DRAINED - Free water remains at or within 12 inches of the surface most of the year. The soils are usually very strongly gleyed. Subsurface horizons usually are of low chroma and yellowish to bluish hues. Mottling may be present but at depth in the profile. Very poorly drained soils usually have a mucky or peaty surface horizon.

34) Average Texture - overall texture of upper 1 m of loose deposit. Given in #27.

MUCK: Dark colored, finely divided, well decomposed organic soil material mixed with mineral soil material. The content of organic matter is more than 20%.

PEAT: Unconsolidated material, largely undecomposed organic matter, that has accumulated under excess moisture.

For Peat deposits use Von Post scale of peat decomposition given in #27.

35) Unvegetated surface - Percentage of surface covered by each category, only including items covering more than 5%.

36) Environmental comments - Additional observations about the plot. Note whether vegetation is homogeneous or made up of distinct units (e.g. hummocks and hollows); evidence of erosion or sedimentation; further observations on inundation, etc.

37) Plot representativeness - Does this plot represent the full variability of the community occurrence? In not, were additional plots done: Note additional species not in plot (use back in separate area if necessary).

C. Environmental Description (Vegetation): (Back of form)

ADD Community Name -. vegetation type name used in state classification.

Plot number, for correlating with site forms and other plots.

Give Plot dimensions used: width and length dimensions for rectangular (or square) plots or radius for circular plots. Choose the appropriate plot size based on the appropriate vegetation. Mueller-Dombois and Ellenberg, 1974, (Source: D. Mueller-Dombois and H. Ellenberg. 1974. Aims and Methods of Vegetation Ecology. John Wiley and Sons. NY.) recommend:

Forest	200 - 500 m^2	Dwarf-shrub heath:	10 -25 m^2
Shrubland	50 - 200 m^2	Moss communities	1 - 4 m^2
Grassland	50 - 200 m^2	Lichen communities	0.1 - 1 m^2

Square, short rectangular, or circular plots are preferred whenever feasible. Because there is a greater potential for edge effects or patchiness in long rectangular plots, use them only when needed to fit in a narrow zone.

41) Leaf type - Select one which best describes the leaf form of the tallest stratum with at least 25% cover..

42) Leaf phenology - Select the type of leaf structure for the dominant stratum with greater then 25% cover.

Perennial - is herbaceous vegetation composed of more than 50% perennial species.
Annual - Herbaceous vegetation composed of more than 50% annual species.

43) Physiognomic type -Select the description that best describes the community structure..

44) Strata / life forms - Visually divide the community into vegetation layers. Indicate the height of the stratum in the first column, and average percent cover of the whole stratum in the second column.

45) Releve Data - list all species and their abundance/cover classes for each stratum, beginning with the tallest. Separate each stratum with a blank line. On the first line of each stratum, record the stratum code (OR Kuchler code), with its total percent cover. Species outside the plot should be listed in parentheses and not counted in the total number of species used in tabular comparison. For tree strata, include diameters (DBH) of several (or all, say which) of the (largest) trees in the plot. IF YOU USE A DIFFERENT APPROACH, MAKE IT VERY CLEAR WHAT YOU HAVE DONE.

Braun-Blanquet

Cover/abundance values:
r one or few individuals
\+ occasional, < 5% cover
1 common, < 5% cover
2- 5-12% cover
2+ 13-25% cover
3 26-50% cover
4 51-75% cover
5 > 75% cover

Sociability scale:
1 growing solitarily, singly
2 small groups, small tussocks
3 small patches, large tussocks
4 large patches, mats
5 great crowds, mats covering whole plot

Kuchler Height Classes

an alternative to the protocol on the back of form 3

Life form Categories

Woody Plants
B Broadleaf evergreen
D Broadleaf deciduous
E Needleleaf evergreen
N Needlaleaf deciduous
S Semideciduous (B+D)
M Mixed (D+E)

Herbaceous Plants
G Graminoids
H Forbs
L Lichens, mosses

Special Life Forms
C Climbers (lianas)
X Epiphytes

Structural Categories

Height (stratification)
8 >35m
7 20 - 35m
6 10 -20m
5 5 - 10m
4 2 - 5m
3 0..5 - 2m
2 0.1- 0.5m (knee high)
1 <0.1m (ankle high)

Coverage (of the layer)
c continuous (>75%)
i interrupted (50 - 75%)
p parklike, patches (25 - 50%)
r rare (5 - 25%)
b barely present, sporadic (1-5%)
a almost absent, scarce, (<1%)

Protocol for Community forms (form 3, back)
January 19, 1996, P. Swain
Using relevé procedures.

Plot sizes vary with the community--generally 20 x 20m or 10 x10m for forest. If necessary subplots can be nested for different layers (5x5m for shrubs, several 1x1m for herbaceous)--label clearly whatever is done.
NOTE: TNC recommends using actual estimated coverages instead of cover classes. If doing that be consistent, and clearly explain what you have done.

Kuchler height class

Species name1	Braun-Blanquet's code	notes (cover . sociability)
Species name2	Braun-Blanquet's code	notes (cover . sociability)

for example: (some people use abbreviations for species in notes, Acsa or Quru

D6c

Acer saccharum	3.1	dbh to 10"
Quercus rubra	1.1	dbh to 8"
Acer rubrum	+.1	dbh to 6"
Fraxinus americana	1.1	dbh to 8", one dead stem

M5p

Tsuga canadensis	2.2
Sassafras albidum	+.1
Betula papyrifera	+.2
Cornus ammomum	1.2
Viburnum lentago	+.1

H2-3c (There's a choice here--call entire layer H and list small Ds and Gs, or separate each growth form. Purists probably separate. I tend to name the layer by appearance, so if grassy looking its G, even if has Hs or if broadleafed herb-y looking its H but includes woody and grassy. Tends to be a long section.)

Aster infirmus +.1 (fl) (There are Lots of +.1, s, probably most common.)
Aster paternus +.2
Viola sp 1.2 (it is best to be as precise as possible on species for the computer)
Eupatorium rugosum +.1
Geum canadense +.1
Osmunda cinnamomea +.2
Acer rubrum +.1
Vaccinium angustifolium 2.4
(Carex stricta 3.4, area near woods, not in plot)

B1r

Mitchella repens	+.2
Gaultheria procumbens	+.2

Note: There's flexibility here. Lump overlapping size classes (ie. D4-5r).
If its a measured plot, say so: if eye balled, say where. And so on.

COMMUNITY FORM 1: TRANSECT, SITE SURVEY SUMMARY

rev. May, 1998

MA Natural Heritage & Endangered Species Program

A. Identifiers

1.Site name:________________________________ 2.Survey site name: ____________________

3.Quad name(s)______________ 4.Quad code(s):__________ 5.County name(s):______________ 6.County code(s): __________

7.Town (LOCALJURIS):________________________ 8.Directions: ____________________________

____

____

____

____

9.Sourcecode: ____________________ 10.Survey date ________ 11.State: ________

12.Surveyors:____

B. Topography 13. Transect___

14.Reconnaissance diagram: Scale:

C. Vegetation / Habitat

15. Observation point 1___	Observation point 2___	Observation point 3___
16. Community name:______________ 17. Additional data: Site form___ form 3 ___	Community name:______________ Additional data: Site form___ form 3___	Community name:______________ Additional data: Site form___ form 3___
18.General description (physiognomy, char./dom spp. of tree, shrub, herb, bryophyte layers)	General description	General description:

Reconnaissance Diagram: Scale:

Observation Point 4 ___	Observation Point 5 ___	Observation Point 6 ___	Observation Point 7 ___
Community name:____________________ Additional data: Site form ___ form 3___	Community name:____________________ Additional data: Site form___ form 3___	Community name:____________________ Additional data: Site form___ form 3___	Community name:____________________ Additional data: Site form___ form 3___
General Description:	General Description:	General Description:	General Description:

Natural Heritage & Endangered Species Program

Massachusetts Natural Heritage & Endangered Species Program
Division of Fisheries & Wildlife
Route 135
Westborough, MA 01581
(508) 792-7270 ext. 200

FORM 2: NATURAL COMMUNITY SUMMARY AND RANKING

(A location map must accompany this form.)

A. Identifiers:

Community Name (MNHESP: Swain & Kearsley, 2000): ____________________

TNC/NVCS Association Name (Optional): ____________________

Survey Date: ____________________ Today's Date: ____________________

Survey Site Name: ____________________

Surveyor Name(s): ____________________

Best Source (Field survey or secondary source used to complete this form): ____________________

____

Transcriber (MNHESP use only. YY-MM-DD XXX): ____________________

USGS Topo Quad Name: ____________________ Town Name: ____________________

Directions to site: ____________________

____

____

____________________ GPS Point(s)___Yes___No

B. Community Description:

Vegetation Description (*EODATA*: **Summarize** the vegetation: dominant and/or characteristic species, indicator species, community structure, variants/microhabitat features, unvegetated surface; spatial distribution (i.e., size, number, and separation distance of patches); intact natural processes, geology, hydrology, topography, and soil properties, especially if relevant to the community identification): ____________

____

____

____

____

____

____

____

____

____

____

____________________ Estimated size (acres)__________

Physical Description (*GENDESC*: Describe the landscape surrounding the community, including the natural area. Both within and surrounding the community, describe: physical structures and land use practices; natural disturbances; embedded, adjacent, and nearby natural communities including aquatic features; notable landforms; scenic qualities): ____________________

____

____

____

____

____

____

____

____

____

____

Is community within a managed conservation area:_______ Managed Area Name:____________________

Evidence of Disturbance/Threats to the Community/Management Recommendations (*MGMTCOM*: Describe the anthropogenic disturbances that have decreased the quality and viability of the community such as hydrologic alterations (ditching, damming, etc.), logging, mining, livestock grazing, plantations, orchards, structures, trampling, and exotic flora or fauna within and surrounding the community. Discuss threats to the site and management implications.):_______________

Protection Comments (*PROTCOM*: Comment on the legal protectability of the site): _______________

General Comments (*COMMENTS*: Note the type of sampling done observation point (form 1), releve plot (form 3), plant list, etc.; note any additional field work needed. Comment on questionable identification.): _______________

Owner's Name: _______________ Telephone: (___)_______________

Address: _______________

Is Owner: aware of community?__yes __no __unknown, protecting community?__yes __no __unknown

Owner Comments (*OWNERCOM*: e.g., contact owner prior to visiting the site):_______________

C: Community Element Occurrence Ranking: (Refer to community ranking specifications for assistance.)

Community Size Rank: (Compare relative size to other known occurrences, configuration, patchiness)

A – Excellent **B** – Good **C** – Marginal **D** - Poor

Comments: _______________

Community Condition Rank: (Consider development/maturity (e.g., old growth), abiotic condition, species and physiognomic diversity, ecological processes, abundance of exotic species, internal connectivity, degree of anthropogenic disturbance including fragmentation).

– Excellent **B** – Good **C** – Marginal **D** - Poor

Comments: _______________

Community Landscape Context Rank: (Consider the size and connectivity of the natural landscape, the position of the community within the landscape, and the landscape condition)

A – Excellent **B** – Good **C** – Marginal **D** - Poor

Comments: _______________

Community EO Rank: (What are the long-term prospects for continued existence of this occurrence at the indicated level of quality? A summary of all factors listed above. Explain the basis of your ranking: range wide, state wide, or locally.)

A – Excellent **B** – Good **C** – Marginal **D** - Poor

Comments (*EORANKCOM:* Summarize the above and justify the EO Rank assigned): _______________

Other rare species and/or natural communities observed at this site (T/U = Transcribed/Updated?):

	SPECIES OR COMMUNITY	T/U?		SPECIES OR COMMUNITY	T/U?
1			4		
2			5		
3			6		

Form 3: Quantitative Community Characterization

rev. May, 1998

MA Natural Heritage & Endangered Species Program

A. Identifiers (general EOR information)

Sci. name: 1.SNAME: ______ 2.GNAME: ______
3.Site name: ______ 4.Survey site name: ______
5.Quad name(s): ______ 6.Quad code(s): ______ 7.County name(s): ______ 8.County code(s): ______
9.Town (LOCALJURIS): ______ 17.State: ______ 10.Lat: N ______ 11.Long ______ W
12. Directions: ______

13.Sourcecode: ______ 14.Survey date ______ 15.Last obs ______ 16.First obs: ______
18 Surveyors: ______

B. Environmental Description

19.Transect / Observation point #

20.Image annotation #

21.Elevation:

22.Topographic position:
____Interfluve ____Backslope
____High slope ____Step in slope
____High level ____Lowslope
____Midslope ____Toeslope
____Low level ____Channel wall
____Channel bed ____Basin floor
____Other

23.Topographic sketch:

24.Slope degrees:_____

25.Slope aspect:_____

26.Parent material:

27.Soil profile description: note depth, texture, and color of each horizon. Note significant changes such as depth to mottling, depth to water table, root penetration depth (SOILCOM)

28.Organic horizon depth:______

29.Organic horizon type:______

30.Average pH of mineral soil:______

31.Soil moisture regime:
____Extremely dry ____Somewhat wet
____Very dry ____Wet
____Dry ____Very wet
____Somewhat moist
____Moist

____Permanently inundated
____Periodically inundated

32.Stoniness:
________Stone free <0.1%
________Moderately stony 0.1-1%
________Stony 3-15%
________Very stony 15-50%
________Exceedingly stony 50-90%
________Stone piles >90%

33.Soil drainage:
____Rapidly drained ____Somewhat poorly drained
____Well drained ____Poorly drained
____Moderately well drained ____Very poorly drained

34.Average texture:
____ sand ____clay loam
____sandy loam ____ clay
____loam ____ peat
____silt loam ____muck
other______

35.Unvegetated surface:
____ % Bedrock
____ % Large rocks (cobbles, boulders > 10 cm)
____ % Small rocks (gravel, 0.2-10 cm)
____ % Sand (0.1-2 mm)
____ % Bare soil
____% Litter, duff
____ % Wood (> 1 cm)
____ % Water
____% Other:______

36.Environmental Comments: vegetation homogeneity, erosion / sedimentation, inundation, etc.

37.Plot representativeness:

C. Vegetation 38.System: __ Terrestrial __ Palustrine __ Estuarine 39.Plot number:___ 40.Plot dimensions:___________

41.Leaf type:
____Broad-leaf
____Semi-broad-leaf
____Semi-needle-leaf
____Needle-leaf
____Graminoid
____Broad-leaf herbaceous
____Pteridophyte

42.Leaf phenology:
____Deciduous
____Semi-deciduous
____Semi-Evergreen
____Evergreen
____Perennial
____Annual

43.Physiognomic type:
____Forest
____Sparse woodland
____Shrubland
____Dwarf shrubland
____Sparse dwarf shrubland
____Herbaceous
____Sparsely vegetated
____Woodland
____Scrub thicket
____Sparse shrubland
____Dwarf scrub thicket
____Non-vascular

44.	height	% cover
T1 Emergent tree		
T2 Tree canopy		
T3 Tree sub- canopy		
S1 Tall shrub		
S2 Short shrub		
H Herbaceous		
N Non-vascular		
E Epiphyte		
V Vine / liana		

45.Species / percent cover: starting with uppermost stratum, list all species and % cover for each in the stratum. For forests and woodlands, list on a separate line below each tree species the DBH of all trees above 10 cm diameter. Separate the measurements with a comma and note whether in cm or inches.

STREAMSIDE BIOSURVEY: HABITAT WALK

Stream Name: ____________________

County: ____________ State: ____________

Investigators: ____________________

____

____

Site (description): ____________________

____

____

____

Latitude: ____________ Longitude: ____________

Site or Map Number: ____________________

Date: ____________ Time: ____________

Weather in past 24 hours:

- ☐ Storm (heavy rain)
- ☐ Rain (steady rain)
- ☐ Showers (intermittent rain)
- ☐ Overcast
- ☐ Clear/Sunny

Weather now:

- ☐ Storm (heavy rain)
- ☐ Rain (steady rain)
- ☐ Showers (intermittent rain)
- ☐ Overcast
- ☐ Clear/Sunny

Source: (Barbour, 1999)

Sketch of site

On your sketch, note features that affect stream habitat, such as: riffles, runs, pools, ditches, wetlands, dams, riprap, outfalls, tributaries, landscape features, logging paths, vegetation, and roads.

PHYSICAL CHARACTERIZATION

In-Stream Characteristics

1. Check which stream habitats are present: Page 73

(You can check more than 1 habitat)

θ Pool(s) θ Riffle(s) θ Run(s)

2. Nature of particles in the stream bottom at site Page 73

	Percent
Silt/Clay/Mud	______
Sand (up to 0.1" in diam.)	______
Gravel (0.1 - 2" in diam.)	______
Cobbles (2 - 10" in diam.)	______
Boulders (over 10" in diam.)	______
Bedrock (solid)	______
TOTAL	*100%*

3. Pick the category that best describes the extent to which gravel, cobbles, and boulders on the stream bottom are embedded (sunk) in silt, sand, or mud. Page 74

θ Somewhat/not embedded (0-25%) θ Mostly embedded (75%)

θ Halfway embedded (50%) θ Completely embedded (100%)

4. Streambank sinks beneath your feet in: Page 74

θ No spots θ A few spots θ Many spots

5. Presence of logs or large woody debris in stream: Page 74

θ None θ Occasional θ Plentiful

6. Presence of naturally-occurring organic material (i.e., leaves and twigs, etc.) in stream: Page 74

θ None θ Occasional θ Plentiful

7. Water appearance: Page 74

θ Clear θ Turbid θ Orange

θ Milky θ Dark brown θ Greenish

θ Foamy θ Oily sheen θ Other ______________

8. Water odor: Page 74

θ Sewage θ Fishy θ None

θ Chlorine θ Rotten eggs θ Other ______________

9. Water temperature: Page 74

________ °C *or* ________ °F

Streambank and Channel Characteristics

10. (a) Approximate depth of run(s): Page 75

θ < 1 ft θ 1-2 ft θ > 2 ft

(b) Approximate depth of pool(s):

θ < 1 ft θ 1-2 ft θ > 2 ft

11. Approximate width of stream channel: Page 75

_________ feet θ measured θ estimated

12. Stream velocity: _________ ft/sec. Page 75

13. Looking upstream (100 yds.), pick the description that best fits the shape of the stream bank and the channel. Page 75

(a) Stream bank:

Left		Right
θ	Vertical/undercut	θ
θ	Steeply sloping (> 30°)	θ
θ	Gradual/no slope (< 30°)	θ

(b) Extent of artificial bank modifications:

Left		Right
θ	Bank 0-25% covered	θ
θ	Bank 25-50% covered	θ
θ	Bank 50-75% covered	θ
θ	Bank 75-100% covered	θ

(c) Shape of the channel:

θ Narrow, deep θ Wide, deep

θ Narrow, shallow θ Wide, shallow

14. Looking upstream (100 yds.), describe the streamside cover Page 76

(a) Along water's edge and stream bank only:

	Left *(Percent)*		Right *(Percent)*
	______	Trees	______
	______	Bushes, shrubs	______
	______	Tall grasses, ferns, etc.	______
	______	Lawn	______
	______	Boulders/rocks	______
	______	Gravel/sand	______
	______	Bare soil	______
	______	Pavement, structures	______
TOTALS	*100%*		*100%*

(b) From the top of the streambank out to 25 yards.

Left *(Percent)*		Right *(Percent)*
______	Trees	______
______	Bushes, shrubs	______
______	Tall grasses, ferns, etc.	______
______	Lawn	______
______	Boulders/rocks	______
______	Gravel/sand	______
______	Bare soil	______
______	Pavement, structures	______
TOTALS *100%*		*100%*

15. Pick the category that best describes the extent to which vegetation shades the stream at your site. Page 77

θ 0% θ 25% θ 50% θ 75% θ 100%

16. Looking upstream, note general conditions. Page 77
Check "1" if present, "2" if severe problem is clearly evident.

Left 1	Left 2		Right 1	Right 2
1	**2**	**Stream Banks**	**1**	**2**
θ	θ	Natural streamside plant cover degraded	θ	θ
θ	θ	Banks collapsed/eroded	θ	θ
θ	θ	Garbage/junk adjacent to the stream	θ	θ
θ	θ	Foam or sheen on bank	θ	θ
1	**2**	**Stream Channel**	**1**	**2**
θ	θ	Mud, silt, or sand in or entering the stream	θ	θ
θ	θ	Garbage/junk in the stream	θ	θ
1	**2**	**Other**	**1**	**2**
θ	θ	Yard waste on bank (grass, clippings, etc.)	θ	θ
θ	θ	Livestock in or with unrestricted access to stream	θ	θ
θ	θ	Actively discharging pipe(s)	θ	θ
θ	θ	Other pipe(s) entering the stream	θ	θ
θ	θ	Ditches entering the stream	θ	θ

Local Watershed Characteristics

(within about 1/4 mile of the site; adjacent and upstream)

17. Land uses in the local watershed can potentially have an impact on a stream. Check "1" if present, "2" if clearly having an impact on the stream. Page 78

1	2	
1	**2**	**Residential**
θ	θ	Single-family housing
θ	θ	Multifamily housing
θ	θ	Lawns
θ	θ	Commercial/institutional
1	**2**	**Roads, etc.**
θ	θ	Paved roads or bridges
θ	θ	Unpaved roads
1	**2**	**Construction underway on:**
θ	θ	Housing development
θ	θ	Commercial development
θ	θ	Road bridge construction/repair
1	**2**	**Agricultural**
θ	θ	Grazing land
θ	θ	Feeding lots or animal holding areas
θ	θ	Cropland
θ	θ	Inactive agricultural land/fields
1	**2**	**Recreation**
θ	θ	Power boating
θ	θ	Golfing
θ	θ	Camping
θ	θ	Swimming/fishing/canoeing
θ	θ	Hiking/paths
1	**2**	**Other**
θ	θ	Mining or gravel pits
θ	θ	Logging
θ	θ	Industry
θ	θ	Oil and gas drilling
θ	θ	Trash dump
θ	θ	Landfills

BIOLOGICAL CHARACTERIZATION

VISUAL BIOLOGICAL SURVEY

18. Fish in the stream? *(Mark all that apply)* Page 78

θ No θ Yes, but rare θ Yes, abundant

θ Small (1-2 in.) θ Medium (3-6 in.) θ Large (7 in. and above)

19. Are there any barriers to fish movement? Page 78

θ Beaver dams θ Waterfalls (>1') θ None

θ Dams θ Road barriers θ Other ________

20. Aquatic plants in the stream. *(Mark all that apply)* Page 78

θ None θ Occasional θ Plentiful

θ Attached θ Free-floating

θ Stream margin θ Pools θ Near riffle

21. Extent of algae in the stream. *(Mark all that apply)* Page 78

(a) Are the submerged stones, twigs, or other material in the stream coated with a layer of algal "slime"?

θ None θ Occasional θ Plentiful

θ Light coating θ Heavy coating

θ Brownish θ Greenish θ Other ________

(b) Are there any filamentous (string-like) algae?

θ None θ Occasional θ Plentiful

θ Brownish θ Greenish θ Other ________

(c) Are any detached "clumps" or "mats" of algae floating on the water's surface?

θ None θ Occasional θ Plentiful

θ Brownish θ Greenish θ Other ________

COMMENTS: *(Note changes or potential problems such as spills, new construction, type of discharging pipes)*

REPRESENTATIVE FORMS FOR THE MID-ATLANTIC REGION

RARE SPECIES REPORTING FORM

Maryland DNR, Wildlife and Heritage Division

Species name: ____________________

Date(s) species was located: ____________________

County name: __________ Directions to the site: ____________________

____

____

Habitat description: ____________________

____

____

Data on species (for example; number seen, age or maturity, breeding behavior, nature of observation - song, tracks, sight record, etc..):

____

____

____

Photograph taken? _____ Yes _____ No Specimen taken? _____ Yes _____ No

if yes, give collection # and repository: ____________________

____

Identification problems? _____ Yes _____No; explain: ____________________

____

Other comments (for example; other people who observed this species, known threats/management needs for species or habitat, land ownership, etc..):

____

____

Reporter's name: ____________________

Address & phone number: ____________________

____

PLEASE ATTACH A LOCATION MAP TO THIS FORM
(e.g., photocopy of ADC book map or USGS quadrangle map with species' location marked.)

Return to: Lynn Davidson
MD Wildlife and Heritage Division
Tawes State Office Bldg, E-1
Annapolis, MD 21401

REPRESENTATIVE FORMS FOR THE SOUTHEAST REGION

FLORIDA NATURAL AREAS INVENTORY FIELD REPORT FORM - **OCCURRENCES OF SPECIAL ANIMALS**

Scientific Name:____ County: __________________________

Common Name: ____ Date observed: _____________

Basis for Identification:_________________________________ Investigator: ______________________

Location of Animal (*please attach map and give specific directions; if possible, mark site on copy of USGS 7.5 minute topo map or draw detailed map on back of this page*):

____

____

____

Describe habitat/plant community, list dominant species:

____

____

____

Extent of this habitat at site that may support animal (e.g., acres, miles) ______________________________

Number of individuals (or nests, burrows, etc.) seen:

____

Estimated no. of individuals in population:

____

Age/population structure (adults, young, etc.)____

Ecological/behavioral notes (e.g., reproductive stage, activity type, feeding, flying, nesting):

____

____

____

Have you seen this species at the same location in the past? Yes _________ No ______

If yes, please give date(s): ________________________________ Previous condition: ___

Is there evidence of disturbance at the site? Yes ________ No _______

If yes, please describe:

____

____

____

Owner(s) of site: ______

Is owner protecting this animal? Yes ___ No _________

Conservation/Management Needs____

____

Comments (other useful information concerning this animal and site - e.g., names and addresses of individuals who might be helpful, publications, museum specimen numbers, etc____

____

(*please include any additional information on the back of this sheet.*)

Additional forms may be obtained upon request. Please send completed field report forms to:

Submitted by: ____

Affiliation: ____

Address: ____

____

Phone ____________________ Date: ____________________

Zoologist
Florida Natural Areas Inventory
1018 Thomasville Rd., Suite 200-C
Tallahassee, FL 32303; ph. (850) 224-8207
Fax (850) 681-9364; dhipes@fnai.org

** note: *each form should include only one species, one locality, and one date*

D

Florida Natural Areas Inventory - Natural Community EOR Form (pg 1 of 2)

Surveysite:______________________________ Surveyors: __________________ Polygon # or ID:__________________ date:______
GPS file #:____________ lat: ____________ long:___________ Photo #:_____________ Comments: ________________________
Directions/locational comments: ____
____

Community type:____ Soil series: ________________ Source:_______

DOMINANT VEGETATION WITHIN 20M RADIUS OF OBSERVATION POINT:

STRATA	cov cl	ht cl	DOMINANT SPECIES COVER: Scientific name - Braun/Blanquet scale
emergent tree			
canopy			
sub-canopy			
tall shrub/ sapling			
short shrub/ sapl, seedl.			
herbaceous tot.			
graminoid			
forb			
fern			
non-vascular			
epiphyte			
vine / liana			
Cover Class - Use Braun/Blanquet scale: 1=0-1% 2=1-5% 3=5-25% 4=25-50% 5=50-75% 6=75-100% Height Class - 1<0.5m 2=0.5-2m 3=2-5m 4=5-10m 5=10-15m 6=15-20m 7=20-35m 8>35m			

SUCCESSION COMMENTS
CANOPY AGE
1 old growth
2 older mature
3 mature
4 younger mature
5 prereproductive trees
6 early successional

SUCCESSION COMMENTS (tree size, structure, age, etc.): ___________________________
____
____
____

NATURE OF DISTURBANCE
1 firebreaks
2 ORV trails or roads
3 agriculture
4 wildlife food plots
5 forestry site prep.
6 logging activities
7 animal digging
8 ditching or hydrologic
9 shrub encroachment
10 exotics encroachment
11 natural disturbances

SEVERITY OF DISTURBANCE
1 light
2 moderate
3 heavy
4 severe

Describe: ___________________
____
____
____
____
____

WEEDY SPECIES
1 absent
2 occasional - <5%
3 common - >5%

List: ___________________
____
____
____

EXOTIC SPECIES
1 absent
2 occasional - <5%
3 common - >5%

List:________________
____
____
____

Disturbance Comments:____
____

HYDROLOGIC ALTERATION
1 shrub encroachment
2 fire breaks
3 ditching
4 roads
5 impoundment
6 dams in watershed
7 canals
8 salt water intrusion
9 groundwater drawdown
10 cause unknown

COMMENTS (Discuss severity for each type and give overall description):
____
____
____
____

PAST FIRE
1 not suppressed
2 suppressed
3 not applicable
4 unknown

Comments/evidence: ____
____
____

MANAGEMENT COMMENTS
____
____
____

OBSERVATION POINT FORM (pg. 2 of 2)

EORANK: (summary of factors such as quality, condition, viability, defensibility, etc.) EORANKDATE:____________________

A Excellent EORANKCOM: ____

B Good ____

C Marginal ____

D Poor ____

COMMUNITY DESCRIPTION (EODATA)

____

____

____

____

____

LANDSCAPE CONTEXT____

____

____

____

____

PLANT CHECKLIST

CANOPY & EMERGENTS	*	%	SHORT SHRUBS	*	%	HERBACEOUS	*	%		*	%
						forb			*graminoid*		
SUB-CANOPY/ TALL SHRUBS											
									fern		
									non-vascular		
									VINES		
									EPIPHYTE		

A=abundant, C=common, O=occasional, R=rare

Revised 9/23/2002

LA NATURAL HERITAGE REPORTING FORM

Mail completed form to:
Louisiana Natural Heritage Program
LA Department of Wildlife & Fisheries
P.O. Box 98000
Baton Rouge, LA 70898
(225) 765-2821

FOR OFFICE USE ONLY
QUADCODE & NAME: ____________
Date received: ____________ (yyyy-mm-dd)
ELCODE: ____________
EOR completed by: ________ (initials) ____________ (date)

We Need Your Help. If you have any information on the location of a rare animal, rare plant or natural ecological community, please complete this form and mail it to us. Thank you!

Species name (scientific & common): ____________________
Natural community type (if known or reporting only a natural community location):
____
Date(s) species located: ____________________
Parish name: ____________ Nearest Town: ____________
Township/Range/Section: ____________ Latitude/Longitude: ____________
*Directions to the site (as detailed as possible): ____________________

____
____
____
____

Habitat Description (plant communities, associated vegetation, topography, surrounding land use):

____
____
____
____
____

Data on species
Number of individuals observed: ____________
Life Stages Present:
For Plants: vegetative ___, in bud ___, flower ___, fruit ___, seedling ___, dormant ___
For Animals: eggs ___, larvae ___, immature ___, adult female ___, adult male ___, adult – sex unknown ___
Other descriptive data on the observation:

____
____
____
____
____

Photograph taken? _____ (If yes, please include a copy for positive identification verification.)
Identification (How was the species identification made? Name identification field guides used or experts consulted. Describe any identification problems): ____________________

Landowner's name, address, & phone if known: ____________________

Ownership comments: ____________________

Disturbance or threats to population: ____________________

Observer's Name, address, & phone: ____________________

* PLEASE ATTACH A LOCATION MAP TO THIS FORM (USGS quadrangle map preferred).

NatureServe

Member Program

The South Carolina Heritage Trust

ELEMENT OCCURRENCE RECORD

EL TYPE __ SUBTYPE __ INDEX CODE ______________ EL OCC NUM ______

*EL NAME ____________________________________ *PRECISION ____

*COUNTY NAME ___________________________ COUNTY CODE ___________

*MAP NAME ____________________________________ MAP NUM ______

LATITUDE ____________________ LONGITUDE ____________________

*SOURCE OF INFO ____________________________________

*DATE ________________ (YYYY-MM-DD) WATERSHED ____________________

LANDOWNER (TYPE) __ (AGENCY) ____ (NAME) ___________________________

SITECODE ______________ SITENAME _________________________________

*DESCRIPTION

____

____

____

____

*Required field

On back of printed form, please copy a topo map showing location.

REPRESENTATIVE FORMS FOR THE MIDWEST REGION

Illinois Natural Heritage Database
Endangered/Threatened Species Occurrence and Sighting Report Form

Name of Species:____

Date Last Observed:_____/_____/_____ | Naturally Occurring ☐ or Introduced Location ☐

Location: (For more accurate mapping, please provide a map showing the exact location)

County:____

Directions from Nearest Landmark:____

____

____

____

Name of Topographic Map(s):____

Legal Description: Township __________ Range__________ Section __________

Site Name:____

Nature of Observation: (number of nests, flowering plants, etc) __________________

____

____

Description of Area:____

____

____

Comments:____

____

____

____

Specimen/Voucher Number(s):____

____

Name of Observer:____

Observer's Phone Number: (______) ________- ______________

____

Return to:
Illinois Department of Natural Resources
Illinois Natural Heritage Database Program Manager
Watershed Managament Section
524 South Second Street
Springfield IL 62701-1787

rev. 2.1: September 6, 2000

MICHIGAN STATE UNIVERSITY EXTENSION

Michigan Natural Features Inventory

ECOLOGICAL COMMUNITY FIELD SURVEY FORM

SURVEY INFORMATION

Survey date: __ __ __ __ - __ __ - __ __ | Time: from ______ am pm to _____ am pm | Sourcecode: F__ __ __ __ __ __ M I U S

Surveyors (principal surveyor first, include first & last name): ____________________

Weather conditions: ____________________

Revisit to this EO needed? ___yes ___no Why?: ____________________

FILING

SURVEYSITE:	SITENAME:
QUADCODE:	QUADNAME:

IDENTIFICATION (Identify community if known positively, or provide closest alliance/association if not known)

Community Name: Data sensitive? Y N | EOID: | Occ # (if known):

Closest Alliance or State/Subnational type ____________________ Data sensitive? Y N

Closest Association or Provisional name ____________________

Classification problems? Y N if Y, explain ____________________

Photo/slide taken? Y N Where has photo/slide been deposited?________________ If associated plot, reference #__________

LOCATIONAL INFORMATION

Was the Landowner contacted? Yes ______ No ______ Landowner Name: ____________________

Owner Type: ____________________ Note: ____________________

DIRECTIONS: Provide detailed directions to the observation (rather than the survey site). Include landmarks, roads, towns, distances, compass directions.

____

Township/Range/Section ____________________

County ____________________ | Managed area ____________________

Was GPS used? Yes ______ No ______ Type of unit ____________________ Unit number __________

Waypoint name/# (when using Garmin) ____________________ File name (when using Trimble) ____________________

OPTIONAL: Latitude ____________________ Longitude ____________________

FEATURE INFORMATION (mandatory) Point: <12.5 m in both dimensions, Line: >12.5 m in one dimension, Polygon: >12.5m in both dimensions

Source Feature: Single Source EO ______ Multi-Source EO ______ Conceptual Feature Type: Point ______ Line ______ Polygon ______

TOPOGRAPHIC MAP (mandatory)

1. Attach a photocopy of the appropriate part of a USGS topographic map (1:24,000 scale if available) and write the map scale on the photocopy. Please do NOT enlarge or reduce the map.
2. Indicate on the map the exact location of the observation(s):
   a. When the observed area is ***no larger than a pen point*** on the map (i.e., extremely small patches), place small points on the map indicating the location(s) of the patches, and label each point with an arrow so they are more easily seen.
   b. When the observed area is ***larger than a pen point*** on the map:
      (1) Draw a thin solid boundary line showing the extent of the observed area for the community.
      (2) Indicate disjunct patches (polygons) by drawing the boundary for each patch separately.
      (3) If the boundary follows the edge of a lake, stream, road, marsh or other feature, draw the boundary precisely on the edge of the feature.
      (4) Where needed, add notes to the map with instructions on where the boundary line is located or if the boundary is shared with other observations.
3. A hand drawn sketch may be included for finer details.
4. Indicate whether aerial photos are available for reference:____________________.

LOCATIONAL CERTAINTY

Is your depiction of the observed area on the map within 6.25 m **(approximately 20ft)** of its actual location on the ground? Y N

If **N,** complete the following:

a. Estimate of uncertainty distance: based on landmarks, elevation, etc., the location of the observed area on the map is accurate to within ___________ meters kilometers feet miles of its actual location on the ground.

b. Is the observed area known to be located within some feature(s) on the map (e.g., wetland boundary, lake, road, trail, highway, contour lines)? Y N

If Y, indicate the boundary within which the observed area is known to be located on the map with a dashed line, and if applicable, identify the feature ________________________________.

FIELD DATA FOR THE ELEMENT

CONFIDENCE EXTENT

Indicate whether there is confidence that the observed area represents the full extent of the community Element at that location. Y N ?

(Y = confidence that the full extent is known; N = confidence that the full extent is not known; ? = uncertainty whether full extent is known)

QUALITATIVE ASSESSMENT OF THE ELEMENT:

Provide a brief "word picture" of the community. Describe variation within the observed area in terms of vegetation structure and environment. Describe dominant and characteristic species and any inclusion communities. If a mosaic, describe spatial distribution and associated community types.

QUALITATIVE DESCRIPTION OF THE ELEMENT:

DBH of several individuals of dominant tree species, include age of cored trees:

Species	DBH(AGE)	DBH(AGE)	DBH(AGE)	DBH(AGE)	DBH(AGE)	DBH(AGE)

Density:

	Tree canopy	Shrub layer	Herb layer
closed			
open			
patchy			
sparse			
absent			

QUANTITATIVE VEGETATION DATA FOR THE ELEMENT:

STRATA	COVER CLASS	DOMINANT SPECIES
T2 -Tree Canopy		
T3 - Subcanopy		
S1 - Tall Shrub		
S2 - Low Shrub		
H - Herb		
N - Nonvascular		
V - Vine		
E - Epiphyte		

Cover Class *

1	trace
2	0.1 – 1%
3	1 – 2%
4	2 – 5%
5	5 – 10%
6	10 – 25%
7	25 – 50%
8	50 – 75%
9	75 – 95%
10	>95%

*this is a widely-used scale included as a guideline

Method used (e.g., ocular estimation, quantitative transect, plot)________________________________

Feature label (e.g., old growth) ________________________________

Note: *For recording more detailed information on species composition and cover/abundance class by stratum, see last page of survey form.*

SIZE - a quantitative measure of the area of the Element at the observed location.

Observed area _____ sq. meters hectares sq. feet sq. yards acres sq. miles *Type of measurement:* precise estimate

Basis for estimate____

CONDITION - an integrated measure of the quality of biotic and abiotic factors, structures and processes within the observed area, and the degree to which they may affect the continued existence of the Element at that location. Components of condition for species are: 1) development/maturity, 2) species composition and biological structure, 3) ecological processes, and 4) abiotic physical/chemical factors. Factors to consider include evidence of stability/presence of old growth, richness/distribution of species, presence of exotic species, degree of disturbance, changes to ecological processes, stability of substrate, and water quality.

Evidence of stability/old growth? Y N if Y, describe ____

____

Evidence of disease, predation, injury to composite species? Y N if Y, describe ____

____

List associated taxa, species, and plant communities within the observed area ____

____

____

Comment on evenness of species distribution within the observed area ____

____

____

____

Natural and Anthropogenic Disturbance: Information on existing disturbance(s) (either natural or caused by humans) within the observed area

- ☐ logging
- ☐ grazing/browsing
- ☐ agriculture
- ☐ mining
- ☐ dumping
- ☐ trails/roads
- ☐ ORV/vehicular disturbance
- ☐ plant disease ____________________
- ☐ insect damage ____________________
- ☐ exotic animal activity (e.g., hog, nutria) ____________________
- ☐ exotic plants ____________________
- ☐ erosion
- ☐ fire
- ☐ wind/ice damage
- ☐ other ____________________

Comment on existing disturbance(s) and changes to ecological processes (e.g., hydrologic and fire regimes) within the observed area ____________________

____

____

____

Comment on exotics present within the observed area and describe resulting impacts ____

____

General Habitat: Information on abiotic physical/chemical factors of specific habitat or microhabitat within the observed area. (check all that apply)

Slope:

Measured Slope ___________° ___________%

☐ Flat	0°	0%
☐ Gentle	0 - 5°	1–9%
☐ Moderate	6 – 14°	10–25%
☐ Somewhat steep	15 – 25°	26–49%
☐ Steep	27 – 45°	50–100%
☐ Very Steep	45 – 69°	101–275%
☐ Abrupt	70 – 100°	276-300%
☐ Overhanging/sheltered	>100°	>300%

Aspect:

Measured Aspect ______° (N = 0°)

☐ Flat	
☐ Variable	
☐ N	338 – 22°
☐ NE	23 – 67°
☐ E	68 – 112°
☐ SE	113 – 157°
☐ S	158 – 202°
☐ SW	203 – 247°
☐ W	248 - 292°
☐ NW	293 - 337°

Topographic position:

- ☐ Ridge, summit, or crest
- ☐ High slope (upper slope, convex slope)
- ☐ Midslope (middle slope)
- ☐ Lowslope (lower slope, footslope)
- ☐ Toeslope (alluvial toeslope)
- ☐ Low level (terrace)
- ☐ Channel
- ☐ other ____________________

Geology:

Igneous Rocks:
- ☐ Granitic (Granite, Schyolite, Syenite, Trachyte)
- ☐ Dioritic (Diorite, Dacite, Andesite)
- ☐ Gabbroic (Gabbro, Basalt, Pyroxenite, Peridotite, Diabase, Traprock)
- ☐ other ______________________

Sedimentary Rocks:
- ☐ Conglomerates and Breccias
- ☐ Sandstone and Conglomerate
- ☐ Siltstone (calcareous or noncalcareous)
- ☐ Limestone and Dolomite
- ☐ Gypsum
- ☐ other ______________________

Metamorphic Rocks:
- ☐ Felsic Gneiss and Schist (Granitic)
- ☐ Mafic Gneiss and Schist
- ☐ Slate and Phyllite
- ☐ Marble
- ☐ Ultramafic (Serpentine)
- ☐ Metasedimentary
- ☐ other______________________

Glacial Deposits:
- ☐ Ice-laid (till)
- ☐ Water-laid (outwash)
- ☐ Lacustrine (lake plain)
- ☐ other ____________

Organic Deposits:
- ☐ Peat (with clear fibric structure)
- ☐ Muck

Slope & Modified Deposits:
- ☐ Talus and scree slopes
- ☐ Colluvial
- ☐ Solifluction, landslide
- ☐ other ____________

Aeolian Deposits:
- ☐ Dunes
- ☐ Aeolian sand flats
- ☐ other ____________

Soil Depth ______ cm (avg)

Surface Soil:
- ☐ Sand
- ☐ Loamy sand
- ☐ Sandy loam
- ☐ Loam
- ☐ Silt loam
- ☐ Sandy Clay loam
- ☐ Clay loam
- ☐ Silty clay loam
- ☐ Sandy clay
- ☐ Clay
- ☐ Silty clay
- ☐ Organic
- ☐ other ____________

Soil Series

Hydrologic Regime:

Wetlands:
- ☐ Intermittently flooded
- ☐ Permanently flooded
- ☐ Semipermanently flooded
- ☐ Temporarily flooded (e.g., floodplains)
- ☐ Seasonally flooded (e.g., seasonal ponds)
- ☐ Saturated (e.g., bogs, perennial seeps)
- ☐ Unknown

Non-Wetlands:
- ☐ Wet Mesic
- ☐ Mesic (moist)
- ☐ Dry-Mesic
- ☐ Xeric (dry)

Groundcover:
(with >5% cover, 20 m x 20 m area)

- _______% Bedrock
- _______% Wood (>1 cm)
- _______% Litter, duff
- _______% Large rocks (cobbles, boulders >10cm)
- _______% Small rocks (gravel, 0.2–10 cm)
- _______% Sand (0.1–2 mm)
- _______% Bare soil
- _______% other
- __________ (total = 100%)

Light:
- ☐ Open
- ☐ Partial
- ☐ Filtered
- ☐ Shade

Cowardin System:
- ☐ Upland
- ☐ Riverine
- ☐ Lacustrine
- ☐ Palustrine

Landform:

Glacial:
- ☐ drumlin
- ☐ end or lateral moraine
- ☐ esker
- ☐ ground moraine
- ☐ kettle-kame topography
- ☐ lake plain
- ☐ outwash channel
- ☐ outwash plain
- ☐ pitted outwash

River / Lakeshore:
- ☐ barrier dune
- ☐ freshwater delta
- ☐ offshore bar
- ☐ riverine estuary
- ☐ sand dune
- ☐ shoreline
- ☐ spit
- ☐ stream bed
- ☐ stream terrace

Other:
- ☐ alluvial fan
- ☐ alluvial flat
- ☐ alluvial terrace
- ☐ cliff
- ☐ cuesta
- ☐ dike
- ☐ hills
- ☐ hills bedrock outcrop
- ☐ hogback
- ☐ ledge
- ☐ plain
- ☐ plateau
- ☐ ravine
- ☐ ridge
- ☐ ridgetop bedrock outcrop
- ☐ rim
- ☐ scarp
- ☐ seep
- ☐ slide
- ☐ talus
- ☐ other ______________________

Describe other abiotic factors <u>within</u> the observed area, including geological formations, aquatic features, and water quality.

____

____

____

____

____

____

LANDSCAPE CONTEXT - an integrated measure of the quality of biotic and abiotic factors, structures and processes <u>surrounding the observed area</u>, and the degree to which they may affect the continued existence of the Element at that location. Components of landscape context for species are: 1) landscape structure and extent, 2) condition of the surrounding landscape (i.e., community development/maturity, species composition and biological structure, ecological processes, and abiotic physical/chemical factors.) Factors to consider include integrity/fragmentation/, stability/old growth, richness/distribution of species, presence of exotic species, degree of disturbance, changes to ecological processes, stability of substrate, and water quality.

Comment on the relative integrity/fragmentation of the Element

List taxa, species, and plant communities in area surrounding the observation

Comment on stability/old growth of communities in area surrounding the observation

Comment on evenness of species distribution in area surrounding the observation

Comment on evidence of existing disturbance (either natural or caused by humans) and changes to ecological processes (e.g., hydrologic and fire regimes) in area surrounding the observation

Comment on exotics present in area surrounding the observation and describe resulting impacts

General Habitat: Describe abiotic factors in area surrounding the observation, such as slope, aspect, topographic position, geology, soils/substrates, hydrologic regime, groundcover, light, Cowardin system, land forms, aquatic features, soils/substrate, geological formations, and water quality.

MISCELLANEOUS DATA

PAST IMPACTS on the Element, both within and surrounding the observed area (e.g., grazing, logging, mining, agriculture, ORVs, dumping)

MANAGEMENT, MONITORING and RESEARCH NEEDS for the Element at this location (e.g., burn periodically, open the canopy, ensure water quality, control exotics, ban ORVs, study effects of browsing)

PROTECTION NEEDS for the Element at this location (e.g., protect the entire marsh, the slope and crest of slope)

ADDITIONAL COMMENTS:

SPECIES COMPOSITION AND COVER/ABUNDANCE CLASS BY STRATUM
(enter values for each stratum AND for Total Cover, columns defined on page 2)

SPECIES	C	Total	T2	T3	S1	S2	H	N	V	E

Cover Class *				
1	trace	6	10 – 25%	
2	0.1 – 1%	7	25 – 50%	
3	1 – 2%	8	50 – 75%	
4	2 – 5%	9	75 – 95%	
5	5 – 10%	10	>95%	*this is a widely-used scale included as a guideline

Use additional pages if necessary.

If you have any questions regarding this form and its methodology please contact MNFI at (517) 373-1552.
P:\nfi\field forms\community_field_form.doc
Rev. 10//2003

MICHIGAN STATE UNIVERSITY EXTENSION

AQUATICS SPECIAL ANIMAL SURVEY FORM

Michigan Natural Features Inventory

SURVEYOR INFORMATION

Survey date: __ __ __ __ - __ __ - __ __	Time from: _______ to: _______ am or pm (circle)	Sourcecode: F __ __ __ __ __ __ M I U S

Surveyors (principal surveyor first, include first & last name): ____________________

____

Weather conditions: ____________________

Revisit to this EO needed? ___yes ___no Why?: ____________________
EO refers to element occurrence i.e. the species this form is reporting on

ELEMENT INFORMATION

Scientific name: Data sensitive? Y N	EOID:	Occ.# (if known):

FILING

SURVEYSITE:	SITENAME:
QUADCODE:	QUADNAME:

LOCATIONAL INFORMATION

Was the Landowner contacted? Yes ______ No ______ Landowner Name:
____

Owner Type: ____________________ Note:____________________

DIRECTIONS: Provide detailed directions to the observation (rather than the survey site). Include landmarks, roads, towns, distances, compass directions.

____

____

____

Township/Range/Section ____________________	Watershed ____________________
County ____________________	Managed area ____________________

Was GPS used? Yes ______ No ______ Type of unit ____________________ Unit number __________

Waypoint name/# (when using Garmin) ____________________ File name (when using Trimble) ____________________

OPTIONAL: Latitude ____________________ Longitude ____________________

FEATURE INFORMATION (mandatory) Point: <12.5 m in both dimensions, Line: >12.5 m in one dimension, Polygon: >12.5m in both dimensions

Source Feature: Single Source EO ______ Multi-Source EO ______ Conceptual Feature Type: Point ______ Line ______ Polygon ______

TOPOGRAPHIC MAP (mandatory, the website topozone.com can be used as a source for these maps)

1. Attach a photocopy of the appropriate part of a USGS topographic map (1:24,000 scale if available) and write the map scale on the photocopy. Please do NOT enlarge or reduce the map.
2. Indicate on the map the exact location of the observation(s):
   a. When the observed area is ***no larger than a pen point*** on the map (i.e., only a small number of individuals or extremely small patches), place small points on the map indicating the location(s) of the individuals or patches, and label each point with an arrow so they are more easily seen.
   b. When the observed area is ***larger than a pen point*** on the map, (e.g., a population of plants, foraging birds):
      (1) Draw a thin solid boundary line showing the extent of the observed area occupied by the individuals.
      (2) Indicate disjunct patches (polygons) by drawing the boundary for each patch separately.
      (3) If the boundary follows the edge of a lake, stream, road, marsh or other feature, draw the boundary precisely on the edge of the feature.
(4) Where needed, add notes to the map with instructions on where the boundary line is located or if the boundary is shared with other observations.
3. A hand drawn sketch may be included for finer details.

LOCATIONAL CERTAINTY
Is your depiction of the observed area on the map within 6.25 **m (approximately 20ft)** of its actual location on the ground? Y N
If **N,** complete the following:

a. Estimate of uncertainty distance: based on landmarks, elevation, etc., the location of the observed area on the map is accurate to within __________ meters kilometers feet miles of its actual location on the ground.

b. Is the observed area known to be located within some feature(s) on the map (e.g., wetland boundary, lake, road, trail, highway, contour lines)? Y N

If Y, indicate the boundary within which the observed area is known to be located on the map line, and if applicable, identify the feature (e.g., marsh).

IDENTIFICATION

Photograph/slide taken? ___yes ___no If yes, will a copy be submitted to Heritage? ___yes ___no MNFI office: Added to collection?____ (check)

Specimen collected? ___yes ___no Collection # and repository: ______________________________

Identification problems? ___yes ___no If necessary, describe the important animal characteristics you used for identification: ______________________________

____

FIELD SURVEY and ELEMENT OCCURRENCE INFORMATION

Type of survey: ___sight ___ netting ___ shock ___ other (explain):______________________________

Gear used (seine, bucket etc.): ______________________________

Time (hours, etc.): ______________________________

Number observed during survey:______________________________

Incidental observed (spent shells, etc.): ______________________________

Population density (if practical): number: ______________ per area unit: ________________ (i.e., $meters^2$, $kilometers^2$, $miles^2$, etc.)

Area of occupancy (fill in one):_______ meters _________acres _________miles Type of measurement (check one): ___ Precise ___ Estimate

CONDITION:

Condition is an integrated measure of the quality of biotic and abiotic factors, structures and processes within the occurrence, and the degree to which they affect the continued existence of the occurrence. Components of condition for species are: 1) reproduction and health, 2) ecological processes, 3) species composition and biological structure, 4) abiotic physical/chemical factors. Factors to consider: evidence of regular successful reproduction, habitat degradation, disturbance, presence of exotic species, the degree to which ecological processes are sustaining the habitat. Where possible include a comparison to other occurrences.

EVIDENCE OF REPRODUCTION (larval, eggs) :______________________________

____

____

EVIDENCE OF DISEASE/PREDATION (parasites, growths) :______________________________

____

ASSOCIATED SPECIES

List other species observed at this site. Note especially listed species and potential competitors, predators, and prey. Mark appropiate columns.

Species	Number Observed	Notes, observations, etc.

EXOTICS:

____yes ____no If yes, describe their impacts to the occurrence.______________________________

____

HABITAT DESCRIPTION: Describe the specific habitat or micro habitat where this animal occurs. Convey a mental image of the habitat and its features including: land forms, aquatic features, vegetation, slope, aspect, soils, associated plant and animal species, natural disturbances.

RIPARIAN DESCRIPTION (trees, shrubs present)

SUBSTRATE (cobble, boulder, aquatic vegetation, etc.)

CURRENT THREATS to this occurrence (i.e., grazing, logging, mining, plantations, ATVs, dumping, etc). *Exotics implied if listed out in previous section.*

POTENTIAL THREATS to this occurrence (erosion, development):

PAST IMPACTS to the occurrence (i.e., logging, , etc.):

Width:	Depth:	Water Clarity:	Flow:
pH:	Conductivity:	Temp:	Other:

MANAGEMENT AND PROTECTION

MANAGEMENT, MONITORING AND RESEARCH NEEDS for this occurrence (e.g. burn periodically, open the canopy, ensure water quality, control exotics, keep out the ATV's, study effects of browsing)

AREAS IN NEED OF PROTECTION: (e.g. the entire marsh, the slope and crest of slope, the fen and upland, etc.)

OTHER FORMS

_____Stream Morphometry _____EPA Habitat _____Mussel Survey _____Fish Survey _____Other:_______________

If you have any questions regarding this form and its methodology please contact MNFI at (517) 373-1552.
P:\nfi\field forms\aquatics_special_animal_form.doc
Rev. 10//2003

MNFI SPECIAL SPECIES FORM

PLEASE ENTER ALL INFORMATION AVAILABLE.

USE THE BACK FOR COMMENTS AS NEEDED.

PLEASE ATTACH A 1:24,000 USGS TOPO MAP SHOWING LOCATION OF ELEMENT.

Source code ______________

Surveysite ______________

Quad code ______________

EO# __________ EOID __________

Surveyor: ______________________________

Phone: ______________ Date: __________

Species identified: ______________________________ **(PERMIT REQUIRED)** Voucher/Collection#: ______________

LOCATION: County ____________________ Town ______ Range ______ sec. ________ ¼ ________

Directions from nearest town or road:

____

____

HABITAT DATA: List associate species. For plants, please list at least 6 species in order of dominance, beginning with overstory if present. Restrict associates to immediate habitat.

Describe microhabitat: Focus on exactly where species occurs and apparent favoring/limiting factors. Include relevant information on soils, micro-topography, moisture conditions, etc.

Estimate of habitat extent: ________________ (acres, sq meters, sq feet?)

POPULATION SIZE, EXTENT AND CONDITION: Total # of individuals: ____________ (Estimate or actual count?)

Phenology (plants): % flowering __________ % fruiting ____________ Apparent vigor (plants): __________

____

Population Age Structure (animals): #adults ___________ #juveniles ___________

Evidence of reproduction:

____

____

CONSERVATION DATA: Overall Site Quality: _____ Excellent _____ Good _____ Fair _____ Poor

Disturbance to organisms or habitat:

____

Threats or need for protection (immediate? long term?):

____

____

Other information needs (survey, monitoring, etc.):

____

____

USE THE BACK FOR COMMENTS AND A MAP SHOWING LOCATION OF ELEMENT

1:24,000 USGS Topo maps can be printed from www.topozone.com

RETURN TO: Michigan Natural Features Inventory, P.O. Box 30444, Lansing, MI 48909-7944

REPRESENTATIVE FORMS FOR THE NORTHWEST REGION

Washington Natural Heritage Program
Rare Plant Sighting Form

0 1 2 3 cm

Please read instructions page. Shaded boxes are for Natural Heritage Staff use only.

Taxon Name: ______________________ EO #

Are you confident of the identification? ☐ yes ☐ no Explain: ______________________

Survey Site Name: ______________________

Surveyor's Name/Phone/Email: ______________________

Survey Date: ______________ (yr-mo-day) County: ______________

Quad Name: ______________ Quad Code:

Township: ________ N Range: ________ Section(s): ________ 1/4 of 1/4: ________ (e.g. NW of NE)

Directions to site: ______________________

<u>Mapping</u> (see instructions): Attach a copy of the USGS 7.5 minute quad with the location and extent of the rare plant population clearly drawn. <u>Do not reduce or enlarge</u> the photocopy or printout of the map. If your map is a different scale (not recommended) please write the scale on the map.
Please answer the following:

1. I used GPS to map the population: ☐ No (skip to #2) ☐ Yes (complete #1 & #3)
   ☐ Coordinates are in electronic file on diskette (preferred) ☐ Coordinates written below or attached
   Description of what coordinates represent: ______________________

   GPS accuracy: ☐ Uncorrected ☐ Corrected to <5m
   GPS datum: ______________________
   GPS coordinates: ______________________

2. I used a topographic map to map the population:
   ☐ yes (complete #2) ☐ no (provide detailed directions & description above, and skip to #3)

   I am confident I have accurately located and mapped the population at map scale: ☐ yes (skip to #3)
   ☐ no, but I am confident the population is within the general area indicated on the map as follows:
   On the same map, use a highlighter to identify the outer boundary of the area where the population could be, given the uncertainties about your exact location.

3. I used the following features on the map to identify my location (stream, shoreline, bridge, road, cliff, etc.):

To the best of my knowledge, I mapped the entire extent of this population
☐ yes ☐ no ☐ unknown If no or unknown, explain: ______________________

Is a revisit needed? ☐ no ☐ yes - if yes, why?: ______________________

Ownership (if known): ______________________

Revised April 2002 - see instructions

Population Size (# of individuals or ramets) or estimate: ______________________________

Population (EO) Data (include population vigor, microhabitat, phenology, etc.): ______________________________

Plant Association (include author, citation, or classification, e.g. Daubenmire): ______________________________

Associated Species (include % cover by layer and by individual species for dominants in each layer):

Lichen/moss layer: ______________________________

Herb layer: ______________________________

Shrub layer(s): ______________________________

Tree layer: ______________________________

General Description (include description of landscape, surrounding plant communities, land forms, land use, etc.):

Minimum elevation (ft.): ______________ Maximum elevation (ft.): ______________

Size (acres): ______________ Aspect: ______________ Slope: ______________

Photo taken? ☐ yes ☐ no

Management Comments (exotics, roads, shape/size, position in landscape, hydrology, adjacent land use, cumulative effects, etc.):

Protection Comments (legal actions/steps/strategies needed to secure protection for the site): ______________________________

Additional Comments (discrepancies, general observations, etc.): ______________________________

Please mail completed form with map:

WASHINGTON NATURAL HERITAGE PROGRAM
DEPARTMENT OF NATURAL RESOURCES
PO BOX 47014, OLYMPIA WA 98504-7014

WASHINGTON STATE DEPARTMENT OF Natural Resources

Instructions for Washington Natural Heritage Program Rare Plant Survey Form
(Form for external data contributors)

Please complete all sections except for the shaded areas. Those will be completed by WNHP staff.

Taxon Name: Please enter a complete scientific name.

Are you confident of the identification? If you had trouble with the identification, please explain why (e.g. immature or senescent plants, similarity to other species, etc.). If a specimen was verified by an expert on the taxon, please indicate, such as "verified by ....".

Survey Site Name: This should be a place name near the population, preferably something that appears on the USGS quad map. It should help someone, not intimately familiar with the area, locate this population.

Surveyor's Name: Enter the name(s) of the person who located the plant. Include their contact information so that they can be contacted if more information is need.

Survey Date: When was the plant located? Please use year-month-day format (e.g. 2001-07-05)

County: In what county is the site located?

Quad Name: Please enter name of the USGS 1:24,000 scale quad map where the site is located.

Township, Range, Section, and _ of _: Enter the legal description of this site. Quarter sections should be entered in the form "NW of SE", which indicates that the site is within the northwest quarter of the southeast quarter-section.

Directions to site: Please explain how someone else could relocate the site, starting from a named paved road.

Mapping: Attach a copy of the USGS 7.5 minute quadrangle map with the location and extent of the rare plant population clearly drawn. Do not reduce or enlarge the photocopy or printout of the map . If you're using a map at a different scale (not recommended) please write the scale on the map. Follow the three steps listed in describing your location. Include detailed comments here; these are useful to us.

1. **GPS:** When mapping with GPS, the best way to submit data to us is to export this data to a floppy disk and mail with your survey from. Submitting a short list of GPS coordinate values is also acceptable. Whether you submit a disk or a list, please provide the accuracy and datum used by your GPS. Also, write a description of what these coordinates represent. For instance, do your GPS points represent the centers of individual patches, each with an estimated size?

2. **Topographic Map:** Submitting this is helpful to interpreting your survey, even if you are submitting data collected via GPS. If neither a map nor GPS was used to collect to the information you are reporting, we will rely on written comments in 'directions to site' and mapping question #3.

   I am confident I have accurately located and mapped the population at map scale: The most common answer is 'no'. When surveying away from roads or mapped streams, one usually cannot reference their position accurately to map scale. Use this rule of thumb: to map at 1:24,000 scale, your marks must be within one pencil line's width of their correct location. Often the field biologist can estimate location to within a small area visible on the map (i.e., 'I know I'm between these two streams and between 1000 and 1400 ft. elevation'). If you can estimate your location, draw this area surrounding your mapped feature.

3. **I used the following features on the map to identify my location**: Please include comments that will help us map the site accurately. If the population is located near or within some feature on the map, please describe. For instance, we want to know if the plants are located within a wetland, at the base of a cliff, on the west bank of a river, or within the littoral zone of a lake.

I mapped the entire extent of the population? Might there be more of these plants in this general area? For instance, did you do an exhaustive survey of all surrounding appropriate habitat, or did you stop at a fence line or ownership boundary.

Is a revisit needed? Check yes if, for instance, identification should be verified at another time, the population should be mapped more accurately, if you did not survey all of the potential habitat, if you think there is some imminent threat, etc.

Ownership: If you know who owns the property, please enter that here.

Population Size: Your count or estimate of the number of individuals or ramets.

Population Data: Describe the population quality and phenology. For example: "45 plants scattered in a wet depression with an area of 10 by 45 meters. Vigorous plants with 30% flowering and 70% vegetative."

Plant Association: If you have access to a vegetation key, please include the plant association of the immediate area along with the author of the key.

Associated Species: Please enter the scientific names of the other plant species that are found in the immediate area and their percent cover, if determined. These should be described by layer as listed on the form.

General Description: Describe the local landscape, including physical land forms, vegetation, and land use.

Minimum & Maximum Elevation: Enter values in feet and a maximum elevation only if this is a large population with a range of elevations.

Size: How many acres does the population cover? If less that 0.1 acre, you can leave this blank.

Aspect: Enter the direction of slope as degrees or as a compass direction such as SW.

Slope: Enter as degrees or percent.

Photo taken? Check yes if you took a photograph of the population, otherwise, check no.

Management Comments: Enter information about land use and threats (exotic species, recreation, road maintenance, grazing, etc.) here as well as recommended changes in site use that will help ensure continued existence of the population.

Protection Comments: Enter any legal steps that you think should be taken to protect the population.

Additional Comments: Enter anything that you think is important about this population that did not fit in any other space on the form.

REPRESENTATIVE FORMS FOR THE SOUTHWEST REGION

COLORADO NATURAL HERITAGE PROGRAM
ELEMENT OCCURRENCE FIELD FORMS

Mailing Address: 8002 Campus Delivery Fort Collins, CO 80523-8002
Physical Address: 254 General Services Bldg., Fort Collins, CO 80523

We Need Your Help. *If you have information on the location of a rare plant, rare animal or ecological community and would like to help us build the Natural Heritage inventory, please complete the forms that follow. - Thank you!*

Field forms for:

Animals

Plants

Natural Communities

Wetland Communities

This box to be completed by CNHP Office
Project name: ______________________________
New: Y N Update: Y N Update eonum: __________

COLORADO NATURAL HERITAGE PROGRAM
ANIMAL ELEMENT OCCURRENCE FIELD FORM

Mailing Address: 8002 Campus Delivery Fort Collins, CO 80523-8002
Physical Address: 254 General Services Bldg., Fort Collins, CO 80523
Attn: Jeremy Siemers

We Need Your Help*. If you have information on the location of a rare plant, rare animal or ecological community and would like to help us build the Natural Heritage inventory, please complete the form below. - Thank you!*

General:
Element Common Name: ______________________________
Element Scientific Name: ______________________________
Observer(s):______________________________ Survey Date: __________

Locational Information:
Quadname: ______________________________ Quadcode (if known): __________
Surveysite Name (from 7.5' Quad):______________________________
County: ______________________________ Elevation (range if applicable):__________
Legal Description (TRS & quarter quarter): ______________________________
UTM Zone: __________ Northing: __________ Easting: __________

Locational Accuracy:
1. Is your depiction of the individuals on the topographic map within 6m (20ft) of their actual location on the ground?
☐ **Yes** ☐ **No** (if no, answer question 2 below)
2. You are accurate to within ____**meters** ____**feet** ___**miles** of the actual location.

Directions:
Driving and hiking directions (please provide a photocopy of map with location of the occurrence marked or outlined:)

____
____
____
____
____
____

Occurrence data (Size, Condition, Landscape Context):
Size of observed feature: ☐ **none (point)** _____**sq. meters** ______**sq. miles** ______ **acres**

NUMBER OF INDIVIDUALS: __________ AGE(S) AND SEX(ES) (if known): __________

REPRODUCTIVE EVIDENCE: ______________________________

EVIDENCE OF DISEASE, PREDATION OR INJURY: ______________________________

ADDITIONAL COMMENTS REGARDING THE OCCURRENCE: ______________________________
____
____

General Habitat Description: (dominant plant community, habitat description, etc.) __________

ASSOCIATED VERTEBRATE TAXA: ______
EXOTIC SPECIES: ______

Management comments (past/present/future recommendations): ______

PREDOMINANT LAND USES:

Protection comments (Are there any protection plans or strategies in place?): ______

Land Owner: ______
Owner comments (special requests, permissions, circumstances): ______

Additional Comments: ______

Photo numbers (if applicable): ______
Specimens: Y N **Collection Numbers**: ______

CNHP Office Below This Line – If no EO Specifications exist
SIZE: A B C D (abundance, density)
Comments______

CONDITION: A B C D (productivity, vigor of individuals)
Comments ______

LANDSCAPE CONTEXT: A B C D (condition and extent of surrounding landscape)
Comments______

Eorank summary comments: ______

Eorank: A B C D E F H X **subrank:** i r **Eorank date:**______

Bestsource: ______

Sourcecode:______COUS

This Box to be completed by CNHP Office
Project name: ______________________
New: Y N   Update: Y N   Update eonum: ________

COLORADO NATURAL HERITAGE PROGRAM
PLANT SPECIES OF SPECIAL CONCERN SURVEY FORM

COLORADO STATE UNIVERSITY-COLLEGE OF NATURAL RESOURCES
Mailing Address: 8002 Campus Delivery, Fort Collins, CO 80523-8002
Physical Address: 254 General Services Bldg., Fort Collins, CO 80523
Attn: Jill Handwerk

***We Need Your Help**. If you have information on the location of a rare plant, rare animal or ecological community and would like to help us build the Natural Heritage inventory, please complete the form below. - Thank you!*

DATE OF SURVEY: ______________
OBSERVER(S): ______________

TAXONOMY
SCIENTIFIC NAME: ______________
COMMON NAME: ______________

LOCATION (attach a copy of pertinent 7.5' or 15' topographic map section with locations of populations/subpopulations outlined, one map for each sensitive species described)
SURVEY SITE NAME: ______________
COUNTY: ______________ USGS QUADRANGLE: ______________
TOWNSHIP: ______________ RANGE: ______________ SECTION: ______________ 1/4 SEC.: ________
ADDITIONAL T/R/S, SECTIONS OR 1/4 SECS.: ______________
UTM ZONE AND COORDINATES: ______________
ELEVATION (at population center and range of population if known): ______________
NATIONAL FOREST/BLM DISTRICT: ______________
LAND OWNERSHIP/MANAGEMENT (if not USFS/BLM): ______________
LOCATIONAL ACCURACY:
1. Is your depiction of the individuals on the topographic map within 6m (20ft) of their actual location on the ground?
☐ Yes ☐ No (if no, answer question 2 below)
2. You are accurate to within ____meters ____feet ___miles of the actual location.
SIZE: Please indicate the estimated size of the area occupied by the animal, plant or community: ______ac or ______sq. m
If the area occupied is long, narrow and less than 12.5 meters wide, please indicate: Length:______(m) Width: ______(m)

DIRECTIONS TO SITE (refer to roads, trails, geographic features, etc): ______________

POPULATION SIZE
ESTIMATED NUMBER OF INDIVIDUALS (or exact count, if feasible; if plants are spreading vegetatively, indicate number of aerial stems): ______________
NUMBER OF SUB POPULATIONS (if applicable): ______________
SIZE OF AREA COVERED BY POPULATION (acres): ______________

BIOLOGY
PHENOLOGY (percentage flowering, fruiting, vegetative): ______________

ANY SYMBIOTIC OR PARASITIC RELATIONSHIPS (e.g. pollinators)? ______________

EVIDENCE OF DISEASE, PREDATION OR INJURY? ______________________________

REPRODUCTIVE SUCCESS (evidence of seed dispersal and establishment): ______________________________

____

HABITAT

VEGETATION STRUCTURE WITHIN POPULATION AREA

TOTAL TREE COVER (%): ______________

TOTAL SHRUB COVER (%): ______________

TOTAL FORB COVER (%): ______________

TOTAL GRAMINOID COVER (%): ______________

TOTAL MOSS/LICHEN COVER (%): ______________

TOTAL BARE GROUND COVER (%): ______________

ASSOCIATED PLANT COMMUNITY (list dominant species currently present, include age structure if known): ________

____

____

HABITAT TYPE: ______________________________

ADDITIONAL ASSOCIATED PLANT SPECIES: ______________________________

____

____

ASPECT (S, SE, NNW, etc.): ______________ % SLOPE: ______________

SLOPE SHAPE (concave, convex, straight, etc.): ______________________________

LIGHT EXPOSURE (open, shaded, partial shade, etc.): ______________________________

MOISTURE (dry, moist, saturated, inundated, seasonal seepage, etc.): ______________________________

PARENT MATERIAL: ______________________________

GEOMORPHIC LAND FORM (e.g. glaciated mountain slopes and ridges, alpine glacial valley, rolling uplands, breaklands, alluvial-colluvial-lacustrine, rockslides): ______________________________

SOIL TEXTURE: ______________________________

EVIDENCE OF THREATS AND DISTURBANCE (e.g. effects on population viability due to mining, recreation, grazing):

____

____

____

____

DOCUMENTATION

PHOTOGRAPH TAKEN (if so, indicate photographer and repository): ______________________________

____

SPECIMEN TAKEN (if so, list collector, collection number, and repository): ______________________________

____

IDENTIFICATION (list name of person making determination, and/or name of flora or book used): ______________

____

COMMENTS:

____

____

____

____

This box to be completed by CNHP Office
Project name: ____________________
New: Y N Update: Y N Update eonum: __________

COLORADO NATURAL HERITAGE PROGRAM
NATURAL COMMUNITY OCCURRENCE FIELD FORM

Mailing Address: 8002 Campus Delivery Fort Collins, CO 80523-8002
Physical Address: 254 General Services Bldg., Fort Collins, CO 80523
Attn: Jodie Bell

We Need Your Help. *If you have information on the location of a rare plant, rare animal or ecological community and would like to help us build the Natural Heritage inventory, please complete the form below. - Thank you!*

Scientific Name: ____________________
Observer(s): ____________________ Survey Date: ____-____-____ (yr-m-d)
Quadname: ____________________ Quadcode (if known): ____________________
Surveysite Name: ____________________ Site Name (if known): ____________________
County: ____________________ Elevation (range if applicable): ____________________
Townrange and Section: ____________________
TRS comments: ____________________
UTM Zone: __________ Northing: ____________________ Easting: ____________________
Size of observed feature: AREA: __________ acres LENGTH: __________ WIDTH: __________
(Pace off or use a measuring tape to obtain length and width)

Locational Accuracy:

1. Is your depiction of the community on the topographic map within 6m (20ft) of its actual location on the ground?
   ___ **Yes** ____ **No** (if no, answer question 2 below)
2. You are accurate to within ____**meters** ____**feet** ___**miles** of the actual location.

Confidence extent: (Y, N, ?): __________
Y = Confidence that the full extent of the Element Occurrence in known.
N = Confidence that the full extent of the Element Occurrence is **not** known.
? = Uncertainty whether the full extent is known.

Directions:
Prominent topographical features: ____________________
____
Driving and hiking directions: ____________________
____
____

Element Ranking Information

EORank: A B C D (Size + Condition + Landscape Context = predicted viability (e.g. "big + not weedy + excellent surroundings = A))
EORankDate: ____-____-____ (yr-m-d)

EORankCom:
Size: A B C D ____________________
(How big is it now?)

Condition: A B C D ____________________
____
____
(Quality of biotic and abiotic features/processes, stand maturity, species composition, stability of substrate, water quality, etc).
Landscape Context : A B C D ____________________
____
____
(Quality of biotic and abiotic factors/processes of surrounding landscape, structure, extent, condition (fragmentation, hydrologic manipulation, etc.)
Other Comments (age class, reproduction, etc.): ____________________

____
____

Community Information and Data

Slope(%):_______ Aspect:_______ Soils:_______________ Geologic Substrate:_______________

GenDesc (site description, environmental information, etc.): ______________________________
____
____
____
____
____

EOData:

Method used:____________________(Ocular estimate, quantitative transect or plot) Total Ground Cover: ______%.

Total Tree cover: ______%.

Tree cover (%) by species: ____
____
____

Total Shrub cover: _____%.

Shrub cover (%) by species____
____
____

Total Graminoid cover: _____%.

Gram cover (%) by species:____
____
____

Total Forb cover: ______%.

Forb cover (%) by species: ____
____
____

Community Description: ____
____
____
____

Management and Protection

Management Urgency:_____ (M1= immediate management need, M2= need w/in 5 years or loss, M3= need w/in 5 years or degrade, M4= future management need, M5= none needed)

MgmtCom (What management actions would help protect this occurrence?): ____________________
____
____

Protection Urgency:_____ (P1= immediate threat, P2= w/in 5 years, P3= not w/in 5 years, P4= no threats, P5= protected)

ProtCom (Known or observed threats to occurrence): ______________________________
____
____

Other Comments: ____
____
____

Owner (Private, USFS, BLM, etc.): ______________________________________

OwnerCom:____

(special requests, permissions, circumstances)

DataSens : ___ (Y/N; Does the landowner request confidentiality?) Photos: __________ (initials, roll #, frame #)

Specimens:____

Bestsource: ____

______________________________ Source Code: ____________________________

This box to be completed by CNHP Office
Project name: ____________________
New: Y N Update: Y N Update eonum: __________

COLORADO NATURAL HERITAGE PROGRAM
NATURAL COMMUNITY OCCURRENCE FIELD FORM–FOR WETLANDS
Mailing Address: 8002 Campus Delivery Fort Collins, CO 80523-8002
Physical Address: 254 General Services Bldg., Fort Collins, CO 80523
Attn: Jodie Bell

We Need Your Help*. If you have information on the location of a rare plant, rare animal or ecological community and would like to help us build the Natural Heritage inventory, please complete the form below. - Thank you!*

Scientific Name: ____________________
Taxonomic Identifiction: Yes No
Observer(s): ____________________ Survey Date: ____-____-____ (yr-m-d)

Locational Information
Quadname: ____________________ Quadcode (if known): ____________________
Surveysite Name: ____________________ Site Name (if known): ____________________
County: ____________________ Elevation (range if applicable): ____________________
Townrange and Section: ____________________
TRS comments: ____________________
UTM Zone: __________ Northing: ____________________ Easting: ____________________

Size of Observed Feature: AREA: __________ acres LENGTH: __________ WIDTH: __________
(Pace off or use a measuring tape to obtain length and width)

Locational Accuracy:
1. Is your depiction of the community on the topographic map within 6m (20ft) of its actual location on the ground?
____**Yes** ____ **No** (if no, answer question 2 below)
2. You are accurate to within ____**meters** ____**feet** ____**miles** of the actual location.

Confidence extent: (Y, N, ?): __________
Y = Confidence that the full extent of the Element Occurrence in known.
N = Confidence that the full extent of the Element Occurrence is **not** known.
? = Uncertainty whether the full extent is known.

Directions:
Prominent topographical features: ____________________

Driving and hiking directions:

Element Ranking Information
EORank: A B C D (Size + Condition + Landscape Context = predicted viability (e.g. "big + not weedy + excellent surroundings = A))
EORankDate: ____-____-____ (yr-m-d)
EORankCom:

Size: A B C D ____________________
(How big is it now?)

Condition: A B C D ____
(Quality of biotic and abiotic features/processes, stand maturity, species composition, stability of substrate, water quality, etc).

Wetland Functions:

- Flood Attenuation and Storage (High, Moderate, Low): ____________________
- Sediment/Shoreline Stabilization (High, Moderate, Low): ____________________
- Groundwater Discharge (Yes, No): ____________________
- Groundwater Recharge (Yes, No): ____________________
- Dynamic Surface Water Storage (High, Moderate, Low): ____________________
- Elemental Cycling (Normal, Disrupted): ____________________
- Removal of Nutrients, Toxicants, and Sediments (High, Moderate, Low): ____________________
- Habitat Diversity (High, Moderate, Low): ____________________
- General Wildlife and Fish Habitat (High, Moderate, Low): ____________________
- Production Export/Food Chain Support (High, Moderate, Low): ____________________
- Uniqueness (High, Moderate, Low): ____________________
- Overall Functional Integrity (At Potential, Below Potential):____________________

Landscape Context : A B C D ____
____
____
(Quality of biotic and abiotic factors/processes of surrounding landscape, structure, extent, condition (fragmentation, hydrologic manipulation, etc.)

Other Comments (age class, reproduction, etc.): ______________________________
____
____

Community and Site Information and Data

Slope(%):_______ Aspect:_______ Soils:_______________ Geologic Substrate:____________________

GenDesc (site and landscape description, landform, **restoration potential**, erosion, animal use, disturbance, etc.): ____________________
____
____
____
____

EO Data: Community Description (vegetation structure e.g., canopy cover, height, density, spatial distribution): ____________________
____
____
____
____

Method used:____________________(Ocular estimate, quantitative transect or plot)

Total Tree cover: ______%.

Tree cover (%) by species: ______________________________
____

Tree cover by size and species (pole, sapling, seedling): ______________________________
____

Total Shrub cover: _____%.

Shrub cover (%) by species: ______________________________
____

Shrub cover by size and species (tall, mid, low): ____

____

Total Forb cover: ______%.
Forb cover (%) by species: ____

____

____

Total Graminoid cover: _____%.
Gram cover (%) by species: ____

____

____

Total Ground Cover:_______%

Management and Protection

Management Urgency:____ (M1= immediate management need, M2= need w/in 5 years or loss, M3= need w/in 5 years or degrade, M4= future management need, M5= none needed)

MgmtCom (What management actions would help protect this occurrence?): ______________________________

____

____

Protection Urgency:____ (P1= protection actions needed immediately; P2= protection actions may be needed within 5 years; P3= Protection actions may be needed, but not within the next 5 years; P4= no protection actions needed in future; P5= land protection is complete)

ProtCom (Known or observed threats to occurrence): ______________________________________

____

____

Other Comments: ____

____

____

Owner (Private, USFS, BLM, etc.): ____

OwnerCom:____
(special requests, permissions, circumstances)

DataSens : ___ (Y/N; Does the landowner request confidentiality?) Photos: __________ (initials, roll #, frame #)

Specimens:____

Bestsource: ____

Source Code: ____

APPENDIX B

LITERATURE REVIEW & INTERPRETATION

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SECTION 1
ECOTOXICOLOGICAL LITERATURE REVIEW

This appendix presents a focused evaluation of selected amphibian ecotoxicological literature, and a database compilation of this literature. The objective of this evaluation is to serve as an initial step in the development a standardized risk assessment protocol for evaluating potential risks to amphibians at sites owned and/or operated by the United States Navy. The first half of this appendix contains a focused literature review for the following 11 constituents: (1) cadmium (2) chromium, (3) copper, (4) lead, (5) mercury, (6) nickel, (7) zinc, (8) PCBs, (9) 4,4 DDT, (10) PAHs, and (11) ordnance and explosives. These constituents were selected because they are commonly identified at CERCLA, RCRA, and other sites being investigated by the Navy under the Installation Restoration (IR) and other environmental programs. For each constituent, a brief profile has been prepared describing the sources, uses, and fate and transport characteristics in terms of its relevance to amphibian toxicity. Following the profile, each constituent-specific sub-section includes a summary of the available amphibian toxicity information.

The ecotoxicological literature review presented in this section focused on acute and chronic immersion laboratory studies with amphibians. Aquatic immersion studies were reviewed (rather than injection studies) since the immersion exposure pathway most closely approximates *in situ* exposure pathways in the natural environment. Contaminant tissue residue studies were not reviewed for the subject constituents, since the majority of these studies simply indicate the body or tissue burden of a constituent, without any indication of effects or ecotoxicological endpoints. FETAX (frog embryo teratogenesis assay *Xenopus*) studies were included in the review. However, it is recognized that there are some uncertainties associated with using this bioassay in a traditional risk assessment context, since it uses a species non-native to North America, there are limited comparative sensitivity data available between native North American species and *Xenopus*, it involves evaluation of limited life stages (often 96-hour studies), and the FETAX bioassay includes endpoints (e.g., teratogenesis) that are not always considered by risk managers when making ecological risk management decisions. When possible, solid phase exposure (e.g., sediment) ecotoxicity data were reviewed independently from aqueous phase studies. Results of aquatic tests did not consistently distinguish between dissolved and total recoverable concentrations.

Ecotoxicological effects data were divided into the following effects categories:

<u>Mortality</u> - These studies included lethal effects studies associated with the death of the target species. Studies review included median lethal concentration (LC_{50}) studies for tests of various durations.

<u>Developmental</u> - Contaminant exposure in these studies was typically associated with disruptions or alterations to various development processes. Endpoints included delayed metamorphosis and polydactyly.

<u>Growth</u> - Growth endpoints included sub-lethal effects on target organisms length and weight.

<u>Behavior</u> - Contaminant exposure in these studies was associated with behavioral observations, including swimming behavior, predator avoidance behavior, and lethargy.

<u>Reproduction</u> - Reproductive endpoints included altered reproductive activity, such as delayed hatching of eggs, and reductions in adult fertility.

Teratogensis – Teratogenic endpoints included developmental effects and subsequent fitness reduction as a result of damage to embryonic cells.

Biochemical /cellular/physiological - A broad array of sub-lethal physiological endpoints were grouped under this category, including enzyme induction, ion balance, ocular responses, and hormone level responses.

Much of the material presented in this chapter was obtained from the following two recently published compilations of amphibian ecotoxicity data:

- Ecotoxicology of Amphibians and Reptiles (Sparling et al., 2000). This resource, published by the Society of Environmental Toxicology and Chemistry (SETAC), provides summaries of several studies that have been conducted with amphibians exposed to a variety of contaminants.
- RATL: A Database of Reptile and Amphibian Toxicology Literature (Pauli, et al., 2000). This resource, published by the Canadian Wildlife Service as a Technical Report, contains numerous data extracted from primary literature for reptiles and amphibians.

When appropriate, focused searches of primary literature were also conducted, and databases such as ECOTOX (www.epa.gov/ecotox) were searched. Much of the data summarized in this chapter are presented in the context of available sediment and surface water quality criteria (e.g., ambient water quality criteria [AWQC]) and guidance values.

SECTION 2
CADMIUM

Cadmium is a silver-white, malleable metal that occurs naturally in small amounts, mainly as a component of the earth crust minerals. According to Eisler (1985), cadmium does not have any known beneficial or essential biological function for animals, but is a minor nutrient for plants at low concentrations (USEPA, 2001b). In the earth's crust, the average concentration of cadmium is 0.18 mg/kg, and soil concentrations range from 0.01 to 1.8 mg/kg (USEPA, 2001b). Cadmium may occur naturally in freshwater at concentrations approaching 0.1 µg/L, but can be several orders of magnitude higher in waters impacted by human activity (USEPA, 2001b).

Cadmium can be released into the environment a number of ways. Anthropogenic activities that may release cadmium include zinc refining, mining activities, sewage and sludge disposal, and burning of fossil fuels. Cadmium is present in fertilizers, pesticides, pigments, and dyes, and is often electroplated to steel as an anticorrosive. Cadmium is also used as a component in alkaline battery and welding electrodes (USEPA, 2001b). Due to the number of ways cadmium can be released to the environment from common items, it is often found on DOD sites in terrestrial and aquatic systems.

2.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

Elemental cadmium in insoluble in water, but cadmium can be present in many forms, primarily sulfate and chloride salts, that are readily soluble in water. Cadmium usually occurs in the divalent state (Cd^{+2}), but may be present as a monovalent metal (Cd^{+1}). According to USEPA (2001b) divalent, free cadmium will be the predominant form in freshwater systems that have low organic carbon content and high dissolved oxygen content. Particulate and dissolved organic material may bind a substantial portion of available cadmium, rendering the metal non-bioavailable. Bioavailability of cadmium is dependent on factors including pH, Eh, and adsorption/desorption rates. Cadmium may be precipitated by hydroxide or carbonate, and may form soluble complexes with hydroxide, carbonate, chloride, and sulfate (USEPA, 2001b).

Cadmium may form a variety of complexes, and there is a general lack of toxicity data correlated to these complexes. USEPA has issued cadmium AWQC based on total recoverable cadmium in the water column (USEPA, 1980a) and acid-soluble cadmium (USEPA, 1985a), but now considers the dissolved fraction of cadmium (able to pass through a 0.45 µm filter) to be the most appropriate approximation of bioavailable cadmium in water. The acute and chronic water quality criteria for freshwater organisms are calculated on a site-specific basis using the hardness (as $CaCO_3$) of the water to adjust the criteria. While several factors do co-vary with hardness, including pH, alkalinity, and ionic strength, USEPA (2001b) considers hardness to be the most appropriate surrogate for the ions that affect cadmium toxicity, and is therefore used as the measure for toxicity adjustment. The toxicity of cadmium to freshwater organisms is significantly and negatively correlated to the hardness of the water (USEPA, 2001b); that is, as the hardness of the water increases, the bioavailability and, therefore, toxicity of the cadmium generally decreases. The source of this correlation may be the competition between calcium, magnesium, and cadmium for binding sites on gills.

Bioavailability of cadmium in sediment and soil is linked to the amount of bioavailable cadmium in the pore water or interstitial water. In aerobic systems (high oxygen), cadmium solubility is controlled by adsorption to clays, organic matter, and manganese and iron oxides (Hem 1985, Alloway 1990). Sorption to organic matter and mineral oxides increases as pH increases (Hatton and Pickering 1980). Cadmium forms weaker bonds with organic matter, clays, and manganese and iron oxides than do other heavy metals such as copper or lead; thus, the presence of other heavy metals such as copper or lead, or divalent cations such as calcium may decrease cadmium sorption (Alloway 1990). Cadmium binds with carbonate, phosphate, and hydroxide ions, forming insoluble minerals. Cadmium carbonate, $CdCO_3$, is the least soluble of these minerals. However, this mineral is not believed to control cadmium solubility in waters with high carbonate or cadmium concentrations (Khalid 1980, Alloway 1990). Khalid (1980) also reported that the formation of insoluble cadmium-organic complexes increased under reducing conditions. Cadmium is less subject to release to overlying waters from sediments maintained under reducing or slightly oxidizing conditions compared to sediments maintained under heavily oxidizing conditions (Khalid 1980).

The USEPA (2000) has incorporated cadmium as one of the divalent cationic metals included in the sediment Equilibrium Partitioning Guideline (ESG) for metals mixtures. The metals mixture ESG is based on equilibrium partitioning (EqP) theory, and considers simultaneously extracted metals (SEM) (cadmium, copper, lead, nickel, silver, and zinc) and acid volatile sulfide (AVS) in sediment and the sediment interstitial water. Metals in sediments will bind to available AVS in order of increasing solubility. Copper, lead, cadmium, zinc, and nickel will bind to available AVS and be sequentially converted to copper sulfide, lead sulfide, cadmium sulfide, zinc sulfide, and nickel sulfide (i.e., in the order of increasing solubility). This reaction takes place as long as sulfides, in particular AVS, are available. If the molar sum of divalent cations (i.e., copper, lead, cadmium, zinc, and nickel) is less than the molar concentration of available AVS, these metals will exist as metal sulfides. Such metal sulfides are insoluble and are not present in sediment pore water. Therefore, sediments with higher concentrations of AVS than metals will tend to exhibit low metals toxicity. Conversely, when the molar sum of the metals is greater than the molar AVS concentration, the portion of the metals in excess of the AVS concentration can potentially exist as free metals, and thus can potentially be bioavailable and toxic.

2.2 Available Aquatic Toxicity Information

The aquatic toxicity information presented in this review comes primarily from one of two sources. Ecotoxicology of Amphibians and Reptiles (Sparling et al., 2000) provides summaries of several studies that have been conducted with amphibians exposed to a variety of contaminants. The Canadian Wildlife Service (Pauli, et al., 2000) has compiled a Database of Reptile and Amphibian Toxicology Literature (RATL). The RATL database includes several studies including acute (lethal) and other endpoints. For the aquatic studies, the data are not normalized to water hardness. Sparling et al. (2000) and the RATL served as secondary sources of cadmium toxicity information and are described in Section 3.0. A limited search of the primary literature was also performed, and the primary literature cited in the secondary sources was obtained for some studies. The following sections describe some of the ecotoxicological data for cadmium in sediment and surface water.

2.2.1 Sediment Exposure Toxicity Data

Several sediment benchmarks have been developed for cadmium. In addition to the draft ESG for metals mixtures described above, bulk sediment screening values are available. These bulk screening benchmarks are summarized in Table 3-1 of the guidance document. The values are based primarily on the potential or observed effects of cadmium to benthic organisms, such as macroinvertebrates. The majority of amphibian toxicity testing data available for cadmium are water-based tests. Few data are available describing the effects of cadmium-contaminated sediments to amphibians. One study was found that exposed tadpoles to cadmium-enriched sediment. Eggs of goldfish (*Carassius auratus*), largemouth bass, and leopard frog (*Rana pipiens*) were exposed to sediment spiked with 1, 10, 100, and 1000 mg/kg cadmium through 4 days post-hatch (Francis et al., 1984). All organisms had low rates of mortality in all sediment exposures, but this mortality was not significantly correlated to either sediment or overlying water cadmium concentration.

2.2.2 Surface Water Exposure Toxicity Data

This section presents toxicity data for amphibians exposed to cadmium in surface water. This presentation includes a summary of data provided by effect category, as well as a summary of the amphibian data included in the USEPA AWQC documentation for cadmium. Table 2-1 summarizes the cadmium amphibian toxicity data discussed in this section.

Federal Ambient Water Quality Criterion Documentation

In 1984, the USEPA issued acute and chronic AWQC for cadmium (USEPA 1985a). In 2001, USEPA updated the cadmium AWQC to reflect a more current understanding of cadmium toxicity in surface water (USEPA, 2001b). Included in the 2001 update are limited acute toxicity data with the African clawed frog (*Xenopus laevis*) and the Northwestern salamander (*Ambystoma gracile*). Of the 55 hardness-normalized (to 50 mg/L $CaCO_3$) genus mean acute values (GMAVs) used in the calculation of the 2001 criteria, these genera ranked 33rd (*Xenopus* GMAV = 1,529 µg/L) and 29th (*Ambystoma* GMAV = 521 µg/L). Genera with lower ranks (e.g., more sensitive to cadmium) included a number of fish and invertebrate species.

Mortality

Toxicity tests conducted with embryos of various amphibian species indicated 24-hour cadmium LC_{50} values ranging from 2,620 (*Microhyla ornata*, the ornate rice frog) to 52,000 µg/L (*Rana clamitans*, the green frog). Nine embryo 24-hour LC_{50} values were reported, and the average concentration of these studies was 13,445 µg/L. Embryo LC_{50} values at 96 hours ranged from 468 (*Ambystoma gracile*, the northwestern salamander) to 15,810 µg/L (*Rana luteiventris*, Columbia spotted frog).

Tadpole LC_{50} values at 48 hours ranged from 470 (*A. mexicanum*) to 32,000 µg/L (*Xenopus laevis*, the African clawed frog). Fourteen embryo 48-hour LC_{50} values were reported, and the average concentration of these studies was 8,486 µg/L. Three 72-hour LC_{50} embryo values were reported, ranging from 2,230 to 7,840 µg/L (*B. arenarum*, the common toad). Thirteen tadpole 96-hour LC_{50} values were reported, and the average concentration of these studies was 4,021 µg/L.

Tests with adults include two 24-hour LC_{50} values of 205 µg/L (*Ambystoma mexicanum*, the axolotl) and 23,494 µg/L (*X. laevis*) and several toxicity tests with adult male and female skipper frogs (*Rana cyanophlyctis*) whereby the duration of lead exposure varied. The 48-hour lead LC_{50} concentrations were 250,000 µg/L for males and 200,000 µg/L for females. The 72-hour LC_{50} values were 146,000 µg/L and 192,000 µg/L for males and females respectively. The adult male and female LC_{50} values at 96 hours were 75,000 µg/L and 56,600 µg/L. The lethal concentrations were not consistently higher for either sex indicating that lethal concentrations are not solely sex-dependent for skipper frogs.

Developmental

Most of the tests with developmental endpoints were conducted with embryonic amphibians, but two studies with adult amphibians were reported. Adverse effects on embryos were noted at concentrations as low as 1 μg/L (deformation) and as high as 4,000 μg/L (abnormalities) for *R. nigromaculata* and *B. arenarum* embryos. A total of eleven studies with developmental effects on embryonic amphibians were found. The average concentration of the studies was 781 μg/L. One study reported no effects to embryonic *X. laevis* exposed to 9 μg/L for 100 days. One study was found with a reported effect concentration for adult amphibians; limb degeneration was noted with adult eastern newt (*Notophthalmus viridescens*) exposed to 2,250 μg/L.

Growth

Three studies were found that reported effects of cadmium on the growth of amphibian tadpoles. *X. laevis* embryos, exposed for 100 days to 30 μg/L exhibited reduced growth. One study, using 3 month old *A. gracile*, reported a NOAEL of 106 μg/L and a LOAEL of 227 μg/L. No duration of exposure was reported for the salamander test.

Behavior

Very little data were found that reported specific adverse effects in the behavior of amphibians exposed to cadmium. One study with *X. laevis* reported a TI_{50} and an $LC_{50}$for inhibition of swimming of 1 and 1.3 μg/L, respectively. No other studies monitoring behavior were noted.

Reproduction

Only one study was found that reported adverse effects specific to reproduction. Egg hatching was reduced in *Gastrophyrne carolinenis* (eastern narrowmouth toad) eggs exposed to 1.34 μg/L. No other studies with direct effects on reproduction were noted.

Biochemical/cellular/physiological

Three studies were found that recorded results at the biochemical or cellular level to amphibians. Organogenesis was noted in *X. laevis* embryos exposed to 2,000 μg/L of cadmium. Primodial germ cell reduction was observed with *R. nigromaculata* (black-spotted frog) eggs exposed to 4,000 μg/L. No effects were observed for *X. laevis* embryos exposed to 300 μg/L of cadmium for 100 days.

Comparative Studies

Birge et al. (2000) compiled cadmium LC_{50} toxicity data for eighteen species of larval amphibians. The LC_{50} values ranged from 10 μg/L (Barbour's smallmouth salamander; *Ambystoma barbouri*) to 5,554 μg/L (red-spotted toad; *Bufo punctatus*). The amphibian LC_{50} data were compared to LC_{50} data for three fish species that are commonly used in toxicity tests. These species included the rainbow trout (*Oncorrhynchus mykiss*), fathead minnow (*Pimephales promelas*), and largemouth bass (*Micropterus salmoides*). With the exception of Fowler's toad (*B. fowleri*) (LC_{50} = 2,530 μg/L) and *B. punctatus* (LC_{50} = 5,554 μg/L), all amphibian LC_{50} values were lower than the minnow and bass LC_{50} (162 and 1,859 μg/L, respectively); all but the two toads and the marbled salamander (*A. opacum*) (142 μg/L), LC_{50} values were lower than the trout LC_{50} (140 μg/L). Ranids (*Rana* sp.) were among the most sensitive species, and toads (*Bufo* sp.) ranked among the least sensitive species.

Table 2-1

Cadmium Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference	
									Primary	Secondary
BEHAVIOR										
Xenopus laevis	African clawed frog	Embryo	1	UG/L	TI50		Swimming	--	Sabourin et al. 1985	RATL
Xenopus laevis	African clawed frog	Embryo	1.3	UG/L	EC50		Swimming	--	Sabourin et al. 1985	RATL
CELLULAR										
<u>NO EFFECT DATA</u>										
Xenopus laevis	African clawed frog	Tadpole	300	UG/L	NOEC	100 D	--	--	Canton and Slooff 1982	Sparling et al. 2000
<u>EFFECT DATA</u>										
Rana nigromaculata	Black-spotted frog	Egg	4,000	UG/L*	LOEC		Primordial germ cell reduction	--	Hah 1978	RATL
Xenopus laevis	African clawed frog	Embryo	2,000	UG/L	EC		Organogenesis	--	Ramusino 1980	RATL
Xenopus laevis	African clawed frog	Embryo	1.1	UG/L*	TI50		Pigmentation	--	Sabourin et al. 1985	RATL
Xenopus laevis	African clawed frog	Embryo	1.2	UG/L*	EC50		Pigmentation	--	Sabourin et al. 1985	RATL
GROWTH										
<u>NO EFFECT DATA</u>										
Xenopus laevis	African clawed frog	Tadpole	30	UG/L	NOEC	100 D	--	--	Canton and Slooff 1982	Sparling et al. 2000
Ambystoma gracile	Northwestern salamander	Larvae	106	UG/L	NOAEL		--	--	Nebeker et al. 1995	RATL
<u>EFFECT DATA</u>										
Ambystoma gracile	Northwestern salamander	Larvae	227	UG/L	LOAEL		--	--	Nebeker et al. 1995	RATL
REPRODUCTIVE										
Gastrophyrne carolinensis	Eastern narrowmouth toad	Egg	1.34	UG/L*	LOEC		Hatch success	Hatch success	Birge et al. 1977	RATL

Table 2-1 (continued)

Cadmium Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference	
									Primary	Secondary
DEVELOPMENTAL										
NO EFFECT DATA										
Xenopus laevis	African clawed frog	Embryo	9	UG/L*	NOEC	100 D	--	--	Canton and Slooff 1982	Sparling et al. 2000
EFFECT DATA										
Xenopus laevis	African clawed frog	Embryo	1	UG/L*	EC		Severve deformity; deformations decreasing with increasing Mg	--	Miller and Landesman 1978	RATL
Bufo arenarum	Common toad	Embryo	30 - 4,000	UG/L*	EC		Delayed development, alterations in gastrulation and neurulation processes	--	Perez-Coll et al. 1985	RATL
Xenopus laevis	African clawed frog	Embryo	1.3	UG/L*	TI50		Malformation	--	Sabourin et al. 1985	RATL
Rana nigromaculata	Black-spotted frog	Embryo	4,000	UG/L*	LOEC		Abnormalities	--	Hah 1978	RATL
Bufo arenarum	Common toad	Embryo	250	UG/L	LOEC	24 HR	15% malformed	100% arrested development	Herkovits and Perez-Coll, 1990	RATL
Ambystoma gracile	Northwestern salamander	Larvae	<2 - 505	UG/L	LOEC	24 DAY	Mean limb degeneration decreased as Cd concentrations increased after 24-D of exposure	--	Nebeker et al. 1994	RATL
Xenopus laevis	African clawed frog	Embryo	1,000	UG/L	LOEC		Developmental	--	Sakamoto et al ?	RATL
Bufo arenarum	Common toad	Tadpole	1,000	UG/L	EC		Physiologic	--	Muino et al. 1990	
Rana sp.	Ranid species	Tadpole	N/A	UG/L	EC		Physiologic	--	Zettergren et al. 1991b	RATL
Notophthalmus viridescens	Eastern newt	Adult	2,250 - 6,750	UG/L*	LOEC		Limb degeneration	--	Manson and O'Flaherty 1978	RATL
Rana catesbeiana	Bullfrog	Adult	5 - 12.5	UM	EC		Eye rod receptor potential suppressed	--	Fox and Sillman 1979	RATL
MORTALITY										
24-HOUR LC50										
Microhyla ornata	Ornate rice frog	Tadpole	2,620	UG/L	LC50	24 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	2,780	UG/L	LC50	24 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Bufo arenarum	Common toad	Tadpole	3,340	UG/L	LC50	24 HR	50% mortality in test organisms	--	Muino et al. 1990	
Xenopus laevis	African clawed frog	Tadpole	4,000	UG/L	LC50	24 HR	50% mortality in test organisms	--	Canton and Slooff 1982	Sparling et al. 2000
Bufo arenarum	Common toad	Tadpole	4,050	UG/L	LC50	24 HR	50% mortality in test organisms	Stage 26; 25oC	Ferrari et al. 1993	RATL
Bufo melanostictus	Black spined toad	Tadpole	19,810	UG/L	LC50	24 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Rana luteiventris	Columbia spotted frog	Tadpole	22,490	UG/L	LC50	24 HR	50% mortality in test organisms	--	Lefcort et al. 1998	
Rana clamitans	Green frog	Tadpole	52,000	UG/L*	LC50	24 HR	50% mortality in test organisms	--	Richard 1993	RATL
Bufo arenarum	Common toad	Tadpole	9,920	UG/L	LC50	24 HR	50% mortality in test organisms	Stage 28; 25oC	Ferrari et al. 1993	RATL
Ambystoma mexicanum	Axolotl	Adult	205	UG/L	LC50	24 HR	50% mortality in test organisms	--	Vaal et al. 1997	RATL
Xenopus laevis	African clawed frog	Adult	23,494	UG/L	LC51	24 HR	50% mortality in test organisms	--	Vaal et al. 1997	RATL

Table 2-1 (continued)

Cadmium Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference	
									Primary	Secondary
48-HOUR LC50										
Ambystoma mexicanum	Axolotl	Embryo	470	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sloof and Baerselman 1980, Sloof et al. 1983	Sparling et al. 2000
Ambystoma mexicanum	Axolotl	Embryo	1,300	UG/L	LC50	48 HR	50% mortality in test organisms	--	Slooff and Baerselman 1980	RATL
Microhyla ornata	Ornate rice frog	Tadpole	2,480	UG/L	LC50	48 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Bufo arenarum	Common toad	Tadpole	2,520	UG/L	LC50	48 HR	50% mortality in test organisms	--	Muino et al. 1990	
Microhyla ornata	Ornate rice frog	Tadpole	2,660	UG/L	LC50	48 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Bufo arenarum	Common toad	Tadpole	3,150	UG/L	LC50	48 HR	50% mortality in test organisms	Stage 26; 25oC	Ferrari et al. 1993	RATL
Xenopus laevis	African clawed frog	Tadpole	3,200	UG/L	LC50	48 HR	50% mortality in test organisms	--	Canton and Slooff 1982	Sparling et al. 2000
Xenopus laevis	African clawed frog	Embryo	7,360	UG/L	LC50	48 HR	50% mortality in test organisms	--	de Zwart and Sloof 1987	Sparling et al. 2000
Xenopus laevis	African clawed frog	Embryo	11,648	UG/L	LC50	48 HR	50% mortality in test organisms	--	Slooff and Baerselman 1980, Sloof et al. 1983	Sparling et al. 2000
Bufo melanostictus	Black spined toad	Tadpole	11,910	UG/L	LC50	48 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Rana luteiventris	Columbia spotted frog	Tadpole	16,590	UG/L	LC50	48 HR	50% mortality in test organisms	--	Lefcort et al. 1998	
Xenopus laevis	African clawed frog	Embryo	20,200	UG/L	LC50	48 HR	50% mortality in test organisms	--	de Zwart and Sloof 1987	Sparling et al. 2000
Xenopus laevis	African clawed frog	Tadpole	32,000	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sloof and Baerselman 1980	Sparling et al. 2000
Bufo arenarum	Common toad	Tadpole	8,600	UG/L	LC50	48 HR	50% mortality in test organisms	Stage 28; 25oC	Ferrari et al. 1993	
Rana cyanophlyctis	Skipper frog	Adult (F)	200,000	UG/L	LC50	48 HR	50% mortality in test organisms	--	Mudgall and Patil, 1985	
Rana cyanophlyctis	Skipper frog	Adult (M)	250,000	UG/L	LC50	48 HR	50% mortality in test organisms	--	Mudgall and Patil, 1985	
72-HOUR LC50										
Bufo arenarum	Common toad	Tadpole	2,230	UG/L	LC50	72 HR	50% mortality in test organisms	--	Muino et al. 1990	
Bufo arenarum	Common toad	Tadpole	2,870	UG/L	LC50	72 HR	50% mortality in test organisms	Stage 26; 25oC	Ferrari et al. 1993	
Bufo arenarum	Common toad	Tadpole	7,840	UG/L	LC50	72 HR	50% mortality in test organisms	Stage 28; 25oC	Ferrari et al. 1993	
Rana cyanophlyctis	Skipper frog	Adult (M)	146,000	UG/L	LC50	72 HR	50% mortality in test organisms	--	Mudgall and Patil, 1985	
Rana cyanophlyctis	Skipper frog	Adult (F)	192,000	UG/L	LC50	72 HR	50% mortality in test organisms	--	Mudgall and Patil, 1985	

Table 2-1 (continued)

Cadmium Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference	
									Primary	Secondary
96-HOUR LC50										
Ambystoma gracile	Northwestern salamander	Larvae	468	UG/L	LC50	96 HR	50% mortality in test organisms	--	Nebeker et al. 1994	Sparling et al. 2000
Xenopus laevis	African clawed frog	Embryo	850	UG/L	LC50	96 HR	50% mortality in test organisms	--	Linder et al. 1991	Sparling et al. 2000
Microhyla ornata	Ornate rice frog	Tadpole	1,580	UG/L	LC50	96 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	1,810	UG/L	LC50	96 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Bufo arenarum	Common toad	Tadpole	2,080	UG/L	LC50	96 HR	50% mortality in test organisms	--	Munio et al. 1990	
Bufo arenarum	Common toad	Tadpole	2,650	UG/L	LC50	96 HR	50% mortality in test organisms	Stage 26; 25oC	Ferrari et al. 1993	
Rana catesbeiana	Bullfrog	Embryo	3,700	UG/L	LC50	96 HR	50% mortality in test organisms	--	Zettergren et al. 1991	Sparling et al. 2000
Rana pipiens	Northern leopard frog	Embryo	3,700	UG/L	LC50	96 HR	50% mortality in test organisms	--	Zettergren et al. 1991	Sparling et al. 2000
Bufo arenarum	Common toad	Tadpole	6,770	UG/L	LC50	96 HR	50% mortality in test organisms	Stage 28; 25oC	Ferrari et al. 1993	
Bufo melanostictus	Black spined toad	Tadpole	8,180	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Rana luteiventris	Columbia spotted frog	Tadpole	15,810	UG/L	LC50	96 HR	50% mortality in test organisms	--	Lefcort et al. 1998	
Rana cyanophlyctis	Skipper frog	Adult (F)	56,600	UG/L	LC50	96 HR	50% mortality in test organisms	--	Mudgall and Patil, 1985	
Rana cyanophlyctis	Skipper frog	Adult (M)	75,000	UG/L	LC50	96 HR	50% mortality in test organisms	--	Mudgall and Patil, 1985	

* units not listed but assumed to be UG/L

SECTION 3
CHROMIUM

Chromium is a naturally occurring element found in rocks, animals, plants, soil, and in volcanic dust and gases (USEPA, 1994). In the natural environment, chromium occurs as two oxidation states: trivalent chromium (chromium III; Cr^{+3}) and hexavalent chromium (chromium VI; Cr^{+6}). Both oxidation states of chromium combine with other elements to produce various compounds (ARB, 1986). Chromium occurs naturally as a trace component in most crude oils. Chromium (III) is a mineral component of most soils, and has been shown to be an essential nutrient for some animals (Eisler, 1986a). The extent to which natural sources of chromium contribute to measured ambient chromium levels is not known (ARB, 1986). In freshwater ecosystems, chromium can exist in several different states, but under strongly oxidizing conditions it may be converted to the hexavalent state (Merck, 1989). Chromium (VI) is virtually always bound to oxygen in ions such as chromates (CrO_4^{-2}) and dichromates ($Cr_2O_7^{-2}$).

Chromium is used for corrosion resistance, steel production, and as protective coating for automotive and equipment accessories. It is a permanent and stable inorganic pigment used for paints, rubber, and plastic products (Howard, 1990). Available information suggests that the chromium is emitted in the trivalent state from oil combustion, sewer sludge incineration, cement production, municipal waste incinerators, and refractories (ARB, 1986). Annual chromium emissions from anthropogenic sources have been estimated between 2,700 – 2,900 tons, of which approximately 35% are released as hexavalent (USEPA, 1990 as cited in ATSDR, 1999). Chromium has been detected but not quantified in motor vehicle exhaust (ARB, 1995a). Chrome plating is a source of chromium (VI) emissions. Chromium VI can be emitted from the firebrick lining of glass furnaces (ARB, 1986). Chromic acid is registered as a fungicide and insecticide, and used for wood and lumber treatment. It may also be used to treat lumber used for pilings for the control of aquatic organisms (DPR, 1996).

3.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

In freshwater ecosystems, precipitation and hydrolysis are the two primary factors affecting the fate and effects of chromium (Eisler, 1986a). Most chromium that enters surface waters binds to inorganic and organic particles and settles to the sediments. Chromium (III) is cationic and adsorbs onto clay particles, organic matter, metal oxyhydroxides, and other negatively charged particles. Chromium (VI) does not interact significantly with clay or organic matter. As a result, chromium (VI) has a higher water-solubility and increased mobility in comparison to chromium (III) (USEPA, 1994). A small amount of chromium may dissolve in water (ATSDR, 1999). Chromium (III) compounds are sparingly soluble in water, while most chromium (VI) compounds are readily soluble in water (USEPA, 1994). The mobility and higher solubility of chromium (VI) renders it more toxic, and hexavalent chromium easily penetrates biological membranes (Eisler, 1986a; ATSDR, 1999).

The factors affecting the valence state of chromium in water and its uptake into animals and plants include organic matter content, ferrous ion content, redox state, and pH (ATSDR, 1999). In general, chromium (VI) is favored by higher pH, aerobic conditions, low amounts of organic matter, and the presence of manganese and iron oxides which oxidize chromium (III).

The USEPA (1980e) issued AWQC for chromium based on total recoverable chromium (III) and total recoverable chromium (VI) in the water column. In the 1985 update to the chromium criteria (USEPA, 1985b), acid-soluble chromium (III) and (VI) were identified as a better measurement. Current USEPA (2002) water quality criteria for chromium (III) and (VI) indicate that the dissolved fraction of chromium (able to pass through a 45 μm filter) should be used to express the criteria.

The chromium (III) acute and chronic water quality criteria for freshwater organisms (USEPA, 2002) are calculated on a site-specific basis using the hardness (as $CaCO_3$) of the water to adjust the criteria. While several factors do co-vary with hardness, including pH, alkalinity, and ionic strength, USEPA (1985b) considers hardness to be the most appropriate surrogate for the ions that affect chromium III toxicity. The toxicity of chromium (III) to freshwater organisms is significantly and negatively correlated to the hardness of the water (USEPA, 1985b); that is, as the hardness of the water increases, the bioavailability and, therefore, toxicity of the chromium (III) generally decreases. Although it has been shown that the toxicity of chromium (VI) to freshwater organisms is dependent on the hardness and pH of the water, the USEPA determined that insufficient information exists for chromium (VI) to develop criteria on the basis of water quality characteristics (USEPA, 1985b).

Bioavailability of chromium in sediment and soil is linked to the amount of bioavailable chromium in the pore water or interstitial water. Sorption to organic matter and mineral oxides increases as pH increases (Eisler, 1986a). As with most heavy metals, chromium is more strongly associated with fine-grained sediments and high TOC concentrations rather than coarse-grained sediments and lower TOC concentrations (Irwin et al., 1997).

3.2 Available Aquatic Toxicity Information

As described above, much of the aquatic toxicity information presented in this review was obtained from two secondary sources: Sparling et al. (2000) and Pauli et al. (2000). In general, these references do not provide water hardness data for the chromium studies. A limited search of the primary literature was also performed, particularly for sediment-associated studies, and the primary literature was reviewed for a number of studies to verify measurement units.

3.2.1 Sediment Exposure Toxicity Data

There were no data found in the literature describing the effects of chromium-contaminated sediments on amphibians.

3.2.2 Surface Water Exposure Toxicity Data

This section presents toxicity data for amphibians exposed to chromium in surface water. This presentation includes a summary of data provided by effect category, as well as a summary of the amphibian data included in the USEPA AWQC documentation for chromium. Table 3-1 summarizes the chromium amphibian toxicity data discussed in this section

Federal Ambient Water Quality Criterion Documentation

In 1985, the USEPA issued the AWQC documentation for chromium. Some amphibian toxicity data were included in the 1985 AWQC document, but these data were not used in the development of the criteria. Included in the 1985 document were trivalent chromium EC_{50} data for death and deformity in embryos of the narrow-mouthed toad (*Gastrophryne carolinensis*) (EC_{50} = 30 μg/L after 7 days) and the marbled salamander (*Ambystoma opacum*) (EC_{50} = 2,130 μg/L after 8 days). The chromium AWQC was updated in 1995, but no amphibian studies were included in the AWQC calculation.

Mortality

Chromium mortality data for eight species of amphibians were located in the literature. Six of these toxicity tests were conducted with tadpoles and two tests were conducted on amphibian embryos.

The two embryo studies include a 7-day LC_{50} value of 30 µg/L for the eastern mouth toad (*Gastrophyrne carolinensis*) and an 8-day LC_{50} value of 2,130 µg/L for the Axolotl (*Ambystoma mexicanum*).

The tadpole studies include one 24-hour chromium LC_{50} value of 57,970 µg/L for the black-spined toad (*Bufo melanostictus*,) tadpole; one 48-hour chromium LC_{50} value of 53,430 µg/L for the ornate rice frog (*Microhyla ornata*,) tadpole; one 72-hour LC_{50} value of 2,000 µg/L for the Asian bull frog (*Rana tigrina*) tadpole; and the following three 96-hour tadpole LC_{50} values: 10,000 µg/L (*R. hexadactyla*, the Indian green frog), 49,290 µg/L (*B. melanosticus*, the black spined toad) and 224,910 µg/L (*Xenopus laevis*, the African clawed frog).

Developmental

Few data were found that reported specific adverse impacts on development of amphibians exposed to chromium. One study with *R. tigris* tadpoles reported greater than 60% malformation during a 72-hour exposure at a concentration of 2,000 µg/L. Malformations were documented for pigmentation, tail fin and the alimentary canal. A study with *X. laevis* tadpole reported a 100-day developmental NOEC of 3,200 µg/L.

Growth

Only one study evaluating chromium impacts on amphibian growth was found. A study with *X. laevis* tadpole reported a 100-day growth NOEC of 3,200 µg/L. No other studies monitoring growth effects were noted.

Behavior

No studies evaluating the effects of chromium on amphibian behavior were found in the literature.

Reproduction

No studies evaluating the effects of chromium on amphibian reproduction were found in the literature.

Biochemical/cellular/physiological

Two studies reported specific adverse effects in amphibians exposed to chromium at the biochemical/cellular level. At 1,000 µg/L chromium, significant numbers of micronucleated red blood cells formed in ribbed newt species (*Pleorodes spp*). Elevated numbers of micronucleated erythrocytes (22 per 1,000) were also documented for spanish ribbed newt (*P. waltl*) following chromium exposure.

Table3-1

Chromium Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference	
									Primary	Secondary
DEVELOPMENTAL										
Xenopus laevis	African clawed frog	Tadpole	3,200	UG/L	NOEC	100 DAY	--	--	Sloof and Canton 1983	Sparling et al. 2000
Rana tigrina	Asian bull frog	Tadpole	2,000	UG/L	EC	72 HR	Abnormalities observed in pigmentation, tail fin and alimentary canal; '>60% malformation	--	Abbasi and Soni 1984	Sparling et al. 2000; RATL
GROWTH										
Xenopus laevis	African clawed frog	Tadpole	3,200	UG/L	NOEC	100 DAY	--	--	Sloof and Canton 1983	Sparling et al. 2000
BIOCEMICAL/CELLULAR/PHYSIOLOGICAL										
Pleorodeles waltl	Spanish ribbed newt	Larvae	125,000	UG/L	EC		High numbers of micronucleated erythrocytes (22 per 1,000)	250 ml/L of river water.	Gauthier et al. 1993	RATL
Pleorodeles spp.	Ribbed newt species	Larvae	0-10,000	UG/L	EC		At 1 ,000 UG/L significant numbers of micronucleated red blood cells formed	--	Godet et al. 1996	RATL
MORTALITY										
24-HOUR LC50										
Bufo melanostictus	Black spined toad	Tadpole	57,970	UG/L	LC50	24 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
48-HOUR LC50										
Microhyla ornata	Ornate rice frog	Tadpole	53,430	UG/L	LC50	48 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
72-HOUR LC50										
Rana tigrina	Asian bull frog	Tadpole	2,000	UG/L	LC50	72 HR	50% mortality in test organisms	--	Abbasi and Soni 1984	RATL
96-HOUR LC50										
Rana hexadactyla	Indian green frog	Tadpole	10,000	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot et al. 1985	Sparling et al. 2000
Bufo melanostictus	Black spined toad	Tadpole	49,290	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Xenopus laevis	African clawed frog	Tadpole	224,910	UG/L*	LC50	96 HR	50% mortality in test organisms	--	Pant and Gill 1982	RATL
OTHER DURATION										
Gastrophyrne carolinensis	Eastern narrowmouth toad	Tadpole	30	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge 1978; Birge et al. 1979	Sparling et al. 2000
Ambystoma mexicanum	Axolotl	Embryo	2,130	UG/L	LC50	8 DAY	50% mortality in test organisms	--	Birge et al. 1978	Sparling et al. 2000

* units not listed but assumed to be UG/L

SECTION 4
COPPER

Copper is reddish in color and is a ductile, malleable metal. Copper is found in its native state in the earth's crust at 70 parts per million (ppm) and in seawater at 0.001 to 0.02 ppm. Copper usually occurs as sulfides or oxides, and occasionally as metallic copper in the rock's and minerals of the earth's crust (Eisler, 1997). Copper is a component of many minerals including azurite, azurmalachite, chalococite, chalcopyrite (copper pyrites) covellite, and cuprite malachite (Merck, 1989). Copper can be found concentrated in clay mineral fractions containing organic carbon (HSDB, 1993). Copper enters into streams or waterways through the natural erosion or weathering of rocks and soil. Anthropogenic activity has significantly increased this load.

Copper is used in electrical wiring, switches, plumbing, heating, roofing and building construction, chemical and pharmaceutical machinery, electroplated coatings, piping, insecticides, catalysts, and in anti-fouling paints (Sax, 1987). It is also used in carbides and high speed steels (HSDB, 1991). Anthropogenic releases of copper into the environment include mining and smelting, industrial emissions and effluents, and municipal wastes and sewage sludge. These releases, primarily to land, may be 2 to 5 times greater than natural loadings. The copper that is introduced to the aquatic environment is mostly bound to particulate matter (ATSDR, 1990). Outside of specific industrial point source releases, run-off is the primary factor contributing to elevated levels detected in many rivers. Copper compounds can be intentionally applied to waterways for use as algaecides, molluscicides, and as anti-fouling agents in paints. Copper sulfate (basic, anhydrous, and pentahydrate) and copper chloride (basic) are registered as fungicides and used on a variety of fruit, vegetable, and ornamental plants for the prevention of fungal and bacterial diseases (DPR, 1996).

4.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

While copper is considered one of the most toxic of the heavy metals to aquatic organisms, it is an essential element that, in small quantities, is vital to the natural growth and metabolic processes of all living organisms (Eisler, 1997). Naturally, copper enters into streams or waterways as particulate matter and settles out or adsorbs to organic matter, hydrous iron and manganese oxides and clays (ATSDR, 1990) rendering it relatively non-bioavailable. Copper bioavailability is modified by biotic as well as abiotic variables. In aquatic ecosystems, dissolved copper concentrations vary with pH, oxidation-reduction potential (ORP), temperature, hardness, suspended matter, rates of sedimentation and concentration of dissolved organics (Eisler, 1997, ATSDR, 1990). Copper speciation in freshwater is important in assessing the bioavailability and toxicity to aquatic organisms and readily changes with varying environmental factors. Free ionic copper (Cu^{2+}) and some copper hydroxyl forms are the most toxic chemical species of copper and are associated with low pH. The concentration of the free cupric ion (Cu^{2+}) is generally low in natural waters. The cupric ion readily forms moderate to strong complexes with both inorganic and organic ligands and precipitates out of the water column (USEPA, 1985c).

USEPA (1980f) originally issued copper AWQC based on total recoverable copper in the water column. In the 1984 update to the copper criterion, USEPA (1985c) determined that acid-soluble copper is a better measurement. Current USEPA (2002) AWQC for copper indicate that the dissolved fraction of copper (able to pass through a 45 μm filter) should be used to express the criteria. The acute and chronic water quality criteria for freshwater organisms are calculated on a site-specific basis using the hardness (as $CaCO_3$) of the water to adjust the criteria. While several factors do co-vary with hardness, including pH, alkalinity, and ionic strength, USEPA (1985c) considers hardness to be the most appropriate surrogate for the ions that affect copper toxicity, and is therefore used as the measure for toxicity adjustment. The toxicity of copper to freshwater organisms is significantly and negatively correlated to the hardness of the water (USEPA, 1985c); that is, as the hardness of the water increases, the bioavailability and, therefore, toxicity of the copper generally decreases.

Sediment is an important sink and reservoir for copper (ATSDR, 1990). Bioavailability of copper in sediment and soil is linked to the amount of bioavailable copper in the pore water or interstitial water. In aerobic systems (high oxygen), the bioavailability of copper is strongly associated with the presence of binding substances and copper speciation. Sorption to organic matter and mineral oxides increases as pH increases (Eisler, 1997). As with most heavy metals, copper is more strongly associated with fine-grained sediments and high TOC concentrations rather than coarse-grained sediments and lower TOC concentrations (Irwin et al., 1997). When sulfide is present, as it is in sediments rich in organic matter, it will bind with the copper in the sediments in a highly insoluble form.

The USEPA (2000) has incorporated copper as one of the divalent cationic metals included in the sediment ESG for metals mixtures. The metals mixture ESG is based on EqP theory, and considers SEM (cadmium, copper, lead, nickel, silver, and zinc) and AVS in sediment. A more detailed description of the mechanism for the metals mixture ESG is presented in Section 2.1.

4.2 Available Aquatic Toxicity Information

As described above, much of the aquatic toxicity information presented in this review was obtained from two secondary sources: Sparling et al. (2000) and Pauli et al. (2000). In general, these references do not provide water hardness data for the copper studies. A limited search of the primary literature was also performed, particularly for sediment-associated studies, and the primary literature was reviewed for a number of studies to verify measurement units.

4.2.1 Sediment Exposure Toxicity Data

There were no data found in the literature describing the effects of copper-contaminated sediments on amphibians.

4.2.2 Surface Water Exposure Toxicity Data

This section presents toxicity data for amphibians exposed to copper in surface water. This presentation includes a summary of data provided by effect category, as well as a summary of the amphibian data included in the USEPA AWQC documentation for chromium. Table 4-1 summarizes the copper amphibian toxicity data discussed in this section

<u>Federal Ambient Water Quality Criterion Documentation</u>

The USEPA published the copper AWQC in 1984, and updated the criteria in 1985, 1995, and 1999. Although some amphibian toxicity data for three species of amphibian are included in the criterion documentation, these data were not included in the development of the AWQC. Studies referenced in the AWQC documentation include: (1) an 80-minute avoidance threshold for the American toad (*Bufo americanus*) of 100 mg/L; and (2) EC_{50} data for death and deformity for embryos of the Southern gray tree frog (*Hyla chrysoscelis*) (EC_{50} = 40 μg/L after 7 minutes), Fowler's toad (*Bufo fowleri*) (EC_{50} = 26,960 μg/L after 7 minutes), the narrow-mouthed toad (*Gastrophryne carolinensis*) (EC_{50} = 40 μg/L after 7 days), the leopard frog (*Rana pipiens*) (EC_{50} = 50 µg/L after 8 days), and the marbled salamander (*Ambystoma opacum*) (EC_{50} = 770 µg/L after 8 days).

Mortality

A number of lethal effects toxicity tests with amphibians were located in the literature. These included frog, toad, and salamander tests of various durations, ranging from 24-hour LC_{50}s to 8-day LC_{50}s.

Embryo tests included two 96-hour LC_{50}'s with values that included 110 µg/L (*X. laevis)* and 315 µg/L for *Ambystoma jeffersonianum*, three 7-day LC_{50} values that ranged from 40 µg/L for *H. chrysoscelis* and *G. carolinensis* to 26,960 µg/L for *Bufo fowleri*, and one 8-day LC_{50} of 770 µg/L *A. opocum*, the marbled salamander).

The 24-hour copper LC_{50} values ranged from 843 µg/L for the black-spined toad (*Bufo melanostictus*,) to 5,610 µg/L (1 week old) and 6,040 µg/L (4 week old) for *Microhyla ornata*, the ornate rice frog.

Tadpole LC_{50} values at 48 hours ranged from 446 (*B. melanostictus*) to 5,740 µg/L (*M. ornata*). Five tadpole 48-hour LC_{50} values were reported, and the average concentration of these studies was 2,775 µg/L.

Three 72-hour LC_{50} values for tadpoles ranged from 150 µg/L for the northern leopard frog (*Rana pipiens*) to 5,140 µg/L (1 week old) and 5,540 µg/L (4 week old) for *M. ornata*.

Tadpole LC_{50} values at 96 hours ranged from 20 µg/L (*Hyla chrysoscelis*, the Cope's gray treefrog) to 5,380 µg/L (*M. ornata*). Seven tadpole 96-hour LC_{50} values were reported, and the average concentration of these studies was 1,562 µg/L.

One adult toxicity test (72-hour LC_{50} of 6,368 µg/L) for *R. pipiens* was located.

Developmental

Effects on amphibian development were observed for western toad (*Bufo boreas*) larvae at copper concentrations between 20 and 3,700 µg/L, while 100% mortality was observed at higher concentrations. No other studies monitoring developmental effects were noted.

Growth

Only one study was found documenting the detrimental effects of copper on amphibian growth. In this study, tadpole growth was inhibited by 0.01% - 0.05% copper concentration on the European common frog (*Rana temporaria*).

Behavior

Only one study was found documenting the effects of copper on amphibian behavior. In this study, the American toad (*Bufo americanus*) avoided copper concentrations of 0.1 mg/L, however was attracted to concentrations 0.93 mg/L. No other data were found documenting the behavioral effects associated with copper exposure to amphibians.

Reproduction

One study was found documenting reproductive effects associated with amphibian exposure to copper in the water column. This study was performed with copper concentrations ranging between 1 ug/L and 25 ug/L, and indicated a reduction in hatching success and an increase in embryonic mortality in Jefferson salamander (*Ambystoma jeffersonianum*) eggs from ponds with the higher copper concentrations.

Biochemical/cellular/physiological

No studies documenting the biochemical or cellular effects of copper on amphibians were found in the literature.

Table 4-1

Copper Toxicity Data for Amphibians

									Reference	
Species	**Common Name**	**Lifestage**	**Concentration**	**Unit**	**Endpoint**	**Duration**	**Endpoint**	**Additional Observations**	**Primary**	**Secondary**
BEHAVIOR										
Bufo americanus	American toad	Tadpole			EC		Avoided 0.1 mg/L, attracted to 0.93 mg/L	--	Birge et al. 1993	RATL
GROWTH										
Rana temporaria	European common frog	Tadpole	0.01- 0.05%	UG/L	EC		Inhibited growth	Pigment in liver and stomach cells; high mortality	Jordan et al. 1977	RATL
DEVELOPMENTAL										
Bufo boreas	Western toad	Tadpole	20 - 3,700	UG/L	EC		At low concentrations all organisms metamorphosed	100% mortality at the high concentrations	Porter and Hakanson 1976	RATL
REPRODUCTION										
Ambystoma jeffersonianum	Jefferson's salamander	Embyos	1 - 25	UG/L	EC		A reduction in hatching success	An increase in embryonic mortality	Horne and Dunson 1995	Eisler, 1998
MORTALITY										
24-HOUR LC50										
Bufo melanostictus	Black spined toad	Tadpole	843	UG/L	LC50	24 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Microhyla ornata	Ornate rice frog	Tadpole	6,040	UG/L	LC50	24 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	5,610	UG/L	LC50	24 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
48-HOUR LC50										
Bufo melanostictus	Black spined toad	Tadpole	446	UG/L	LC50	48 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Xenopus laevis	African clawed frog	Tadpole	677	UG/L	LC50	48 HR	50% mortality in test organisms	--	de Zwart and Sloof 1987	Sparling et al. 2000
Xenopus laevis	African clawed frog	Tadpole	1,700	UG/L	LC50	48 HR	50% mortality in test organisms	--	de Zwart and Sloof 1987	Sparling et al. 2000
Microhyla ornata	Ornate rice frog	Tadpole	5,310	UG/L	LC50	48 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	5,740	UG/L	LC50	48 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
72-HOUR LC50										
Rana pipiens	Northern leopard frog	Tadpole	150	UG/L	LC50	72 HR	50% mortality in test organisms	--	Lande and Guttman 1973	Sparling et al. 2000
Microhyla ornata	Ornate rice frog	Tadpole	5,140	UG/L	LC50	72 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	5,540	UG/L	LC50	72 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Rana pipiens	Northern leopard frog	Adult	6,368	UG/L	LC50	72 HR	50% mortality in test organisms	--	Kaplan and Yoh 1961	Sparling et al. 2000
96-HOUR LC50										
Xenopus laevis	African clawed frog	Embryo	110	UG/L	LC50	96 HR	50% mortality in test organisms	--	Linder et al. 1991	Sparling et al. 2000
Ambystoma jeffersonianum	Jefferson's salamander	Embryo	315	UG/L	LC50	96 HR	50% mortality in test organisms	--	Horne and Dunson 1994	Sparling et al. 2000
Hyla chrysoscelis	Cope's gray treefrog	Tadpole	20	UG/L	LC50	96 HR	50% mortality in test organisms	--	Gottscalk 1995	
Rana hexadactyla	Indian green frog	Tadpole	39	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot et al. 1985	Sparling et al. 2000
Rana pipiens	Northern leopard frog	Tadpole	60	UG/L	LC50	96 HR	50% mortality in test organisms	--	Lande and Guttman 1973	Sparling et al. 2000
Rana pipiens	Northern leopard frog	Tadpole	76.1	UG/L*	LC50	96 HR	50% mortality in test organisms	--	Gottscalk 1995	

Table 4-1 (continued)

Copper Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference	
									Primary	Secondary
Bufo melanostictus	Black spined toad	Tadpole	320	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Microhyla ornata	Ornate rice frog	Tadpole	5,040	UG/L	LC50	96 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	5,380	UG/L	LC50	96 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
OTHER DURATION										
Gastrophyrne carolinensis	Eastern narrowmouth toad	embryo-post hatch	20	UG/L	LC50	7 DAY	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Gastrophyrne carolinensis	Eastern narrowmouth toad	Embryo	40	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge 1978; Birge and Black 1979, Birge et al. 1979	Sparling et al. 2000
Hyla chrysoscelis	Cope's gray treefrog	Embryo	40	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge and Black 1979; Birge et al. 1979	Sparling et al. 2000
Pseudacris crucifer	Spring peeper	embryo-post hatch	50	UG/L	LC50	7 DAY	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Bufo fowleri	Fowler's toad	Embryo	26,960	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge and Black 1979	Sparling et al. 2000
Bufo fowleri	Fowler's toad	embryo-post hatch	27000	UG/L	LC50	7 DAY	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Ambystoma opocum	Marbled salamander	Embryo	770	UG/L	LC50	8 DAY	50% mortality in test organisms	--	Birge et al. 1978; Birge and Black 1979	Sparling et al. 2000
Ambystoma opocum	Marbled salamander	embryo-post hatch	1630	UG/L	LC50	9-10 DAY	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Rana palustris	Pickeral frog	embryo-post hatch	20	UG/L	LC50	10 DAY	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Rana pipiens	Northern leopard frog	embryo-post hatch	50	UG/L	LC50	10 DAY	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Ambystoma texanum	Small-mouthed salamander	embryo-post hatch	380	UG/L	LC50	10-11 DAY	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Rana catesbeiana	Bullfrog	embryo-post hatch	20	UG/L	LC50	10-12 DAYS	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Ambystoma jeffersonianum	Jefferson's salamander	embryo-post hatch	370	UG/L	LC50	10-12 DAYS	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Ambystoma maculatum	Spotted salamander	embryo-post hatch	480	UG/L	LC50	10-12 DAYS	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Ambystoma t. tigrinum	Eastern tiger salamander	embryo-post hatch	500	UG/L	LC50	10-12 DAYS	50% mortality in test organisms	--	D. W. Sparling et al. 2000	
Ambystoma barbouri	Streamedside salamander	embryo-post hatch	250	UG/L	LC50	11-12 DAY	50% mortality in test organisms	--	D. W. Sparling et al. 2000	

* units not listed but assumed to be UG/L

SECTION 5
LEAD

Lead is a bluish-gray, noncombustible metal that occurs naturally in the earth's crust. Approximately 10 to 17 mg/kg or 0.001 to 0.007% of the earth's crust is comprised of lead (ARB, 1993; Merck, 1989). Lead occurs in the earth's crust as the end-product of the radiometric decay of three naturally-occurring radioactive elements: uranium, thorium, and actinium (Sax, 1987). A natural means of releasing lead to the atmosphere is via windborne dusts created by the weathering of deposits. Other natural sources of lead emissions are sea and salt lake aerosols, forest fires, and volcanic eruptions (HSDB, 1995).

Although lead is a naturally occurring element, its distribution in the environment is predominantly a result of anthropogenic activities (ATSDR, 1998a). Historically, the primary source of lead to the environment has been through the anthropogenic emissions to the atmosphere. Urban runoff contributes primarily to the particulate and bound forms of lead to the aquatic environment while the labile forms are generally the result of atmospheric deposition (Eisler, 1988). Direct sources of lead to aquatic ecosystems are largely due to releases from the steel and iron industries and from lead production and processing operations.

Lead compounds are used in construction materials for tank linings, piping, equipment for handling corrosive gases and liquids used in petroleum refining, halogenation, sulfonation, extraction, condensation, metallurgy, and for pigments for paints. It is also used in ceramics, plastics, electronic devices, as a component of lead batteries, and in the production of ammunition, solder, cable covering, and sheet lead (HSDB, 1995). Lead was a common component of gasoline until the mid-1970's. Since that time, lead in ambient air has decreased significantly. However, inorganic lead emission may accumulate in soils for many years (ARB, 1997b).

5.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

Lead reaching surface waters is predominantly sorbed to suspended solids and sediments. As with most heavy metals, the dissolved form of lead is more toxic than the total lead and the organic forms are more toxic than the inorganic forms. The soluble and bioavailable portion of lead in surface waters is enhanced by low levels of pH, organic matter, suspended sediments, and dissolved salt concentration (Eisler, 1988). The amount of dissolved lead in surface waters is generally low, since lead readily forms compounds with anions such as hydroxides, carbonates, sulfates, and phosphates that have low solubilities and settle out of the water column. The ratio of lead in suspended solids to dissolved lead has been found to vary from 4:1 in rural areas to 27:1 in urban streams (ATSDR, 1998a). Sulfates limit the dissolved content of lead at pH below 5.4, while carbonate forms predominate at pH greater than 5.4. In the aquatic environment, the divalent form (Pb^{2+}) is the stable form.

As with most heavy metals, higher concentrations of lead are associated with fine-grained sediments and high TOC concentrations (Irwin, et al., 1997). Lead is mobilized and released from sediments when ionic composition changes or with a drop in pH (Eisler, 1988). Transport and speciation of lead is heavily influenced by water flow rate (Eisler, 1988). At higher flows, particulate and labile forms increase, while in areas of low flow lead, quickly settles out of the water column. Average lead concentrations in river sediments are 20 mg/kg (USEPA, 1982 as cited in ATSDR, 1998a).

The USEPA (2000) has incorporated lead as one of the divalent cationic metals included in the sediment ESG for metals mixtures. The metals mixture ESG is based on EqP theory, and considers SEM (cadmium, copper, lead, nickel, silver, and zinc) and AVS in sediment. A more detailed description of the mechanism for the metals mixture ESG is presented in Section 2.1.

Lead is not believed to essential or beneficial to any aquatic organisms and all measured effects from lead have been adverse (Eisler, 1988). Lead is toxic to all phyla of aquatic biota; however, effects vary with changes in biotic and abiotic parameters (Eisler, 1988). Lead bioaccumulates in the tissues of living organisms and highest concentrations are associated with older organisms. The numeric aquatic life criteria developed by the USEPA were designed to be protective of aquatic life and although not designed specifically for wetlands, are generally applicable to most wetland types (USEPA, 1990). The concentration of lead in surface waters is dependent on pollution sources, concentration of lead in the sediments and local environmental characteristics of the water body (e.g. pH, alkalinity, etc.). Typical levels of lead in surface waters throughout the U.S. range between 5 μg/L and 30 μg/L (USEPA, 1986b).

In general, there is a lack of literature information documenting the toxicity associated with the various forms of lead.

The USEPA (1980g) lead AWQC was based on total recoverable lead in the water column. In the 1984 update to the lead criteria (USEPA, 1985d), USEPA determined that acid-soluble lead is a better measurement. Current USEPA (1999a) water quality criteria for lead indicates that the dissolved fraction of lead (able to pass through a 45 μm filter) should be used to express the criteria. The toxicity of lead in freshwater organisms is significantly and negatively correlated to the hardness of the water (USEPA, 1985d). Several factors co-vary with hardness, including pH, alkalinity, and ionic strength. However, USEPA (1985d) considers hardness to be the most appropriate surrogate for the ions that affect lead toxicity, and is therefore used as the measure for toxicity adjustment.

5.2 Available Aquatic Toxicity Information

As described above, much of the aquatic toxicity information presented in this review was obtained from two secondary sources: Sparling et al. (2000) and Pauli et al. (2000). In general, these references do not provide water hardness data for the lead studies. A limited search of the primary literature was also performed, particularly for sediment-associated studies, and the primary literature was reviewed for a number of studies to verify measurement units.

5.2.1 Sediment Exposure Toxicity Data

There were no data found in the literature describing the effects of lead-contaminated sediments on amphibians.

5.2.2 Surface Water Exposure Toxicity Data

This section presents toxicity data for amphibians exposed to lead in surface water. This presentation includes a summary of data provided by effect category, as well as a summary of the amphibian data included in the USEPA AWQC documentation for lead. Table 5-1 summarizes the lead amphibian toxicity data discussed in this section

Federal Ambient Water Quality Criterion Documentation

USEPA published acute and chronic freshwater AWQC for lead in 1985. The lead AWQC documentation included some toxicity data for three species of amphibian, but these data were not included in the development of the criteria. Adult leopard frog (*Rana pipiens*) exhibited mortality when exposed to 100 μg/L lead for thirty days. EC_{50} data for death and deformity were included for embryos of the narrow-mouthed toad (*Gastrophryne carolinensis*) (EC_{50} = 40 μg/L after 7 days) and the marbled salamander (*Ambystoma opacum*) (EC_{50} = 1,460 µg/L after 8 days).

Mortality

A number of lethal effects toxicity tests with amphibians were located in the literature. These included frog, toad, and salamander tests of various durations, ranging from 24-hour LC_{50}s to 30-day LC_{50}s.

Embryo mortality tests included one 48-hour LC_{50} value between 470 - 900 µg/L (*Bufo arenarum*, the common toad), one 7-day LC_{50} value of 40 µg/L (*Gastrophyrne carolinensis*, the eastern narrowmouth toad), and one 8-day LC_{50} of 1,460 µg/L (*A. opocum,* the marbled salamander).

Only one 96-hour tadpole LC_{50} was reported with a concentration of 33,280 µg/L for the Indian green frog (*Rana hexadactyla*). A 30-day LC_{50} value for *R. pipiens* was 105,000 µg/L, but some deaths and elevated concentrations of lead in the liver were found at concentrations as low as 25,000 µg/L

Several toxicity tests were conducted with adult male and female skipper frogs (*Rana cyanophlyctis*). The 24-hour lead LC_{50} concentrations were 1,895,800 µg/L for males and 1,688,500 µg/L for females. LC_{50} values at 48 hours were 1,583,300 ug/L for males and 1,770,800 µg/L for females. The 72-hour LC_{50} values were 1,542,700 µg/L and 1,625,000 µg/L for males and females respectively. The adult male and female LC_{50} values at 96 hours were 1,540,700 µg/L and 1,625,300 µg/L. The lethal concentrations were consistently higher for females than for males, indicating a higher tolerance for females to the lethal effects of lead.

Developmental

The effects of lead exposure on the development of amphibians were observed for the eggs of the black spotted frog (*Rana nigromaculata*) at 70 µg/L, where a partial reduction in primordial germ cells at the 9 - 12 mm body length stage was observed. These developmental effects were lethal to tadpoles. The larvae of *R. pipiens* stages 10 – 20 were exposed to lead concentrations of 100, 500, 1,000, and 1,500 µg/L. Delayed metamorphosis was noted; however, no morphological changes were observed and the size of the thyroid gland and follicle were reduced at the higher concentrations. Embryos from *B. arenarum* exposed to concentrations of 1,000 µg/L reported developmental effects that varied with stage. At the completion of development, 80% of the individuals were malformed. Embryos from *X. laevus* experienced developmental effects at concentrations as low as 1 µg/L, which increased in severity with decreasing concentrations of magnesium.

Growth

No studies evaluating the effects of chromium on amphibian growth were found in the literature.

Behavior

As documented in Table 5-1 learning and memory was effected in green frog tadpoles (*Rana clamitans*) at concentrations of 750 µg/L. Exposure concentrations for *R. clamitans* tadpoles between 0 – 1,000 µg/L resulted in greater variability of activity at concentrations between 500 – 1,000 µg/L and variability in locomotor activity between lead concentrations of 750 – 1,000 µg/L; no mortality was observed at these exposure concentrations. Increased latencies and fewer avoidance's were observed in the bull frog (*R. catesbiana*) at unreported lead concentrations. No indication of stress was observed for the American toad *(B. americanus*) exposed to lead concentrations between 500 – 1,000 µg/L in a plume.

Reproduction

No studies evaluating the effects of lead on amphibian reproduction were found in the literature.

Biochemical/cellular/physiological

Few data were found documenting adverse biochemical/cellular effects associated with lead exposure to amphibians. In one study, a 9% and 20% decrease in rod response was observed for adult bullfrogs (*R. catesbiana*) at concentrations of 5 and 12.5 µM. Effects on calcium metabolism were observed at concentrations of 1,000 µg/L in the bullfrog (*Rana catesbiana*). In tadpoles of *R. utricularia*, thyroid histopathological effects were recorded following exposure to 500 µg/L lead.

Table 5-1

Lead Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoints	Additional Observations	Reference: Primary	Reference: Secondary
BEHAVIOR										
NO EFFECT DATA										
Bufo americanus	American toad	Tadpole	500 - 1,000	UG/L	EC		No indication of behavioral stress with contact of plume	--	Steele et al. 1991	RATL
EFFECT DATA										
Rana clamitens	Green frog	Tadpole	0 - 1,000	UG/L	EC		Greater variablity in activity at 500 - 1000 ug/L	--	Steele et al. 1989	RATL
Rana clamitens	Green frog	Tadpole	0 - 1,000	UG/L	EC		variability in locomotor activity occurred at 750 - 1,000 ug/L	0% Mortality	Taylor et al 1990	RATL
Rana clamitens	Green frog	Tadpole	750	UG/L	EC		Learning and memory acquisition affected	--	Strickler-Shaw and Taylor 1990	RATL
DEVELOPMENTAL										
Rana nigromaculata	Black-spotted frog	Egg	70	UG/L	EC		Partial reduction in primordial germ cells at the 9 - 12 mm body length stage	Lethal to tadpoles	Hah 1978	RATL
Rana pipiens	Northern leopard frog	Tadpole	100	UG/L	EC		Delayed metamorphosis occurred related to Pb	--	Yeung 1978	RATL; Eisler, 1988
Rana pipiens	Northern leopard frog	Tadpole	500	UG/L	EC		concentrations however, no morphological changes were	--	Yeung 1978	RATL
Rana pipiens	Northern leopard frog	Tadpole	1,000	UG/L	EC		observed. The size of the thyroid gland and follicle were	--	Yeung 1978	RATL
Rana pipiens	Northern leopard frog	Tadpole	1,500	UG/L	EC		reduced for higher Pb concentrations.	--	Yeung 1978	RATL; Eisler, 1988
Bufo arenarum	Common toad	Embryo	1,000	UG/L	EC		80% malformations observed at the completion of development	Susceptibility was stage dependant	Perez-Coll and Herkovits 1990	
Xenopus laevis	African clawed frog	Embryo	1 - 10,000	UG/L	EC		Low Mg and exposure to Pb resulted in severe deformities	10,000 ppb Pb = 100% mortality	Miller and Landesman 1978	RATL
BIOCEMICAL/CELLULAR/PHYSIOLOGICAL										
Rana utricularia	Southern leopard frog	Tadpole	500	UG/L	EC		Thyroid histopathology was recorded	Delay in metamorphosis	Yeung 1978	Eisler, 1988
Rana catesbeiana	Bullfrog	Adult	1000	UG/L	EC		Synoptic transmissions of competitive inhibition of calcium were blocked	--	Kober and Cooper, 1976	Eisler, 1988
MORTALITY										
24-HOUR LC50										
Rana cyanophlyctis	Skipper frog	Adult (M)	1,687,500	UG/L	LC50	24 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (F)	1,895,800	UG/L	LC50	24 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
48-HOUR LC50										
Bufo arenarum	Common toad	Embryo	470 - 900	UG/L	LC50	48 HR	50% mortality in test organisms	--	Perez-Coll et al. 1988	Sparling et al. 2000
Rana cyanophlyctis	Skipper frog	Adult (M)	1,583,300	UG/L	LC50	48 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (F)	1,770,800	UG/L	LC50	48 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
72-HOUR LC50										
Rana cyanophlyctis	Skipper frog	Adult (M)	1,541,700	UG/L	LC50	72 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (F)	1,625,000	UG/L	LC50	72 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
96-HOUR LC50										
Rana hexadactyla	Indian green frog	Tadpole	33,280	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khargarot et al. 1985	Sparling et al. 2000
Rana cyanophlyctis	Skipper frog	Adult (M)	1,540,700	UG/L	LC50	96 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (F)	1,632,300	UG/L	LC50	96 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
OTHER DURATION										
Gastrophyrne carolinensis	Eastern narrowmouth toad	Embryo	40	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge 1978; Birge et al. 1979	Sparling et al. 2000
Ambystoma opacum	Marbled salamander	Embryo	1,460	UG/L	LC50	8 DAY	50% mortality in test organisms	99 mg CaCO3	Birge et al. 1978; EPA 1995	Sparling et al. 2000; Eisler, 1988
Rana pipiens	Northern leopard frog	Adult	105,000	UG/L	LC50	30 DAY	50% mortality in test organisms	Some deaths as low as 25,000 UG/L	Kaplan et al., 1967	Eisler, 1988

* units not listed but assumed to be UG/L

SECTION 6
MERCURY

Mercury is a naturally occurring substance that is found in the earth's crust at approximately 0.5 ppm (Merck, 1989). Mercury is a unique metal in that it is a dense silver-colored liquid at ambient temperature with a relatively high vapor pressure. Mercury occurs naturally in rocks, soils, and water and is ubiquitous in the aquatic environment. It is found in rock and ores such as limestone, calcareous shales, sandstone, serpentine, chert andesite, basalt, and rhyolite. It is recovered primarily from cinnabar although elemental mercury occurs in other ores. Fossil fuels such as coal and crude petroleum can contain mercury (HSDB, 1991). Naturally, mercury is released into the air by out-gassing of soil, transpiration, decay of vegetation, as well as volcanoes and hot springs.

Mercury is used in measuring devices (barometers, thermometers, hydrometers, and pyrometers), the manufacture of dry cell batteries, fluorescent light bulbs, mercury salts, mirrors, agricultural poisons, anti-fouling paint, electrical apparatus, mercury vapor and arc lamps, and dental amalgams. It is also used in the electrolytic preparation of chlorine and caustic soda, as a catalyst in the oxidation of organic compounds, in extracting gold and silver from ores, in pharmaceuticals, and in mercury boilers (Merck, 1989; HSDB, 1991). The primary stationary sources that have reported emissions of mercury in California are electrical services, hydraulic cement manufacturing sites, and petroleum production facilities (ARB, 1997a). Mercuric chloride is used in the manufacture of calomel, disinfectants, chemical reagents, metallurgy, tanning, as a catalyst for vinyl chloride, in embalming, as an intensifier in photography, in electroplating, and to free gold from lead. It is also used as an inorganic reagent (Merck, 1989).

Approximately 80% of the anthropogenic sources of mercury to the environment are emissions of elemental mercury to the air, primarily from fossil fuel combustion, mining, smelting, and from solid waste incineration. Another 15% of mercury emissions is from the application of fertilizers and fungicides, and municipal solid waste (e.g., batteries and thermometers), and an additional 5% of mercury emissions occurs via direct discharge of commercial effluent to water bodies (Stein et al., 1996).

6.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

The toxicity of mercury in the aquatic environment is influenced by a variety of environmental factors that alter the chemical speciation of mercury (Eisler, 1987a). Mercury speciation in freshwater systems depends on the mercury loadings, sedimentation rates, microbial activity, pH, nutrient content, redox, and suspended matter, as well as other factors (Eisler, 1987a). Mercury is usually discharged into aquatic ecosystems as elemental mercury, inorganic divalent mercury, phenylmercury or alkoxyalkyl.

The dominant process affecting the distribution of mercury and mercury compounds in the environment is the sorption to particulates, primarily organics (ATSDR, 1998b). Once in an aquatic system, ionic mercury can partition between the dissolved and particulate phases. The fraction of mercury associated with filterable particles can often be large (Gill and Bruland, 1990). Because of the strong association of Hg^{2+} with filterable particles, the distribution of inorganic mercury in the environment is often controlled by physical transport mechanisms governing sediment transport. Mercury that has formed some compound or is bound to organic or inorganic ligands has varying degrees of stability depending on the strength of the associated bond.

In general, organometallic ions are much more toxic than inorganic metal compounds because of their ability to transfer ions across biological membranes, greater solubility in lipid tissue, and tendency to bioconcentrate and bioaccumulate (Grandjean, 1984). While methylmercury has been detected in precipitation and in air (Hall et al., 1995), the atmospheric concentration of methylmercury, and the levels of methylmercury as a percentage of total mercury, are generally low.

Ionic mercury can be transformed to the more toxic methylmercury form, and the availability of the Hg^{2+} can largely affect the level of methylmercury in an aquatic environment. Increased levels of dissolved organic carbon (DOC) have been shown to reduce mercury methylation by limiting the availability of inorganic mercury to the methylation site (Miskimmin et al., 1992). Inorganic mercury ions can bind with sulfide under anoxic conditions and precipitate mercury as a sulfide complex, limiting the availability of mercury for methylation. Sulfide has a very strong affinity for ionic mercury and this precipitation can effectively remove the mercury from the system.

Gilmour et al. (1992) suggests that anaerobic sulfur-reducing bacteria (SRB) produce methyl mercury as a byproduct of their natural sulfur chemistry and that methylation can result in remobilization of sorbed or precipitated mercury. Methylmercury is kinetically inert toward decomposition and is water-soluble; thus, it is bioavailable for uptake by aquatic plants or animals (ATSDR, 1998b). If environmental conditions are able to support SRB activity, and mercury is present in the system, reduced oxygen levels can lead to an increase in methylmercury due to SRB (Gilmour et al., 1992).

Once methylmercury is produced it can either enter into the food chain or be demethylated. Upon entering the food chain, methylmercury tends to accumulate via trophic transfer. This bioaccumulation process is driven by the low methylmercury loss rate. Body burden mercury concentrations will increase up the food chain and older organisms tend to have higher body burdens than younger ones. Essentially all of the mercury in freshwater fish tissue is methylmercury (99%, Grieb et al., 1990; >95% Surma-Aho et al., 1986).

Several studies (e.g., St. Louis et al., 1994) concluded that wetlands are an important source of methylmercury and that yields of methylmercury from catchments containing wetlands were significantly higher (5 to 14 fold) than from purely upland catchments. In particular, wetlands appear to be key environments for microbially enhanced conversion of mercury into methylmercury. Once in aquatic systems, mercury can exist in dissolved or particulate forms and can undergo a number of chemical transformations. Contaminated sediments at the bottom of surface waters can serve as an important mercury reservoir, with sediment-bound mercury recycling back into the aquatic ecosystem for decades or longer (USEPA, 2001a).

6.2 Available Aquatic Toxicity Information

As described above, much of the aquatic toxicity information presented in this review was obtained from two secondary sources: Sparling et al. (2000) and Pauli et al. (2000). A limited search of the primary literature was also performed, particularly for sediment-associated studies, and the primary literature was reviewed for a number of studies to verify measurement units.

6.2.1 Sediment Exposure Toxicity Data

There were no data found in the literature describing the effects of mercury-contaminated sediments on amphibians.

6.2.2 Surface Water Exposure Toxicity Data

This section presents toxicity data for amphibians exposed to mercury in surface water. This presentation includes a summary of data provided by effect category, as well as a summary of the amphibian data included in the USEPA AWQC documentation for mercury. Table 6-1 summarizes the mercury amphibian toxicity data discussed in this section

Federal Ambient Water Quality Criterion Documentation

USEPA published acute and chronic freshwater AWQC for mercury in 1984; the AWQC was revised in 1995 and 1999. The 1984 mercury AWQC (USEPA, 1985e) documentation included some toxicity data for three species of amphibian, but these data were not included in the development of the criteria at that time or in the subsequent revisions. Data summarized in the 1984 mercury AWQC documentation indicated that leopard frog (*Rana pipiens*) died (LC_{100}) after 48 hours when exposed to 50-100 µg/L inorganic mercury, and failed to metamorphose after 4 months of exposure to 1-10 µg/L. Three life stages of leopard frog, blastula embryo, gastrula embryo, and neural plate embryo, were exposed to mercury. For each of these embryos, 5-day LC_{50} values were reported as 12-16, 8-12, and 12-16 µg/L, respectively, and 96-hour EC_{50} data for teratogenesis were 0-4, 8-12, and 12 µg/L, respectively. Death was noted in studies with a newt (*Triturus viridescens*) after 8 days at 1000 µg/L and after 17 days at 300 µg/L. After 2 days, newts exposed to 8 µg/L inorganic mercury exhibited delayed limb regeneration.

Mortality

A number of lethal effects mercury toxicity tests with amphibians were located in the literature. These included frog, toad, and salamander tests of various durations, ranging from 3-hour LC_{50}s to 8-day LC_{50}s.

Embryo studies included a 3-hour mercury LC_{50} value of 1,430 µg/L for embryos of the Indian green frog (*Rana hexadactyla*). Two 24-hour LC_{50} values were located: 7.3 µg/L and 65.9 µg/L for embryos from the northern leopard frog (*Rana pipiens*) and Fowler's toad (*Bufo fowleri*), respectively. Three 72-hour LC_{50} values for embryos ranged from 1 µg/L (*Gastrophyrne carolinensis*, the eastern narrowmouth toad) to 25 µg/L (*Bufo punctatus*, the red-spotted toad). Three embryo LC_{50}'s were also reported for 96-hour duration and ranged from 126 µg/L for a gastrulation-staged ornate rice frogs (*Microhyla ornata*) to 502 µg/L for the river frog (*Rana heckscheri*). One 3-day LC_{50} was documented for the embryos of the squirrel tree frog (*Hyla squirella*) at a concentration of 5 µg/L. Three six day LC_{50}'s ranged from 10 µg/L for the northern leopard frog (R. pipiens) to 75 µg/L for the pig frog (*R. grylio*) and river frog (*R. heckscheri).*

Embryo/embryo-larvae LC_{50} values at 7-days ranged from 1.0 (*G. carolinensis*) to 107.5 µg/L (*Amolops poecilus*, the Poecilus sucker frog). Fourteen embryo/embryo-larvae 7-day LC_{50} values were reported, and the average concentration of these studies was 29.2 µg/L. One 8-day embryo LC_{50} for the marbled salamander (*Ambystoma opacum*) was 110 µg/L.

Larval 48-hour LC_{50}s ranged from 100 µg/L (3 – 4 wk old *X. laevis*) to 400 µg/L (*A. mexicanum*, the Axolotyl). The three 24-hour LC_{50} values for tadpoles were 52.8 µg/L (*B. melanostictus*, the black-spined toad) and 2,040 µg/L and 2,410 µg/L for *M. ornata*. Five tadpole 48-hour LC_{50} values ranged from 45.6 µg/L for *B. melanostictus* to 2,070 µg/L for *M. ornata*. Only one tadpole 72-hour LC_{50} value was reported for the Fowler's toad (*B. fowleri*) at a concentration of 25 µg/L. Twelve 96-hour LC_{50} values were reported for tadpoles and ranged from 43.6 µg/L (B. *melanostictus)* to 1,430 µg/L (*M. ornata*), with an average of 325 µg/L. Two 5-day LC_{50} values of 1,000 µg/L were reported for tadpoles of the *R. catesbiana* (bullfrog) and *R. pipiens*.

Several adult amphibian toxicity tests were located with adult male and female skipper frogs (*R. cyanophlyctis*) and Asian bullfrogs (*R. tigrina*) as the test species. Test durations for these studies ranged from 24-hour LC_{50}s to 96-hour LC_{50}s. The lethal concentrations were consistently higher for females over males, suggesting a higher tolerance to mercury exposure for adult female frogs. Additional adult LC_{50}s included two 48-hour LC_{50} values that ranged from 100 µg/L (X. *laevis*) to 350 µg/L (*A. mexicanum*); one 96-hour LC_{50} value of 3,252 for *R. heckscheri* (river frog); and one 8-day LC_{50} value of 10,000 for *R. pipiens*.

Developmental

Mercury exposure to gametes, eggs, embryos, and tadpoles effected the development of various amphibian species. Although specific effects were not noted in the gametes of the Indian green frog (*R. hexadactyla*), development was altered at concentrations between 0 – 5,000 µg/L. Eggs from the eastern narrowmouth toad (*G. carolinensis*) illustrated signs of abnormal development at concentrations between 0.146 - 122.83 µg/L resulting in 41 – 49% larvae mortality at hatching. Damage to primordial germ cells was observed in eggs from the black-spotted frog (*R. nigromaculata*) at mercury concentrations of 800 µg/L. Eggs from the African clawed frog (*X. laevis*) exposed to concentrations of 20 – 100 µg/L either expired or the survivors were deformed. Various deformities of the eyes, heart, tail and intestines were noted. Embryos from the African clawed frog (*X. laevis*) experienced abnormal development at concentrations of 1 µg/L and expired at concentrations of 1,000 µg/L. In this study, deformities increased with increasing concentrations however, magnesium decreased the toxic effects of mercury. Delayed and irregular development was observed in embryos of the common toad (*B. arenarum*) exposed to mercury concentrations between 0 – 500 µg/L. One study researching the effects of mercury on the development of black-spotted frog (*R. nigromaculata*) tadpoles documented that concentrations of 400 µg/L and 800 µg/L caused abnormalities and were also lethal.

Growth

Growth was retarded and various abnormalities observed in adult ornate rice frogs (*M. ornata*) exposed to mercury concentrations between 50 – 250 µg/L for 72 to 96 hours. No other data was found documenting the effects on amphibian growth as a result of mercury exposure.

Behavior

No studies evaluating the effects of mercury on amphibian behavior were found in the literature.

Reproduction

Only one study was found that reported the adverse effects related to mercury exposure on amphibian reproduction. In this study, adult *X. laevis* exposed to mercury concentrations of 0.49 µg/L resulted in gonadal residue associated with reproductive dysfunction. In addition, gametes were defective and early life survival was reduced.

Biochemical/cellular/physiological

Few studies were found documenting the effects of mercury at the biochemical or cellular level of amphibians. One study documented an irreversible decrease in rod response in the adult bullfrog (*R. catesbiana*) at undocumented mercury concentrations.

Table 6-1

Mercury Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference Primary	Reference Secondary
DEVELOPMENTAL										
Rana nigromaculata	Black-spotted frog	Egg	800	UG/L*	EC		Damage to primordial germ cells; slower proliferation rate	--	Hah 1978	RATL
Gastrophyrne carolinensis	Eastern narrowmouth toad	Egg	0.15 - 122.8	UG/L*	EC		--	41-49% mortality at hatching.	Birge et al. 1977	RATL
Rana hexadactyla	Indian green frog	Gametes	0 - 5,000	UG/L	EC		--	--	Punzo 1993a	RATL
Xenopus laevis	African clawed frog	Egg	20-100	UG/L	EC		Retarded development of survivors, deformities of eyes, heart, tail and intestine	Mortality	Schowing and Boverio 1979	RATL
Gastrophryne carolinensis	Eastern narrowmouth toad	Embryo	1	UG/L		7 DAY	>10% malformation	--	Birge 1978; Birge et al. 1979	Sparling et al. 2000
Gastrophryne carolinensis	Eastern narrowmouth toad	Embryo	2	UG/L		7 DAY	>10% malformation	--	Birge et al. 1983	Sparling et al. 2000
Rana pipiens	Northern leopard frog	Embryo	2	UG/L		7 DAY	>7% malformation	--	Birge et al 1983	Sparling et al. 2000
Hyla chysocelis	Cope's gray treefrog	Embryo	2.4	UG/L		7 DAY	>10% malformation	--	Birge et al. 1979;1983	Sparling et al. 2000
Hyla chysocelis	Cope's gray treefrog	Embryo	5	UG/L		7 DAY	>10% malformation	--	Birge et al. 1983	Sparling et al. 2000
Bufo punctatus	Baird's spotted toad	Embryo	25	UG/L		7 DAY	>10% malformation	--	Birge et al. 1983	Sparling et al. 2000
Bufo fowleri	Fowler's toad	Embryo	25	UG/L		7-8 DAY	>7% malformation	--	Birge et al 1983	Sparling et al. 2000
Rana grylio	Pig frog	Embryo	75	UG/L		7 DAY	5% malformation	--	Birge et al 1983	Sparling et al. 2000
Bufo arenarum	Common toad	Embryo	0 - 500	UG/L	EC		Delayed and irregular development	--	Rengel and Pisano 1989	RATL
Xenopus laevis	African clawed frog	Embryo	1 - 1,000	UG/L	EC		Increased Hg concentrations resulted in moderate to severe deformities	1,000 ppb lethal; Mg decreases toxic effects of Hg	Miller and Landesman 1978	RATL
Rana nigromaculata	Black-spotted frog	Tadpole	400 - 800	UG/L*	EC		0.4 and 0.8 caused abnormalities	Induced mortality	Hah 1978	RATL
GROWTH										
Microhyla ornata	Ornate rice frog	Adult	50 - 250	UG/L	EC	72-96 HR	Retarded growth and caused various abnomalities	--	Ghate and Mulherkar 1980	
REPRODUCTIVE										
Xenopus laevis	African clawed frog	Adult	0.49	UG/L	EC		Gonadal residue associated with reproductive dysfunction	Defective gametes and reduced early life survival	Sparling et al., 2000	
BIOCHEMICAL/CELLULAR/PHYSIOLOGICAL										
Rana catesbeiana	Bullfrog	Adult	NA		EC		Irreversible decrease in rod response.	--	Fox and Sillman 1979	RATL

Table 6-1 (continued)

Mercury Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference Primary	Reference Secondary
MORTALITY										
24-HOUR LC50										
Bufo fowleri	Fowler's toad	Embryo	65.9	UG/L*	LC50	24 HR	50% mortality in test organisms	--	Birge et al. 1983	RATL
Rana pipiens	Northern leopard frog	Embryo	7.3	UG/L*	LC50	24 HR	50% mortality in test organisms	--	Birge et al. 1983	RATL
Bufo melanostictus	Black spined toad	Tadpole	52.8	UG/L	LC50	24 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Microhyla ornata	Ornate rice frog	Tadpole	2,040	UG/L	LC50	24 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	RATL
Microhyla ornata	Ornate rice frog	Tadpole	2,410	UG/L	LC50	24 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	RATL
Ambystoma mexicanum	Axolotl	Adult	Log 0.17	U/MOL	LC50	24 HR	50% mortality in test organisms	--	Vaal et al 1997	RATL
Xenopus laevis	African clawed frog	Adult	Log 0.46	U/MOL	LC50	24 HR	50% mortality in test organisms	--	Vaal et al 1997	RATL
Rana tigrina	Asian bullfrog	Adult (F)	19,020	UG/L	LC50	48 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana tigrina	Asian bullfrog	Adult (M)	18,300	UG/L	LC50	48 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (F)	3,830	UG/L	LC50	24 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (M)	3,350	UG/L	LC50	24 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
48-HOUR LC50										
Xenopus laevis	African clawed frog	Tadpole	100	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sloof and Baerselman 1980	
Ambystoma mexicanum	Axolotl	Larvae	259	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sloof and Baerelman 1980	Sparling et al. 2000
Ambystoma mexicanum	Axolotl	Larvae	296	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sloof et al. 1983	Sparling et al. 2000
Ambystoma mexicanum	Axolotl	Larvae	400	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sloof and Baerselman 1980	
Bufo melanostictus	Black spined toad	Tadpole	45.6	UG/L	LC50	48 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	RATL
Xenopus laevis	African clawed frog	Tadpole	74	UG/L	LC50	48 HR	50% mortality in test organisms	--	de Zwart and Sloof 1987; Sloof et al 1983	Sparling et al. 2000
Bufo japonicus		Tadpole	120	UG/L	LC50	48 HR	50% mortality in test organisms	--	Hashimoto and Nishiuchi 1981	Sparling et al. 2000
Microhyla ornata	Ornate rice frog	Tadpole	1,680	UG/L	LC50	48 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	2,070	UG/L	LC50	48 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Ambystoma mexicanum	Axolotl	Adult	350	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sloof et al. 1983	
Xenopus laevis	African clawed frog	Adult	100	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sloof et al. 1983	
Rana tigrina	Asian bullfrog	Adult (F)	18,040	UG/L	LC50	48 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana tigrina	Asian bullfrog	Adult (M)	18,950	UG/L	LC50	48 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (F)	3,330	UG/L	LC50	48 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	
Rana cyanophlyctis	Skipper frog	Adult (M)	3,050	UG/L	LC50	48 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	

Table 6-1 (continued)

Mercury Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference Primary	Reference Secondary
72-HOUR LC50										
Hyla chysocephala		Embryo	5	UG/L	LC50	72 HR	50% mortality in test organisms	--	Birge and Black 1977	Sparling et al. 2000
Bufo punctatus	Red-spotted toad	Embryo	25	UG/L	LC50	72 HR	50% mortality in test organisms	--	Birge and Black 1979	Sparling et al. 2000
Gastrophyrne carolinensis	Eastern narrowmouth toad	Embryo	1	UG/L	LC50	72 HR	50% mortality in test organisms	--	Birge and Black 1977	Sparling et al. 2000
Bufo fowleri	Fowler's toad	Tadpole	25	UG/L	LC50	72 HR	50% mortality in test organisms	--	Birge and Black 1977	Sparling et al. 2000
Rana tigrina	Asian bullfrog	Adult (F)	18,500	UG/L	LC50	72 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana tigrina	Asian bullfrog	Adult (M)	16,740	UG/L	LC50	72 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (F)	3,160	UG/L	LC50	72 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (M)	2,900	UG/L	LC50	72 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
96-HOUR LC50										
Microhyla ornata	Ornate rice frog	Embryo	170.4	UG/L*	LC50	96 HR	50% mortality in test organisms	--	Ghate and Mulherkar 1980	
Microhyla ornata	Ornate rice frog	Embryo	126	UG/L	LC50	96 HR	50% mortality in test organisms	--	Ghate and Mulherkar 1980	Sparling et al. 2000
Rana heckscheri	River frog	Embryo	502	UG/L	LC50	96 HR	50% mortality in test organisms	--	Punzo 1993	Sparling et al. 2000
Bufo melanostictus	Black spined toad	Tadpole	43.6	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Bufo melanostictus	Black spined toad	Tadpole	44	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	Sparling et al. 2000
Rana hexadactyla	Indian green frog	Tadpole	51	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot et al. 1985	Sparling et al. 2000
Bufo melanostictus	Black spined toad	Tadpole	56	UG/L	LC50	96 HR	50% mortality in test organisms	--	Paulose 1988	Sparling et al. 2000
Rana breviceps		Tadpole	60	UG/L	LC50	96 HR	50% mortality in test organisms	--	Paulose 1988	Sparling et al. 2000
Microhyla ornata	Ornate rice frog	Tadpole	88	UG/L	LC50	96 HR	50% mortality in test organisms	--	Ghate and Mulherkar 1980	Sparling et al. 2000
Microhyla ornata	Ornate rice frog	Tadpole	118.4	UG/L*	LC50	96 HR	50% mortality in test organisms	--	Ghate and Mulherkar 1980	
Bufo melanostictus	Black spined toad	Tadpole	185	UG/L	LC50	96 HR	50% mortality in test organisms	--	Paulose 1988	Sparling et al. 2000
Rana breviceps		Tadpole	207	UG/L	LC50	96 HR	50% mortality in test organisms	--	Paulose 1988	Sparling et al. 2000
Rana heckscheri	River frog	Tadpole	502	UG/L	LC50	96 HR	50% mortality in test organisms	--	Punzo 1993	Sparling et al. 2000
Microhyla ornata	Ornate rice frog	Tadpole	1,120	UG/L	LC50	96 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	1,430	UG/L	LC50	96 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Rana hexadactyla	Indian green frog	Juvenile	680	UG/L	LC50	96 HR	50% mortality in test organisms	--	Punzo 1993a	RATL
Rana heckscheri	River frog	Adult	3,252	UG/L	LC50	96 HR	50% mortality in test organisms	--	Punzo 1993	Sparling et al. 2000
Rana tigrina	Asian bullfrog	Adult (F)	18,300	UG/L	LC50	96 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana tigrina	Asian bullfrog	Adult (M)	16,100	UG/L	LC50	96 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (F)	3,160	UG/L	LC50	96 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL
Rana cyanophlyctis	Skipper frog	Adult (M)	2,500	UG/L	LC50	96 HR	50% mortality in test organisms	--	Mudgall and Patil 1988	RATL

Table 6-1 (continued)

Mercury Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference: Primary	Reference: Secondary
OTHER DURATION										
Rana hexadactyla	Indian green frog	Embryo	1,430	UG/L	LC50	3 HR	50% mortality in test organisms	--	Punzo 1993a	RATL
Hyla squirella	Squirrel treefrog	Embryo	5	UG/L	LC50	3 DAY	50% mortality in test organisms	--	Birge and Black 1977	Sparling et al. 2000
Rana catesbeiana	Bullfrog	Tadpole	1000	UG/L	LC50	5 DAY	50% mortality in test organisms	--	Birge and Just 1973; 1975	Sparling et al. 2000
Rana pipians	Northern leopard frog	Tadpole	1000	UG/L	LC50	5 DAY	50% mortality in test organisms	--	Birge and Just 1973; 1975	Sparling et al. 2000
Rana grylio	Pig frog	Embryo	75	UG/L	LC50	6 DAY	50% mortality in test organisms	--	Birge and Just 1973; 1975	Sparling et al. 2000
Rana heckscheri	River frog	Embryo	75	UG/L	LC50	6 DAY	50% mortality in test organisms	--	Birge and Just 1973; 1975	Sparling et al. 2000
Rana pipians	Northern leopard frog	Embryo	10	UG/L	LC50	6 DAY	50% mortality in test organisms	--	Birge and Just 1973; 1976	Sparling et al. 2001
Ambystoma opacum	Marbled salamander	Embryo	107.5	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al 1979	
Acris crepitans	Northern cricket frog	Tadpole	10.4	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Bufo debilis debilis	Eastern green toad	Tadpole	40	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Bufo punctatus	Baird's spotted toad	Tadpole	36.8	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Gastrophyrne carolinensis	Eastern narrowmouth toad	Tadpole	1.3	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Hyla chrysoscelis	Cope's gray treefrog	Tadpole	2.4	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Hyla gratiosa	Barking treefrog	Tadpole	2.5	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Hyla squirella	Squirrel treefrog	Tadpole	2.4	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Hyla versicolor	Gray treefrog	Tadpole	2.6	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Pseudacris crucifer	Spring Peeper	Tadpole	2.8	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Rana grylio	Pig frog	Tadpole	67.2	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Rana heckscheri	River frog	Tadpole	65.9	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Rana hexadactyla	Indian green frog	Tadpole	59.9	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Rana pipiens	Northern leopard frog	Tadpole	7.3	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge et al. 1979	
Ambystoma opacum	Marbled salamander	Embryo	110	UG/L	LC50	8 DAY	50% mortality in test organisms	--	Birge et al 1978	Sparling et al. 2000
Rana pipiens	Northern leopard frog	Adult	10,000	UG/L	LC50	8 DAY	50% mortality in test organisms	--	Birge and Just 1975a	RATL

* units not listed but assumed to be UG/L

SECTION 7
NICKEL

Nickel is an odorless, dark gray silvery metal, which occurs naturally in the earth's crust (Eisler, 1998; USEPA, 1986a). The predominant form of nickel are nickel sulfate and nickel oxides (USEPA, 1994). Chief sources of these forms of nickel include chalcopyrite, pyrrhotite, pentlandite, garnierite, nicolite, and millerite. The natural release of nickel to the surrounding environment includes erosion of rocks, precipitation, inflow of particulate matter, soil, sea spray, volcanoes, forest fires, and vegetation. Nickel and nickel compounds constitute 0.03 percent of the particulate matter suspended in the atmosphere. Wind erosion and volcanic activity contribute 40 to 50 percent of the atmospheric nickel from natural sources (ARB, 1991).

Nickel is commonly introduced in the environment as a byproduct from anthropogenic activity. Nickel is primarily used for the production of various metal alloys, cast irons, and electroplated goods (ARB, 1991; ATSDR, 1996). In addition, nickel carbonyl is used as a catalyst in the petroleum, plastic, and rubber industries (ARB, 1991). Nickel is also released into the atmosphere in motor vehicle exhaust (ARB, 1995b); fuel combustion (residential oil, distillate oil, coke and coal) is responsible for the majority of the emissions of nickel.

The majority of nickel that is released to the environment is released to land or water (ATSDR, 1996). The nickel that is released to surface waters is primarily discharged by Publicly Owned Treatment Works (POTW). Additional sources of nickel to the environment include the disposal of domestic and commercial trash, which may be recycled, landfilled or incinerated. The form of nickel emitted to the atmosphere varies with the source, but generally include complex nickel oxides, nickel sulfate and metallic nickel associated with combustion, incineration and metals smelting and refining. Due to the common use of nickel in various applications, it is often found in wetland and terrestrial habitats on DOD sites.

7.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

Elemental nickel is insoluble in water; however, in various other forms it is one of the most common metals present in surface waters (USEPA, 1986a). Nickel may exist in several oxidation states. The divalent cation is the predominant form of nickel, and is considered the most toxic. The bioavailability and toxicity of nickel in the aquatic environment is dependent on interactions with alkalinity, hardness, salinity, pH, temperature, and complexing agents such as humic acids. Nickel that is occluded in minerals, clay, and sand or that is strongly sorbed to particulate matter, is generally not bioavailable and not likely to become toxic under natural conditions. Mixtures of metals containing nickel salts are more toxic to daphnids and fishes than are predicted based on the individual components (Eisler, 1998).

USEPA (1980c) issued nickel AWQC based on total recoverable nickel in the water column and as acid-soluble nickel (USEPA, 1986a). In the 1999 Update to the AWQC (USEPA, 1999a), the USEPA indicated that the dissolved fraction of nickel (able to pass through a 0.45 μm filter) is the most appropriate approximation of bioavailable nickel in water. The acute and chronic water quality criteria for freshwater organisms are calculated on a site-specific basis using the hardness (as $CaCO_3$) of the water to adjust the criteria. While several factors do co-vary with hardness, including pH, alkalinity, and ionic strength, USEPA (1986a) considers hardness to be the most appropriate surrogate for the ions that affect nickel toxicity, and is therefore used as the measure for toxicity adjustment. The toxicity of nickel to freshwater organisms is significantly and negatively correlated to the hardness of the water (USEPA, 1986a); that is, as the hardness of the water increases, the bioavailability and, therefore, toxicity of the nickel generally decreases.

Bioavailability of nickel in sediment and soil is linked to the amount of bioavailable nickel in the pore water or interstitial water. In aerobic systems (high oxygen), the bioavailability of nickel is strongly associated with the presence of binding substances and nickel speciation. Sorption to organic matter and mineral oxides increases as pH increases (Eisler, 1998). As with most heavy metals, nickel is more strongly associated with fine-grained sediments and high TOC concentrations rather than coarse-grained sediments and lower TOC concentrations (Irwin et al., 1997). Nickel binds with carbonate, phosphate, and hydroxide ions, forming insoluble minerals.

The USEPA (2000) has incorporated nickel as one of the divalent cationic metals included in the sediment ESG for metals mixtures. The metals mixture ESG is based on EqP theory, and considers SEM (cadmium, copper, lead, nickel, silver, and zinc) and AVS in sediment. A more detailed description of the mechanism for the metals mixture ESG is presented in Section 2.1.

7.2 Available Aquatic Toxicity Information

As described above, much of the aquatic toxicity information presented in this review was obtained from two secondary sources: Sparling et al. (2000) and Pauli et al. (2000). In general, these references do not provide water hardness data for the nickel studies. A limited search of the primary literature was also performed, particularly for sediment-associated studies, and the primary literature was reviewed for a number of studies to verify measurement units.

7.2.1 Sediment Exposure Toxicity Data

There were no data found in the literature describing the effects of nickel-contaminated sediments on amphibians.

7.2.2 Surface Water Exposure Toxicity Data

This section presents toxicity data for amphibians exposed to nickel in surface water. This presentation includes a summary of data provided by effect category, as well as a summary of the amphibian data included in the USEPA AWQC documentation for nickel. Table 7-1 summarizes the nickel amphibian toxicity data discussed in this section

<u>Federal Ambient Water Quality Criterion Documentation</u>

The USEPA issued the nickel freshwater AWQC in 1986, and revised this value in 1995 and 1999. The 1986 criteria document included limited toxicity data for three species of amphibian, but these data were not included in the development of the original criterion or in any of the subsequent revisions. Data documented in the 1986 AWQC publication included EC_{50} data for death and deformity for embryos of Fowler's toad (*Bufo fowleri*) (EC_{50} = 11,030 μg/L after 7 days), the narrow-mouthed toad (*Gastrophryne carolinensis*) (EC_{50} = 50 μg/L after 7 days), and the marbled salamander (*Ambystoma opacum*) (EC_{50} = 420 μg/L after 8 days).

<u>Mortality</u>

Several lethal effects nickel toxicity tests with amphibians were located in the literature. These included frog, toad, and salamander tests of various durations, ranging from 24-hour LC_{50}s to 8-day LC_{50}'s.

Embryo 96-hour median lethal concentrations (LC_{50}) ranged from 146 μg/L to greater than 21,000 μg/L in *Xenopus laevis* embryo; these tests were conducted under a range of pH and hardness regimes. Two 7- and one 8- day embryo toxicity tests indicated LC_{50} values for *Bufo fowleri* (7-day), *Gastrophryne carolinensis* (7-day) and *Ambystoma opacum* ranging from 50 µg/L in *G. carolinensis* to 11,000 µg/L for *B. fowleri*.

One larval amphibian 24-hour LC_{50} was located (53,210 µg/L) for the black-spine toad (*Bufo melanostictus*). Two 48-hour larval LC_{50} values were 34,300 µg/L (*B. melanostictus*) and 261.18 µg/L (*Rana limnocharis*, adult Indian rice frog). Nickel sulfate toxicity tests on *B. melanostictus* tadpoles resulted in a 96-hour LC_{50} greater than 25,000 µg/L.

Growth

No studies evaluating the effects of nickel on amphibian growth were found in the literature.

Behavior

No studies evaluating the effects of nickel on amphibian behavior were found in the literature.

Reproduction

No studies evaluating the effects of nickel on amphibian reproduction were found in the literature.

Biochemical/Cellular

Relatively few data were available for the toxic effects of nickel at the biochemical and cellular level. One study reported that 10^{-4} M of nickel decreased membrane potential in *Cynops pyrrhogaster* (Japanese firebelly newt) by up to 82% in comparison to the control value. No other studies were noted.

Table 7-1

Nickel Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference: Primary	Reference: Secondary
BIOCHEMICAL/CELLULAR/PHYSIOLOGICAL										
Cynops pyrrhogaster	Japanese firebelly newt	Tadpole	10^{-4}	M	EC		Decreased membrane potential	--	Kanno et al. 1978	RATL
MORTALITY										
24 HOUR LC50										
Bufo melanostictus	Black spined toad	Tadpole	53,210	UG/L	LC50	24 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	RATL
48 HOUR LC50										
Bufo melanostictus	Black spined toad	Tadpole	34,300	UG/L	LC50	48 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	RATL
Rana limnocharis	Indian rice frog	Adult	261	UG/L	LC50	48 HR	50% mortality in test organisms	--	Pan and Liang 1993	RATL
96 HOUR LC50										
Xenopus laevis	African clawed frog	Embryo	300	UG/L	LC50	96 HR	50% mortality in test organisms	--	Linder et al. 1991	Sparling et al. 2000
Xenopus laevis	African clawed frog	Embryo	1,800	UG/L	LC50	96 HR	50% mortality in test organisms	pH of 6.0	Linder et al. 1991	Sparling et al. 2000
Xenopus laevis	African clawed frog	Embryo	1,700	UG/L	LC50	96 HR	50% mortality in test organisms	pH of 6.0	Linder et al. 1991	Sparling et al. 2000
Xenopus laevis	African clawed frog	Embryo	21,429	UG/L	LC50	96 HR	50% mortality in test organisms	pH of 6.8	Hopfer et al. 1991	Sparling et al. 2000
Bufo melanostictus	Black spined toad	Tadpole	25,320	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
OTHER DURATION										
Gastrophryne carolinensis	Eastern narrowmouth toad	Embryo	50	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge 1978; Birge et al 1979; Birge and Black 1980	
Bufo fowleri	Fowler's toad	Embryo	11,030	UG/L	LC50	7 DAY	50% mortality in test organisms	--	Birge and Black 1980	
Ambystoma opacum	Marbled salamander	Embryo	420	UG/L	LC50	8 DAY	50% mortality in test organisms	--	Birge et al. 1978	Sparling et al. 2000

SECTION 8
ZINC

Elemental zinc is a bluish-white, lustrous metal that occurs naturally as a sulfide, oxide or carbonate (Eisler, 1993). Zinc is widely distributed in nature, making up between 0.0005% - 0.02% of the Earth's crust (Irwin, et al., 1997). Zinc occurs naturally in smithsonite, sphalterite, wurtzite, zinc blende, zincite, willemite, franklinite, and gahnite ores. In sediments, zinc predominately exists in the forms of zinc hydroxide, ferric and manganic oxyhydroxide, insoluble organic complexes, and soluble sulfides (Eisler, 1993).

Anthropogenic activities account for greater than 96% of the total zinc released into the environment (Eisler, 1993). Zinc is used in alloys, galvanizing iron and other metals, electroplating, metal spraying, auto parts, electrical fuses, batteries, engravers' plates, cable wrappings, organ pipes, extracting gold, purifying fats for soaps, and railroad car linings (Merck, 1989). Zinc (metallic zinc) is a very minor component of a fungicide product composed of mancozeb, cymoxanil, and manganese sulfate. Zinc chloride is registered as a herbicide used to control lichen and moss growing on the roofs of houses and other domestic dwellings, along walks, driveways, fences, and wherever moss grows (DPR, 1996). Zinc phosphide is registered as a rodenticide for the control of mice, rats, gophers, squirrels, and other pestiferous rodents. Zinc oxide is used in paints, ointments, cosmetics, cement, glass, automobile tires, fabricated rubber products, plumbing fixtures, glue, matches, tiles, ceramics and porcelains, feed additives, seed treatment, inks, zinc green, electrostatic copying paper and color photography, flame retardant, semiconductor manufacturing, and as an ultraviolet absorber in plastics (Merck, 1989; Sax, 1987).

8.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

Zinc is a trace essential element required in the metabolism of most organisms (USEPA, 1980d; Irwin, et al., 1997). In aquatic organisms, zinc toxicity is most commonly the result of direct contact with high concentrations in surface water, rather than accumulation through the food chain (Irwin, et al., 1997).

In general, background concentrations of zinc in surface waters is usually less than 50 µg/L (USEPA, 1980d), significantly lower than the current AWQC for zinc. USEPA issued zinc AWQC based on total recoverable zinc in the water column (USEPA, 1980d). USEPA (1999a; 2002) considers the dissolved fraction of zinc (able to pass through a 0.45 µm filter) to be the most appropriate approximation of bioavailable zinc in water. The acute and chronic water quality criteria for freshwater organisms are calculated on a site-specific basis using the hardness (as $CaCO_3$) of the water to adjust the criteria. While several factors do co-vary with hardness, including pH, alkalinity, and ionic strength, USEPA (1980d) considers hardness to be the most appropriate surrogate for the ions that affect zinc toxicity, and is therefore used as the measure for toxicity adjustment. The toxicity of zinc to freshwater organisms is significantly and negatively correlated to the hardness of the water (USEPA, 1980d); that is, as the hardness of the water increases, the bioavailability and, therefore, toxicity of the zinc generally decreases.

Dissolved zinc usually consists of the toxic aquo ion ($(Zn(H_2O)_6)^{2+}$) (in the absence of other adsorbing or complexing parameters) and various organic and inorganic complexes (Eisler, 1993). Data compiled by Eisler (1993) reveals that in freshwater systems where pHs fall between 4 and 7, the aquo ion form dominates almost exclusively. Typically in rivers, 90% of the zinc is in the form of aquo ion while the remaining 10 percent is present as zinc carbonate, zinc sulfate, and the monohydroxide ion (Spear, 1981). The toxicity of aquo ions and other toxic forms on aquatic organisms is increased when ambient conditions are characterized by low pH, low alkalinity, low dissolved oxygen, and elevated temperatures (Eisler, 1993; Irwin, et al., 1997).

Zinc interacts with many chemicals, and these interactions may have a distinct effect on aquatic ecosystems (Eisler, 1993). For example, waterborne solutions of zinc-cadmium mixtures were usually additive in toxicity to aquatic organisms and mixtures of zinc and copper are generally acknowledged to be more-than-additive in toxicity to a wide variety of aquatic organisms. Zinc toxicity is also confounded by the observation that organisms inhabiting zinc-polluted areas or that are chronically exposed to zinc exhibit a higher tolerance for zinc in comparison to organisms occupying non-contaminated habitats.

Most of the zinc introduced into the aquatic environment is adsorbed by organic matter or inorganic substances such as mineral particles, clays, hydrous oxides of manganese, and iron, which partitions to the sediments or suspended solids (Eisler, 1993; Irwin, 1997; ATSDR, 1994). The release of zinc from the sediments and its mobility in the freshwater ecosystems is enhanced by low pH, high dissolved oxygen, and low alkalinity (Eisler, 1993; Irwin, et al., 1997).

Concentrations of zinc in the sediment interstitial pore waters have a positive correlation with the concentrations of dissolved zinc in the overlying surface waters (Eisler, 1993; Irwin, et al., 1997). The bioavailability of zinc to aquatic life is strongly associated with the presence of binding substances and zinc speciation and is further modified by the environmental factors discussed above. As with most heavy metals, zinc is more strongly associated with fine-grained sediments and high TOC concentrations rather than coarse-grained sediments and lower TOC concentrations (ATSDR, 1990). In addition, when sulfide is present, as it is in sediments rich in organic matter, it will bind with the zinc in the sediments in a highly insoluble form.

The USEPA (2000) has incorporated zinc as one of the divalent cationic metals included in the sediment Equilibrium Partitioning Guideline (ESG) for metals mixtures. The metals mixture ESG is based on EqP theory, and considers SEM (cadmium, copper, lead, nickel, silver, and zinc) and AVS in sediment. A more detailed description of the mechanism for the metals mixture ESG is presented in Section 2.1.

8.2 Available Aquatic Toxicity Information

As described above, much of the aquatic toxicity information presented in this review was obtained from two secondary sources: Sparling et al. (2000) and Pauli et al. (2000). In general, these references do not provide water hardness data for the zinc studies. A limited search of the primary literature was also performed, particularly for sediment-associated studies, and the primary literature was reviewed for a number of studies to verify measurement units.

8.2.1 Sediment Exposure Toxicity Data

There were no data found in the literature describing the effects of zinc-contaminated sediments on amphibians.

8.2.2 Surface Water Exposure Toxicity Data

This section presents toxicity data for amphibians exposed to zinc in surface water. This presentation includes a summary of data provided by effect category, as well as a summary of the amphibian data included in the USEPA AWQC documentation for zinc. Table 8-1 summarizes the zinc amphibian toxicity data discussed in this section

Federal Ambient Water Quality Criterion Documentation

The 1995 USEPA zinc AWQC includes results from tests with African clawed frog (*Xenopus laevis*). Of the 100 hardness-normalized (to 50 mg/L $CaCO_3$) genus mean acute values (GMAVs) used in the calculation of the 1995 criteria, this genera ranked 34th (*Xenopus* GMAV = 19,176 µg/L). Genera with lower ranks (e.g., more sensitive to zinc) included numerous species of fish and invertebrates.

Mortality

Several lethal effects zinc toxicity tests with amphibians were located in the literature. These included frog and toad tests of various durations, ranging from 24-hour LC_{50}s to 7-day LC_{50}s.

Tests with embryos include four 96-hour LC_{50} values reported for *M. ornata*, which ranged from 1,300 to 34,500 µg/L, and one 7-day LC_{50} value of 10 µg/L reported for the eastern narrow mouth toad (*Gastrophyrene carolinensis*).

One study reported the LC_{50} values of 1 week and 4 week old tadpoles of the ornate rice frog (*Microhyla ornata*) at several exposure durations with the following results; 24-hour LC_{50} values of 25,420 (4 wk) and 24,060 (1 wk) µg/L; 48-hour values LC_{50} of 24,380 (4 wk) and 23,420 (1 wk) µg/L; 72-hour LC_{50} values of 23,510 (4 wk) and 23,070 (1 wk) µg/L; and 96-hour LC_{50} values of 23,080 (4 wk) and 22,410 (1 wk) µg/L. The same LC_{50} value of 28,380 µg/L was reported for the Columbian spotted frog tadpoles (*R. luteiventris*) for 24-, 48-, 72- and 96-hour exposures. Additional lethal concentration values for tadpoles include one 24-hour LC_{50} value of 47,260 µg/L and 48-hour LC_{50} value of 25,650 µg/L for *B. melanostictus* and four 96-hour LC_{50} values that ranged from 2,100 (*R. hexadactyla*, the Indian green frog) to 19,860 µg/L (*B. melanostictus*).

Only one study was found in the literature documenting the lethal zinc concentrations for adult amphibians. The 48-hour LC_{50} for the Indian rice frog (*R. limnocharis*) was 71,870 µg/L.

Developmental

Adverse development resulting from zinc exposure was observed in amphibian eggs and tadpoles. Although specific development malformations were not documented in the eggs of the eastern narrow mouth toad (*Gastrophyrne carolinensis*), the malformations resulted in a 5 – 14% increase in mortality within 4 days of hatching at concentrations between 100 and 100,000 µg/L. After 3 months of exposure to zinc, tadpole survivors were stunted and did not develop limb buds. In contrast, western toad larvae (*B. boreas*) exhibited no effects on development at concentrations between 100 µg/L; however, 100% mortality was observed at concentrations of 39,000 µg/L. Zinc had a protective affect in *B. arenarum* embryos against spontaneous malformations and lethality.

Growth

No studies evaluating the effects of zinc on amphibian growth were found in the literature.

Behavior

No studies evaluating the effects of zinc on amphibian behavior were found in the literature.

Reproduction

No studies evaluating the effects of zinc on amphibian reproduction were found in the literature.

Biochemical/cellular/physiological

No studies evaluating the effects of zinc at the biochemical or cellular level were found in the literature.

Table 8-1

Zinc Toxicity Data for Amphibians

Species	Common Name	Lifestage	Age	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference: Primary	Reference: Secondary
DEVELOPMENTAL											
NO EFFECT DATA											
Bufo arenarum	Common toad	Embryo		1,000	UG/L	EC		Zn has protective affect in embryos against spontaneous malformations and lethality		Herkovits et al. 1989	RATL
Bufo boreas	Western toad	Tadpole		100-39,000	UG/L	EC		At 100 ug/L all larvae metamorphosed	100% mortality in 39,000 ug/L within 24 HR	Porter and Hakanson 1976	RATL
EFFECT DATA											
Gastrophyrne carolinensis	Eastern narrowmouth toad	Egg		100 - 100,000	UG/L*	EC		3 - 7% mortality and teratogenisis at hatching	5 - 14 % mortality at 4 day post hatching	Birge et al. 1977	RATL
Xenopus laevis	African clawed frog	Embryo		3,600	UG/L	EC50	96 HR	--	--	Dawson et al. 1988	Sparling et al. 2000
BIOCHEMICAL/CELLULAR/PHYSIOLOGICAL											
Pleorodeles spp	Ribbed newt species	Larvae		0-10,000	UG/L	NOEC		No genotoxicity observed	--	Godet et al. 1996	RATL
MORTALITY											
24-HOUR LC50											
Microhyla ornata	Ornate rice frog	Tadpole	4 WK	25,420	UG/L	LC50	24 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	1 WK	24,060	UG/L	LC50	24 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Rana luteiventris	Columbia spotted frog	Tadpole		28,380	UG/L	LC50	24 HR	50% mortality in test organisms	--	Lefcort et al. 1998	
Bufo melanostictus	Black spined toad	Tadpole		47,260	UG/L	LC50	24 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Rana catesbeiana	Bullfrog			130,000	UG/L	LC50	24 HR	50% mortality in test organisms	--	ECOTOX	USEPA, 1997
48-HOUR LC50											
Microhyla ornata	Ornate rice frog	Tadpole	4 WK	24,380	UG/L	LC50	48 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	1 WK	23,420	UG/L	LC50	48 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Bufo melanostictus	Black spined toad	Tadpole		25,650	UG/L	LC50	48 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	
Rana luteiventris	Columbia spotted frog	Tadpole		28,380	UG/L	LC50	48 HR	50% mortality in test organisms	--	Lefcort et al. 1998	
Rana limnocharis	Indian rice frog	Adult		71,870	UG/L*	LC50	48 HR	50% mortality in test organisms	--	Pan and Liang 1993	RATL
Rana catesbeiana	Bullfrog			110,000	UG/L	LC50	48 HR	50% mortality in test organisms	--	ECOTOX	USEPA, 1997
72-HOUR LC50											
Rana luteiventris	Columbia spotted frog	Tadpole		28,380	UG/L	LC50	72 HR	50% mortality in test organisms	--	Lefcort et al. 1998	
Microhyla ornata	Ornate rice frog	Tadpole	1 WK	23,070	UG/L	LC50	72 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	4 WK	23,510	UG/L	LC50	72 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	

Table 8-1 (continued)

Zinc Toxicity Data for Amphibians

Species	Common Name	Lifestage	Age	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference	
										Primary	Secondary
<u>96-HOUR LC50</u>											
Xenopus laevis	African clawed frog	Embryo		1,300	UG/L	LC50	96 HR	50% mortality in test organisms	--	Linder et al. 1991	Sparling et al. 2000
Xenopus laevis	African clawed frog	Embryo		13,689	UG/L	LC50	96 HR	50% mortality in test organisms	--	Fort et al. 1989	Sparling et al. 2000
Xenopus laevis	African clawed frog	Embryo		14,175	UG/L	LC50	96 HR	50% mortality in test organisms	--	Fort et al. 1989	Sparling et al. 2000
Xenopus laevis	African clawed frog	Embryo		34,500	UG/L	LC50	96 HR	50% mortality in test organisms	--	Dawson et al. 1988	Sparling et al. 2000
Rana luteiventris	Columbia spotted frog	Tadpole		28,380	UG/L	LC50	96 HR	50% mortality in test organisms	--	Lefcort et al. 1998	
Rana hexadactyla	Indian green frog	Tadpole		2,100	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot et al. 1985	Sparling et al. 2000
Hyla chrysoscelis	Cope's gray treefrog	Tadpole		4,700	UG/L	LC50	96 HR	50% mortality in test organisms	--	Gottschalk 1995	RATL
Rana pipiens	Northern leopard frog	Tadpole		10,200	UG/L	LC50	96 HR	50% mortality in test organisms	--	Gottschalk 1995	RATL
Bufo melanostictus	Black spined toad	Tadpole		19,860	UG/L	LC50	96 HR	50% mortality in test organisms	--	Khangarot and Ray 1987	Sparling et al. 2000
Microhyla ornata	Ornate rice frog	Tadpole	1 WK	22,410	UG/L	LC50	96 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Microhyla ornata	Ornate rice frog	Tadpole	4 WK	23,080	UG/L	LC50	96 HR	50% mortality in test organisms	--	Rao and Madhyastha 1987	
Rana catesbeiana	Bullfrog			70,000	UG/L	LC50	96 HR	50% mortality in test organisms	--	ECOTOX	USEPA, 1997
<u>OTHER DURATION</u>											
Gastrophyrne carolinensis	Eastern narrowmouth toad	Embryo		10	UG/L	LC50	7 DY	50% mortality in test organisms	--	Birge 1978; Birge et al. 1979	Sparling et al. 2000
Ambystoma opacum	Marbled salamander	Embryo		2,380	UG/L	LC50	8 DAY	50% mortality in test organisms	--	Birge et al. 1978	Sparling et al. 2000
Rana pipiens	Northern leopard frog	Adult		155	UG/L	LC50	15 DAY	50% mortality in test organisms	--	Kaplan and Glaczenski 1965	RATL

* units not listed but assumed to be UG/L

SECTION 9
POLYCHLORINATED BIPHENYLS

Polychlorinated Biphenyls (PCBs) are commercially produced organic compounds that do not occur naturally (Eisler, 1986b). There are 209 possible PCB isomers, with ten possible degrees of chlorination (i.e., ten homologues). PCBs vary in appearance from mobile, oily liquids to white, crystalline solids to hard, non-crystalline resins. Since 1974, all uses of PCBs in the United States have been confined to closed systems such as electrical capacitors, electrical transformers, vacuum pumps, and gas-transmission turbines. PCBs are no longer produced in the United States except for limited research and development applications (NTP, 1991). Sources of PCBs to the environment include landfills containing PCB waste materials and products, destruction of manufactured articles containing PCBs in municipal and industrial waste disposal burners, and gradual wear and weathering of PCB-containing products (HSDB, 1991). PCB contamination is highest in surface waters with a history of anthropogenic discharge and near-shore waters. In the environment, PCBs occur as mixtures of congeners, but their composition differs from the commercial mixtures (often called aroclors). After release to the environment, the composition of PCB mixtures changes over time through partitioning, chemical transformation, and preferential bioaccumulation and degradation of certain congeners (USEPA, 1999b).

9.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

The environmental fate, transport, and the toxic properties of PCBs in the environment are determined by the properties of the individual congeners (ATSDR, 1997). Bioavailability of PCBs to aquatic biota significantly vary between different organisms as well as the number and arrangement of chlorine atoms (Eisler, 1986b). According to Eisler (1986b), the PCB congeners associated with high octanol-water partitioning coefficient (K_{ow}) values and high numbers of substituted chlorines in adjacent positions pose the greatest risk to the environment including aquatic organisms.

The primary current source of PCBs to aquatic environments is through the environmental cycling process between the atmosphere and aquatic ecosystems (ATSDR, 1997). The solubility of PCB isomers in surface waters decreases with increasing chlorine content and chlorination (Eisler, 1986). In freshwater systems, PCBs are also partitioned from water to aquatic organisms (ATSDR, 1997). PCBs are highly lipophillic, with the greatest concentrations concentrated in fatty tissues (Eisler, 1986). Removal of PCBs is slow and there is evidence of biomagnification from lower trophic level organisms to higher ones for aquatic organisms (ATSDR, 1997).

PCBs are persistent in the environment and are resistant to biological and chemical degradation (ATSDR, 1997). However, studies illustrate that microbial degradation may break down higher chlorinated congeners to lower chlorinated congeners under anaerobic conditions. PCBs, especially those associated with a higher number of chlorinated congeners, strongly sorb to soil, sediment and particulates. PCBs are more strongly associated with finer-grained sediments, clay, and high TOC concentrations (Eisler, 1986b; Irwin et al., 1997). Volatilization of PCBs from surface waters and sorption of PCBs to bottom sediments can be significant processes that remove PCBs from surface waters.

PCBs in soils, sediments, and aquatic systems can be biodegraded under both aerobic and anaerobic conditions. In general, under aerobic conditions, soil bacteria have been reported to degrade only the lower chlorinated

PCB congeners (mono- to tetra-chloro PCBs). Speed of biodegradation increases as the number of chlorines on the molecule decreases (Abramowicz 1990). While highly chlorinated PCBs congeners (hepta- to deca-chloro PCBs) are generally not biodegraded aerobically, they can be degraded by bacteria under anaerobic conditions. Through a process known as "reductive dechlorination", bacteria remove chlorines from the PCB molecule, but do not alter the molecule's biphenyl backbone. The products of reductive dechlorination are less chlorinated PCBs which can be biodegraded aerobically. Reductive dechlorination requires highly reduced environmental conditions which would be most likely found in flooded soils and anaerobic sediments (Abramowicz 1990, Mohn 1992).

9.2 Available Aquatic Toxicity Information

As described above, much of the aquatic toxicity information presented in this review was obtained from two secondary sources: Sparling et al. (2000) and Pauli et al. (2000). A limited search of the primary literature was also performed, particularly for sediment-associated studies, and the primary literature was reviewed for a number of studies to verify measurement units.

9.2.1 Sediment Exposure Toxicity Data

One study was identified that evaluated the effects of PCB-contaminated sediments on amphibians. Savage et al. (2002) conducted tests with field-collected sediments containing PCBs. They used wood frog (*R. sylvatica)* to assess acute and chronic effects in a 42-day test with sediments containing 325 mg/kg PCBs. Some tadpoles were exposed directly to the sediment and others were suspended above the sediment in mesh containers to avoid direct contact. The results demonstrated that survivorship was significantly reduced by exposure to PCB-contaminated sediment. Decreased activity levels and swimming speeds were also observed. Impacts were more significant for the direct exposure scenarios.

9.2.2 Surface Water Exposure Toxicity Data

This section presents toxicity data for amphibians exposed to PCBs in surface water. This presentation includes a summary of data provided by effect category, as well as a summary of the amphibian data included in the USEPA AWQC documentation for PCBs. Table 9-1 summarizes the PCB amphibian toxicity data discussed in this section.

Federal Ambient Water Quality Criterion Documentation

USEPA (1980h) had recommended chronic AWQC for PCBs based on tissue residue data. These criteria were subsequently revoked (USEPA, 1999a,b), and no toxicity based chronic AWQC currently exist for PCB toxicity to aquatic organisms.

Mortality

Few lethal effects PCB studies were located. Early life stages of the northern leopard frog (*R. pipiens*) exposed to PCBs had LC_{50} values ranging from 1,030 to 6,950 µg/L, while early life stages of the Fowlers toad (*Bufo fowleri*) reported LC_{50} PCB concentrations ranging between 2,950 to 7,680 µg/L. In both studies, the duration of exposure was not documented.

Growth

No studies evaluating the effects of PCBs on amphibian growth were found in the literature.

Behavior

No studies evaluating the effects of PCBs on amphibian behavior were found in the literature.

Reproduction

No studies evaluating the effects of PCBs on amphibian reproduction were found in the literature.

Biochemical/cellular/physiological

One biomarker amphibian PCB study was located. Antibodies usually produced in response to heat shock were produced by contamination stress associated with PCB exposure concentrations of 0.1 µg/L in bullfrog tadpoles (*Rana catesbiana*). No other studies were found documenting the effects of PCB exposure at the biochemical/cellular level.

Table 9-1

PCB Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference Primary	Reference Secondary
BIOCHEMICAL/CELLULAR										
Rana catesbeiana	Bullfrog	Tadpoles	0.1	UG/L	EC		Antibodies usually produced in response to heat shock were produced by contamination stress	Exposure to Aroclor 1254	Dunlap and Matsumura 1997	RATL
MORTALITY										
Rana pipiens	Northern leopard frog	Early life-stage	2,870	UG/L	LC50	10 DAY	50% mortality in test organisms	Exposure to Capacitor 21	Sparling et al. 2000	
Rana pipiens	Northern leopard frog	Early life-stage	6,190	UG/L	LC50	10 DAY	50% mortality in test organisms	Exposure to Aroclor 1016	Sparling et al. 2000	
Rana pipiens	Northern leopard frog	Early life-stage	2,130	UG/L	LC50	10 DAY	50% mortality in test organisms	Exposure to Aroclor 1242	Sparling et al. 2000	
Rana pipiens	Northern leopard frog	Early life-stage	1,030	UG/L	LC50	10 DAY	50% mortality in test organisms	Exposure to Aroclor 1254	Sparling et al. 2000	
Bufo americanus	Common toad	Early life-stage	9,970	UG/L	LC50	10 DAY	50% mortality in test organisms	Exposure to Capacitor 21	Sparling et al. 2000	
Bufo americanus	Common toad	Early life-stage	7,160	UG/L	LC50	10 DAY	50% mortality in test organisms	Exposure to Aroclor 1016	Sparling et al. 2000	
Bufo americanus	Common toad	Early life-stage	2,710	UG/L	LC50	10 DAY	50% mortality in test organisms	Exposure to Aroclor 1242	Sparling et al. 2000	
Bufo americanus	Common toad	Early life-stage	2,020	UG/L	LC50	10 DAY	50% mortality in test organisms	Exposure to Aroclor 1254	Sparling et al. 2000	
Bufo fowleri	Fowler's toad	Early life-stage	28,000	UG/L	LC50	7 DAY	50% mortality in test organisms	Exposure to Capacitor 21	Sparling et al. 2000	
Bufo fowleri	Fowler's toad	Early life-stage	27,700	UG/L	LC50	7 DAY	50% mortality in test organisms	Exposure to Aroclor 1016	Sparling et al. 2000	
Bufo fowleri	Fowler's toad	Early life-stage	12,100	UG/L	LC50	7 DAY	50% mortality in test organisms	Exposure to Aroclor 1242	Sparling et al. 2000	
Bufo fowleri	Fowler's toad	Early life-stage	3,740	UG/L	LC50	7 DAY	50% mortality in test organisms	Exposure to Aroclor 1254	Sparling et al. 2000	
Rana pipiens	Northern leopard frog	Early life-stage	3,630	UG/L	LC50		50% mortality in test organisms	--	Sparling et al. 2000	
Rana pipiens	Northern leopard frog	Early life-stage	4,440	UG/L	LC50		50% mortality in test organisms	--	Sparling et al. 2000	
Rana pipiens	Northern leopard frog	Early life-stage	6,950	UG/L	LC50		50% mortality in test organisms	--	Sparling et al. 2000	
Bufo fowleri	Fowler's toad	Early life-stage	2,950	UG/L	LC50		50% mortality in test organisms	--	Sparling et al. 2000	
Bufo fowleri	Fowler's toad	Early life-stage	3,740	UG/L	LC50		50% mortality in test organisms	--	Sparling et al. 2000	
Bufo fowleri	Fowler's toad	Early life-stage	3,880	UG/L	LC50		50% mortality in test organisms	--	Sparling et al. 2000	
Bufo fowleri	Fowler's toad	Early life-stage	7,680	UG/L	LC50		50% mortality in test organisms	--	Sparling et al. 2000	
Pleorodeles waltl	Spanish ribbed newt	erythrocytes	50	UG/L	LC50		50% mortality in test organisms	Exposure to Aroclor 1254	Fernandez and l'Haridan 1989	Sparling et al. 2000

SECTION 10
DDT

DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)) is an organochlorine pesticide that was commonly used as an insecticide in the United States until 1973 (ATSDR, 2001). Technical grade DDT may contain its metabolites, DDE (1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene) and DDD (1,1-dichloro-2,2-bis(p-phlorophenyl)ethane) as contaminants. DDT and its metabolites do not occur naturally in the environment. Use of DDT peaked in the 1960's and declined until it was banned in the United States. The production and exporting of DDT in the United States continued until the 1980's; however, DDT is still manufactured and used in other countries, posing the potential for further global contamination.

Historically DDT was used as an insecticide on agricultural crops to control the damage caused by insects (ATSDR, 2001). DDT was also used extensively in the military to protect soldiers enlisted in World War II from diseases transmitted by insects including typhus and malaria. DDT was commonly used to control forests pests that were threatening the native populations of many trees. Since its ban in the United States, the presence of DDT in the environment is declining; however, its persistent and bioaccumulative properties have slowed any natural remedial processes (ATSDR, 1991). Sources of DDT in aquatic ecosystems are the result of pesticide application near surface waters, runoff, atmospheric deposition, and direct atmospheric exchange. Once in surface waters DDT, strongly adsorbs to sediments and particulate matter.

For some organisms, toxic effects associated with the parent compound, DDT, are less severe than the effects associated with its metabolites (e.g. DDD, DDE) (Sparling 2000). DDT contamination is not limited to ecosystems adjacent to its production or use. Although DDT typically enters the atmosphere through direct application and by revolatilization of residues in surface water or soil, DDT residues have been detected in the ice, soil and tissues of wildlife as far away as the Arctic and Antarctic.

10.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

Sediments are a sink for DDT, but a small portion may remain dissolved in the surface water where it is available for uptake by aquatic organisms. The DDT partitioned to the sediment may remain in depositional areas, degrade, or it may be ingested, resuspended or redistributed by benthic organisms. DDT is lipophilic as reflected by K_{ow}s ranging between 5.87 and 6.91. As a result, DDT bioaccumulates in the tissues of aquatic organisms. Concentrations of DDT in aquatic organisms are highest in higher trophic level organisms due to biomagnification.

In sediments, DDT may be photooxidized. DDT is biodegraded into DDD and DDE, which may further be degraded, although the extent and rate are determined by local sediment conditions. Biodegradation may occur under aerobic and anaerobic conditions by fungi, bacteria, and algae. DDT, DDE, and DDD can be broken down by a process called cometabolism whereby microbes derive alternative nutrient sources other than the usual compound. This process is longer for DDE than for DDD or DDT. Some studies have indicated that degradation is more rapid with sediments associated with higher organic carbon content and metals.

Several studies have documented the bioaccumulation of chlorinated hydrocarbon pesticides such as DDT. Bioconcentration factors and body burdens have been measured, but little information exists on the effects this exposure has on the viability of amphibian populations. Some data were available documenting the effects from direct exposure to DDT. The consensus of a few studies indicate that the ability of amphibians to metabolize organic compounds is most similar to that of fish (Sparling, 2000).

10.2 Available Aquatic Toxicity Information

As described above, much of the aquatic toxicity information presented in this review was obtained from two secondary sources: Sparling et al. (2000) and Pauli et al. (2000). A limited search of the primary literature was also performed, particularly for sediment-associated studies, and the primary literature was reviewed for a number of studies to verify measurement units.

10.2.1 Sediment Exposure Toxicity Data

There were no data found in the literature describing the effects of DDT-contaminated sediments on amphibians.

10.2.2 Surface Water Exposure Toxicity Data

This section presents toxicity data for amphibians exposed to DDT in surface water. This presentation includes a summary of data provided by effect category, as well as a summary of the amphibian data included in the USEPA AWQC documentation for DDT. Table 10-1 summarizes the DDT amphibian toxicity data discussed in this section.

Federal Ambient Water Quality Criterion Documentation

USEPA (1980i) had recommended chronic water quality criteria for DDT and DDE based on tissue residue data. These criteria were subsequently revoked (USEPA, 1999a), and no toxicity based chronic criteria currently exist for DDT or DDE toxicity to aquatic organisms.

Mortality

Several lethal effects DDT toxicity tests with amphibians were located in the literature. These included frog and toad tests of various durations, ranging from 24-hour LC_{50}s to 96-hour LC_{50}s. The majority of reported studies used tadpoles as the test organisms; no DDT embryo studies were reported.

The 24-hour LC_{50} tadpole studies included values ranging from 700 µg/L (*B. boreas*, the western toad) to 5,400 µg/L (*Bufo woodhousii*, the woodhouse's toad). The 48-hour tadpole LC_{50} values ranged from 410 µg/L (*B. woodhousii*) to 31,000 µg/L *(B. juxtasper*, the Sunge tawan toad), and the 96-hour LC_{50} values ranged from 30 µg/L *for B. woodhousii* to 1,400 µg/L for *P. triserata.*

Studies documenting the adult lethal concentrations as a result of DDT exposure include one 36-hour LC_{50} value between 400 and 50,000 µg/L for *B. woodhousii* and one 48-hour LC_{50} value of 380 µg/L for *Rana limnocharis* (Indian rice frog).

Developmental

Adverse development resulting from the exposure to DDT was observed in the early life stages of three amphibian species. A reduced time to metamorphosis was observed in embryos of the common toad (*B. arenarum*) exposed to DDT concentrations of 1,000 µg/L. Reduced tail regeneration was observed in tadpoles of the northern leopard frog (*R. pipiens*) exposed to DDT concentrations of 5 and 25 µg/L. In tadpoles of *R. temporaria* (European common frog), 29% developed abnormal snouts at 100 µg/L. In addition, 3% died and all affected individuals that reached tail resorption stage died.

Growth

No studies were found that documented the effects DDT may cause on the growth of amphibians.

Behavior

DDT exposure modified the behavior of spawn, larvae, tadpoles and adult amphibians. Spawn of *R. temporaria* exposed to DDT concentrations of 500 μg/L resulted in hyperactive behavior exhibited 8 – 13 days post hatch and development and weight gain were also retarded. Frantic behavior was observed at concentrations of 5 μg/L in the larvae of *Triturus vulgaris* (smooth newt). In the same study, DDT concentrations of 500 μg/L also increased *T. vulgaris* larval mortality by 10% and 35% at exposure durations of 24 hours and 48 hours, respectively. Hyperactivity was observed in tadpoles of the *R. temporaria* at concentrations as low as 100 μg/L. The tadpoles tended to float near the surface and smaller tadpoles were deformed. Hyperactivity and abnormal snouts were also noted in tadpoles of *R. temporaria* exposed to DDT concentrations between 20 – 500 μg/L

Reproduction

The hatching success of wood frog (*Rana sylvatica*) embryos was modified at DDT exposure concentrations of 25 μg/L.

Biochemical/cellular/physiological

Only one study was found documenting any abnormal activity at the cellular or biochemical level as a result of DDT exposure. In this study, a loss of intracellular potassium was observed at concentrations of 35 μg/L in adults of the American toad (*B. americanus*).

Table 10-1

DDT Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference Primary	Reference Secondary
BEHAVIOR										
Rana temporaria	European common frog	Egg	500	UG/L	EC		Hyperactive 8 - 13 d post-hatch	Development and weight gain were retarded	Cooke 1972b	RATL
Bufo arenarum	Common toad	Tadpole	1	UG/L	EC		Increased activity	--	Juarez and Guzman 1986	RATL
Triturus vulgaris	Smooth newt	Larvae	5	UG/L	EC	48 HR	Frantic Behavior	500 ppb = 10% mortality observed after 24 hours & 35% mortality observed after 48 hours	Cooke 1972b	RATL
Rana temporaria	European common frog	Tadpole	100	UG/L*	EC		Hyperactivity	Tendency to float near surface; deformities in small tadpoles	Cooke 1979	RATL
Rana temporaria	European common frog	Tadpole	20 - 500	UG/L	EC		Hyperactivity	Abnormal snouts noted in tadpoles treated with 20 - 500 ug/L	Cooke 1972b	RATL
BIOCHEMICAL/CELLULAR/PHYSIOLOGICAL										
Bufo americanus	American toad	Adult	35	UG/L*	EC		Loss of intracellular potassium	--	Sides and Finn	RATL
Rana tigrina	Asian bullfrog	Adult	0.1 - 0.3	%	EC	24-96 HR	Decreased vitamin A storage in liver	--	Keshavan and Deshmukh 1984	RATL
REPRODUCTIVE										
Rana sylvatica	Wood frog	Embryo	25	UG/L	EC		Hatch success impaired	--	Licht 1985	RATL
DEVELOPMENTAL										
Rana pipiens	Northern leopard frog	Tadpole	5	UG/L	EC		Reduced tail regeneration	--	Weis 1975	RATL
Rana pipiens	Northern leopard frog	Tadpole	25	UG/L	EC		Reduced tail regeneration	--	Weis 1975	RATL
Rana temporaria	European common frog	Tadpole	100	UG/L	EC	48 HR	29% developed snout abnormalities	3% died; all affected individuals that reached tail resorbtion stage died	Osborn et al. 1981	
Bufo arenarum	Common toad	Embryo	1,000	UG/L	EC		Reduced time to metamorphosis	Higher concentrations were lethal	Juarez and Guzman 1984a	RATL
MORTALITY										
24-HOUR LC50										
Bufo boreas	Western toad	Tadpole	700	UG/L	LC50	24 HR	50% mortality in test organisms	--	Marchal-Srgaut 1976	RATL
Pseudacris triserata	Western chorus frog	Tadpole	1,400	UG/L	LC50	24 HR	50% mortality in test organisms	--	Sanders 1970	RATL
Bufo woodhousii fowleri	Fowler's toad	Tadpole	1,400	UG/L	LC50	24 HR	50% mortality in test organisms	--	Sanders 1970	RATL
Bufo woodhousii fowleri	Fowler's toad	Tadpole	2,200	UG/L	LC50	24 HR	50% mortality in test organisms	--	Sanders 1970	RATL
Bufo woodhousii fowleri	Fowler's toad	Tadpole	2,400	UG/L	LC50	24 HR	50% mortality in test organisms	--	Sanders 1970	RATL
Bufo woodhousii fowleri	Fowler's toad	Tadpole	5,300	UG/L	LC50	24 HR	50% mortality in test organisms	--	Sanders 1970	RATL
Bufo woodhousii fowleri	Fowler's toad	Tadpole	5,400	UG/L	LC50	24 HR	50% mortality in test organisms	--	Sanders 1970	RATL

Table 10-1 (continued)

DDT Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference	
									Primary	Secondary
<u>48-HOUR LC50</u>										
Bufo woodhousii fowleri	Fowler's toad	Tadpole	410	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sanders 1970	
Bufo boreas	Western toad	Tadpole	500	UG/L	LC50	48 HR	50% mortality in test organisms	--	Marchal-Srgaut 1976	RATL
Bufo woodhousii fowleri	Fowler's toad	Tadpole	750	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sanders 1970	
Pseudacris triserata	Western chorus frog	Tadpole	900	UG/L	LT50	48 HR	50% mortality in test organisms	--	Sanders 1970	
Bufo woodhousii fowleri	Fowler's toad	Tadpole	1,000	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sanders 1970	
Bufo woodhousii fowleri	Fowler's toad	Tadpole	1,300	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sanders 1970	
Bufo woodhousii fowleri	Fowler's toad	Tadpole	1,800	UG/L	LC50	48 HR	50% mortality in test organisms	--	Sanders 1970	
Bufo woodhousii fowleri	Fowler's toad	Tadpole	1,500	UG/L	LT50	48 HR	50% mortality in test organisms	--	Sanders 1970	
Bufo juxtasper	Sungei tawan toad	Tadpole	31,000	UG/L*	LC50	48 HR	50% mortality in test organisms	--	Hashimoto and Nishiuchi	RATL
Rana limnocharis	Indian rice frog	Adult	380	UG/L*	LC50	48 HR	50% mortality in test organisms	--	Pan and Liang 1993	RATL
<u>72-HOUR LC50</u>										
Bufo boreas	Western toad	Tadpole	400	UG/L	LC50	72 HR	50% mortality in test organisms	--	Marchal-Srgaut 1976	RATL
<u>96-HOUR LC50</u>										
Pseudacris triserata	Western chorus frog	Tadpole	800	UG/L	LT50	96 HR	50% mortality in test organisms	--	Sanders 1970	
Bufo woodhousii fowleri	Fowler's toad	Tadpole	30	UG/L	LC50	96 HR	50% mortality in test organisms	--	Sanders 1970	
Bufo woodhousii fowleri	Fowler's toad	Tadpole	100	UG/L	LC50	96 HR	50% mortality in test organisms	--	Sanders 1970	
Bufo woodhousii fowleri	Fowler's toad	Tadpole	750	UG/L	LC50	96 HR	50% mortality in test organisms	--	Sanders 1970	
Bufo woodhousii fowleri	Fowler's toad	Tadpole	1000	UG/L	LC50	96 HR	50% mortality in test organisms	--	Sanders 1970	
<u>OTHER DURATION</u>										
Bufo arenarum	Common toad	Embryo	15000	UG/L	LC50	12 DAY	50% mortality in test organisms	--	Juarez and Guzman 1984a	RATL
Bufo arenarum	Common toad	Embryo	5000	UG/L	LC50	16 DAY	50% mortality in test organisms	--	Juarez and Guzman 1984a	RATL
Bufo woodhousii	Woodhouse's toad	young adult	400 - 50,000	UG/L	LC50	36 HR	50% mortality in test organisms	--	Ferguson and Gilbert 1967	RATL

No appropriate data found for DDD or DDE; all data presented are DDT

* units not listed but assumed to be UG/L

SECTION 11
POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic Aromatic Hydrocarbons (PAHs) are a combination of hydrogen and carbon arranged in two or more benzene rings (Eisler, 1987b). There are thousands of different PAH compounds that differ according to the number and position of aromatic rings and the position of substituents on the basic ring system. PAHs are formed by the incomplete combustion of organic substances under low oxygenated conditions (USEPA, 1980b), and are introduced to the environment through natural forces, such as forest fires, volcanic activity, natural petroleum seeps, and microbial synthesis (Eisler, 1987b). PAHs are found in soil, sediment, air, water, and plant and animal tissues as a result of anthropogenic activities and natural processes (ATSDR, 1995; Eilser, 1987b).

Prior to the 1900's, PAH contribution to the environment was in balance with the natural breakdown (Eisler, 1987b). The increased use of fossil fuels with the onset of the industrial resolution increased the load of PAHs to the environment, surpassing the amount removed from the natural remediation processes. Although PAHs are ubiquitous in the environment, releases from anthropogenic activities contribute to locally high concentrations and contamination. PAHs are released to the environment from the residential burning of wood, controlled refuse incineration, the emissions from vehicles used for transportation, and the generation of heat and power (Eisler, 1987b, ATSDR, 1995). Industrial sources of PAHs to the environment include coke production in the iron and steel industries, catalytic cracking in the petroleum industry, and the manufacturing of carbon black, coal tar pitch and asphalt. Municipal wastewater discharge, domestic and industrial effluents, oil spills, runoff, and atmospheric deposition sources contribute to the concentrations of PAHs in the aquatic environment.

11.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

PAHs enter the aquatic environment primarily through effluents, runoff and atmospheric deposition. As a result, PAH concentrations and speciation vary depending on the predominant source to each system. The fate and transport processes of PAHs in surface waters may include volatilization, photodegradation, oxidation, biodegradation, adsorption to particulates, or accumulation in aquatic organisms (ATSDR, 1995). In general, PAHs have low solubility, and the solubility of PAHs increases with decreasing molecular weight (ATSDR, 1995). Dissolved PAHs are quickly degraded primarily via photooxidation. Degradation of PAHs increases with increasing temperatures, oxygen, and at higher frequencies of solar radiation.

The properties that determine PAH compound transport and partitioning in the environment are roughly correlated with their molecular weight. Low molecular weight PAHs, such as napthalene, may pose the greatest threat to the environment due to their mobility (Eisler, 1987b). Di-aromatic (2-ring) hydrocarbons such as napthalene are associated with acute toxicity in surface water and pose a significant hazard to aquatic organisms. It is suggested that acute PAH toxicity is associated with photo-toxicity from the combined effects of high UV radiation and oxidation (Sparling, 2000). Photo-toxicity may be the result of the breakdown of PAHs into more toxic intermediates. Life stages vulnerable to photo enhanced effects from PAHs include amphibian larvae and eggs deposited in shallow water or at the surface microlayer (Irwin et al., 1997).

In the aquatic environment, PAH concentrations are highest in depositional areas associated with fine grains and high TOC concentrations (Eisler, 1987b). Most of the PAHs entering aquatic ecosystems partition to particulate matter and either remain in suspension or settle into the sediments (EPA, 1980b; Eisler, 1987b). PAHs are presumably degraded or biotransfered in the sediments by benthic organisms. The breakdown rate of PAH compounds varies and is slowest under low oxygen conditions or in the absence of penetrating solar radiation (EPA, 1980b). The consensus of a few studies indicate that the ability of amphibians to metabolize organic compounds is most similar to that of fish (Sparling, 2000).

PAHs typically occur in the environment as complex mixtures, rather than as single chemicals. Given that the mode of toxicity of individual PAHs is similar and is assumed to be additive, evaluation of the sum of PAHs (tPAHs), rather than individual PAHs, has been theorized to provide the most realistic estimate of potential toxicity to ecological receptors. USEPA is currently developing an ESG for PAHs using the theory of the additivity of PAH toxicity (DiToro and McGrath, 1999; Swartz, et al. 1995).

11.2 Available Aquatic Toxicity Information

As described above, much of the aquatic toxicity information presented in this review was obtained from two secondary sources: Sparling et al. (2000) and Pauli et al. (2000). A limited search of the primary literature was also performed, particularly for sediment-associated studies, and the primary literature was reviewed for a number of studies to verify measurement units.

11.2.1 Sediment Exposure Toxicity Data

There were no data found in the literature describing the effects of PAH-contaminated sediments to amphibians.

11.2.2 Surface Water Exposure Toxicity Data

This section presents toxicity data for amphibians exposed to PAHs in surface water. This presentation includes a summary of data provided by effect category. Table 11-1 summarizes the PAH amphibian toxicity data discussed in this section.

Federal Ambient Water Quality Criterion Documentation

There are no national freshwater AWQC for total PAHs in surface waters. USEPA (1993) calculated final chronic values (FCV) for three PAHs (naphthalene, phenanthrene, and anthracene) to support the derivation of sediment quality criteria (SQC), that have since been revoked.

Mortality

Lethal concentrations of various PAHs varied with species and life-stage, as well as the specific PAH compound utilized in the study.

A thirty–minute LC_{50} value of 65 µg/L (anthracene) was reported for embryos of the R. pipiens. Two 1-hour LC_{50} values of 90 and 140 µg/L (fluoranthene and pyrene, respectively) were reported for the embryos of *R. pipiens* and *X. laevis* respectively. A 5-hour LC_{50} (anthracene) value of 25 µg/L was reported for *R. pipiens* embryos and a 96-hour LC_{50} value of 2,100 µg/L was reported for *X. laevis* larvae.

Several lethal concentrations were also reported for unreported durations, as documented in Table 11-1.

Biochemical/cellular/physiological

Elevated frequencies of micronucleated erythrocytes were reported at various PAH concentrations ranging from 12.5 to 4,000 µg/L for larvae and tadpoles of *P. waltl* and *X. laevis*. Reductions in DNA adducts and micronuclei at 31 and 348 nM of PAHs were also noted.

Table 11-1

PAH Toxicity Data for Amphibians

Species	Common Name	Lifestage	Concentration	Unit	Endpoint	Duration	Endpoint	Additional Observations	Reference	
									Primary	**Secondary**
BEHAVIORAL										
Rana catesbeiana	Bullfrog		37.97	UG/L	LOEC	96 HR		Fluoranthene	Walker, et al., 1998	AQUIRE
Rana catesbeiana	Bullfrog		10.97	UG/L	NOEC	96 HR		Fluoranthene	Walker, et al., 1998	AQUIRE
Rana catesbeiana	Bullfrog		10.97 - 59.48	UG/L	NOEC			Fluoranthene	Walker, et al., 1998	AQUIRE
DEVELOPMENTAL										
Ambystoma maculatum	Spotted salamander		247	UG/L	EC50	288 HR		Fluoranthene	Hatch, A.C., 1998	AQUIRE
Rana pipiens	Leopard frog		276	UG/L	EC50	96 HR		Fluoranthene	Hatch, A.C., 1998	AQUIRE
GROWTH										
Ambystoma maculatum	Spotted salamander		17.2 - 906.1	UG/L		288 HR		Fluoranthene	Hatch, A.C., 1998	AQUIRE
Rana pipiens	Leopard frog		17.6 - 602.8	UG/L		96 HR		Fluoranthene	Hatch, A.C., 1998	AQUIRE
BIOCHEMICAL/CELLULAR/PHYSIOLOGICAL										
Pleorodeles waltl	Spanish ribbed newt	Larvae	0 - 12.5	UG/L	EC		Frequency of micronucleated erythrocytes: 0 - 6.25 ppb = 15-17/1000;	12.5 ppb resulted in death; Anthracene with UV	Fernandez and L'Haridon, 1994	RATL
Pleorodeles waltl	Spanish ribbed newt	Larvae	25 - 100	UG/L	EC		Frequency of micronucleated erythrocytes: 25 ppb = 27; 100 ppb = 304	Benzo(a)pyrene	Fernandez et al., 1989	RATL
Pleorodeles waltl	Spanish ribbed newt	Larvae	35 - 200	UG/L	EC		Frequency of micronucleated erythrocytes: 35 ppb = 10/1000; 200 ppb = 22/1000	Pyrene	Fernandez et al., 1989	RATL
Pleorodeles waltl	Spanish ribbed newt		200	UG/L		48 HR	Biochemical	Benzo(a)pyrene	Marty, et al. 1989	AQUIRE
Pleorodeles waltl	Spanish ribbed newt		2500 - 10000	UG/L		12 DAY	Cellular change	Anthracene	Djomo, et al., 1995	AQUIRE
Pleorodeles waltl	Spanish ribbed newt		4 - 200	UG/L		12 DAY	Increased cellular activity	Benzo(a)pyrene	Djomo, et al., 1995	AQUIRE
Pleorodeles waltl	Spanish ribbed newt		125 - 500	UG/L		12 DAY	Cellular change	Naphthalene	Djomo, et al., 1995	AQUIRE
Pleorodeles waltl	Spanish ribbed newt		1 - 4	UG/L		12 DAY	Cellular change	Phenanthrene	Djomo, et al., 1995	AQUIRE
Pleorodeles waltl	Spanish ribbed newt		25	UG/L		12 DAY	Decreased cellular activity	Benzo(a)pyrene	Godet, et al., 1996	AQUIRE
Xenopus laevis	African clawed frog	Tadpole	0 - 4,000	UG/L	EC		Frequency of micronucleated erythrocytes: 0.5 ppm = 68/1000; 1,000 ppb = 26/1000	Benzo(a)pyrene	Van Hummelen et al., 1989	RATL
Xenopus laevis	African clawed frog	Tadpole	31 - 248	nM	EC		Mean numbers of microcnuleated erythrocytes were 1.7, 6.3, and 16.4/1000; DNA adducts and micronuclei reduced at 31 and 348 nM, but assayed at metamorphosis	Benzo(a)pyrene	Sadinski et al., 1995	RATL
MORTALITY										
Ambystoma maculatum	Spotted salamander	Embryo	1,250	UG/L	LC5		5% mortality in test organisms	Fluoranthene	Hatch and Burton, 1996	RATL
Ambystoma maculatum	Spotted salamander	Embryo	125	UG/L	LC10		10% mortality in test organisms	Fluoranthene	Hatch and Burton, 1996	RATL
Rana pipiens	Northern leopard frog	Embryo	125	UG/L	LC15		15% mortality in test organisms	Fluoranthene	Hatch and Burton, 1996	RATL
Rana pipiens	Northern leopard frog	Embryo	625	UG/L	LC20		20% mortality in test organisms	Fluoranthene	Hatch and Burton, 1996	RATL
Rana pipiens	Northern leopard frog	Embryo	65	UG/L	LC50	30 MIN	50% mortality in test organisms	Anthracene with UV	Kagan et al. 1984	RATL
Xenopus laevis	African clawed frog	Embryo	140	UG/L*	LC50	1 HR	50% mortality in test organisms	Pyrene	Kagan et al. 1985	RATL
Rana pipiens	Northern leopard frog	Embryo	90	UG/L	LC50	1 HR	50% mortality in test organisms	Fluoranthene	Kagan et al. 1984	RATL
Rana pipiens	Northern leopard frog	Embryo	25	UG/L	LC50	5 HR	50% mortality in test organisms	Anthracene with UV	Kagan et al. 1984	RATL
Xenopus laevis	African clawed frog	Tadpole	2,100	UG/L*	LC50	96 HR	50% mortality in test organisms	Naphthalene	Edisten and Bantle, 1982	RATL
Ambystoma maculatum	Spotted salamander		247	UG/L	LC50	96 HR		Fluoranthene	Hatch, A.C., 1998	AQUIRE
Rana pipiens	Leopard frog		366	UG/L	LC50	288 HR		Fluoranthene	Hatch, A.C., 1998	AQUIRE
Rana pipiens	Northern leopard frog	Embryo	625	UG/L	LC80		80% mortality in test organisms	Fluoranthene	Hatch and Burton, 1996	RATL
Ambystoma maculatum	Spotted salamander	Embryo	625	UG/L	LC100		100% mortality in test organisms	Fluoranthene	Hatch and Burton, 1996	RATL
Pleorodeles waltl	Spanish ribbed newt	Embryo	25	UG/L			At 25 ppb BaP, 24% mortality; enchanced by UV	Benzo(a)pyrene	Fernandez and L'Haridon, 1994	RATL
Pleorodeles waltl	Spanish ribbed newt	Larvae	12.5 - 500	UG/L			At >25 ppb BaP, 90% mortality at 50ppb BaP+UV; BaP 4-fold less genotoxic than non-irradiated BaP	Benzo(a)pyrene	Fernandez and L'Haridon, 1994	RATL

* units not listed but presumed to be UG/L

SECTION 12
ORDNANCE AND EXPLOSIVES

Ordnance and Explosives (OE) consists of a group of nitroaromatic chemicals that may be released to the environment during manufacturing and handling. Among explosives and metabolites are 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine (HMX), n-methyl-n,2,4,6-tetranitroaniline (tetryl), 1,3,5-trinitrobenzene (TNB), 1,3-dinitrobenzene (DNB), nitrobenzene (NB), nitrocellulose, 2-amino-4,6-dinitrotoluene (2-A), 4-amino-2,6-dinitrotoluene (4-A), 2,4-diamino-6-nitrotoluene (2,4-DA), 2,6-diamino-4-nitrotoluene (2,6-DA), 3,5-dinitroaniline (DNA), 2,2',6,6'-tetranitro-4,4'-azoxytoluene (4,4'-AZ), 4,4',6,6'-tetranitro-2,2'-azoxytoluene (2,2'-AZ), 2',4,6,6'-tetranitro-2,4'-azoxytoluene (2,4'-AZ), and 2-amino-4,6-dinitrotoluene (2-ADNT).

OE compounds are found in a variety of applications associated with explosives. For example, RDX and tetryl are used as detonators in bombs and blasting caps, TNT is used in propellants in bombs, and 2,4-DNT is used as propellant in dynamite. OE compounds are formulated to be easily transportable, with less potential for random ignition than other explosives, such as nitroglycerin or lead azide (U.S. Army, 1993).

OE compounds have been detected in abiotic media at a large number of military institutions. In 1993, the U.S. Army Environmental Center and Environmental Hygiene Agency presented a briefing at Fort Devens, Massachusetts. As part of the presentation, the number of DOD facilities where explosives had been detected in soil and groundwater was tabulated. In soil and groundwater, respectively, TNT was detected at 15 and 7 facilities; RDX at 14 and 6 facilities; 2,4-DNT at 10 and 6 facilities; 2,6-DNT at 9 and 3 facilities; HMX at 14 and 2 facilities; and tetryl at 5 and 0 facilities. Disposal of OE compounds may occur in one of several ways including open burning, open detonation, or incineration. Of the facilities where groundwater was analyzed for OE compounds, none were found in groundwater where open detonation had occurred and OE compounds were detected in groundwater at approximately half the facilities where open burning on the ground had occurred. The detection of OE in groundwater was also correlated to the precipitation:evaporation ratio; OE were not detected when evaporation exceeded precipitation.

There are many of DOD sites where OE may have been released to the environment during load, assemble, and pack (LAP), manufacturing, and demilitarization activities. In general, many of these DOD sites are located on expanses of undisturbed land that include viable ecological habitats and many aquatic and terrestrial receptors. In recognition of the potential exposure to a vast array of potential receptors, Oak Ridge National Laboratory (Talmage et al., 1999) worked with the U.S. Army and the USEPA to develop aquatic and terrestrial OE screening criteria and benchmarks. No benchmarks, however, were developed for amphibian exposure.

The degradation potential of OE compounds varies significantly. Photolysis is a major contributing factor for the degradation of several OE compounds including TNT, RDX, HMX, 2,4-DNT, 2,6-DNT and TNB. The half-life of TNT under ultraviolet (UV) lights was estimated to be 0.5 – 22 hours (Talmage et al., 1999). Some OE compounds degrade by hydrolysis. The hydrolytic half-life of tetryl is approximately 10 months (U.S. Army, 1993).

All OE compounds appear to undergo some degree of anaerobic or aerobic biodegradation.

12.1 Factors Affecting Bioavailability and Toxicity in Freshwater Systems

Nitroaromatic munition compounds generally have low solubility in water. TNT is one of the most soluble of the OE compounds in this review (130 g/L (Talmage et al., 1999)); the other compounds are orders of magnitude less soluble. In general, OE compounds have low Kow values, indicating that they are not likely to bind to organic particles in sediment or surface water. The compounds are quite stable when not subjected to water or light and are not considered to be bioaccumulative or volatile.

12.2 Available Aquatic Toxicity Information

No amphibian aquatic toxicity information were found in the two sources of information used extensively for the other constituents reviewed in the report: Sparling et al. (2000) or Pauli et al. (2000). A search was performed using the USEPA's on-line database ECOTOX (http://www.epa.gov/ecotox/). Few data were found. A limited search of the primary literature was also performed, and the primary literature cited in the secondary sources was obtained for some studies. The following sections describe some of the ecotoxicological data for OE compounds in sediment and surface.

12.2.1 Sediment Exposure Toxicity Data

One amphibian study evaluating impacts on the tiger salamander (*Ambystoma maculatum*) from TNT exposure in soil and food items was located (Johnson et al., 2000). This study evaluated the fate and biochemical effects of TNT to identify biochemical indicators of exposure. No lethal or sub-lethal effects other than cytochrome P450 induction were observed following 14-day exposure to treated soil and food. Treated soil at study commencement contained up to 1,200 mg/kg dry weight TNT. Natural attenuation of TNT in soil was observed throughout the study, with soil concentrations dropping an order of magnitude and significant concentrations of monamino TNT reduction products present. Concentrations of TNT and its breakdown products were reported in a variety of tiger salamander tissues.

12.2.2 Surface Water Exposure Toxicity Data

No surface water amphibian OE toxicity studies were located.

SECTION 13
FURTHER EVALUATION OF SELECTED COMPOUNDS

Five constituents (cadmium, copper, mercury, zinc, and DDT) were selected for further evaluation of lethal effects data: the lethal effects data for these five analytes represent the more robust of the amphibian data sets available. In order to establish preliminary effects concentrations for these chemicals in water, the 10^{th} centile and 50^{th} centile of the toxicity distribution were calculated using methods described by Solomon et al. (2001). Observed lethal effects endpoints (LC_{50} values) from all species and measured effects were incorporated into the dataset for the 10^{th} and 50^{th} centile calculations. No adjustment was made to account for the hardness of the water, which, as described in Section 3, may affect the sensitivity of aquatic organisms to some metals.

A lethal effect concentration was estimated for each species in each of the chemical data sets. To maintain the most robust data sets possible, studies of various durations and lifestages were included. Tests for any single species may include several test durations and lifestages of amphibians; no attempt was made to estimate the most sensitive lifestage. The geometric mean of all available LC_{50} values for each species was calculated and used to estimate the species mean acute value (SMAV).

Data were ranked from low to high, and the percentile for each concentration calculated as [100 * i/(n+1)], where i is the rank of the datum and n is the number of data points in the set. Log-normalized concentration data and the calculated concentration percentile were plotted, and linear regressions were performed. Attachment B-1 presents the regression analyses performed for the five chemicals.

The following text provides a summary of the SMAVs for the five constituents evaluated. The USEPA AWQC and the calculated 10^{th} and 50^{th} centile thresholds for cadmium, copper, mercury, zinc, and DDT are presented in Table 13-1. With the exception of the chronic/10^{th} centile values for zinc, all thresholds calculated using the available amphibian mortality data are higher than their respective acute and chronic AWQC.

13.1 Cadmium

The cadmium dataset included eleven SMAVs. The resulting regression equation is:

$$y = 35.448x - 83.829$$
$$R^2 = 0.9452$$

From this equation, the concentration associated with the 10^{th} and 50^{th} percentiles of the data could be estimated. The resulting values (10^{th} percentile = 444 μg/L and 50^{th} percentile = 5,962 μg/L) are several orders of magnitude higher than the USEPA 2001 cadmium (dissolved) AWQC (CCC = 0.938 μg/L and CMC = 0.973 μg/L at 50 mg/L $CaCO_3$). The cadmium SMAVs and calculated percentile thresholds are presented in Figure 13-1.

13.2 Copper

The copper dataset included seventeen SMAVs. The resulting regression equation is:

$$y = 30.45x - 22.662$$
$$R^2 = 0.8914$$

From this equation, the concentration associated with the 10^{th} and 50^{th} percentiles of the data could be estimated. The resulting values (10^{th} percentile = 12 μg/L and 50^{th} percentile = 243 μg/L) are higher than the

USEPA 2002 copper (dissolved) AWQC (CCC = 9 µg/L and CMC = 13 µg/L at 50 mg/L $CaCO_3$). The copper SMAVs and calculated percentile thresholds are presented in Figure 13-2.

13.3 Mercury

The mercury dataset included twenty-five SMAVs. The resulting regression equation is:

$$y = 25.773x + 5.3403$$
$$R^2 = 0.9409$$

From this equation, the concentration associated with the 10th and 50th percentiles of the data could be estimated. The resulting values (10th percentile = 1.52 µg/L and 50th percentile = 54 µg/L) are higher than the USEPA 2002 mercury (dissolved) AWQC (CCC = 0.77 µg/L and CMC = 1.4 µg/L). The mercury SMAVs and calculated percentile thresholds are presented in Figure 13-3.

13.4 Zinc

The zinc dataset included eleven SMAVs. The resulting regression equation is:

$$y = 22.139x – 33.725$$
$$R^2 = 0.8042$$

From this equation, the concentration associated with the 10th and 50th percentiles of the data could be estimated. The resulting 10th percentile (94 µg/L) is lower than the USEPA 2002 chronic zinc AWQC (CCC = 120 µg/L, dissolved zinc at 50 mg/L $CaCO_3$). The 50th percentile (6,050 µg/L) is higher than the USEPA 2002 zinc acute AWQC (CMC = 120 µg/L dissolved zinc at 50 mg/L $CaCO_3$). The zinc SMAVs and calculated percentile thresholds are presented in Figure 13-4.

13.5 DDT

The DDT dataset included seven SMAVs. The resulting regression equation is:

$$y = 34.075x – 59.121$$
$$R^2 = 0.8928$$

From this equation, the concentration associated with the 10th and 50th percentiles of the data could be estimated. The resulting values (10th percentile = 107 µg/L and 50th percentile = 1,594 µg/L) are higher than the USEPA 2002 DDT AWQC (CCC = 0.001 µg/L and CMC = 1.1 µg/L). The DDT SMAVs and calculated percentile thresholds are presented in Figure 13-5.

13.6 Genus Mean Acute Values

To evaluate whether or not there were any observable phylogenetic trends in amphibian contaminant sensitivity, genus mean acute values (GMAV) were calculated as the geometric mean of all SMAVs from the same genus. A total of eight genera were represented in the data sets for the five chemicals. Of these eight, only two (*Rana* and *Bufo*) included studies for each of the five chemicals. GMAVs are presented in Table 13-2.

The chemical-specific GMAVs were compared to the 10th and 50th percentile thresholds. For each genus, the number of chemical-specific GMAVs exceeding their respective thresholds and those that do not exceed their respective thresholds were tabulated. Figure 13-6 presents this information. For two of three chemicals (no data were available for cadmium and DDT), *Gastrophryne* GMAVs were lower than the 10th percentile thresholds. The GMAVs for all other chemicals exceeded their respective 10th percentile thresholds.

All *Gastrophryne* and *Hyla* GMAVs were lower than the calculated 50th percentile thresholds. Two of three *Pseudacris* GMAVs, two of four *Ambystoma* GMAVs, two of five *Bufo* GMAVs, two of five *Rana* GMAVs, and one of four *Microhyla* GMAVs were also lower than their respective chemical 50th percentile thresholds. All *Xenopus* GMAVs exceeded the 50th percentile thresholds.

These data are consistent with the findings of Birge et al. (2000), who compared the sensitivity of fish to twenty-one amphibian species to metals and eight amphibian species to organics. The results of these studies are presented in Table 13-3. The classification of sensitivity was assigned as a function of the ratio of amphibian LC_{50} to the concurrently-tested fish (rainbow trout, *Oncorhynchus mykiss*) LC_{50}. Amphibian and fish embryos were exposed from fertilization to four days post-hatch. Exposure duration varied among species, but common endpoints in developmental stage were used for comparison.

Although there are numerous uncertainties with the interpretation of these limited data, several trends may be worth further investigation. In general, *Gastrophryne* (narrow-mouth toad) and *Hyla* (treefrog) species appear to be more sensitive to metals, and *Bufo* (toad) species appear to be among the least sensitive. *Ranid* and *Ambystomid* species appear to fall in the mid-range of sensitivity, with some species showing greater sensitivity than others. Based on the limited available data, *Xenopus* may be more tolerant to contaminant exposure that the native amphibians included in this evaluation.

Fewer species were exposed by Birge et al. (2000) to organic contaminants, but the sensitivity also appears to be consistent with the GMAVs calculated from this database. In the Birge et al. (2000) investigation, the single Ambystomid species was the most sensitive, Ranids and *Bufo* species sensitivity rankings were scattered in the mid-range, and *Xenopus* were highly tolerant.

13.7 Summary

Using a consistent method of interpretation, five sets of compound-specific threshold values were calculated for amphibians. Although there are considerable uncertainties associated with this approach (e.g., differences in test species, duration, exposure conditions, and general test methods can produce highly variable lethal (or sub-lethal) thresholds for any single chemical), evaluation of these thresholds indicates that amphibians may be sensitive to mercury and zinc contamination, and relatively insensitive to cadmium contamination (Table 13-1). Amphibian thresholds were generally much higher than the AWQC; however, it is important to recognize that this evaluation considered only lethal effects data. It is possible that the results would differ markedly for sub-lethal effects data, or if exposure duration and life stage data were explicitly considered.

Table 13-1

Comparison of Surface Water Screening Benchmarks to Calculated Centiles

Analyte (ppb)	Chronic Values		Acute Values	
	Chronic AWQC	Calculated 10th Centile	Acute AWQC	Calculated 50th Centile
Inorganics				
Cadmium	0.25	444	2	5,962
Copper	9	11.8	13	243
Mercury	0.77	1.52	1.4	54
Zinc	120	94	120	6,050
Organics				
DDT	0.001	107	1.1	1,594

AWQC - Ambient Water Quality Criteria (USEPA, 2002).

Table 13-2

Genus Mean Acute Values

Genus	Chemical: Cadmium (mg/L)	Copper (mg/L)	DDT (mg/L)	Mercury (mg/L)	Zinc (mg/L)
Ambystoma	484	454	--	187	2,380
Bufo	1,372	494	2,116	51	28,875
Gastrophryne	--	28	--	1.14	10
Hyla	--	28	--	3.06	4,700
Microhyla	2,272	5,467	--	678	23,653
Pseudacris	--	50	1,618	2.80	--
Rana	12,564	41	380	485	14,005
Xenopus	7,833	502	--	90	9,659

Table 13-3

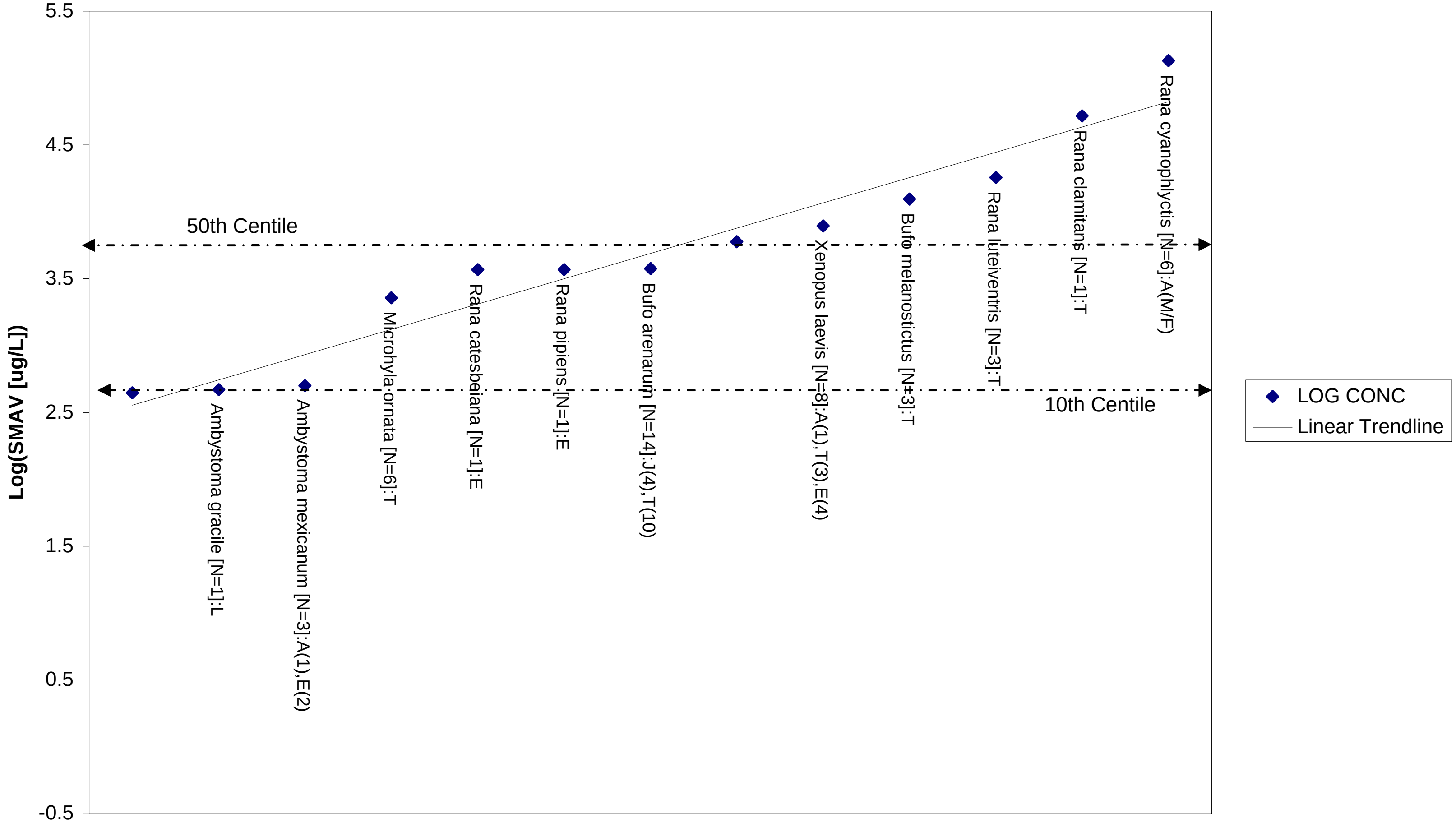
Relative Sensitivity of Amphibian Species

Tolerance Classification [1]			
Very sensitive	**Sensitive**	**Moderately Tolerant**	**Tolerant**
<u>Metals</u>			
Pseudacris crucifer	*Hyla gratiosa*	*Ambystoma maculatum*	*Bufo fowleri*
Rana palustris	*Hyla aquirella*	*Rana heckscheri*	*Ambystoma opacum*
Rana pipiens	*Ambystoma barbouri*	*Rana grylio*	*Bufo debilis debilis*
Gastrophryne carolinensis	*Acris crepitans blanchardi*	*Ambystoma t. tigrinum*	*Bufo punctatus*
Hyla chrysoscelis	*Ambystoma jeffersonianum*		
Hyla versicolor	*Ambystoma texanum*		
Rana catesbeiana			
<u>Organic Constituents</u>			
--	*Rana temporaria*	*Rana catesbeiana*	*Bufo fowleri*
	Ambystoma gracile	*Rana pipiens*	*Rana palustris*
			Bufo americanus
			Xenopus laevis

(1) Tolerance classifications assigned by Birge et al. (2000) based on geometric mean of amphibian LC50 values relative to rainbow trout LC50 values.

Figure 13-1

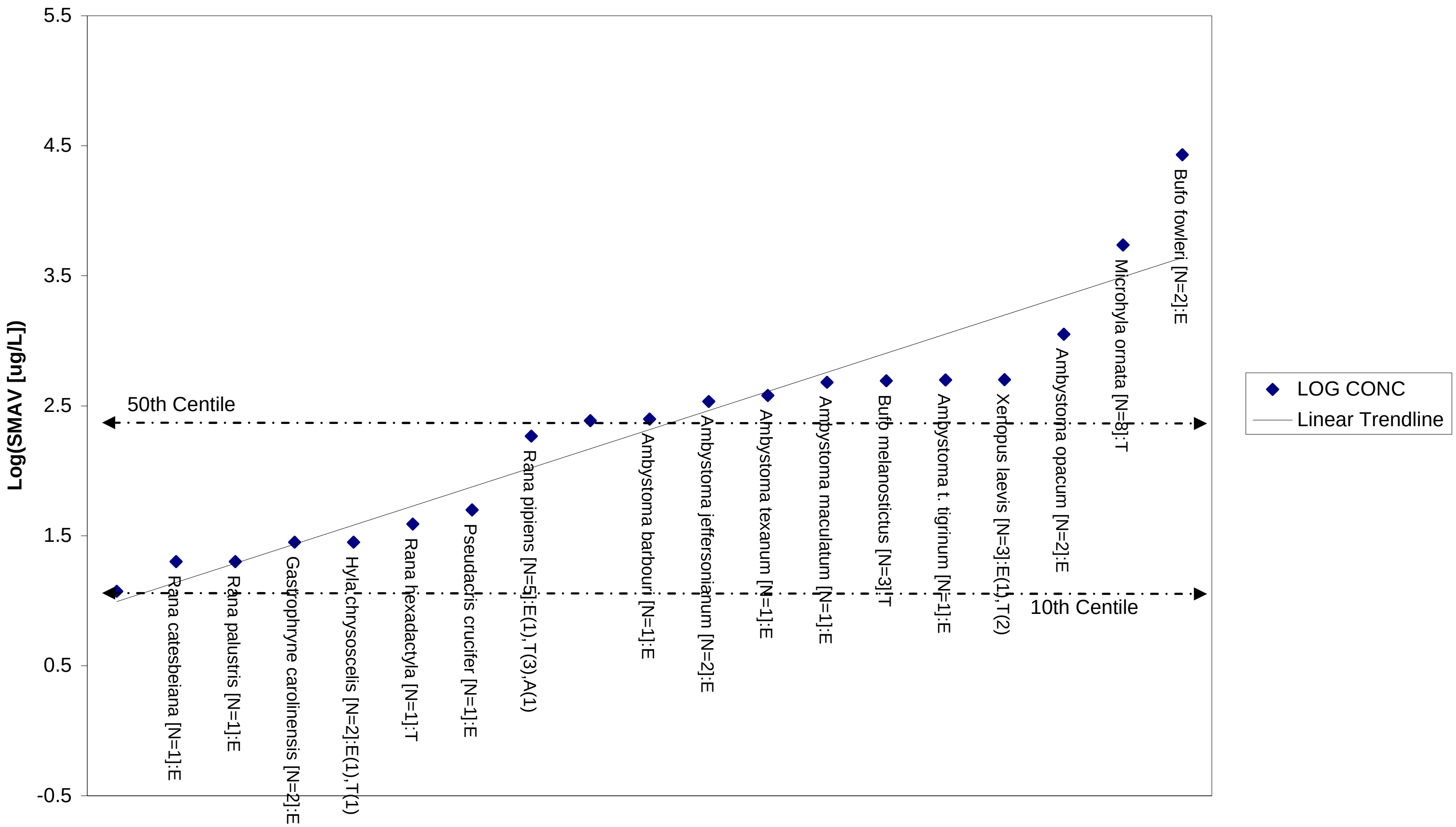
Cadmium SMAVs and Centile Thresholds



Species names are followed by number of studies and lifestages:
A = adult
M/F = male and female
J = juvenile
T = tadpole
L = larvae
E = embryo

Figure 13-2

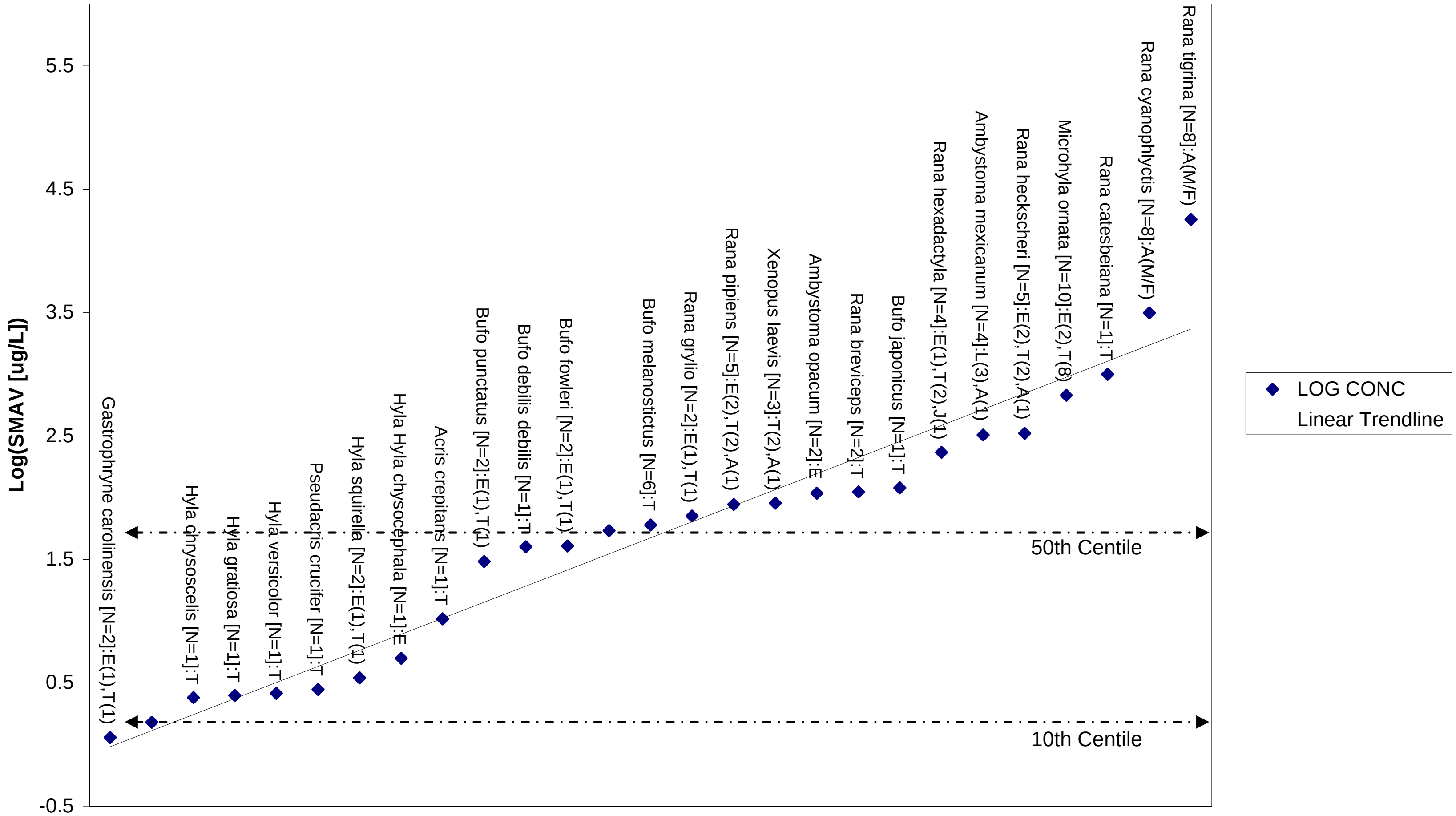
Copper SMAVs and Centile Thresholds



Species names are followed by number of studies and lifestages:
A = adult
M/F = male and female
J = juvenile
T = tadpole
L = larvae
E = embryo

Figure 13-3

Mercury SMAVs and Centile Thresholds

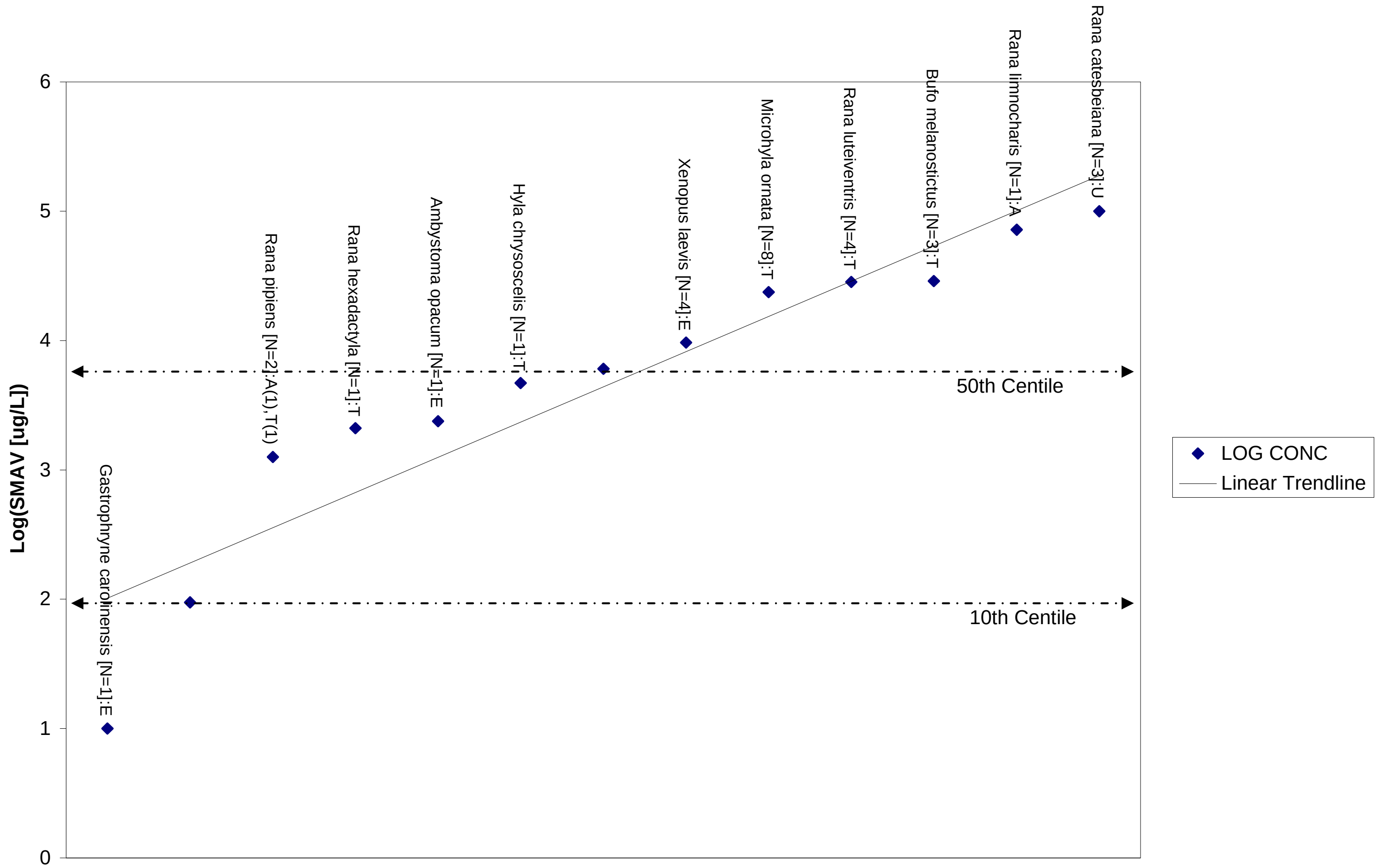


Species names are followed by number of studies and lifestages:
A = adult
M/F = male and female
J = juvenile

T = tadpole
L = larvae
E = embryo

Figure 13-4

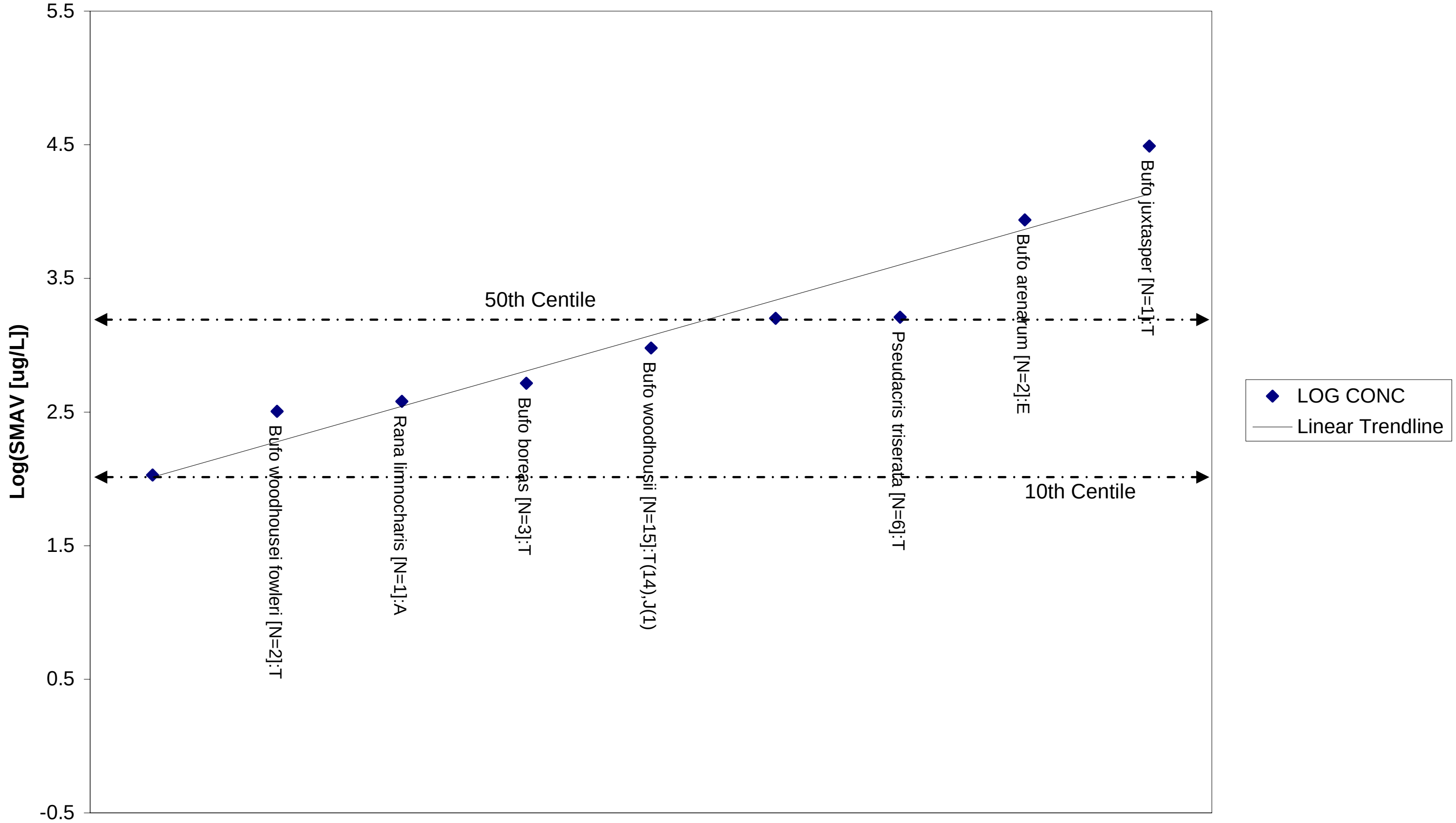
Zinc SMAVs and Centile Thresholds



Species names are followed by number of studies and lifestages:
A = adult
M/F = male and female
J = juvenile
T = tadpole
L = larvae
E = embryo

Figure 13-5

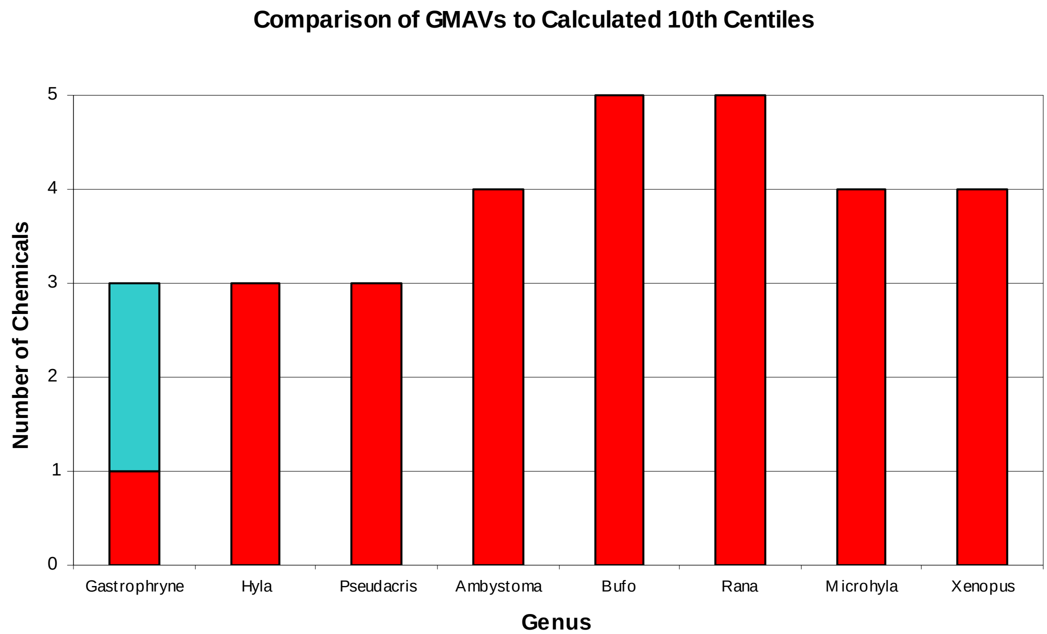
DDT SMAVs and Centile Thresholds

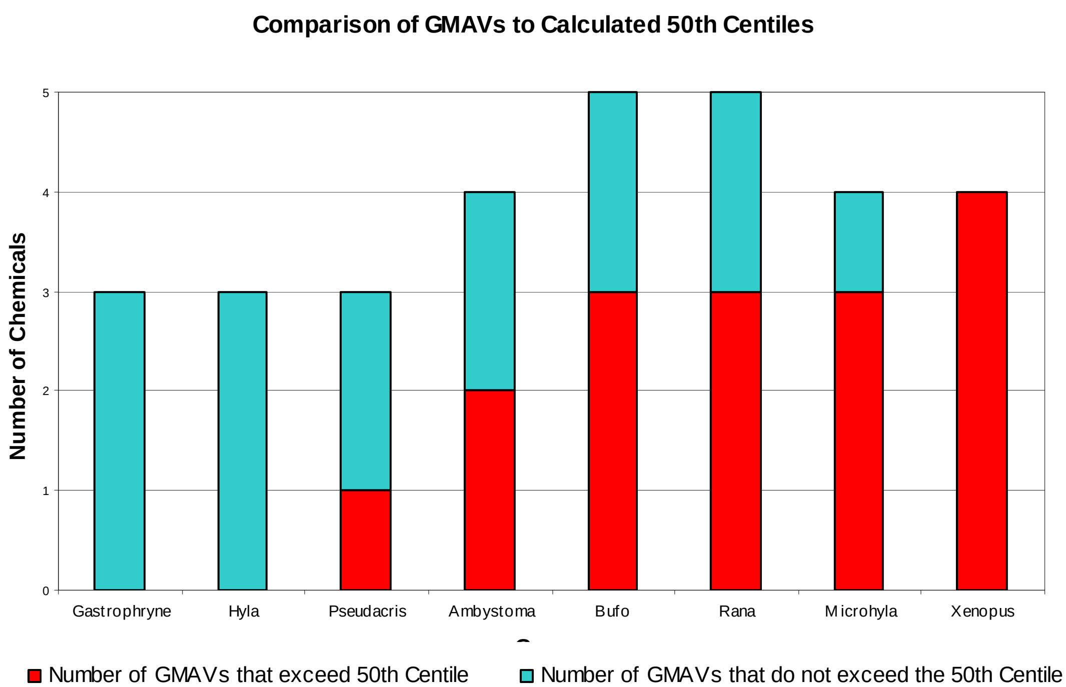


Species names are followed by number of studies and lifestages:
A = adult
M/F = male and female
J = juvenile
T = tadpole
L = larvae
E = embryo

Figure 13-6

Comparison of Chemical-Specific Genus Mean Acute Values to Calculated Centiles





SECTION 14
REFERENCES

Abramowicz, D.A. 1990. Aerobic and anaerobic degradation of PCBs: A review. Critical Reviews in Biotechnology 10:241-251.

Agency for Toxic Substances and Disease Registry (ATSDR). 1990. Toxicological Profile for Copper. U.S. Public Health Service. U.S. Department of Health and Human Services. Atlanta, Georgia.

Agency for Toxic Substances and Disease Registry (ATSDR). 1995. Toxicological Profile for Polycylic Aromatic Hydrocarbons (PAHs). U.S. Public Health Service. U.S. Department of Health and Human Services. Atlanta, Georgia.

Agency for Toxic Substances and Disease Registry (ATSDR). 1996. Toxicological Profile for Nickel. U.S. Public Health Service. U.S. Department of Health and Human Services. Atlanta, Georgia.

Agency for Toxic Substances and Disease Registry (ATSDR). 1997. Toxicological Profile for PCBs. U.S. Public Health Service. U.S. Department of Health and Human Services. Atlanta, Georgia.

Agency for Toxic Substances and Disease Registry (ATSDR). 1998a. Toxicological Profile for Lead. U.S. Public Health Service. U.S. Department of Health and Human Services. Atlanta, Georgia.

Agency for Toxic Substances and Disease Registry (ATSDR). 1998b. Toxicological Profile for Mercury. U.S. Public Health Service. U.S. Department of Health and Human Services. Atlanta, Georgia.

Agency for Toxic Substances and Disease Registry (ATSDR). 1999. Toxicological Profile for Chromium. U.S. Public Health Service, U.S. Department of Health and Human Services. Atlanta, Georgia.

Agency for Toxic Substances and Disease Registry (ATSDR). 2001. Toxicological Profile for DDT/DDD/DDE. U.S. Public Health Service. U.S. Department of Health and Human Services. Atlanta, Georgia.

Air Resources Board (ARB). 1986. Public Hearing to Consider the Adoption of a Regulatory Amendment Identifying Hexavalent Chromium as a Toxic Air Contaminant. Stationary Source Division. Sacramento, California.

Air Resources Board (ARB). 1991. Proposed Identification of Nickel as a Toxic Air Contaminant. Technical Support Document. Final Report. Stationary Source Division. Sacramento, California.

Air Resources Board (ARB). 1993. Proposed Airborne Toxic Control Measure for Emissions of Toxic Metals from Non-Ferrous Metal Melting. Stationary Source Division. Sacramento, California.

Air Resources Board (ARB). 1995a. Data extracted from the Statewide Summary of Ambient Toxic Metals. Technical Services Division. Sacramento, California.

Air Resources Board (ARB). 1995b. Personal communication from Jim Shikiya, Monitoring and Laboratory Division to Kitty Martin, Stationary Source Division. Data from the ARB Surveillance Vehicle Program. Sacramento, California.

Air Resources Board (ARB). 1997a. Data retrieved from ATEDS (Air Toxics Emission Data System). Run date: July 11, 1997. Technical Support Division, Special Pollutants Emission Inventory Section. Sacramento, California.

Air Resources Board (ARB). 1997b. Proposed Identification of Inorganic Lead as a Toxic Air Contaminant. Stationary Source Division. Sacramento, California.

Alloway, B.J. (ed.) 1990. Heavy Metals in Soils. John Wiley & Sons, New York.

Birge, W.J. and J.A. Black, 1980. Aquatic Toxicology of Nickel. *In:* Nickel in the Environment Ed. J.O. Nriagu. John Wiley and Sons, Inc. pp. 349-366

Birge, W.J., J.A. Black, A.G. Westerman, and J.E. Hudson, 1979. The effects of mercury on the reproduction of fish and amphibians. *In:* The biogeochemistry of mercury in the environment. Ed. J.O. Nriagu. Elsevier/North Holland Biomedical Press.

Department of Pesticide Regulation (DPR). 1996. Correspondence in November 1996 with the Environmental Monitoring and Pest Management Branch, Department of Pesticide Regulation, 1020 N St., Sacramento, California.

DiToro, D.M and, J.A. McGrath, 1999. Technical basis for narcosis chemicals and PAH criteria. I. Sediment and mixtures. Environ. Tox. Chem.

Eisler, R. 1985. Cadmium hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service, Biological Science Report 85(1.2).

Eisler, R. 1986a. Chromium hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service, Biological Science Report 85(1.6). 60pp.

Eisler, R. 1986b. Polychlorinated biphenyl hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR--1998-0002. Report # 7.

Eisler, R. 1987a. Mercury hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR--1998-0002. Report # 34.

Eisler, R. 1987b. Polyaromatic Hydrocarbons hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR--1998-0002. Report # 7.

Eisler, R. 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish Wildl. Serv. Biol. Rep. 85(1.14). 134 pp.

Eisler, R. 1993. Zinc hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR--1998-0002. Report # 34.

Eisler, R. 1997. Copper hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR--1998-0002. 98

Eisler, R. 1998. Nickel hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR--1998-0002. Report # 34.

Eisler, R. and A.A. Belisle. 1996. Planar PCB Hazards to Fish, Wildlife, and Invertebrates: a Synoptic Review. National Biological Service Biological Report 31, U.S. Department of the Interior. August.

Ferrari, L., A. Salibian and C. V. Muino. 1993. Selective protection of temperature against cadmium acute toxicity to *Bufo arenarum* tadpoles. Bull.Environ.Contam.Toxicol.. 50: 212-218.

Francis, P.C., W.J. Birge and J.A. Black. 1984. Effects of cadmium-enriched sediment on fish and amphibian embryo-larval stages. Ecotoxicol.Environ.Saf. 8: 378-387.

Ghate, H.V. and L. Mulherkar, 1980. Effect of Mercuric Chloride on Embryonic Development of the Frog *Microhyla ornata*. Indian Journal of Experimental Biology Vol. 18, pp. 1094-1096.

Gill, G.A. and K.W. Bruland, 1990. Mercury Speciation in Surface Freshwater Systems in California and other Areas. Environ. Sci. Technil., 24, 1392-1400.

Gilmour, C.C., E.A. Henry and R. Mitchell. 1992. Sulfate Stimulation of Mercury Methylation in Freshwater Sediments. Envir. Sci. Technol., 26, 2281-2287.

Gottschalk, J.A. 1995. Copper and Zinc Toxicity to the Gray tree frog *Hyla chysocelis*. MSc. Thesis Clemson University.

Grandjean, P. 1984. Biological Effects of Organolead Compounds. CRC Press, Boca Raton, Florida.

Grieb, T.M., C.T. Driscoll, S.P. Gloss, C.L. Shofield, G.L. Bowie and D.B. Porcella, 1990. Factors affecting mercury accumulation in fish in the upper Michigan peninsula. Envir. Toxicol. Chem., 9: 919-930.

Hall, B., N.S. Bloom, J. Munthe, 1995. An experimental study of tow potential methylation agents of mercury in the atmosphere: CH3I and DMS, Water, Air, and Soil Pollution, 80, 337-341, 1995.

Hatton, D. and W.F. Pickering. 1980. The effect of pH on the retention of copper, lead, zinc, and cadmium by clay-humic acid mixtures. Water Air Soil pollution. 14: 13-21.

Hazardous Substance Data Bank (HSDB). 1991. U.S. Department of Health and Human Services. National Toxicology Information Program. National Library of Medicine. Washington, D.C.

Hazardous Substance Data Bank (HSDB), online database, 1993. U.S. Department of Health and Human Services. National Toxicology Information Program. National Library of Medicine. Washington, D.C.

Hazardous Substance Data Bank (HSDB). 1995. U.S. Department of Health and Human Services. National Toxicology Information Program. National Library of Medicine. Bethesda, Maryland (CD ROM version), Micromedex, Denver, Colorado.

Howard, P.H. 1990. Handbook of Environmental Fate and Exposure for Organic Chemicals. Volumes 1-4. Lewis Publishers. Chelsea, Michigan.

Hunt, R. J. 1996. Do Created Wetlands Replace the Wetlands that are Destroyed? Fact Sheet. U.S. Geological Survey. Madison, WI. http://wi.water.usgs.gov/pubs/FS-246-96/

Irwin, R.J., M. VanMouwerik, L. Stevens, M.D. Seese, and W. Basham. 1997. Environmental Contaminants Encyclopedia. National Park Service, Water Resources Division, Fort Collins, Colorado.

Johnson, M.S., S.D. Holladay, K.S. Lippenholz, J.L. Jenkins, and W.C. McCain. 2000. Effects of 2,4,6-trinitrotoluene in a holistic environmental exposure regime to a terrestrial salamander: *Ambystoma tigrinum*. Toxicological Pathol. 28(2):334-341.

Khalid, R.A. 1980. Chemical Mobility of cadmium in sediment-water systems. Chapter 8 in "Cadmium in the Environment: Part I: Ecological Cycling" J.O. Nriagu (ed.), John Wiley & Sons, New York.

Khangarot, B.S. and P.K. Ray 1987 Sensitivity of toad tadpoles, *Bufo melaostictus* (Schneider), to Heavy Metals. Bull Environ. Contam. Toxicol. 38: 523-527.

Lefcort, H., R.A. Meguire, L.H. Wilson, and W.F. Ettinger, 1998. Heavy metals alter the survival, growth, metamorphosis, and Antipredatory Behavior of Columbia Spetted Frog (*Rana luteiventris)* tadpoles Arch.

Merck and Company, 1989. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals 11th edition. Ed. S. Budavari. Merck and Co., Rahway, N.J.

Miskimmin, B.M., J.W.M. Rudd, and C.A. Kelly, 1992. Influence of Dissolved Organic Carbon, pH, and Microbial Respiration Rates on Mercury Methylation and Demethylation in Lake Water. Can. J. Fish and Aquat. Sci. Vol. 49.

Mohn, W.W. and J.J. Tiedge. 1992. Mirobial reductive dehalogenation. Microbiological Reviews 56:482-507.

Mudgal, C.F and H.S. Patil, 1985. Sex related toxicity of mercury and cadmium to Frog *Rana cyanophlyctis.* Environment and Ecology, Vol. 3 No. 3

Muino, C.V., L. Ferrari and A. Salibian, 1990. Protective action of ions against cadmium toxicity to young *Bufo arenarum* tadpoles. Bull. Environ. Contam. Toxicol. 45:313-319.

National Toxicology Program (NTP). 1991. Sixth Annual Report on Carcinogens: 1991 Summary. U.S. Department of Health and Human Services. Research Triangle Park, North Carolina.

Osborn, D. A.S. Cooke, and S. Freestone. 1981. Histology of a teratogenic effect of DDT on Rana temporaria tadpoles. Environ. Pollut. Series A. 25: 305-319.

Pauli, B.D., J.A. Perrault, and S.L. Money, 2000. RATL: A Database of Reptile and Amphibian Toxicology Literature. National Wildlife Research Centre 2000, Canadian Wildlife Service, Environmental Conservation Branch. Technical Report Series Numer 357.

Perez-Coll, C.S. and J. Herkovits, 1990. Stage dependent susceptibility to lead in *Bufo arenarum* embryos. Environ. Pollut. 63:239-245

Rao, J.I. and M.N. Madhyastha, 1987. Toxicities of some heavy metals to the tadpoles of frog *Microhyla ornata* (Dumeril and Bibron). Toxicology Letters, 36 pp. 205-208.

Sanders, H.O.. 1970. Pesticide toxicities to tadpoles of the Western chorus frog *Pseudacris triseriata* and Fowler's toad *Bufo woodhousii fowleri*. Copeia.1970.2.246-251

Savage, W.K., F.W. Quimby, and A.P. DeCaprio. 2002. Lethal and sublethal effects of polychlorinated biphenyls on *Rana sylvatica* tadpoles. Envir. Tox. and Chem., Vol 21, No. 1, pp. 168-174.

Sax, N.I., Lewis, R.J., Sr. 1987. Hawley's Condensed Chemical Dictionary. 11th edition. Van Nostrand Reinhold. New York, New York.

Sloof, W. and R. Baerselman. 1980. Comparison of usefulness of the Mexican Axolotl (*Ambystoma mexicanum*) and the Clawed Toad (*Xenopus laevis*) in toxicological bioassays. Bull Environ. Contam. Toxicol. 24: 439-443

Solomon, K.R., J.M. Giddings, and S.J. Maund 2001. Probabilistic Risk Assessment of Cotton Pyrethroids: I. Distributional Analyses of Laboratory Aquatic Toxicity Data, Envir. Tox. and Chem., Vol 20, No. 3, pp. 652-659.

Sparling, D.W., 2000. Ecotoxicolgy of Organic Contaminants to Amphibians. *In:* Sparling, D.W., G. Linder and C.A. Bishop, editors. 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Sparling, D.W., G. Linder and C.A. Bishop editors, 2000. Ecotoxicology of Amphibians and Reptiles. Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC).

Spear, P.A., 1981. Zinc in the aquatic environment: chemistry, distribution, and toxicology. National Research Council of Canada Publication NRCC 17589. 145 pp.

St. Louis, V.L., J.W.M. Rudd, C.A. Kelly, K.G. Beaty, N.S. Bloom and R.J. Flett. 1994. Importance of wetlands as sources of methylmercury to Boreal Forest Ecosystems. Can. J. Fish and Aquat. Sci. Vol. 29; 1685-1690.

Stein, E.D., Y. Cohen, and A. M. Winer. 1996. Environmental Distribution and Transformation of Mercury Compounds. Critical Reviews in Environmental Science and Technology, 26(1):1-43.

Surma-Aho, K., J. Paasivirta, S. Rekolainen, and M. Verta, 1986. Organic and inorganic mercury in the food chain of some lakes and reservoirs in Finland. Chemosphere,15(3):353-372. 1986.

Swartz, R.C., D.W. Schults, R.J. Ozretich, J.O. Lamberson, F.A. Cole, T.H. DeWitt, M.S. Redmond, and S.P. Ferraro. 1995. Sum PAH: A model to predict the toxicity of field-collected marine sediment contaminated by polynuclear aromatic hydrocarbons. Environ. Toxicol. Chem. 14:1977-1987

Talmage, S.S., D.M. Opresko, C.J. Maxwell, C.J.E.Welsh, F.M. Cretella, P.H. Reno, and F.B. Daniel, 1999. Nitroaromatic Munition Compounds: Environmental Effects and Screening Values. Rev. Environ. Contam. Toxicol. 161:1-156.

U.S. Army, 1993. Explosives Information Briefing. A presentation by U.S. Army Environmental Center and U.S. Army Environmental Hygiene Agency. March 18, 1993.

USEPA, 1980a. Ambient Water Quality Criteria for Cadmium. Office of Water. EPA 440-5-80-025.

USEPA, 1980b. Ambient Water Quality Criteria for Polynuclear Aromatic Hydrocarbons. Office of Water. EPA 440-5-80-070. October 1980.

USEPA. 1980c. Ambient Water Quality Criteria for Nickel. Office of Water. EPA 440/5-80-060

USEPA. 1980d. Ambient Water Quality Criteria for Zinc. Office of Water. EPA 440/5-80-060

USEPA. 1980e. Ambient Water Quality Criteria for Chromium - 1980. Office of Water. EPA 440/5-80-035

USEPA. 1980f. Ambient Water Quality Criteria for Copper - 1980. Office of Water. EPA 440/5-80-036

USEPA. 1980g. Ambient Water Quality Criteria for Lead - 1980. Office of Water. EPA 440/5-84-057.

USEPA. 1980h. Ambient Water Quality Criteria for Polychlorinated Biphenyls - 1980. Office of Water. EPA 440/5-84-068.

USEPA. 1980i. Ambient Water Quality Criteria for DDT - 1980. Office of Water. EPA 440/5-84-038.

USEPA. 1985a. Ambient Water Quality Criteria for Cadmium - 1984. Office of Water. EPA 440/5-84-032.

USEPA. 1985b. Ambient Water Quality Criteria for Chromium - 1984. Office of Water. EPA 440/5-84-029 January 1985

USEPA. 1985c. Ambient Water Quality Criteria for Copper - 1984. Office of Water. EPA 440/5-84-031 January 1985

USEPA. 1985d. Ambient Water Quality Criteria for Lead - 1984. Office of Water. EPA 440/5-84-027 January 1985

USEPA. 1985e. Ambient Water Quality Criteria for Mercury - 1984. Office of Water. EPA 440/5-84-026 January 1985

USEPA. 1986a. Ambient Water Quality Criteria for Nickel - 1986. Office of Water. EPA 440/5-86-004 September 1986

USEPA. 1986b. Air quality criteria for lead. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. EPA 600/8-83-028F

USEPA 1990. National Guidance: Water Quality Standards for Wetlands. Prepared by: U.S. Environmental Protection Agency Office of Water Regulations and Standards Office of Wetlands Protection. July, 1990. http://www.epa.gov/owow/wetlands/regs/quality.html

USEPA, 1994. Review Draft of the Health Effects Notebook for Hazardous Air Pollutants. Air Risk Information Support Center (Air RISC), Research Triangle Park, North Carolina. Contract No. 68-D2-0065.

USEPA. 1996. 1995 Updates: Water Quality Criteria Documents for the Protection of Aquatic Life in Ambient Water. Office of Water. EPA 820-B-96-001. September 1996.

USEPA, 1999a. National Recommended Water Quality Criteria – Correction. Office of Water. EPA 822-2-99-001. April 1999.

USEPA, 1999b. Water Quality Standards; Establishment of Numeric Criteria for Priority Toxic Pollutants; States' Compliance--Revision of Polychlorinated Biphenyls (PCBs) Criteria; Final Rule Federal Register: November 9, 1999 (Volume 64, Number 216)]

USEPA, 2000. Draft Equilibrium Partitioning Sediment Guidelines (ESGs) for the Protection of Benthic Organisms: Metal Mixtures. USEPA, Office of Science and Technology and Office of Research and Development.

USEPA. 2001a. Water Quality Criteria: Notice of Availability of Water Quality Criterion for the Protection of Human Health: Methylmercury. Federal Register January 8, 2001 (Volume 66, Number 5).

USEPA 2001b. 2001 Update of Ambient Water Quality Criteria for Cadmium. Office of Water. EPA 822-R-01-001. April, 2001.

USEPA, 2002. National Recommended Water Quality Criteria: 2002. EPA-822-R-02-047. November 2002.

ATTACHMENT B-1

CALCULATION OF LINEAR REGRESSION

LINEAR REGRESSION
SUMMARY OUTPUT - CADMIUM

Regression Statistics	
Multiple R	0.97222
R Square	0.94520
Adjusted R Square	0.93912
Standard Error	6.81978
Observations	11

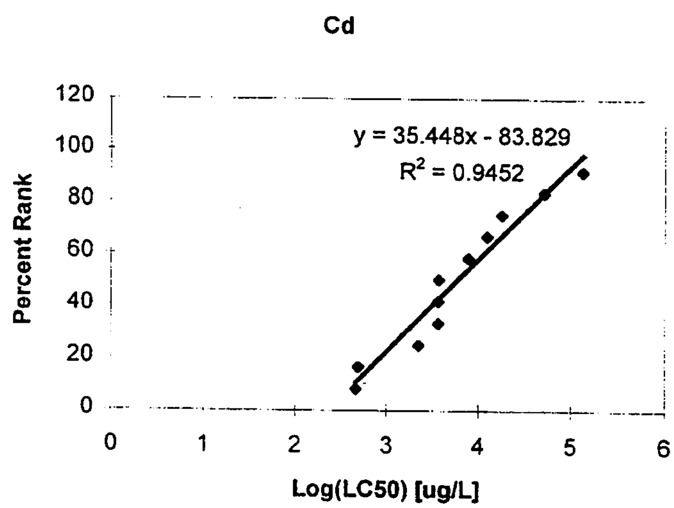
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	7220.30452	7220.30452	155.24407	0.00000
Residual	9	418.58437	46.50937		
Total	10	7638.888889			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*pper 95.0%*
Intercept	-83.82885	10.93599	-7.66541	0.00003	-108.56780	-59.08990	-108.56780	-59.08990
X Variable 1	35.44786	2.84500	12.45970	0.00000	29.01201	41.88370	29.01201	41.88370

RESIDUAL OUTPUT

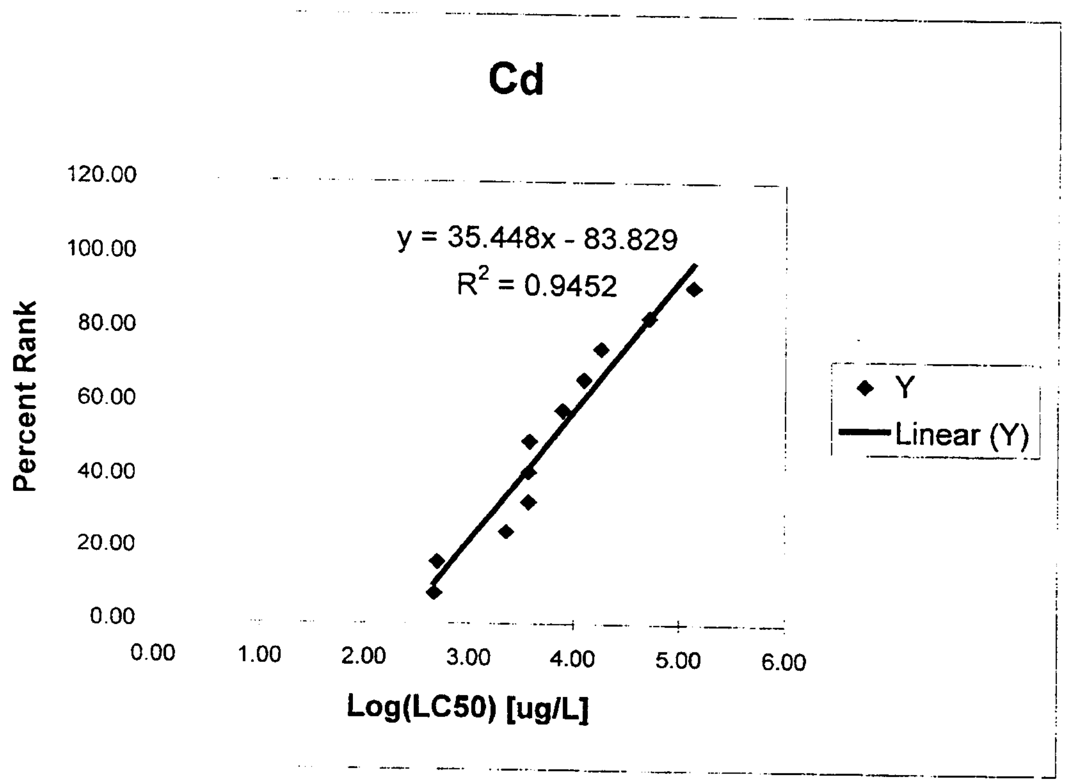
Observation	*Predicted Y*	*Residuals*
1	10.82564	-2.49231
2	11.85431	4.81236
3	35.14606	-10.14606
4	42.65625	-9.32292
5	42.65625	-0.98958
6	42.92379	7.07621
7	54.20163	4.13170
8	61.33661	5.33005
9	67.06993	7.93007
10	83.34336	-0.01002
11	97.98617	-6.31950



Compound	Genus	Species	Conc (ug/L)	Rank	Log Conc	Percentile
Cadmium	Ambystoma	gracile	468	1	2.67	8.33
Cadmium	Ambystoma	mexicanum	500	2	2.70	16.67
Cadmium	Microhyla	ornata	2272	3	3.36	25.00
Cadmium	Rana	catesbeiana	3700	4	3.57	33.33
Cadmium	Rana	pipiens	3700	5	3.57	41.67
Cadmium	Bufo	arenarum	3765	6	3.58	50.00
Cadmium	Xenopus	laevis	7833	7	3.89	58.33
Cadmium	Bufo	melanostictus	12450	8	4.10	66.67
Cadmium	Rana	luteiventris	18069	9	4.26	75.00
Cadmium	Rana	clamitans	52000	10	4.72	83.33
Cadmium	Rana	cyanophlyctis	134612	11	5.13	91.67

y = 35.448x - 83.829

	10th	50th
log	2.65	3.78
ug/L	444	5962



LINEAR REGRESSION
SUMMARY OUTPUT - COPPER

Regression Statistics	
Multiple R	0.94413
R Square	0.89139
Adjusted R Square	0.88415
Standard Error	9.54877
Observations	17

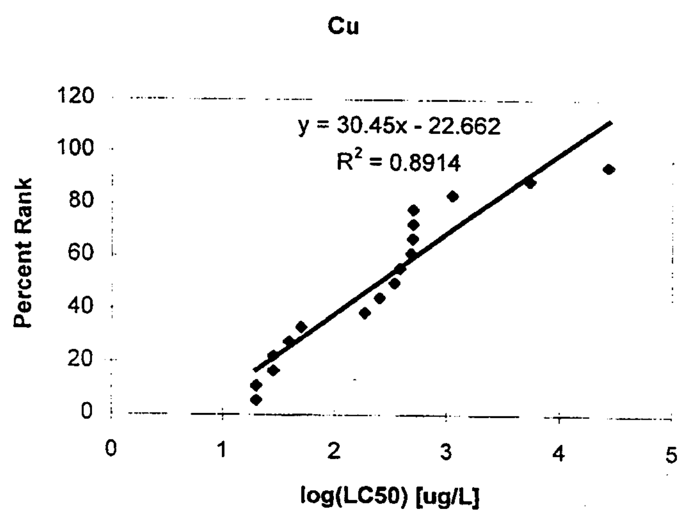
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	11224.90692	11224.90692	123.10841	0.00000
Residua:	15	1367.68567	91.17904		
Total	16	12592.59259			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*pper 95.0%*
Intercept	-22.66193	6.94626	-3.26247	0.00525	-37.46755	-7.85632	-37.46755	-7.85632
X Variable 1	30.45012	2.74439	11.09542	0.00000	24.60060	36.29964	24.60060	36.29964

RESIDUAL OUTPUT

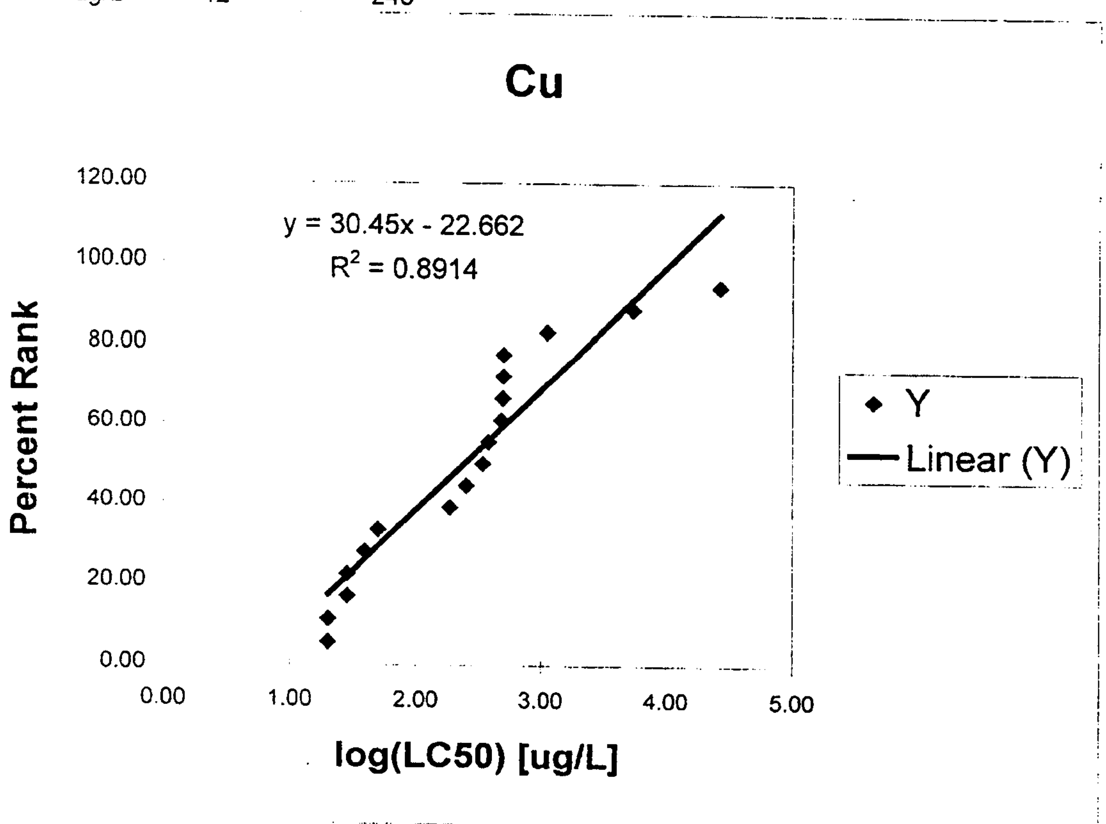
Observation	*Predicted Y*	*Residuals*
1	16.95459	-11.39903
2	16.95459	-5.84348
3	21.53779	-4.87112
4	21.53779	0.68444
5	25.78618	1.99160
6	29.07191	4.26143
7	46.39042	-7.50153
8	50.35563	-5.91119
9	54.47602	-4.47602
10	55.89279	-0.33723
11	58.98219	2.12892
12	59.35356	7.31310
13	59.52203	12.70019
14	59.57806	18.19972
15	70.19081	13.14252
16	91.15183	-2.26294
17	112.26383	-17.81938



Compound	Genus	Species	Conc (ug/L)	Rank	Log Conc	Percentile
Copper	Rana	catesbeiana	20	1	1.30	5.56
Copper	Rana	palustris	20	2	1.30	11.11
Copper	Gastrophryne	carolinensis	28	3	1.45	16.67
Copper	Hyla	chrysoscelis	28	4	1.45	22.22
Copper	Rana	hexadactyla	39	5	1.59	27.78
Copper	Pseudacris	crucifer	50	6	1.70	33.33
Copper	Rana	pipiens	185	7	2.27	38.89
Copper	Ambystoma	barbouri	250	8	2.40	44.44
Copper	Ambystoma	jeffersonianum	341	9	2.53	50.00
Copper	Ambystoma	texanum	380	10	2.58	55.56
Copper	Ambystoma	maculatum	480	11	2.68	61.11
Copper	Bufo	melanostictus	494	12	2.69	66.67
Copper	Ambystoma	t. tigrinum	500	13	2.70	72.22
Copper	Xenopus	laevis	502	14	2.70	77.78
Copper	Ambystoma	opacum	1120	15	3.05	83.33
Copper	Microhyla	ornata	5467	16	3.74	88.89
Copper	Bufo	fowleri	26980	17	4.43	94.44

y = 30.45x - 22.662

	10th	50th
log	1.07	2.39
ug/L	12	243



LINEAR REGRESSION
SUMMARY OUTPUT - MERCURY

Regression Statistics	
Multiple R	0.97002
R Square	0.94093
Adjusted R Square	0.93837
Standard Error	7.02752
Observations	25

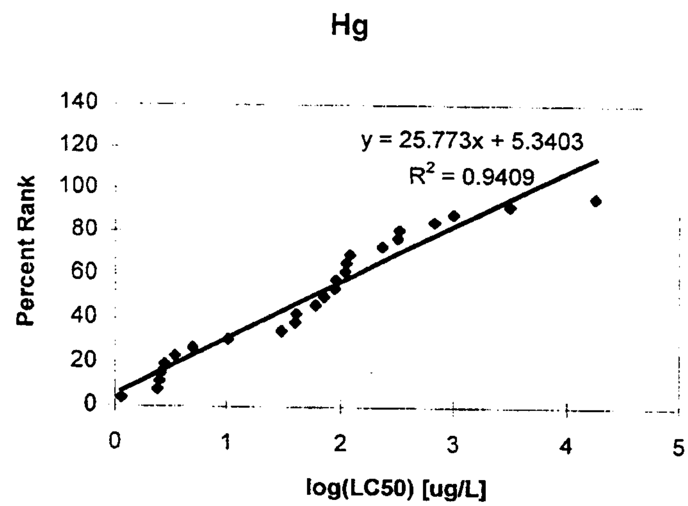
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	18094.88998	18094.88998	366.39675	0.00000
Residual	23	1135.87925	49.38605		
Total	24	19230.76923			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*pper 95.0%*
Intercept	5.34026	2.72378	1.96061	0.06215	-0.29430	10.97482	-0.29430	10.97482
X Variable 1	25.77329	1.34646	19.14149	0.00000	22.98792	28.55865	22.98792	28.55865

RESIDUAL OUTPUT

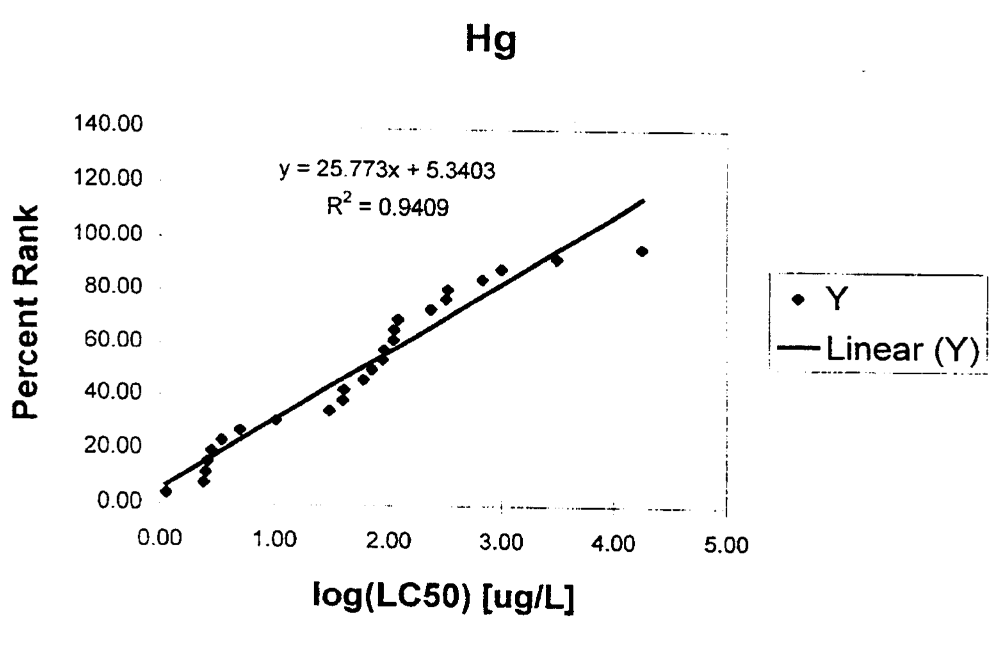
Observation	*Predicted Y*	*Residuals*
1	6.80860	-2.96245
2	15.13955	-7.44724
3	15.59648	-4.05802
4	16.03548	-0.65087
5	16.86499	2.36578
6	19.24728	3.82964
7	23.35501	3.56806
8	31.55255	-0.78332
9	43.53354	-8.91815
10	46.63061	-8.16907
11	46.79434	-4.48665
12	51.19923	-5.04539
13	53.05216	-3.05216
14	55.47778	-1.63163
15	55.76339	1.92892
16	57.82499	3.71347
17	58.09974	7.28487
18	58.92759	10.30318
19	66.37683	6.70010
20	69.97141	6.95167
21	70.32763	10.44161
22	78.30365	6.31174
23	82.66012	5.80142
24	95.46525	-3.15756
25	114.99178	-18.83793



Compound	Genus	Species	Conc (ug/L)	Rank	Log Conc	Percentile
Mercury	Gastrophryne	carolinensis	1.1402	1	0.06	3.85
Mercury	Hyla	chrysoscelis	2.4000	2	0.38	7.69
Mercury	Hyla	gratiosa	2.5000	3	0.40	11.54
Mercury	Hyla	versicolor	2.6000	4	0.41	15.38
Mercury	Pseudacris	crucifer	2.8000	5	0.45	19.23
Mercury	Hyla	squirella	3.4641	6	0.54	23.08
Mercury	Hyla	chysocephala	5.0000	7	0.70	26.92
Mercury	Acris	crepitans	10.4000	8	1.02	30.77
Mercury	Bufo	punctatus	30.3315	9	1.48	34.62
Mercury	Bufo	debilis debilis	40.0000	10	1.60	38.46
Mercury	Bufo	fowleri	40.5894	11	1.61	42.31
Mercury	Bufo	melanostictus	60.1620	12	1.78	46.15
Mercury	Rana	grylio	70.9930	13	1.85	50.00
Mercury	Rana	pipiens	88.1717	14	1.95	53.85
Mercury	Xenopus	laevis	90.4504	15	1.96	57.69
Mercury	Ambystoma	opacum	108.7428	16	2.04	61.54
Mercury	Rana	breviceps	111.4451	17	2.05	65.38
Mercury	Bufo	japonicus	120.0000	18	2.08	69.23
Mercury	Rana	hexadactyla	233.4589	19	2.37	73.08
Mercury	Ambystoma	mexicanum	321.8695	20	2.51	76.92
Mercury	Rana	heckscheri	332.2775	21	2.52	80.77
Mercury	Microhyla	ornata	677.5938	22	2.83	84.62
Mercury	Rana	catesbeiana	1000.0000	23	3.00	88.46
Mercury	Rana	cyanophlyctis	3139.3337	24	3.50	92.31
Mercury	Rana	tigrina	17966.5188	25	4.25	96.15

y = 25.773x + 5.3403

	10th	50th
log	0.18	1.73
ug/L	1.52	54



LINEAR REGRESSION
SUMMARY OUTPUT - ZINC

Regression Statistics	
Multiple R	0.89678
R Square	0.80422
Adjustec R Square	0.78246
Standard Error	12.89084
Observations	11

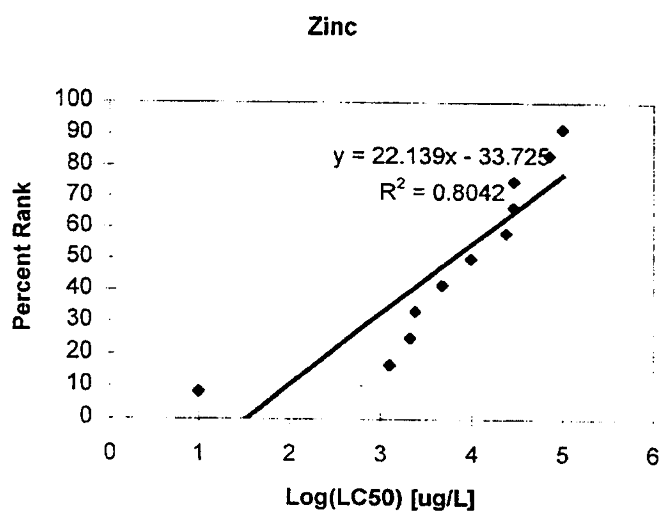
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	6143.32600	6143.32600	36.96931	0.00018
Residua	9	1495.56289	166.17365		
Total	10	7638.888889			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*pper 95.0%*
Intercept	-33.72457	14.30797	-2.35705	0.04281	-66.09148	-1.35766	-66.09148	-1.35766
X Variable 1	22.13904	3.64115	6.08024	0.00018	13.90219	30.37589	13.90219	30.37589

RESIDUAL OUTPUT

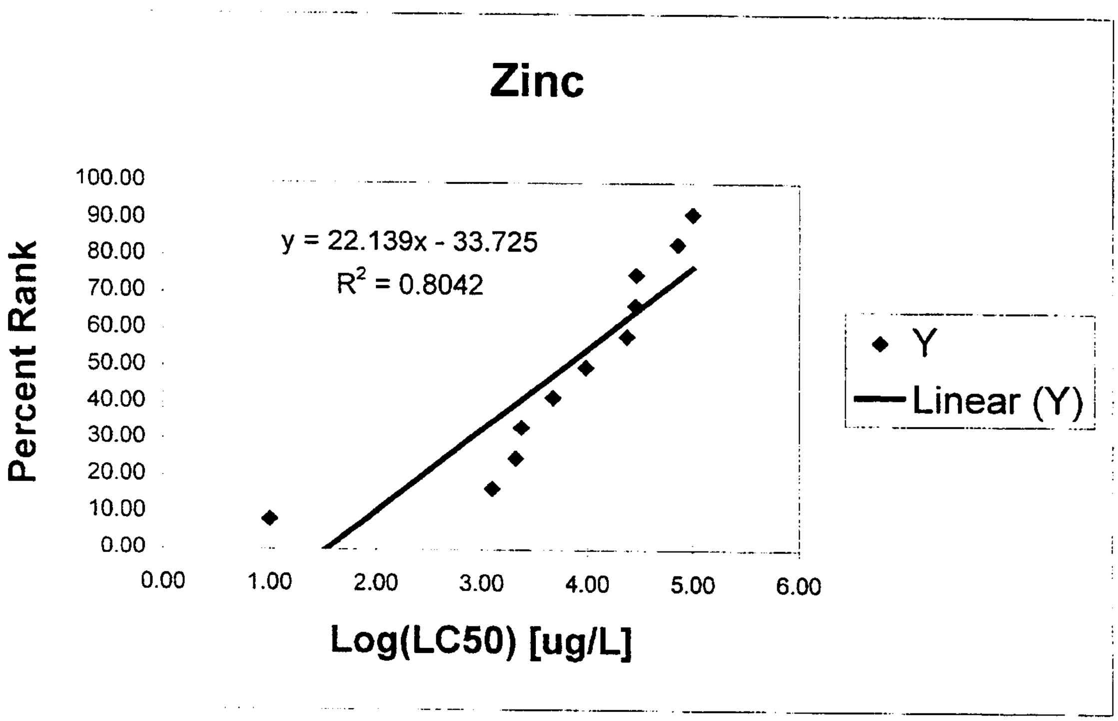
Observation	*Predicted Y*	*Residuals*
1	-11.58553	19.91886
2	34.89462	-18.22795
3	39.82617	-14.82617
4	41.02959	-7.69626
5	47.57214	-5.90547
6	54.49759	-4.49759
7	63.10900	-4.77567
8	64.86083	1.80583
9	65.02705	9.97295
10	73.79472	9.53862
11	76.97382	14.69285



Compound	Genus	Species	Conc (ug/L)	Rank	Log Conc	Percentile
Zinc	Gastrophryne	carolinensis	10	1	1.00	8.33
Zinc	Rana	pipiens	1257	2	3.10	16.67
Zinc	Rana	hexadactyla	2100	3	3.32	25.00
Zinc	Ambystoma	opacum	2380	4	3.38	33.33
Zinc	Hyla	chrysoscelis	4700	5	3.67	41.67
Zinc	Xenopus	laevis	9659	6	3.98	50.00
Zinc	Microhyla	ornata	23653	7	4.37	58.33
Zinc	Rana	luteiventris	28380	8	4.45	66.67
Zinc	Bufo	melanostictus	28875	9	4.46	75.00
Zinc	Rana	limnocharis	71870	10	4.86	83.33
Zinc	Rana	catesbeiana	100033	11	5.00	91.67

y = 22.139x - 33.725

	10th	50th
log	1.98	3.78
ug/L	94	6050



LINEAR REGRESSION
SUMMARY OUTPUT - DDT

Regression Statistics	
Multiple R	0.94488
R Square	0.89281
Adjusted R Square	0.87137
Standard Error	9.68469
Observations	7

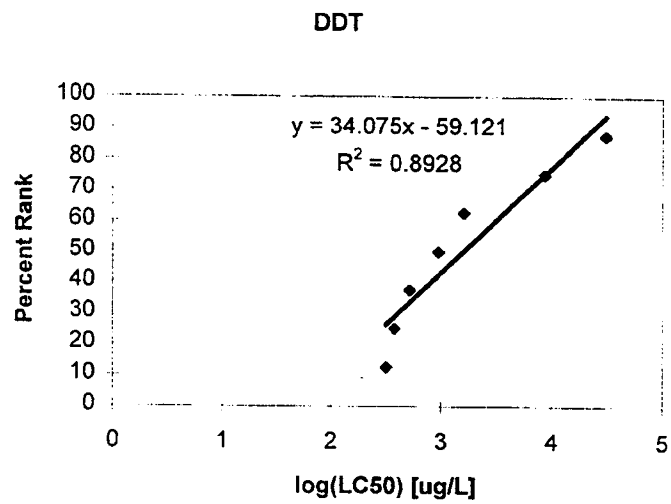
ANOVA

	df	SS	MS	F	Significance F
Regression	1	3906.03351	3906.03351	41.64512	0.00133
Residual	5	468.96649	93.79330		
Total	6	4375			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	pper 95.0%
Intercept	-59.12116	17.30102	-3.41721	0.01890	-103.59476	-14.64756	-103.59476	-14.64756
X Variable 1	34.07546	5.28031	6.45330	0.00133	20.50200	47.64891	20.50200	47.64891

RESIDUAL OUTPUT

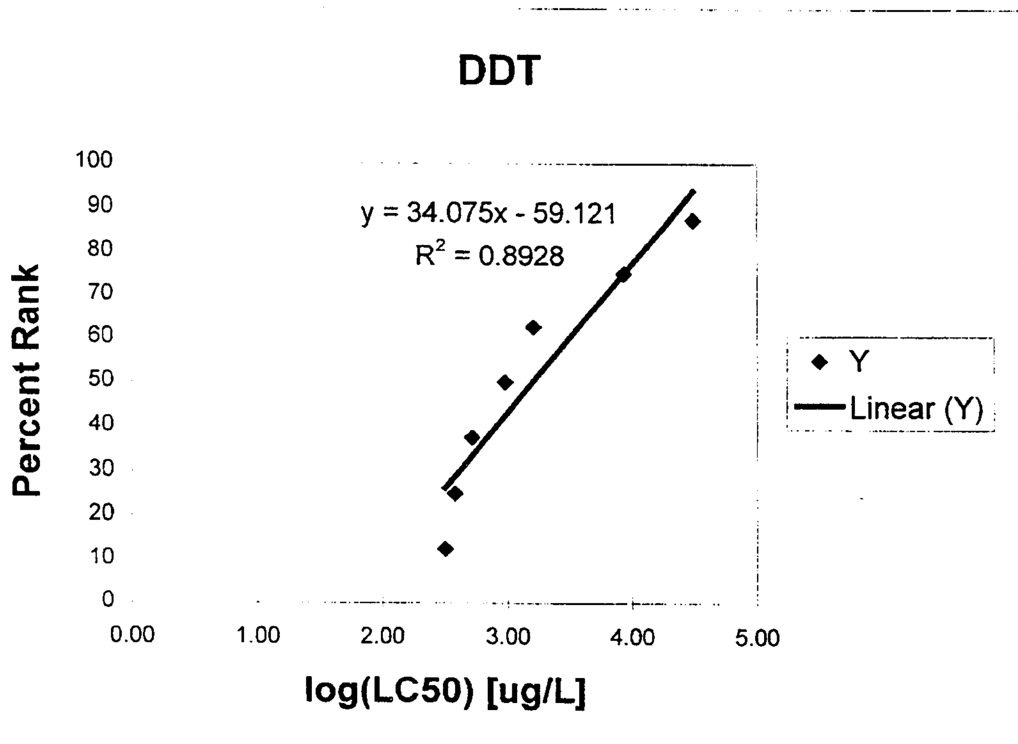
Observation	Predicted Y	Residuals
1	26.21401	-13.71401
2	28.78614	-3.78614
3	33.40651	4.09349
4	42.38990	7.61010
5	50.22741	12.27259
6	75.05199	-0.05199
7	93.92404	-6.42404



Compound	Genus	Species	Conc (ug/L)	Rank	Log Conc	Percentile
DDT	Bufo	woodhousei fowleri	319	1	2.50	12.5
DDT	Rana	limnocharis	380	2	2.58	25
DDT	Bufo	boreas	519	3	2.72	37.5
DDT	Bufo	woodhousii	953	4	2.98	50
DDT	Pseudacris	triserata	1618	5	3.21	62.5
DDT	Bufo	arenarum	8660	6	3.94	75
DDT	Bufo	juxtasper	31000	7	4.49	87.5

y = 34.075x - 59.121

	Percentiles	
	10th	50th
log	2.03	3.20
ug/L	106.78	1594



APPENDIX C

SOP DEVELOPMENT

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SECTION 1
INTRODUCTION

This appendix describes the development of a laboratory toxicity testing procedure to evaluate the potential effects of sediment/ hydric soil exposure to early life stage amphibians. This test development is part of an overall evaluation of the use of amphibian testing as a risk assessment tool at sites owned and/or operated by the United States Navy.

1.1 Project Scope

This appendix describes the first two experimental phases of the project, which are 1) Test Development and 2) Test Refinement. The goal of these experimental phases was to collect data necessary for the completion of a Standard Operating Procedure (SOP) for conducting sediment toxicity tests with amphibians. To achieve this goal, several factors were investigated, including:

- Organism handling and maintenance, including:
    - Holding conditions
    - Water type
    - Food
    - Temperature
- Acceptable control sediment
- Tolerance limits for ammonia
- Effects of various toxicants on tadpoles
- Most sensitive sublethal endpoint
- Most sensitive organism age
- Appropriate test length

These factors were investigated using two different anuran taxa in a series of studies conducted over several months.

1.2 Appendix Organization

This appendix is organized in the following manner:

- Section 2 describes the factors evaluated during method development;
- Section 3 presents a discussion of the laboratory test results;
- Section 4 includes a summary and conclusions;
- Section 5 includes a list of references cited in this report; and
- Attachment C-1 presents the SOP developed to evaluate the sediment toxicity with early life stage amphibians.

SECTION 2
METHOD DEVELOPMENT

In order to develop the SOP for conducting sediment toxicity tests with amphibians, a number of factors were evaluated. This section describes the series of studies conducted over several months to evaluate these factors and develop the SOP.

2.1 Test Organisms

Many of the standard test organisms used to conduct freshwater and marine toxicity testing are readily available year-round. Culturing methods have been developed and perfected for animals such as fathead minnows (*Pimephales promelas*), water fleas (*Ceriodaphnia dubia*, *Daphnia pulex*, *Daphnia magna*), mysid shrimp (*Americamysis bahia*), and others. Methods for breeding a variety of amphibians are far less established. The most commonly tested amphibian species is probably the African clawed frog (*Xenopus laevis)*, which is generally available year-round and can be bred and easily raised in a laboratory setting. Other amphibians are also often available from commercial suppliers, including the bullfrog (*Rana catesbeiana*), green tree frog (*Hyla cinerea*), dwarf clawed frog (*Hymenochirus spp.*), giant toad (*Bufo marinus*), and mud puppy (*Necturus maculosus*). However, these are generally available only as adult organisms and may not be native to North America.

The taxa used in the studies – the Northern Leopard Frog (*Rana pipiens)* and the American Toad (*Bufo americanus)* – were obtained from a commercial supplier or wild-caught. *Rana pipiens* was selected as a test species because it is native to North American and found in wetlands in many areas of the country, and its eggs are commercially available for several months of the year. Small *Bufo* species are also relatively ubiquitous and can be easily obtained from the wild. Seasonal availability of test organisms may limit the application of this test method and suppliers should be investigated as early as possible.

For all the tests conducted during this study, organisms were received as eggs. In some cases the eggs were very near hatching when received, while other eggs were held for two to three days before hatching. Most eggs were obtained from Carolina Biological Supply Company (Burlington, NC). Eggs were also obtained from Nasco (Fort Atkinson, WI), and field collected in southeastern Massachusetts.

From November through approximately early March, *Rana pipiens* are induced to lay eggs in the commercial laboratories. After March, laboratory-produced eggs become scarce and wild-collected eggs are available. Many of the tests conducted during this study used animals hatched from wild-collected eggs. The use of wild-collected organisms adds genetic variability to the pool of test animals, and therefore may also result in greater response variation. However, there are still many test species, particularly marine organisms (e.g., *Rhepoxynius abronius*) that are not generally bred in the laboratory and are collected in the wild for each test.

Tests were conducted during two time periods: 1) May – June 2001 (Phase I Test Development) and 2) December 2001 – February 2002 (Phase II Test Refinement). A single batch of *Rana* sp. was received from Carolina Biological Supply at the ENSR Fort Collins Environmental Toxicology Laboratory (FCETL) on May 11, 2001. Although *R. pipiens* was requested, the exact species of *Rana* cannot be stated with certainty since these were wild-collected organisms. The eggs hatched on May 12, 2001 (Figure 2-1). On June 1, 2001 a batch of *Bufo americanus* eggs were received, having been collected in the wild by ENSR personnel from the Westford, MA office. Those eggs hatched on June 3, 2001. The two species were kept in separate aquaria in a water bath at 23°C until use. Feeding began when the tadpoles reached stage 25. Tadpoles were initially fed a combination of foods (see Section 2.3) during the holding period (TetraMin, TetraMin:trout chow mix, frog chow). After the food preference study, frog chow was not fed to the tadpoles being held because of the poor growth response observed.

Figure 2-1 Rana sp. tadpoles just prior to hatching.

The organisms used for tests during the Test Refinement stage were obtained from Carolina Biological Supply and from Nasco. All of the eggs received for these studies were obtained through artificial fertilization of *Rana pipiens* eggs in the laboratory. Therefore, the organisms used in these tests are considered to be *Rana pipiens*. Eggs from Carolina Biological Supply were received in plastic bags injected with oxygen. The first batches were opened and eggs were immediately transferred to an aquarium. This resulted in very high egg mortality. Subsequent batches were left in the bags in a temperature-controlled water bath (23°C) until they began to hatch; the embryos were then transferred to an aquarium with Horsetooth Reservoir water. Hatch rate using this method was generally high (>70%). A single batch of eggs was obtained from Nasco on December 13, 2001. Those eggs were in a small bag with no liquid and were immediately transferred to a shallow dish containing water from Horsetooth Reservoir. Less than 50% of those eggs hatched. All of the batches received are listed in Table 2-1.

2.1.1 Tadpole Development

Gosner (1960) developed a table for staging of anuran embryos, particularly *Rana pipiens.* The classification includes 46 stages from fertilized egg to air-breathing adult. The first 25 nonfeeding stages are based upon a scheme developed by Shumway (1940). From stage 25 until adulthood, stage is generally identified by limb bud development and, in later stages, reabsorption of the tail and mouth size

Eggs of both *Rana* and *Bufo* hatch about two weeks after fertilization. Upon hatching, tadpoles have external gill filaments on either side of their body. However, these are quickly covered by the operculum. By stage 25, evidence of the external gills is gone and organisms are ready to begin feeding (Shumway, 1940; Gosner, 1960). Tadpoles are omnivores, feeding on algae, plants, and dead organisms, including other tadpoles. At hatch, tadpoles are at stage 20 and achieve stage 25 within a couple days. Tadpoles complete the metamorphosis to adults in 10 to 13 weeks, but this is somewhat dependent upon temperature and availability of food. For more information on *Rana* and *Bufo* development and ecology, see the Standard Operating Procedure for conducting sediment tests (Attachment C-1).

2.2 Control Sediment Preference and Test System

Like all scientific studies, toxicity investigations must include a negative control, that is, a control where the organisms should not show an adverse response. For sediment tests, that means a sediment in which the organisms will survive and grow normally. There is no standard control material for sediment tests. Laboratories around the country use different materials that have been shown to be effective negative controls. At the ENSR toxicology laboratory, two types of control sediment have been used for tests with the amphipod, *Hyalella azteca*, and the dipteran midge, *Chironomus tentans*. One is a natural sediment collected from the Cache la Poudre River in the foothills of the Rocky Mountains northeast of Fort Collins. The other is a formulated sediment prepared at the ENSR laboratory.

Before use, the sediment from the Cache la Poudre River (Poudre sediment) was rinsed with filtered lake water (from Horsetooth Reservoir) until the rinsate ran clean; it was then dried at 100 ± 2°C. The formulated sediment was prepared according to Walsh et al. 1992, as shown in Table 2-2.

Evaluation of the suitability of the control sediment was tested in conjunction with a study of the appropriate test system. Many laboratories conduct sediment toxicity tests with the amphipod, *Hyalella azteca*, and the midge, *Chironomus tentans*, using a static-renewal system, where water is replaced twice a day using a "renewal box." Overlying water in sediment tests is seldom siphoned off (such as in a water column test) because of the resulting disturbance and potential loss of sediment. The potential problems with using a static-renewal system for the amphibian studies include depressed dissolved oxygen levels and higher ammonia concentrations because of the larger size (relative to *Hyalella* and *Chironomus*) and rapid metabolism and growth of the test organism. Therefore, both static-renewal and continuous flow-through systems were studied."

Each type of control sediment (formulated and Poudre) was placed in three flow-through, 5-liter test chambers. An additional three chambers contained only water, for a total of nine flow-through chambers. Water from Horsetooth Reservoir was fed, via gravity, into each flow-through chamber (Figure 2-2).

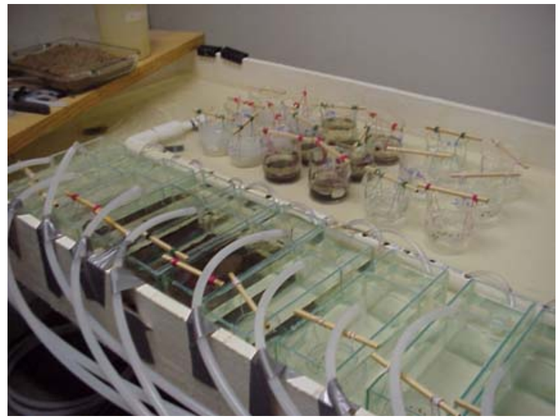


Figure 2-2 Flow-through chambers (bottom) and static-renewal chambers (top) used during test development

Each type of control sediment was also placed in 500 ml beakers for a static-renewal test (Figure 2-2). In this type of test, there is not a continuous flow of water into and out of the chamber, but fresh water is added daily. Exiting water flows out of the beaker through a hole in the side of the chamber which is covered by a fine-mesh Nitex screen. For each type of control sediment, three beakers were prepared with Horsetooth Reservoir water and three were prepared with moderately hard (Mod Hard) laboratory water. Mod Hard is a reconstituted water prepared by adding certain salts to pure (deionized) water (USEPA, 1993). Therefore, there were a total of 12 static chambers with sediment:

- Formulated sediment and Mod Hard water
- Formulated sediment and Horsetooth Reservoir water
- Poudre sediment and Mod Hard water
- Poudre sediment and Horsetooth Reservoir water

In addition, three water-only chambers were prepared with Mod Hard and three with Horsetooth Reservoir water.

Ten *Rana pipiens* eggs were placed in baskets suspended in each chamber (Figure 2-3). The eggs were only 24 hours away from hatch. Two days after hatch (approximately three days after test initiation), the tadpoles were removed from the baskets so they could have direct exposure to the sediment and feeding was initiated. Each of the three replicates containing a sediment and water (or water-only) treatment was fed a different food as described in Section 2.3.

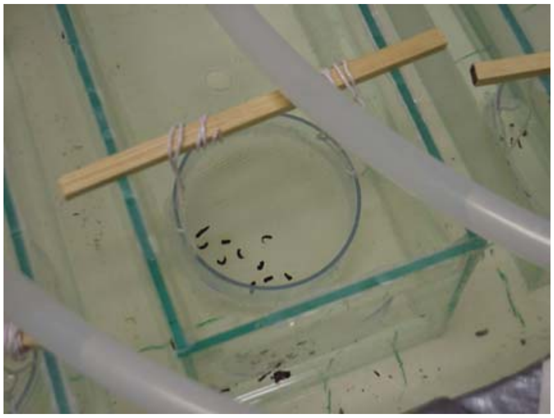


Figure 2-3 Rana tadpoles in baskets in test chambers

2.3 Food Preference

Different researchers use different foods in amphibian tests. Possible foods considered for this study included boiled spinach, boiled lettuce, fish flakes, TetraMin®, Yeast/Trout Chow/Cereal flakes (YTC), or a combination of these. Three foods were tested to determine which resulted in better tadpole growth: TetraMin®, a TetraMin®:YTC Mix, and frog chow (from Carolina Biological Supply). These foods were selected primarily because of their availability and/or existing use in laboratories. TetraMin®, for example, is commonly used to feed midges (*Chironomus tentans*) during sediment tests. YTC is prepared to feed (along with algae) cladocerans and the amphipod, *Hyalella azteca*, during sediment tests. These food combinations were fed to groups of *R. pipiens* exposed to different sediment and water exposures, as described in Section 2.2. Test chamber A in all exposure groups received frog chow (FC); test chamber B received TetraMin®, and test chamber C received a 50:50 mix of TetraMin® and YTC.

Chambers were fed daily. The amount of food placed in each test chamber was reduced from an initial amount of 1/16 of a teaspoon to 1/32 of a teaspoon (approximately 90 mg), and finally to about ½ this amount (45 mg). This reduction was based on excess food observed in test chambers and concerns about dissolved oxygen and ammonia levels. Tadpoles were observed eating the food either off the bottom or, more commonly, by turning over and eating upside-down from the surface.

2.4 Ammonia Tolerance

Aquatic toxicity tests, including those with sediment, are generally conducted under either static-renewal or flow-through conditions. In static-renewal tests the overlying water is replaced once or twice daily but no additional water is added over the 24-hour period. In flow-through tests there is a continuous stream or drip of water into the test chambers. Excess water drains from the chambers via some mechanism. As described in Section 2.1, both systems were evaluated for the amphibian sediment test.

One of the problems with conducting static-renewal in sediment tests with amphibians is that they are much larger than any other organisms used in sediment tests (*H. azteca* or *C. tentans*), they grow rapidly, and process large amounts of food quickly. The associated wastes often result cause ammonia levels to increase rapidly. In tests with *Rana* using sediments collected from a historical mining site in California, ammonia levels in static tests were measured as high as 10.3 mg/L. In flow-through tests, ammonia levels remained at less than 1.0 mg/L. There was mortality of test organisms in the test chambers that had measured ammonia levels of approximately 10 mg/L. Although it could not be shown conclusively that the observed mortality was due to ammonia, concentrations at this level can cause toxicity to fish. From this example it is clear that the use of static test conditions may be detrimental to the outcome of the assay and may indicate toxicity that is not necessarily related to the sediment exposure itself.

To determine their tolerance of ammonia, *Rana* tadpoles were exposed to nominal ammonia concentrations ranging from about 2 mg/L to 50 mg/L. The ammonia solutions were prepared by adding reagent-grade ammonium chloride to moderately hard laboratory water. Test duration was seven days. Actual ammonia concentrations were measured in each treatment on days 0, 1, 4, 6, and 7 using an ammonia-specific probe and an Orion 720A meter. Survival was documented daily. At the end of the test, body width, distance between eyes, total length, body length, dry weight, and metamorphic stage were determined. Statistically significant differences were determined using Toxstat Version 3.5 (WEST and Gulley 1996).

2.5 Determination of Appropriate Sublethal Endpoints

Even though an effluent or test material may not cause acute toxicity (death) to a test organism, the organism can be affected in other, sublethal, ways that may impair its growth or otherwise affect the ability of the population to successfully survive and/or reproduce in the environment. For example, in short-term chronic toxicity tests with fish growth (weight) is used as the sublethal endpoint, while reproduction is monitored in assays using the parthenogenic cladoceran, *Ceriodaphnia dubia*. Researchers have monitored numerous sublethal factors in amphibians, including biochemistry of body fluids. Measurement of some biochemical markers is time-consuming and expensive. Since the purpose of these studies was to develop a test that could be conducted by most laboratories at a reasonable cost, sublethal metrics were restricted to those that could be quantified without too much difficulty. For these studies, the following sublethal measurements were made:

- Body width;
- Total length;
- Body length;
- Metamorphic stage; and
- Dry weight.

The distance between eyes was also measured at the end of some tests.

2.5.1 Test Materials

There are literally hundreds of substances that could be used to test the sensitivity of various sublethal measurements. For this evaluation, seven toxicants were selected:

- Copper, as $CuCl_2$;
- NaCl;
- $MgCl_2$;
- $CaCl_2$;
- FreezGard (a commercially available $MgCl_2$-based deicer);
- Hydromelt (a commercially available $MgCl_2$-based deicer); and
- CF-7 (a commercially available potassium acetate-based deicer).

The toxicants were chosen because they were readily available in the laboratory and they represent a range of toxicant types ranging from common salts, to a trace metal, to an organic material. In addition, the inclusion of copper and the salts addresses the need to establish an appropriate reference toxicant for amphibian studies (KCl and $CdCl_2$ were also included for this reason in Phase II testing). Reference toxicants are used in most laboratories to track the historical sensitivity of the species used in toxicity testing. A steady increase or decrease in organism sensitivity suggests a problem with the organisms or an error in chemical analysis. Copper and salts (particularly NaCl) are commonly-used reference toxicants.

Studies for the Test Development Phase of the project were conducted in June 2001. Test Refinement Studies were conducted from December 2001 through February 2002, and also evaluated sublethal endpoints (as well as appropriate test length and organism age).

Phase II Test Refinement Studies are described in Section 2.6.

By the time the June 2001 tests were ready to be initiated, the original *Rana* tadpoles (hatched on May 12, 2001) had grown considerably and most were nearing the end of the metamorphosis into an adult frog. Only a few remained small enough for use in testing. Therefore, only one test (with copper) was conducted with *Rana*. All other tests were conducted with *Bufo*. The tests were 7 days in duration. Test dates and organism ages are listed in Table 2-3.

2.5.2 Test Methods

Test chambers were 500 to 600 ml beakers containing 200 to 300 ml of test solution. For all tests with salts and commercial deicers, five organisms were placed into each test chamber with three replicates per treatment. For the copper tests with *Rana*, only three organisms were placed in each chamber because of their size. Only four *Bufo* were placed in each test chamber for the second copper test with *Bufo*, also because of the large size of the tadpoles. Chambers were renewed daily with fresh solution. Toxicant concentrations were verified analytically. Chloride salts, FreezGard, and Hydromelt (magnesium chloride-based deicers) were quantified by measuring the concentration of chloride in the test solutions using a Hach Digital Titrator with mercuric nitrate titration. The concentration of CF-7 (potassium acetate deicer) was verified by measuring the concentration of potassium using Trace ICP (SW-846 method 6010B) following digestion using method 3005A. Copper concentrations were verified using atomic absorption spectroscopy/graphite furnace. Each chamber received approximately 90 mg of a 50:50 mix of TetraMin® and trout chow two hours before solution renewal on a daily basis. Survival was documented daily. At the end of the test, body width, distance between eyes, total length, body length, dry weight, and metamorphic stage were determined. Statistically significant differences among treatments were determined using Toxstat Version 3.5 (WEST and Gulley, 1996).

2.6 Effects of Age and Test Length

Following the Phase I tests, some questions remained regarding specific parameters of the test protocol. In particular, the Phase I tests did not address 1) effects of organism age at test initiation and 2) impacts of different test lengths. These factors were evaluated during the Test Refinement Phase of this research. Test Refinement studies commenced in December 2001. These tests evaluated the effects of copper, cadmium, salts, and commercial deicers on *R. pipiens*.

Because the organisms used during the Test Refinement stage came from eggs laid and fertilized in the laboratory (Carolina Biological Supply or Nasco), they were known to be *Rana pipiens*. All tests were water-only exposures, conducted in 500 ml beakers with 200 to 300 ml of test solution in each beaker. Five organisms were placed in each test chamber with four replicates of each treatment. Tadpoles were fed approximately 4 mg of TetraMin® daily after they reached stage 25. Test solutions were renewed daily.

Since one of the goals of this project phase was to evaluate the effects of test duration as well as the sensitivity of organisms of different ages, some tests were 1) initiated at the same time but maintained for different durations or 2) maintained for the same length of time but initiated with organisms of different ages from the same batch. Test dates and organism ages are listed in Table 2-4.

One test with copper was conducted at 20°C rather than 23°C to evaluate the effects of a different test temperature. Survival was documented daily and at the end of the test, body width, total length, body length, dry weight, and metamorphic stage were determined. Statistically significant differences among treatments were determined using Toxstat Version 3.5 (WEST and Gulley, 1996).

Table 2-1
Frog and Toad Eggs Received

Species	Batch Number	Date Received	Date Hatched	Source
Rana sp.	01-022	5/11/01	5/12/01	Carolina Biological
Bufo americanus	None	6/1/01	6/3/01	ENSR (wild-collected)
Rana pipiens	01-061	11/7/01	NA[a]	Carolina Biological
Rana pipiens	01-062	11/14/01	11/17/01	Carolina Biological
Rana pipiens	01-064	12/6/01	12/9/01	Carolina Biological
Rana pipiens	01-066	12/13/01	12/15/01	Nasco
Rana pipiens	02-03	1/8/02	1/11/02	Carolina Biological

[a] All eggs died before hatching.

Table 2-2
Artificial Sediment Used as Controls

Ingredient	Quantity (g)
Rinsed, #20 Silica Sand	850
Clay/Silt Mixture (ASP 400)	150
Dolomite	0.5
Humic Acid (Sodium Salt)	0.1
Sieved Sphagnum Moss	22

Table 2-3
Tests Conducted During Phase I (Test Development)

Test Material	Taxa	Test Dates	Organism Age at Initiation (days)
NaCl	*Bufo*	6/12 – 6/19/01	9
$MgCl_2$	*Bufo*	6/12 – 6/19/01	9
$CaCl_2$	*Bufo*	6/12 – 6/19/01	9
FreezGard	*Bufo*	6/8 – 6/15/01	5
HydroMelt	*Bufo*	6/8 – 6/15/01	5
Cryotech CF-7	*Bufo*	6/8 – 6/15/01	5
$CuCl_2$	*Rana*	6/7 – 6/14/01	26
$CuCl_2$	*Bufo*	6/6 – 6/13/01	3
$CuCl_2$	*Bufo*	6/25 – 7/2/01	22

Table 2-4
Test Length and Organism Age in All Tests Conducted During Phase II (Test Refinement)

Test Material	Test Dates	Length (Days)	Organism Age at Initiation (Days)
$CuCl_2$	12/17 – 12/24/01	7	1-2
$CuCl_2$	1/11 – 1/18/02	7	<1
$CuCl_2$	1/11 – 1/25/02	14	<1
$CuCl_2$	1/11 – 2/1/02	21	<1
$CuCl_2$	1/11 – 1/18/02[a]	7	<1
$CuCl_2$	1/15 – 1/22/02	7	4
$CdCl_2$	1/15 – 1/22/02	7	4
$CuCl_2$	1/21 – 1/28/02	7	10
NaCl	12/10 – 12/17/02	7	1
$MgCl_2$	12/10 – 12/17/02	7	1
NaCl	12/14 – 12/21/02	7	5
$MgCl_2$	12/14 – 12/21/02	7	5
NaCl	12/20 – 12/27/02	7	11
$MgCl_2$	12/20 – 12/27/02	7	11
$CaCl_2$	1/11 – 1/18/02	7	<1
$CaCl_2$	1/11 – 1/25/02	14	<1
KCl	1/11 – 1/18/02	7	<1
KCl	1/11 – 1/18/02	14	<1
$CaCl_2$	1/21 – 1/28/02	7	10
FreezGard	1/21 – 1/28/02	7	10
HydroMelt	2/1 – 2/8/02	7	21
CF-7	2/1 – 2/8/02	7	21

[a] Test was run at 20°C; all other tests conducted at 23°C.

SECTION 3
RESULTS

3.1 Sediment Preference and Test System

Identical treatment replicates were not conducted in the food and sediment preference tests, so statistical analysis was not possible. However, trends are apparent in the data. Because of the loss of some organisms due to over-topping of the beakers (the drain screens became clogged), accurate estimates of organism survival were not possible. However, it appeared that survival was not adversely affected by any tested sediment. However, some of the sublethal measurements indicated adverse effects, specifically total length, body width, and metamorphic stage (Figures 3-1 to 3-3). In all cases, tadpoles grew better in the Poudre River sediment than in either formulated sediment or in water alone. The tadpoles in the formulated sediment were strikingly smaller than those in the other exposures.

Water quality was poorer in the static-renewal beakers, relative to the flow-through chambers, and degraded as the test progressed and the organisms grew. Dissolved oxygen concentrations were lower in the static-renewal beakers, sometimes dropping below 2.0 mg/L. In addition, ammonia concentrations increased to as much as 10 mg/L. The water in the static-renewal chambers also had a tendency to become cloudy. Conversely, in the flow-through chambers water was clear with dissolved oxygen concentrations being in excess of 6.0 mg/L and ammonia was not detectable. These results strongly indicated that a continuous flow-through system would be the best way to maintain acceptable quality of the overlying water in sediment tests.

In addition to the test renewal systems, two mesh sizes were also evaluated. The mesh covers a hole in the side of the beakers used to drain excess water during renewals. A 300 µm nylon monofilament mesh available from Wildco was selected for use during the test validation phase. The mesh size could be increased to 500 µm if there are problems with screens clogging (reducing the renewal flow and grinding food into smaller particle as also recommended).

3.2 Food Preference

While survival was not apparently affect by any food type, the studies did indicate that tadpoles grew better when they were fed TetraMin® or a 50:50 mixture of TetraMin® and trout chow (Figures 3-1 to 3-3). The effect of food on metamorphic state was less apparent than on either total length or body width.

3.3 Ammonia Tolerance

In the ammonia tolerance evaluation, 95% of the *Rana* tadpoles were still alive after seven days in vessels with measured total ammonia concentrations of approximately 13.6 mg/L. However, there was significant mortality at 32.8 (47% survival) and 17.1 mg/L (60% survival), and total mortality at 47.7 mg/L (Figure 3-4). The survival No Observed Effect Concentration (NOEC) was 13.6 mg/L. A general decrease was observed in several sublethal measurements (body width and body length) with increasing ammonia concentration (Figures 3-5 and 3-6), although only body width indicated effects at a lower concentration, specifically, a NOEC of 6.1 mg/L.

3.4 Determination of Appropriate Sublethal Endpoints

3.4.1 Toxicity of Copper

In the first set of Phase I studies conducted in June 2001, only three tests with copper (as $CuCl_2$) were conducted, one with *Rana* and two with *Bufo* (at early and late stages of development). In these tests, *Rana* was much less sensitive to Cu than *Bufo*. In the test with *Rana*, 100% mortality was observed in 295.5 μg/L of Cu, but *Bufo* experienced 100% mortality at 69 μg/L Cu in the first study (early-stage) and 94.5 μg/L Cu in the second study (late stage)(Figures 3-7 to 3-9). The survival NOEC for *Rana* was 167.8 μg/L, while the survival NOECs for the first and second *Bufo* tests were 23.7 and 52.4 μg/L Cu, respectively.

The sublethal measurements did not indicate an increased level of sensitivity to copper. For the *Rana* study and the early-stage *Bufo* study, the growth NOECs were the same as the survival NOECs (167.8 μg/L for *Rana* and 23.7 μg/L for *Bufo*). In the second test using late-stage *Bufo*, the growth NOEC was reduced to 32.1 μg/L, compared to the survival NOEC of 52.4 μg/L.

3.4.2 Toxicity of Salts and Commercial Deicers

For tests with NaCl, $MgCl_2$, and $CaCl_2$,, there was a clear concentration/response curve (Figures 3-10 to 3-12). For all three salts, at least two of the sublethal measurements were more sensitive than survival. The body width NOEC was lower for all three salts and the total length NOEC was lower for the NaCl and $MgCl_2$ tests. Body length and metamorphic stage NOECs were also lower for at least one of the salts.

Of the three deicers tested, Hydromelt was the least toxic as a neat product. The survival NOEC of Hydromelt was 12,500 mg/L while the NOECs for FreezGard and CF-7 were 6,250 and 3,125 mg/L neat product, respectively. When compared to the laboratory –prepared $MgCl_2$ solution, FreezGard demonstrated similar toxicity (Figure 3-13) while Hydromelt was less toxic than the neat $MgCl_2$ solution (Figure 3-14). For both Hydromelt and FreezGard, at least some of the NOECs for the sublethal measurements were lower than the survival NOECs, indicating the sublethal endpoints were more sensitive than survival alone. The sublethal NOECs for CF-7, however, were not any lower than the survival NOEC (Figure 3-15).

3.5 Summary of the Evaluation of Sublethal Endpoints – Phase I Testing

For the studies with ammonia, copper, salts, and deicers, NOECs and LOECs were calculated for both survival and the sublethal endpoints (selected results summarized in Table 3-1). Out of 10 studies (not including the sediment and food preference studies), body width resulted in lower (relative to survival) NOECs in six studies and total length resulted in lower NOECs in five studies. Body length, metamorphic stage, and dry weight per surviving organism resulted in lower NOECs in three studies, while the eye width NOEC was lower in only two studies.

3.6 Effects of Age and Test Length

3.6.1 Organism Age

Tests were initiated with tadpoles of various ages, varying from <24 hours old to 26 days old. Tests with NaCl, $MgCl_2$, and copper were initiated with tadpoles of three different ages: 1-day old, 4 or 5 days old, and 10 or 11 days old. In addition, the copper tests initiated during the Test Refinement phase can be compared with the copper test conducted with *Rana* during the Test Development phase. That test was begun with 26-day old organisms. Two $CaCl_2$ tests were conducted, one with 1-day old tadpoles and the other with 10-day old tadpoles.

In the NaCl, $CaCl_2$, and copper tests, the older tadpoles were substantially less sensitive than the younger animals, both for acute and chronic endpoints. For example, the survival NOECs in the copper studies were 38.8, 77.9, 163.4, and 167.8 μg/L Cu for 1-, 4-, 10-, and 26-day old tadpoles, respectively (Figure 3-16). Similar results were observed for the sublethal metrics as well. It is interesting to note that in tests initiated with older organisms, the number of cases where the sublethal NOECs were lower than the survival NOEC drops. For example, in the test started with approximately 1-day old tadpoles, all five of the sublethal NOECs were at least one test concentration lower (19.0 μg/L) than the survival NOEC (39.0 μg/L) and two sublethal NOECs were less than the lowest tested concentration. For the 4-day old organisms, four sublethal NOECs were less than the survival NOEC; for the 10-day old organisms, only two sublethal NOECs were less than the survival NOEC. Finally, in tests initiated with 26-day old organisms, none of the sublethal NOECs were less than the survival NOEC.

In tests with NaCl and $CaCl_2$, the NOECs from studies with older organisms were always greater (less toxic) than the NOECs from the younger organisms, with one exception (Figures 3-17 and 3-18). The survival NOEC for $CaCl_2$ was slightly higher for the younger tadpoles. However, like with copper, the sublethal NOECs generated using the 1-day old *Rana* tadpoles were all lower than the survival NOEC. The pattern of the $MgCl_2$ test was not the same as with the other toxicants in that NOECs from the test with 2-day old tadpoles were higher than the NOECs from the test with 5-day old organisms (Figure 3-19). However, the highest NOECs were nevertheless generated from the test with 11-day old tadpoles. These data generated with *Rana* during the Test Refinement phase support the data from the earlier *Bufo* tests which showed that younger tadpoles were more sensitive than older tadpoles when exposed to copper.

3.6.2 Test Length

For copper, three tests were initiated using the same batch of 1-day old tadpoles. The tests were terminated after 7, 14, or 21 days. In addition, $CaCl_2$ and KCl tests were conducted for 7 and 14 days using the same batch of <24-hour old tadpoles. In the $CaCl_2$ and copper studies, the NOECs for the acute and sublethal metrics were either approximately the same, or higher, in the 14- or 21-day studies relative to the 7-day study (Figures 3-20 and 3-21), indicating that running the tests for two or three weeks did not result in greater toxicity to the organisms. However, the 14-day NOECs in the KCl study were lower; indicating greater toxicity (Figure 3-22).

3.7 Interspecies Sensitivity

Evaluating the variability in sensitivities between anuran species was not an *a priori* goal of this research. However, sufficient data were collected on both *Rana* and *Bufo* that some comparisons can be made. During Phase I of this project, *Bufo* was the primary test species while *Rana* was the only species evaluated during Phase II. In no cases were the organisms of the exact same age used to test any particular toxicant, although in some cases the ages of the two taxa were quite similar. Copper, as $CuCl_2$, was tested with both young and old tadpoles of both *Rana* and *Bufo.* In both cases, *Bufo* was substantially more sensitive (Figure 3-23). *Bufo* was also slightly more sensitive to NaCl. However, for all of the other salts and the commercial deicers, *Rana* was more sensitive, with survival NOECs for *Bufo* often being over twice those of *Rana*. In tests with commercial deicers, even though *Bufo* were younger than *Rana*, they were less sensitive, with consistently higher NOECs.

3.8 Summary of the Evaluation of Sublethal Endpoints – Phase II Testing

Twenty-two studies were conducted during the Test Refinement stage of this research. In 10 of the nineteen tests conducted with chlorides, a lower sublethal NOEC was calculated (relative to the survival NOEC) when body width or body length were used (Table 3-2). In nine cases the total length NOEC was lower than the survival NOEC. The weight and metamorphic stage NOECs were lower in eight and seven cases respectively.

3.9 Statistical Analysis

Because of the number of variables that were examined during the test development phase, not all tests included replication such that hypothesis testing could be conducted. For those studies where at least three replicates were included, statistical analysis was completed using Toxstat Version 3.5 (WEST, Inc. and Gulley 1996). Survival data were entered as proportional results and first treated with an arcsine square root transformation. Data were analyzed to determine if they meet the requirements for parametric analysis (normality and homogeneity of variance). If the data did meet the parametric assumptions, then they were analyzed with a parametric test (α=0.05) such as Dunnett's test. If the data did not meet the parametric assumptions, they were analyzed using a nonparametric test such as Steel's Many-One Rank test.

3.10 Testing Costs and Report Production

The cost of a short-term chronic test with amphibian tadpoles will vary according to the laboratory conducting the study. Costs will, of course, also vary with the amount of preparation and monitoring needed for the studies. For the purpose of estimating a test price, the following assumptions are considered:

- The cost of collecting samples is not included
- Test organisms will be available from commercial suppliers or collected opportunistically from the field (costs are not included for a specific collection trip)
- The test will be of bulk sediment, without dilution with nontoxic sediment
- No more than eight replicates will be tested with each sediment
- A reference toxicant test will be included for each different batch of organisms used
- Analytical chemistry (other than basic measurements such as pH, temperature, ammonia, and dissolved oxygen) costs are not included
- All toxicity test data will undergo a full quality assurance review
- A study report will be written

Given these assumptions, the cost of conducting short-term chronic test should range from $750 to $1,200.

It is estimated that a laboratory could produce a draft report for submission within three weeks of completion of the proposed sediment toxicity test. Therefore, given the test period of 10 days, it would take about 4.5 weeks from test initiation to produce a draft report. The time from sample collection to report delivery will depend upon how soon a sample arrives at the laboratory and how quickly a test can be initiated, which will be dependent upon the laboratory schedule and organism availability.

Table 3-1
Summary of Selected Phase I Results

Figure Number	Test Organism	Compound	Endpoint	NOEC	LOEC	Units
3-4	*Rana*	Ammonia	Survival	13.6	17.1	mg/L ammonia
3-5	*Rana*	Ammonia	Body Width	6.1	9.7	mg/L ammonia
3-6	*Rana*	Ammonia	Body Length	32.8	47.7	mg/L ammonia
3-7	*Rana*	Copper Chloride	Survival	167.8	29.5	ug/L Cu
3-8	*Bufo*	Copper Chloride	Survival	23.7	38.5	ug/L Cu
3-9	*Bufo*	Copper Chloride	Survival	52.4	94.5	ug/L Cu
3-10	*Bufo*	Sodium Chloride	Survival	4,204	8,407	ug/L NaCl
3-11	*Bufo*	Magnesium Chloride	Survival	2,182	4,364	ug/L $MgCl_2$
3-12	*Bufo*	Calcium Chloride	Survival	5,009	10,018	ug/L $CaCl_2$
3-13	*Bufo*	FreezGard as Magnesium Chloride	Survival	3,961	7,923	mg/L $MgCl_2$
3-14	*Bufo*	Hydromelt as Magnesium Chloride	Survival	4,364	8,729	mg/L $MgCl_2$
3-15	*Bufo*	CF-7	Survival	3,125	6,250	mg/L neat product

NOEC - No observed effect concentration

LOEC - Lowest observed effect concentration.

Table 3-2
Summary of Sublethal NOECs vs Survival NOECs

Toxicant	Body Width NOEC	Total Length NOEC	Body Length NOEC	Stage NOEC	Weight NOEC
$CuCl_2$	N	Y	Y	N	Y
$CuCl_2$	Y	Y	Y	Y	Y
$CuCl_2$	Y	Y	Y	Y	Y
$CuCl_2$	Y	Y	Y	Y	N
$CuCl_2$	Y	Y	Y	Y	Y
$CuCl_2$	Y	N	Y	Y	Y
$CdCl_2$	N	N	N	N	N
CuCl2	N	Y	N	N	Y
NaCl	N	N	N	N	N
$MgCl_2$	N	N	N	N	N
NaCl	Y	N	Y	N	Y
$MgCl_2$	N	N	N	N	N
NaCl	N	N	N	N	N
$MgCl_2$	Y	N	N	N	N
$CaCl_2$	Y	Y	Y	Y	Y
$CaCl_2$	Y	Y	Y	N	N
KCl	N	N	N	N	N
KCl	Y	Y	Y	Y	N
$CaCl_2$	N	N	N	N	N
Number of Tests Where the Sublethal NOECs Were More Sensitive Than the Survival NOEC					
	10	9	10	7	8

NOEC - No observed effect concentration

Y = Sublethal NOEC was lower than survival NOEC.

N = Sublethal NOEC was the same as survival NOEC

Figure 3-1
Effects of Sediment and Food Type on Total Length of Rana Tadpoles.

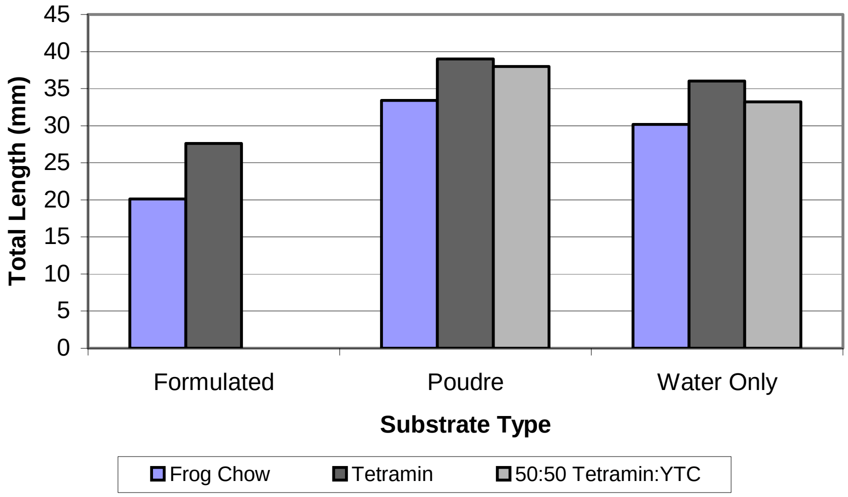


Figure 3-2
Effect of Sediment and Food Type on Body Width of Rana Tadpoles

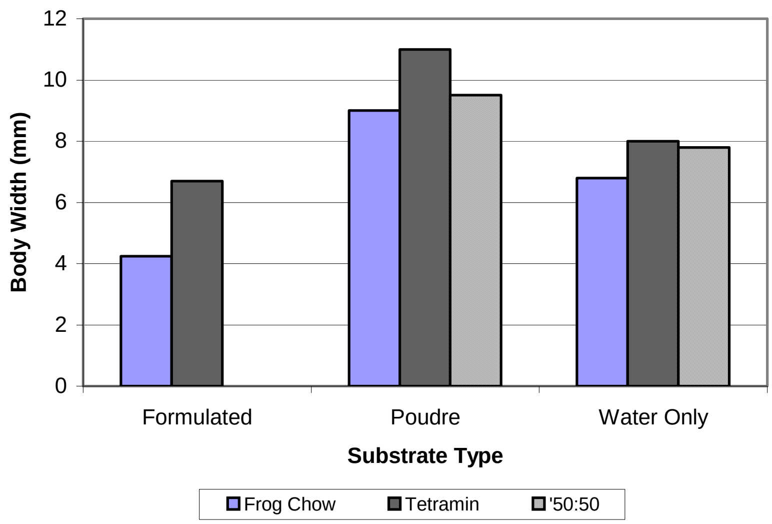


Figure 3-3
Effects of Sediment and Food Types on Metamorphic Stage of Rana Tadpoles

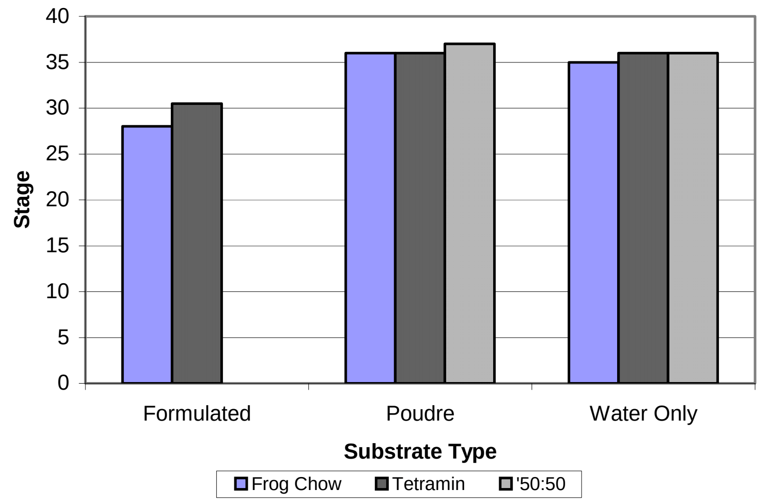


Figure 3-4
Acute Toxicity of Ammonia to Rana Tadpoles

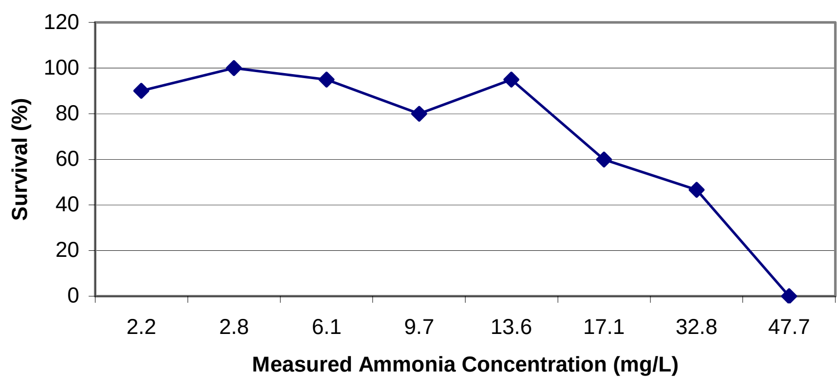


Figure 3-5
Effect of Ammonia on Body Width of Rana Tadpoles

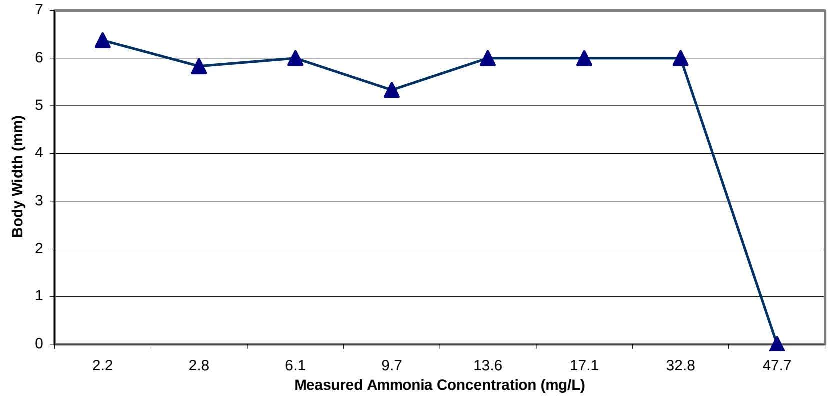


Figure 3-6
Effect of Ammonia on Body Length of Rana Tadpoles

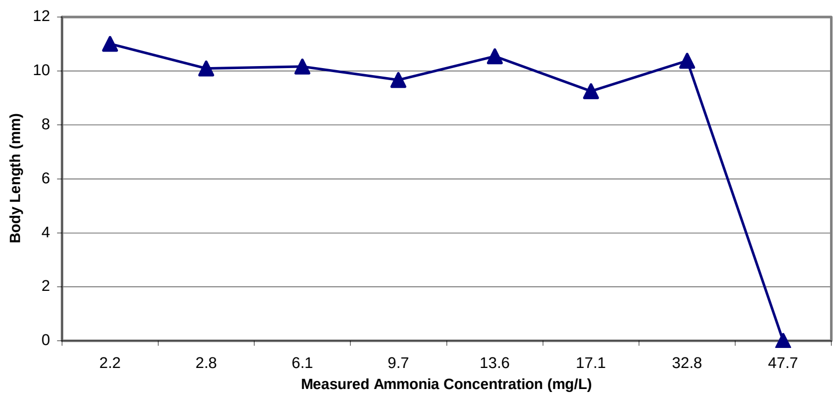


Figure 3-7
Acute Toxicity of Copper to **Rana** *Tadpoles*

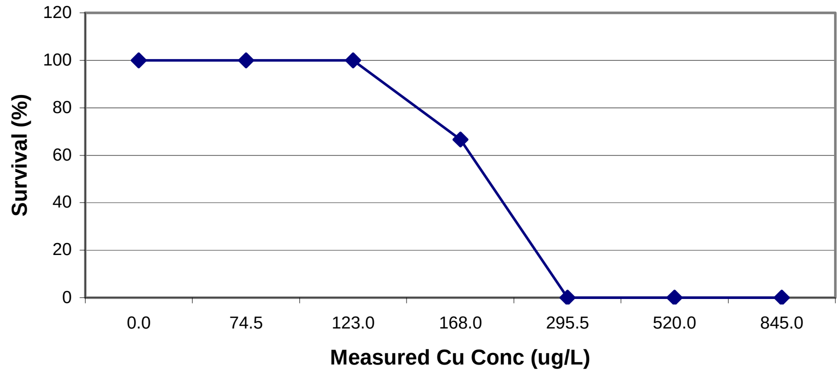


Figure 3-8
Acute Toxicity of Copper to **Bufo** *Early-Stage Tadpoles*

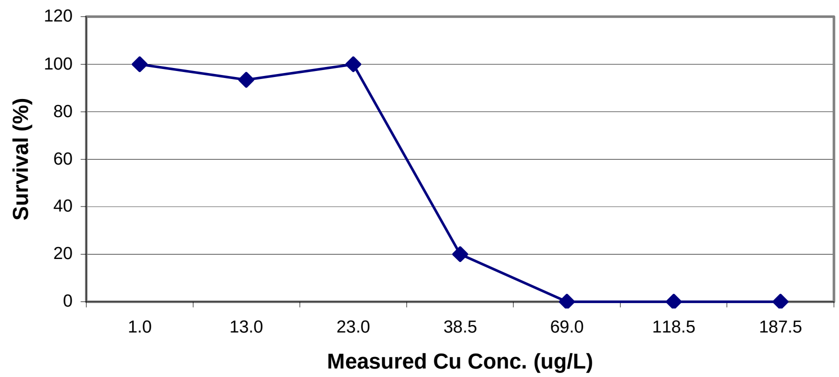


Figure 3-9
Acute Toxicity of Copper to **Bufo** *Late-Stage Tadpoles*

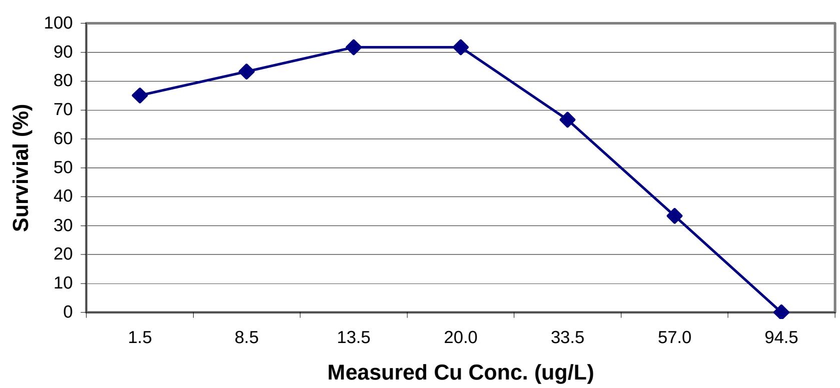


Figure 3-10
Acute Effect of Sodium Chloride on* Bufo *Tadpoles

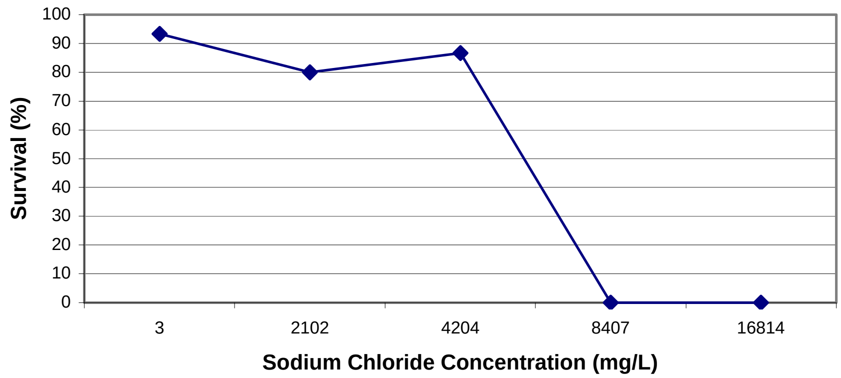


Figure 3-11
Acute Effect of Magnesium Chloride on* Bufo *Tadpoles

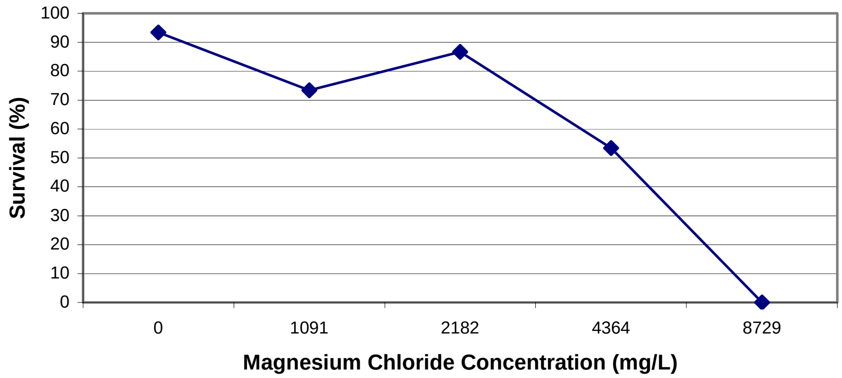


Figure 3-12
Acute Effect of Calcium Chloride on* Bufo *Tadpoles

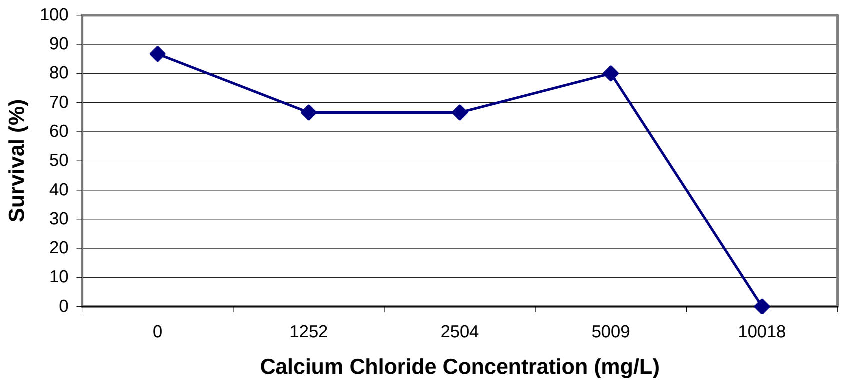


Figure 3-13
Acute Effect of FreezGard Deicer on Bufo *Tadpoles*

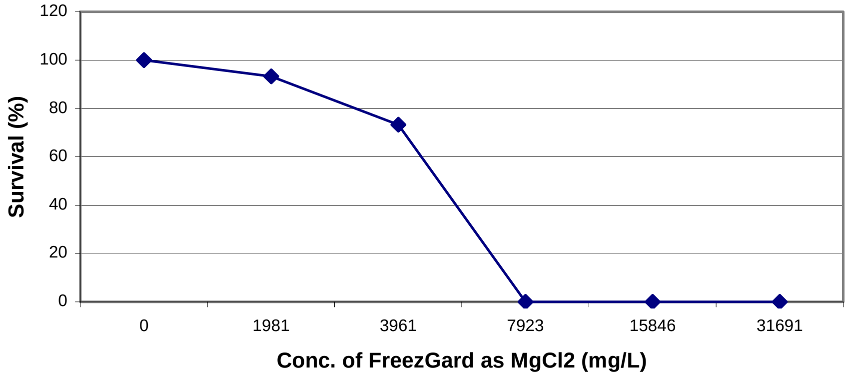


Figure 3-14
Acute Effect of Hydromelt Deicer on Bufo *Tadpoles*

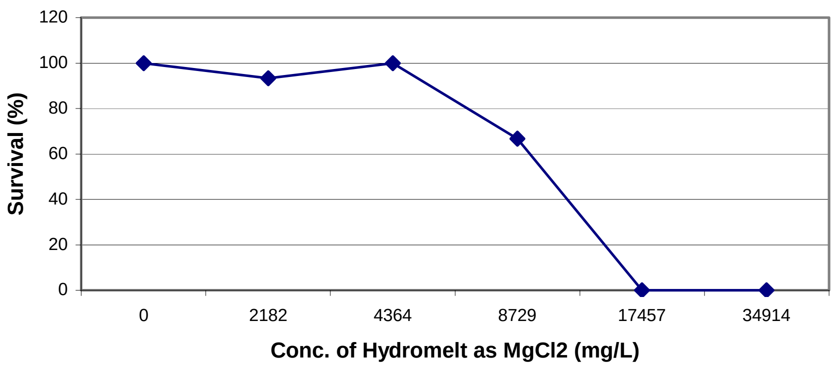


Figure 3-15
Acute Effect of Cryotech CF-7 Deicer on Bufo *Tadpoles*

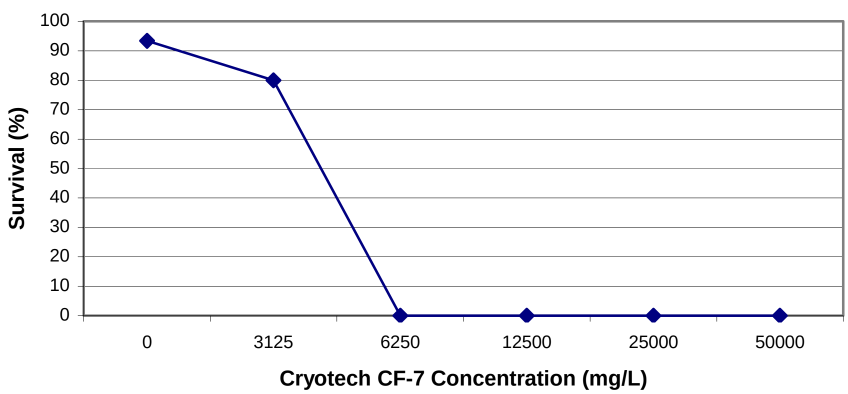


Figure 3-16

Changes in Acute and Chronic Copper NOECs with Increasing Organism Age at Test Initiation

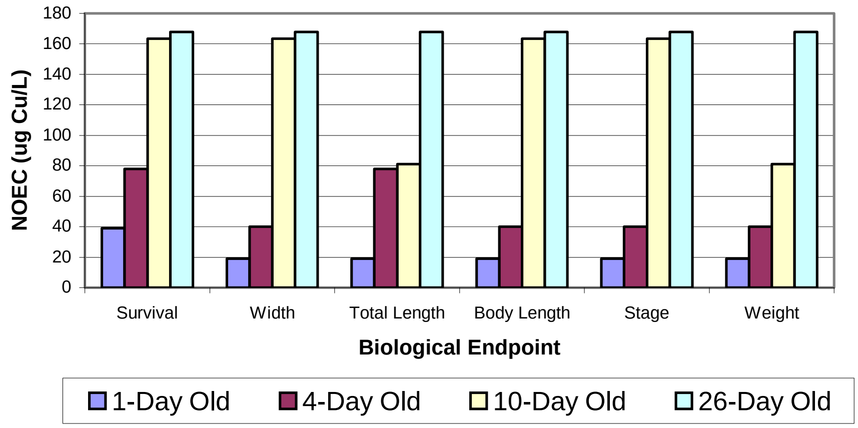


Figure 3-17

Changes in Acute and Chronic NaCl NOECs with Increasing Organism Age at Test Initiation

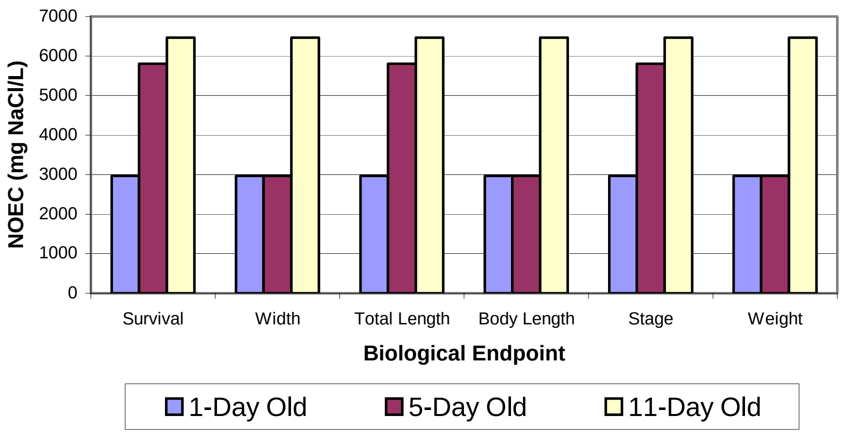


Figure 3-18

Changes in Acute and Chronic $CaCl_2$ NOECs with Increasing Organism Age at Test Initiation

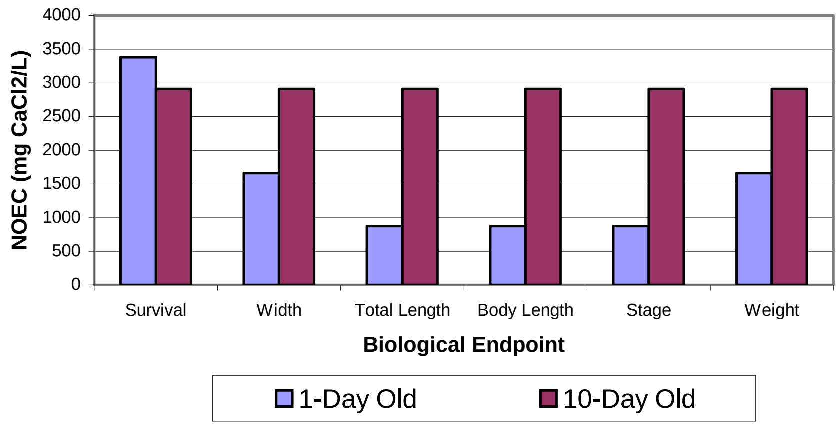


Figure 3-19

Changes in Acute and Chronic $MgCl_2$ NOECs with Increasing Organism Age at Test Initiation

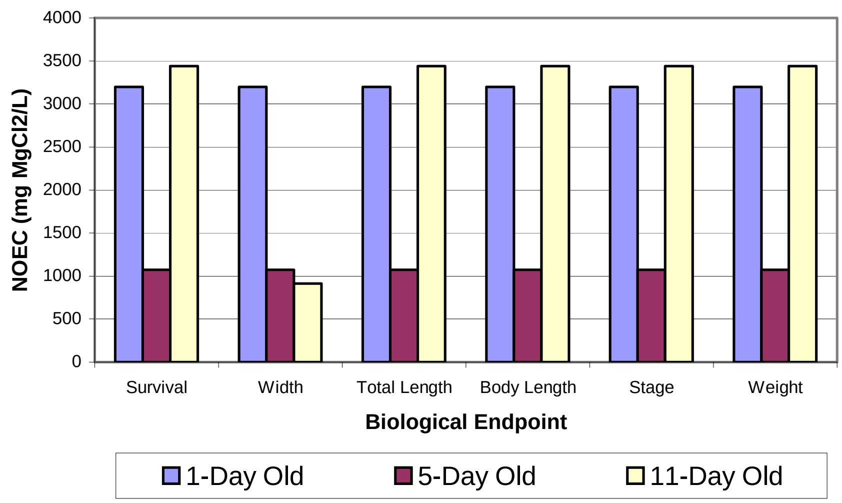


Figure 3-20
Changes in Acute and Chronic $CaCl_2$ NOECs with Increasing Test Duration

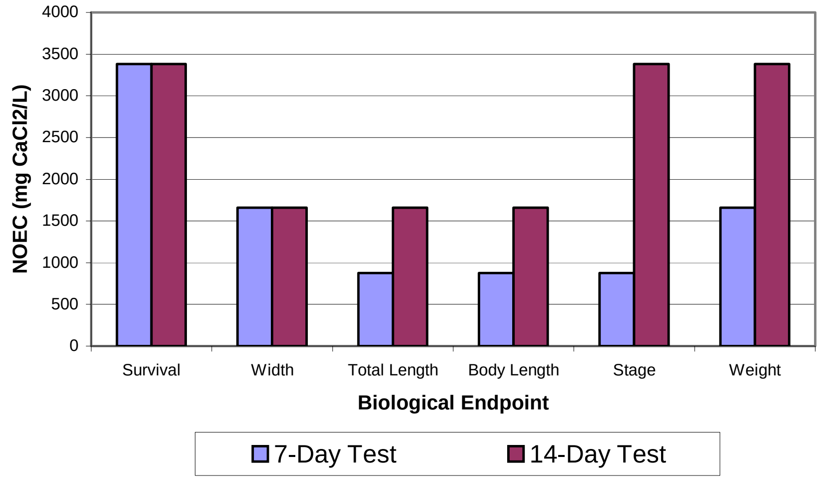


Figure 3-21
Changes in Acute and Chronic Cu NOECs with Increasing Test Duration

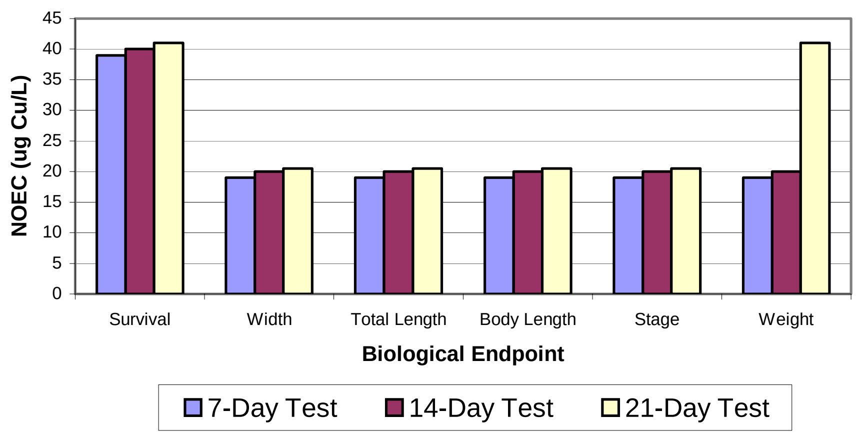


Figure 3-22
Changes in Acute and Chronic KCI NOECs with Increasing Test Duration

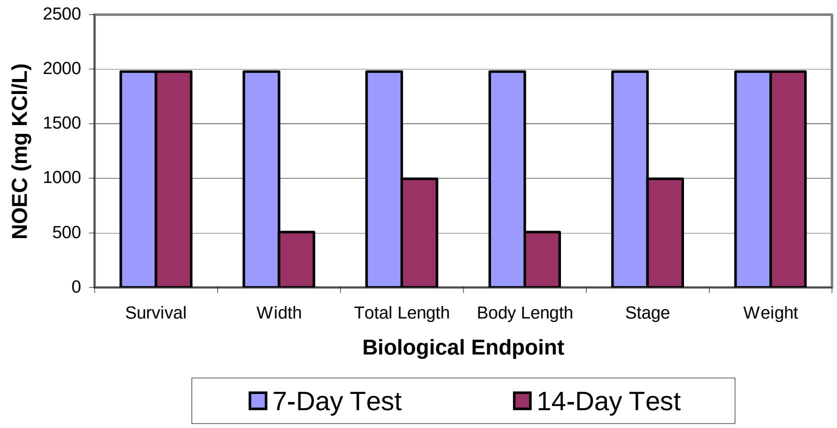
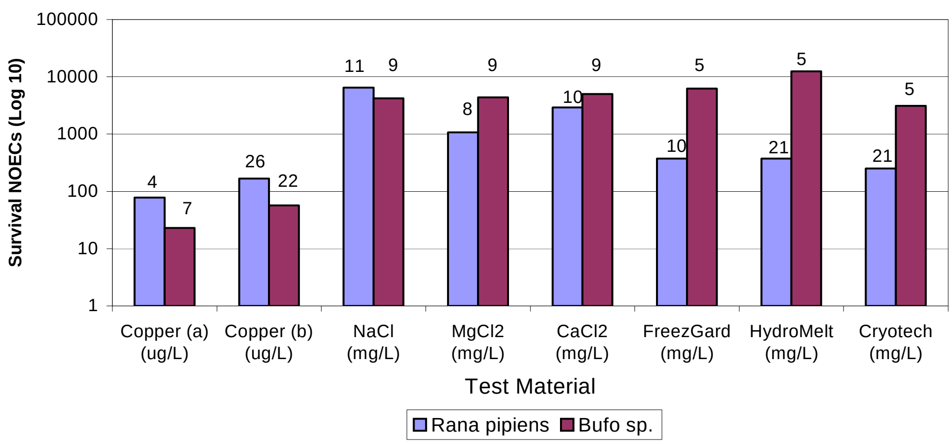


Figure 3-23
Comparison of Survival NOECs between **Rana** *and* **Bufo** *for Various Toxicants*



Numbers above bars indicate age or organisms at test initiation.

SECTION 4
SUMMARY AND CONCLUSIONS

This report presents a focused evaluation of a variety of laboratory test conditions, including varied exposure durations, different sub-lethal endpoints, and life stages of test organisms. The purpose of these studies with tadpoles was to develop and refine a test methodology that can be incorporated into the development a standardized risk assessment protocol for evaluating potential risks to amphibians at sites owned and/or operated by the U.S. Navy. Several areas were addressed including:

- Test containers
- Test temperature
- Control sediment
- Food source
- Tolerance to ammonia
- Sublethal endpoints
- Interspecies sensitivity

In order to address these issues, over 31 studies were conducted. The following conclusions can be drawn from the studies:

- Tadpoles of *Rana pipiens* can be easily obtained from commercial suppliers from about early November through late March. Between late March and mid-May field collected tadpoles of *Rana* and *Bufo* can be obtained and do well in the laboratory.
- Tadpoles grow better exposed to a natural control sediment rather than a formulated sediment.
- Tadpoles grow better when fed TetraMin® or a TetraMin®mix rather than the frog food available commercially. No information is available regarding their relative performance when given other foods such as boiled spinach or lettuce.
- Sediment tests in flow-through chambers are preferable over static-renewal systems because of the buildup of ammonia.
- Ammonia concentrations in excess of 5 mg/L could cause sublethal effects to anurans.
- Organisms grow adequately and remain healthy when tested at a temperature of 23°C.
- Younger organisms are generally more sensitive to toxicants than older organisms. For sediment tests, organisms should not be older than about 72 hours at test initiation.
- Conducting tests for longer periods of time does not result in substantially lower statistical endpoints (e.g., NOECs).
- *Bufo* may be more sensitive to copper than *Rana*, but less sensitive to chloride salts and commercial deicers.

Given the information derived from these studies, recommendations can be made as to a protocol or standard operating procedure for conducting sediment toxicity tests with amphibians. The parameters listed below have been incorporated into the standard operating procedure presented in Attachment C-1.

Test Length	10 days
Test Temperature	23°C
Test Chambers	500-1000 ml beakers or aquaria with an overflow pipe or other outflow system
Sediment Volume	≥100 mls
Age at Test Initiation	≤72 hours
Food	Approximately 4 mg dry TetraMin®in each test chamber after organisms reach stage 25
Endpoints	Survival, body width and body length
Test Acceptability	80% survival in the controls and measurable growth in the controls

The purpose of the SOP is to help predict possible effects of chemical stressors in sediments and hydric soils on amphibians in natural ecosystems. This test method uses an early life stage of a native North American species, and lethal and sub-lethal toxicity endpoints that are relevant to typical assessment endpoints considered by the Navy in their ecological risk assessments.

SECTION 5
REFERENCES

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica. 16:183-190.

Shumway, W. 1940, Stages in the normal development of *Rana pipiens*. The Anatomical Record. 78:139-147.

USEPA. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth Edition. EPA/600/4-90/027F.

WEST, Inc. and D.D. Gulley. 1996. Toxstat Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, WY.

ATTACHMENT C-1

SOP

C-1
STANDARD OPERATING PROCEDURES

TEST METHOD FOR CONDUCTING WHOLE SEDIMENT TOXICITY TESTS WITH AMPHIBIANS

1.0 Purpose and Applicability

Amphibians are often a major ecosystem component of wetlands around the world. Concern over the state of amphibian species has increased in recent years due to recorded declines in populations around the world. Although some of this decline is attributed to habitat disturbance and destruction and the introduction of exotic species, some effects may also be due to environmental contaminants, including those deposited in sediments. While federal criteria and state standards exist that define acute and chronic "safe" levels in the water column, effects levels in the sediment are poorly defined and may be dependent upon numerous modifying factors. Therefore, simply measuring the concentration of a chemical in the sediment is often insufficient to evaluate its actual environmental toxicity. Laboratory studies are one way of assessing toxicity directly. The purpose of this standard operating procedure (SOP) is to provide guidance for initiating, conducting, and terminating sediment toxicity tests with amphibians. This SOP should be followed to conduct a 10-day test with *Rana pipiens* or *Bufo americanus*. Other species may be used if sufficient data on handling, feeding, and sensitivity are available.

2.0 Definitions

Control Sediment – a sediment that is essentially free of contaminants and in which organisms should experience no significant acute or chronic effects. Control sediment may come from any appropriate location, such as a river, lake, or pond. It can also be a formulated sediment prepared in the laboratory. However, studies have shown that tadpoles may grow better in a natural sediment. Control sediment should be tested independently before use in an actual study.

EC_{50} – Median effective concentration. The concentration at which 50% of the test organisms experience a designated effect. The effect is usually a non-lethal one, such as growth.

IC_{25} – 25% inhibition concentration. Concentration at which there is a 25% reduction in organism performance, relative to the control. Performance may be survival or a sublethal measurement such as growth.

LC_{50} – Median lethal concentration. Concentration at which 50% of the test organisms die.

LOEC – Lowest observed effect concentration. Lowest concentration at which there is a significant difference, relative to the control.

NOEC – No observed effect concentration. Highest concentration at which there is no significant difference, relative to the control.

Overlying Water – Water that is placed over the sediment for the duration of the study. Overlying water may be surface water collected from a lake or reservoir, or reconstituted water prepared in the laboratory (e.g., moderately hard water [USEPA 1994a]). Site water could be used but would require shipping a large volume of water to the laboratory.

Test Sediment – Sediment that may contain contaminants, which is being evaluated using this test procedure.

3.0 Health and Safety Considerations

Some test materials, as well as some materials used to preserve test organisms, may be inherently hazardous. Caution should be used when handling these materials. When working with any potentially hazardous materials, including those used for analytical measurements (e.g., acid used during alkalinity titrations), users should wear appropriate protective equipment (e.g., safety glasses and gloves). All laboratory-specific health and safety considerations must be followed.

4.0 Quality Assurance Planning Considerations

Testing procedures should be consistent with the requirements described in this SOP (e.g., test organism age, replicates, etc.). However, study-specific modifications may be necessary and acceptable as long as they do not compromise the integrity of the study.

4.1 Reference Toxicant Testing

It is usually desirable for laboratories to conduct periodic reference toxicant tests with test organisms. Reference toxicant tests involve exposing organisms that are used to start a sediment study to a known toxicant at known concentrations in water-only exposures. Organisms of a given species should demonstrate a consistent response to a reference toxicant. Since the procedure described in this SOP will be a new study for most laboratories, historical data on the response of anurans to toxicants are generally not available. However, some toxicity data can be found in the literature and can be compared to a reference toxicant test until the laboratory generates several data points.

If the reference toxicity results from a given study fall outside the "expected" range (± 2 standard deviations), the sensitivity of the organisms and the credibility of the study may be in question. However, reference toxicant data outside of the acceptable range does not necessarily indicate an unacceptable sediment toxicant test. In such a case, test procedures should be examined for any serious defects. If serious problems are not found, then the test may be acceptable.

Reference toxicant performance should improve with experience. Control limits should narrow with time as statistics stabilize and the impact of a single datum decreases. Nevertheless, 95% control limits will be exceeded, by definition, 5% of the time. The width of the control limits should be considered when decisions are made regarding acceptance or rejection of data.

There are several chemicals that are used as reference toxicants. In studies conducted during the development of this SOP copper, as $CuCl_2$, was found to produce consistent responses from the test organisms, provided organism age and test water were held constant. The sensitivity of frog and toad tadpoles decreases dramatically as organisms age. In addition, dissolved organic carbon greatly reduces the bioavailability of copper.

5.0 Responsibilities

The Study Director is responsible or ensuring that tests are conducted correctly. Each technician performing this procedure is responsible for understanding and following this SOP.

6.0 Training and Qualifications

Personnel performing this procedure must be trained in these and all other applicable laboratory methods or receive supervision when conducting them. Personnel should be familiar with other specific SOPs that are applicable to these studies but not explicitly described in this SOP.

7.0 Required Materials

The following materials are required for this procedure:

Sample Collection

- Decontaminated sampling equipment (e.g., corer, Ponar dredge, Ekman dredge, stainless steel shovel, etc.)
- Clean sample containers (e.g., wide-mouth high-density polyethylene jars)
- Labels
- Coolers for sample transport

Testing

- Stainless steel spoon or auger to homogenize sediment
- Testing chambers (usually 300-500 ml beaker with a small-mesh (300 µm) screen covering a hole drilled in the side of the beaker (secured with nontoxic silicone adhesive))
- Transfer pipettes
- Small nets
- Dissolved oxygen meter
- Conductivity meter
- pH meter
- Ammonia meter
- Reagents and equipment for hardness and alkalinity determinations
- 23 ± 1°C temperature-controlled water bath or environmental chamber
- Flow-through water delivery system
- 3-aminobenzoic acid ethyl ester, methanesulfonate salt (MS-222 anesthetic)
- Food (TetraMin®)
- Appropriate data forms
- Metric ruler
- Forceps
- Statistical software (e.g., Toxstat Version 3.5 [WEST and Gulley, 1996] and Statistix Version 7.0 [Analytical Software, 2000])

8.0 Organisms

Test organisms are recently hatched tadpoles of small North American anurans. The preferred species are the Northern Leopard Frog, *Rana pipiens*, or the American Toad, *Bufo americanus*. Handling and culturing methods for these two species were well studied during development of this SOP and the response of these two species to various toxicants has been studied and documented. Other species may be used for testing if handling and holding conditions are known.

A number of websites that contain information on amphibians were identified during this project. Information presented in this section regarding frog and toad lifestages and habitats was obtained from some of the following Internet sites:

- www.npwrc.usgs.gov/narcam/idguide
- www.library.thinkquest.org
- www.dnr.state.wi.us/org/caer/ce/eek/critter/amphibian
- www.raysweb.net/specialplaces/pages/frog.html
- www.allaboutfrogs.org/info/species/leopard.html
- www.alienexplorer.com/ecology
- www.museum.gov.ns.ca/mnh/nature/frogs
- www.frogs.org
- www.knapp.home.midsoring.com
- www.uri.edu/cels/ms/patron/LH_pifr.html
- www.myherp.com/michigan/frogtoad.html

As an adult, *R. pipiens* (also referred to as the grass frog and meadow frog) is a small- to medium-sized frog, with a total body length of 5 to 9 cm. Body coloration is green to light brown. Yellow-outlined, oval, black spots cover the back of *R. pipiens*. It also has two lightly colored lines on ridges that run the length of the back (Figure 1).

Figure 1 Adult Northern Leopard Frog

(www.museum.gov.ns.ca/mnh/nature/frogs/north.htm)

The Northern Leopard Frog is found over a large area of North America, from the Atlantic Coast to eastern California, Oregon, and Washington. It is found from northern Canada to as far south as southern New Mexico, although it is not found in the southeastern United States (Figure 2).

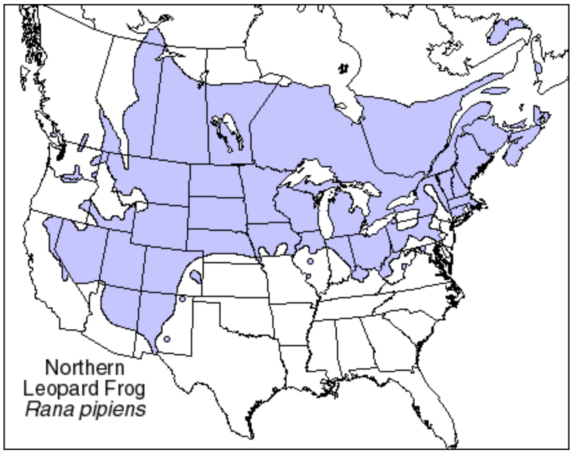


Figure 2 Range of the Northern Leopard Frog in North America

(www.npwrc.usgs.gov/narcam/idguide/rpipiens.htm).

Adult *R. pipiens* overwinter in the mud at the bottom of lakes and ponds and emerge in the spring when air temperature reaches approximately 10°C. The breeding season runs from March through May, depending upon the latitude within the animal's range. A female lays up to 6,000 eggs that form a large floating mass. The eggs hatch in about two weeks. Tadpoles are omnivores, feeding on algae, plants, and dead organisms, including other tadpoles. Tadpoles complete the metamorphosis to adults in 10 to 13 weeks, but this is somewhat dependent upon temperature and availability of food.

Gosner (1960) developed a table for staging of anuran embryos, particularly *Rana pipiens.* The classification includes 46 stages from fertilized egg to air-breathing adult. The first 25 nonfeeding stages are based upon a scheme developed by Shumway (1940). Eggs hatch at approximately stage 20, which occurs approximately six days after fertilization (at 18°C). Stage 25 can be identified by the complete loss of external gills (right operculum closes last). From stage 25 until adulthood, stage is generally identified by limb bud development and, in later stages, reabsorption of the tail and mouth size (Figure 3).

Figure 3 Rana pipiens tadpole stages from approximately stage 25-27 to stage 46 (young adult froglet).

There are other frog species that are very similar to the Northern Leopard Frog in appearance as tadpoles and adults. The Southern Leopard Frog (*Rana sphenocephala*) and Pickerel Frog (*Rana palustris*) are similar to *R. pipiens*, although there are slight differences. The spots of *R. palustris* are nearly square while the spots on *R. sphenocephala* tend to be smaller and there are fewer of them. The Southern Leopard Frog ranges over throughout the southeast United States and Atlantic Coast, although it may overlap with *R. pipiens* in some areas (Figure 4). Where overlap does occur, hybridization may be possible.

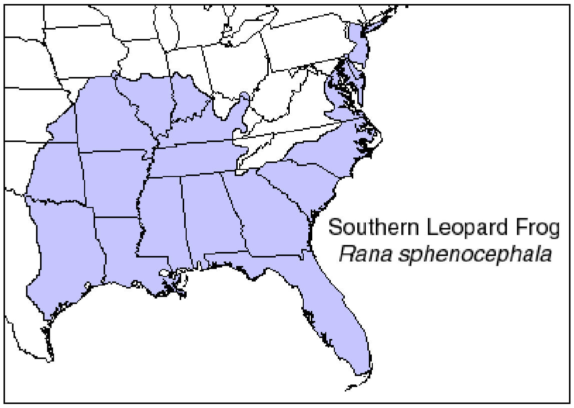


Figure 4 Range of the Southern Leopard Frog in North America.

(www.npwrc.usgs.gov/narcam/idguide/ranaut.htm)

The American Toad (*Bufo americanus)* may also be used for testing. Like *R. pipiens*, *B. americanus* is a small to medium-sized anuran with a relatively short tadpole phase. Two subspecies of *B. americanus* are found in North America. The Eastern American Toad (*B. americanus americanus)* is found throughout New England and southeast Canada. The range of the Dwarf American Toad (*B. americanus charlesmith*) is generally restricted to a smaller area in the southwest corner of the range of *B. a. americanus* (Figure 5).

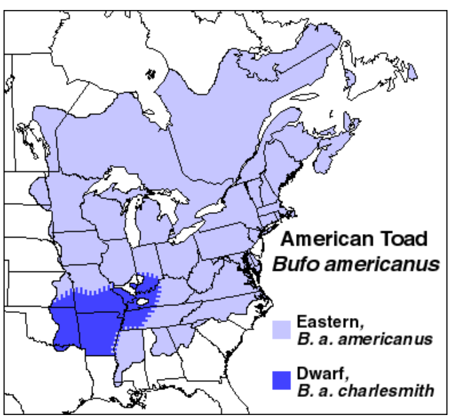


Figure 5 Range of the American Toad in North America

(www.npwrc.usgs.gov/narcam/idguide/american.htm)

The adult Eastern American Toad is slightly larger (5.1 to 8.9 cm) than the Dwarf American Toad. The appearance of the American Toad is somewhat variable, with colors can ranging from brown to red to olive. Generally, the skin is dark and the chest and abdomen are covered with warts (Figure 6).

Like most anurans, American Toads need shallow water for breeding, but will spend most of their lives in moist, humid environments. Breeding takes place from April to July. Eggs are laid in strings around vegetation.

Bufo americanus develops in the same manner, and at about the same rate, as *R. pipiens*. Limbaugh and Volpe (1957) identified the metamorphic stages of the Gulf Coast Toad (*B. valliceps*) which is similar to *B. americanus*.

Figure 6 Adult Eastern American Toad

(http://museum.gov.ns.ca/mnh/nature/frogs/toad.htm)

8.1 Source of Test Organisms

While adults of several species of toads and frogs are available for most of the year from commercial suppliers of living organisms, availability of eggs is more limited. Eggs of *Rana pipiens* and *Bufo americanus* can be collected in the wild during the spring. Since it may be difficult to distinguish between the eggs of related *Rana* and *Bufo* species, collectors should be well-trained in species' habitats and identification. If possible, adult animals should also be collected for identification in the same area that eggs are being collected.

Eggs of *Rana pipiens* can be obtained from at least two commercial suppliers from approximately November until March. These eggs are produced and fertilized in the laboratory and therefore it can be assumed that taxonomy is accurate. The contact information for two suppliers is given in Table 1. However, researchers are encouraged to use available resources, including the Internet, to find other suppliers.

Eggs received from commercial suppliers or collected in the wild should be subjected to a minimum of handling. Suppliers, like Carolina Biological, package and ship eggs in bags that have been injected with oxygen. Upon receipt these bags should be allowed to rise to test temperature (avoid rapid temperature changes) and placed in an environmental chamber or water bath at test temperature to hatch. Time to hatch will depend upon age at the time of shipping. Once the young embryos have developed into a recognizable tadpole and are actively moving, the bag can be opened and the eggs placed in an aquarium or other large chamber. If eggs are received in a container that has not been injected with oxygen, then the eggs should be carefully transferred to an aquarium. If the eggs have been cooled then they should be allowed to come up to room temperature in the original container before transfer. Always wear laboratory gloves (e.g., latex) when handling eggs, and gently pour the eggs to transfer. Once embryos have reached a distinctive tadpole shape, they are less prone to mortality from handling.

Table 1 Suppliers of Rana pipiens Eggs

Carolina Biological Supply Company 2700 York Road Burlington, NC 27215-3398 800-334-5551 Fax: 800-222-7112 www.carolina.com	Nasco 901 Janesville Ave. Fort Atkinson, WI 53538-0901 920-563-2446 Fax: 920-563-8296 www.enasco.com

9.0 Methods

9.1 Collection, Storage, and Manipulation of Sediment Samples

The method and number of samples (replicates) collected will be dependent upon site conditions. In shallow riverine and lentic systems it may be possible to wade to the collection location. However, sediment should be collected with as little disturbance as possible. Therefore, the number of field personnel wading in the water should be minimized. In a riverine system, a sample location should be approached from downstream so suspended material will be carried downstream, away from the sample site. It may be preferable to collect sediments from a boat (even if wading is possible) to minimize sediment disruption. Since the distribution of contaminants in sediment matrices can demonstrate a great deal of spatial variability, it may be preferable to collect multiple replicates. At a minimum, multiple samples should be collected and composited in the field so the sample better represents environmental conditions. Large pieces of plant material should be removed during collection. The exact collection procedures will depend upon study design. The statistical analyses that will be applied to the data should be considered during the study planning phase.

Sediment can be collected using several methods. In shallow water, sediment can be collected by hand, although the collector must wear durable, waterproof gloves that will prevent sample contamination as well protect the collector from chemical and physical injuries. If depth-specific testing is desired, a coring device may be required that maintains the integrity of the sediment profile. Grabs and dredges (e.g., Ekman, Ponar, Petersen, Van Veen) are often useful for collecting large amounts of sediment from deep water. The top of an Ekman grab can be opened to retrieve only the upper-most sediment layer (5-15 cm), which is usually the most biologically active. However, the effectiveness of Ekman grabs generally decreases as particle size increases. Highly unconsolidated sediment can be difficult to collect by any method.

Ten-day sediment toxicity tests with amphibians or other species require a minimum of 800 ml. Since samples will settle during storage and transport, at least one liter should be collected for each planned test. Since this amount does not allow for accidental loss, spillage, analytical chemistry, or test reruns, a minimum of two liters is recommended. The most convenient sample containers are wide-mouth, high-density polyethylene (HDPE) bottles. These are available from several distributors. Glass jars may be preferred for studies; however, these require greater care in handling and packing for shipment. If possible, samples should be cooled to 4°C before shipping and when not being used. Samples should not be frozen.

It is desirable to initiate tests as soon as possible following field collection of sediments. Some labile chemicals can degrade or volatize during storage. For these materials, a maximum holding time of two weeks (from the time of sample collection to test initiation) is recommended (Sarda and Burton, 1995). However, sediments can be stable for very long periods of time with little change in toxicity. Holding times should be specified in the project study plan.

Prior to test initiation, the sediment must be homogenized, even if it was already mixed in the field. Homogenization can be accomplished by using a tumbling or rolling mixer or other suitable apparatus. It can also be done using a stainless steel auger and drill or simply by hand with a stainless steel spoon. A minimum interval (at least three minutes) should be established for mixing each sample. A more heterogeneous sample would indicate the need for a longer mixing time. Augers, spoons, etc. must be washed and decontaminated between samples.

9.2 Testing

The standard study length is 10 days long. Savage et al. (2002) reported that mortality to *Rana sylvatica* continued to increase up to about 20 days of a 42-day exposure to PCB-contaminated sediment. However, a comparison of amphibian studies up to 21 days long, completed during development of this SOP, indicated that longer study durations do not necessarily result in greater effects (lower statistical endpoints such as a NOEC), using survival, total and body length, body width, weight, or metamorphic stage. In summary, young tadpoles are placed in beakers containing sediment and overlying water. The overlying water in each beaker is replaced continuously via a flow-through delivery system. The beakers are placed in a water bath or environmental chamber that is held constant at 23 ± 1°C. Water chemistry (e.g., pH, dissolved oxygen, temperature, etc.) is measured on the appropriate days. When the tadpoles reach stage 25 (all external evidence of gills is gone), they are fed a small amount of TetraMin® on a daily basis.

Beakers are examined daily for live organisms. If a cursory examination seems to indicate possible mortality in any one beaker, then all of the beakers in that treatment should be removed from the bath or environmental chamber and examined for dead organisms. Dead tadpoles must be removed. Live tadpoles are left in the chamber and it is placed back into the water bath or environmental chamber.

At the end of the test (10 days), final overlying water chemistry samples are collected and measured. These parameters include, at a minimum, temperature, DO, pH, and conductivity in, and, at the Study Director's discretion, hardness and alkalinity. All living organisms are counted and removed for sublethal (width and body length) measurements. Sediment and/or water can be collected for chemical analysis, if necessary.

Test specifications are listed in Table 2 and specific daily activities are listed below.

Day –1

Place 100 ml of homogenized sediment, including control sediment, in each of the test chambers. Add 175 ml of overlying water to each chamber. Add the water carefully to avoid, as much as possible, suspension of sediment. Do not start flow-through system yet.

Day 0

Begin flow-through system. Water flow rate should be slow, so as not to disturb the sediment in the test beakers. Set the rate so that the test chamber volume is replaced two to four times during each 24-hour period. After at least one hour collect overlying water for initial water characterization (dissolved oxygen [DO] temperature, pH, conductivity, hardness, alkalinity, ammonia, and total residual chlorine).

If DO in any test chamber is less than 3.0 mg/L, increase the flow rate of the incoming water slightly. This must be done for all test chambers. After one hour, recheck the DO, if it is still low, begin aeration of all test chambers. Set aeration tubes or pipettes (Pasteur pipettes work well) so that the tip is no more than 0.5 cm under the water's surface. After aerating the test chambers for approximately 30 minutes, recheck the DO to ensure that the level has increased to >3.0 mg/L.

If total ammonia concentrations are >5.0 mg/L, a second sample should be collected and retested. If ammonia levels are still high, then the test can proceed but a notation should be made of the high levels. Ammonia concentrations >5.0 mg/L may be high enough to cause adverse effects to the test organisms.

Add five tadpoles to each test chamber. At ≤72 hours in age, all tadpoles should be very close in size; avoid using animals that are noticeably small or large. Also, do not use animals that exhibit unusual behavior or are deformed. To transfer organisms, use a glass pipette and gently place them in the test chambers. Release organisms under the water's surface. Minimize the amount of water transferred with the organisms. Rinse the pipette with deionized water before obtaining more organisms.

After all organisms have been placed in the test chambers, return the chambers to the water bath or environmental chamber. Check DO within one to two hours after the organisms have been added to the chambers. If DO is low (< 3.0 mg/L) follow the procedures described above for increasing flow or adding aeration.

From the remaining batch of tadpoles, select 5 to 10 for possible examination of metamorphic stage. These organisms should be preserved with 70% isopropanol or 10% formalin. If the tissue concentration of specific chemicals is to be measured, additional organisms must be collected for determination of initial concentration. The amount of tissue needed for analysis varies with the specific analyte. Check with the analytical laboratory to determine how much tissue will be needed. Animals for tissue analysis must be frozen unless they are processed and analyzed immediately.

Days 1-9

Examine organisms from at least three beakers each day to determine metamorphic stage. At hatch, tadpoles are at stage 20. It takes approximately 4 to 6 days for hatched tadpoles to reach stage 25, when feeding begins. Therefore, if tests are initiated with <24 h-old organisms, feeding will begin about midway through the test. However, if tests are initiated with 72-hour organisms, feeding may begin on day 1 or 2. If organisms are at stage 25, feeding should begin with approximately 4 mg of ground, dry TetraMin® per chamber. Adding excess food should be avoided since it can cause a reduction in DO concentrations that may result in mortality.

Each chamber should be examined for living organisms each day. If no organisms are seen swimming, then the chamber should be removed and examined carefully. Dead organisms must be removed.

The following water characterizations are made:

- Temperature: continuously in the water bath or environmental chamber and in each treatment (one replicate only) on days 3, 6, and 9.
- Dissolved oxygen: daily in each treatment (one replicate only) and in any chamber where mortality has occurred or where water quality is in question.
- pH: in each treatment (one replicate only) on days 3, 6, and 9 and in any chamber where mortality has occurred or where water quality is in question.
- Ammonia: at least twice in each treatment during the course of the study. For example, days 3 and 7.

Day 10

Final water characterizations are made:

- Temperature, DO, pH, conductivity in each test treatment. At the Study Director's discretion, hardness and alkalinity may be measured as well.

Remove live organisms from each test chamber and transfer them to small beakers (glass or plastic) containing 10 to 20 ml of clean (unchlorinated) water. Tadpoles can easily blend in with some sediment and often move very little, even with prodding. Test chambers should be examined thoroughly to find any live organisms. When pouring out water for chemistry or disposal, pour the water through a net to catch any tadpoles that may have been missed.

Live tadpoles must be anesthetized or killed before sublethal measurements can be made. The use of 3-aminobenzoic acid ethyl ester (MS-222) is recommended. To each of the small beakers containing tadpoles, add approximately 1 ml of a stock solution (2 g/liter) of MS-222. If organisms continue to move after several minutes, add a few additional drops of the anesthetic. Tadpoles should not be left in the MS-222 solution for an extended period of time since tadpoles will begin to fall apart.

Using a clear metric ruler, measure the maximum body width and body length. The maximum body width is the widest part of the cephalothorax (excluding the tail). Body length is the distance from snout to the base of the tail where it emerges from the body (Figure 7).

Figure 7 Measurement of Body Width

Table 2
Test Specifications

Test Organism	*Rana pipiens* or small *Bufo* species
Test Organism Age	≤72 hours
Test Duration	10 days
Test Chambers	500 ml beakers or chambers with drainage system
Vol. of Sediment	100 mls
Vol. of Overlying Water	175 mls
Replicates	Minimum of 8
Organisms/replicates	Minimum of 5
Control Sediment	Uncontaminated natural sediment or formulated sediment that has been shown to have no adverse effects on test organisms over the study period
Overlying Water	Site water, site water match (hardness and alkalinity), natural lake or groundwater, or reconstituted laboratory water (e.g., moderately hard (USEPA 1994a))
Test Temperature	23 ± 1°C
Dissolved Oxygen	≥3.0 mg/L
Solution Renewal	Continuous flow-through
Feeding	4 mg TetraMin per vessel daily after tadpoles reach stage 25
Test Endpoints	Survival, body width, and body length
Acceptability	Mean control survival of at least 80%

9.3 Data Analysis

Mortality or apparent size reduction in any sediment treatment is not necessarily an indication of toxicity. Statistical analysis must be used to determine if apparent differences are significant. Organism response to test sediments is typically compared to the control response. If a reference sediment (e.g., upstream of a study site) was also collected, then the Study Director or Study Sponsor may choose to compare test sediments against the reference sediment. Two types of data are obtained from the toxicity test: acute (mortality) and chronic (width and length). Each data type should be analyzed independently. If other measurements are also obtained (e.g., weight or tissue burden) then those data can also be analyzed separately.

Data analysis is in two general forms: hypothesis testing and point estimation. Hypothesis testing involves assigning an alpha level for the analysis and then, using that criterion, determining which treatments are significantly different from the control. If only bulk sediment is tested, then data analysis will consist only of hypothesis testing. If however, a series of sediment dilutions were prepared (i.e., mixing test sediment with control sediment at fixed percentages [6.25, 12.5, 25, 50]), or if sediment samples represented a true concentration gradient for a chemical of concern, then point estimates can be made. A point estimate, such as an LC_{50}, is a concentration of test media at which a certain effect (e.g., half the test organisms die) is determined to occur. General guidance for conducting these analyses is given in the following sections.

9.3.1 Hypothesis Testing

Hypothesis testing should follow the same general structure as described by USEPA (1994a; 2000). In summary, mortality/survival data are analyzed first. If there is a significant reduction in survival in any treatment, that treatment is dropped from analysis of sublethal data. Determination of significant effects is dependent upon the predetermined alpha level. The alpha level, or α, is defined as the probability of committing a Type I statistical error - rejecting the null hypothesis (H_o) of no effect, even if H_o is true. That is, concluding a sample is toxic, even when it isn't (Table 3).

Table 3 Statistical Errors

Decision	If H_o is True	If H_o is False
H_o Rejected	Type I error (α)	No error
H_o Accepted	No error	Type II error (β)

The majority of studies in environmental toxicology are analyzed with an α of 0.05, which means there is a theoretical 5% chance that a Type I error will be committed. The α level is not fixed and can be changed, depending upon the objectives of the study. A lower α - 0.01 for example – will reduce the likelihood of a Type I error. However, it will also increase the likelihood of a Type II error (β), that is, concluding that a sample is not toxic when it, in fact, is. Historically, β and its inverse (1-β), which is the associated power of the test, have generally been ignored by environmental researchers. However, because the power of a test is defined as the probability of correctly detecting a true toxic effect, considering β may be important in designing a study. If α is held constant, for example, β decreases (and test power increases) as the sample size increases and variance decreases (Denton and Norberg-King 1996).

Since survival data often demonstrate non-normal distributions, proportional survival data are first transformed using an arc sine-squareroot transformation. The normality and homogeneity of variance are then evaluated using tests such as Shapiro-Wilk's and Bartlett's, respectively. If data are found to meet the normality and homogeneity of variance requirements of parametric tests, then differences from the control can be analyzed with Dunnett's Procedure (for an equal number of replicates) or a T-Test with Bonferroni adjustments (for unequal replicates). If data do not meet the assumptions for a parametric test, then nonparametric (rank) tests have to be used. The most common tests are Steel's Many-One Rank Test (for equal replicates) or Wilcoxon Rank Sum Test with Bonferroni adjustments (for unequal replicates).

While these statistical tests are the ones most commonly used in the analysis of toxicity data, they are not the only ones available. For example, the Study Director may want to determine if test sediments are significantly different from each other, as well as from the control. In that case, analysis of variance with Tukey's multiple range test (parametric) or a Kruskal-Wallis test (nonparametric) may be appropriate. Because of the many tests that are available, it is important that the project goals be thoroughly defined before data are collected.

Sublethal effects are analyzed after acute effects have been evaluated. For any replicate, individual sublethal measurements are averaged to produce a mean width and length (per surviving organism) for each replicate. For example, if there are four surviving organisms in one replicate and the measured widths are 3.5, 4.0. 4.0, and 4.5 mm, then the mean width for the replicate is 4.0 mm. If there was significant mortality in any test treatment, that treatment is typically dropped from analysis of sublethal effects. Sublethal measurements are continuous data and therefore do not need to be transformed (arc sine-squareroot) before analysis. With that exception, the analysis of sublethal endpoints is the same as for survival.

9.3.2 Point Estimates

Point estimations are seldom used in sediment tests because there is generally no known concentration gradient of a particular chemical of concern. In addition, sediments may contain multiple toxicants that could act independently or have synergistic, additive, or antagonistic effects. For example, if a sediment (e.g., from a historical mining district) has high concentrations of copper, zinc, and cadmium, all of which may be at toxic levels, a point estimate based on the concentration of any one metal may be meaningless because of the presence of the other metals. However, point estimates could be calculated based upon the percent (weight or volume) of a test sediment mixed with a nontoxic control sediment. If this method is used, then both sediments should have approximately the same moisture fraction so that the percentage estimates are reasonably accurate. Point estimates could also be used if samples are collected along a known concentration gradient for one particular chemical and no other chemicals of concern are present. Finally, if spiked sediment tests are conducted where different treatments of sediment contain variable but known quantities of a particular chemical, then point estimates can be made.

Any of the point estimation procedures calculate a concentration (mass per volume or percent) at which a certain effect will occur. An LC_{50}, for example, is the concentration at which 50% of the organisms are expected to die while an IC_{25} is the concentration which causes a 25% reduction in the endpoint of interest. The manner in which LC_{50}s (or EC_{50}s which are the same thing except with an endpoint other than death) are calculated varies with the structure of the data. For example, if the responses in the test treatments are all or nothing (either everything is alive or everything is dead), than the simplest method – graphical – is used. LC_{50}s using the graphical method, like the name implies, are calculated on graph paper, although a simpler method is simply calculating the geometric mean of the highest "all-alive" concentration and the lowest "all-dead" concentration. If there is partial mortality in any test treatment then a Spearman-Karber, Trimmed Spearman-Karber, or Probit method must be used. These methods are described in detail in Section 11 of USEPA (1993). In brief, if there are two or more treatments with partial mortality, then use of the Probit method (parametric) is indicated. In situations where the Probit method is inappropriate due to non-normal or significantly heterogeneous data, the Trimmed Spearman-Karber or Spearman-Karber Methods may be used. These LC_{50} procedures are available with a variety of computer software programs (e.g., USEPA 1994b).

LC_{50} models, by definition, are used to calculate point estimates for mortality endpoints, although they can also be used to calculate point estimates for nonlethal endpoints (EC_{50}). The Linear Interpolation Method was developed for the general application to data generated during chronic toxicity tests. The endpoint generated by the Linear Interpolation Method is an ICp value, where IC = Inhibition Concentration and p is the percent effect. The value of p can be adjusted, although the most typical values are 25 and 50. The Linear Interpolation Model assumes a linear response from one concentration to the next and assumes that the mean response of the next higher concentration will be equal to or less than the preceding concentration. If this is not the case, the data are adjusted by smoothing. A more thorough discussion of the Linear Interpolation Model is provided by Norberg-King (1993).

10.0 Quality Control Checks and Acceptance Criteria

- If survival in the control treatment is less than 80%, then the test data should be carefully examined to determine if it is acceptable. Survival in controls sometimes does not meet the acceptability criterion, especially in sediment tests. However, even if control survival is <80%, test data may still be valuable and yield important results. The following test data should be examined:
- Survival in all test treatments. If survival in test treatments is greater than in the control, then it can be concluded that field-collected sediments are not acutely toxic.
- Variability within a treatment. If mortality is highly variable and scattered throughout the test, then the test might not be acceptable. Highly variable survival may be due to variations in water chemistry (e.g., low DOs or elevated ammonia due to excess food in some chambers), variability in organism health, or differences in how chambers were treated (e.g., different amounts of food or flow rates of overlying water).
- Water chemistry. Highly variable water chemistry may indicate the sediment was not sufficiently homogenized or differences in flow rates.

It may be noted that there are no specific acceptability requirements for survival in test treatments collected from reference stations. However if survival is significantly reduced, then questions are raised as to the appropriateness of the reference site.

Reference toxicant data for a given batch of organisms should fall within the historical 95% limits for that species. However, data falling outside the range does not necessarily indicate automatic rejection of the data (see Section 4.0).

11.0 Documentation

Chemical and biological monitoring information must be recorded on appropriate data sheets.

12.0 References

Analytical Software. 2000. Statistix 7. Analytical Software, Tallahassee, FL.

Denton, D.L. and T.J. Norberg-King. 1996. Whole Effluent Toxicity Statistics: A

Regulatory Perspective. Ppp. 83-102 *in* Dr. Grothe, K.L. Dickson, and D.K. Reed-Judkins, eds., *Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving System Impacts*. SETAC Press, Pensacola.

Gosner, K.L. 1960. A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. Herpetologica. 16:183-190.

Limbaugh, B.A. and E. P. Volpe. 1957. Early Development of the Gulf Coast Toad, Bufo valliceps Wiegmann. American Museum Novitates, 1842:1-32.

Norberg-King, T.J. 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0). NETAC Technical Report 03-93, United States Environmental Protection Agency, Duluth, MN.

Sarda, N. and G. A. Burton, Jr. 1995. Ammonia Variation in Sediments: Spatial, Temporal and Method-Related Effects. Environ. Toxicol. Chem. 14:1499-1506.

Savage, W.K., F.W. Quimby, and A.P. DeCaprio. 2002. Lethal and Sublethal Effects of Polychlorinated Biphenyls on *Rana sylvatica* tadpoles. Environ. Toxicol. Chem. 21:168-174.

Shumway, W. 1940. Stages in the Normal Development of *Rana pipiens*. The Anatomical Record. 78:139-147.

USEPA. 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fourth Edition. EPA/600/4-90/027F.

USEPA. 1994a. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Third Edition. EPA/600/4-91/002.

USEPA. 1994b. USEPA Toxicity Data Analysis Software. Version 1.5. United States Environmental Protection Agency, Cincinnati, OH.

USEPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates. Second Edition. EPA/600/R-99/064.

WEST, Inc. and D.D. Gulley. 1996. Toxstat Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, WY.

APPENDIX D

SOP VALIDATION

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SECTION 1
INTRODUCTION

This appendix describes the validation of a laboratory toxicity testing technique designed to evaluate the potential effects of sediment/hydric soil exposure to early life stage amphibians. This validation was part of an overall evaluation of the use of amphibian testing as a risk assessment tool at sites owned and/or operated by the United States Navy.

1.1 Project Scope

This phase of the project involves validating the laboratory toxicity testing technique described in the Standard Operating Procedure (SOP), entitled *Development of a Short Term Chronic Sediment Toxicity Test Using Early Life Stage Amphibians*, and presented in Attachment C-1 of this guidance document. The validation process involved conducting amphibian toxicity testing according to the procedures presented in the SOP, conducting a series of tests with matrix spikes of cadmium, copper, lead, and/or zinc, and evaluating the responses of the amphibians and any relationships among a sub-set of test variables (i.e., metals concentration, total organic carbon, dissolved organic carbon, hardness). The primary objective of this phase of work was to provide the Navy with a series of dose-response curves whereby effect concentrations can be estimated based on ambient conditions. In addition, the validation testing resulted in a limited data set that can be used to evaluate media concentrations (e.g., in sediment, water, and tissue) that may be associated with adverse impacts to amphibians.

1.2 Appendix Organization

This appendix is organized in the following manner:

- Section 2 provides a summary of existing amphibian toxicity testing methodologies referenced in scientific literature;
- Section 3 presents the project-specific laboratory test conditions used in the validation phase of this YO817 project;
- Section 4 presents a discussion of the laboratory tests;
- Section 5 presents a discussion of the results;
- Section 6 includes a summary and conclusions; and
- Section 7 includes a list of references cited in this report.

SECTION 2
LITERATURE REVIEW: AMPHIBIAN TOXICITY TESTING LABORATORY METHODS

Toxicity testing has been used to evaluate the effects of water on aquatic species for several decades. Generally, these testing procedures have focused on the use of fish and other aquatic species in effluent testing and testing the toxicity of specific chemicals. However, the importance of sediments as a potential contributor of environmental contamination has triggered the development of test procedures for evaluating sediment toxicity. Recently published methods for freshwater testing (USEPA, 2000; ASTM, 2001a) include tests for an amphipod (*Hyalella azteca*), a dipteran midge (*Chironomus tentans*), and an oligochaete (*Lumbriculus variegatus*). Currently, USEPA and ASTM do not present standardized sediment test methods for amphibians. However, as described in the following text some semi-standardized amphibian toxicity test methods do exist and research scientists have developed a variety of testing methods to support specific research needs.

2.1 Established Amphibian Test Methods

ASTM provides two laboratory toxicity testing methods that can use amphibians, one for ambient water samples and effluents (1192-97) and one for test materials (729-96) (ASTM, 2001b; and ASTM, 2001c). These methods are both intended for evaluating the exposure of amphibians in a liquid matrix. ASTM also publishes the guide for conducting the Frog-Embryo Teratogenesis Assay-Xenopus (FETAX) (ASTM, 2001d). This study procedure includes the exposure of African clawed frog (*X. laevis)* embryos to a test solution to which some test material has been added. This method was developed as a water-only exposure. In addition, this assay is conducted with an exotic species and, even though endpoints (e.g., development and teratogenesis) are sublethal, it is only a 96-hour study and thus may not be representative of more commonly experienced chronic exposures. Lastly, *X. laevis* may be a more tolerant species that is less suitable for routine use in toxicity testing associated with development of aquatic life criteria (Birge, et al, 2000; ENSR, 2001).

The USEPA Office of Prevention, Pesticides, and Toxic Substances (OPPTS) publishes test guidelines "for use in the testing of pesticides and toxic substances and the development of test data that must be submitted to the Agency for review under Federal regulations." OPPTS 850.1800 is the guidance for conducting sediment tests with tadpoles (USEPA, 1996). The guidance is intended for use when a sediment or slurry has been spiked with a chemical and exposes older tadpoles (i.e., with hind legs already emerged) for a 30-day exposure duration. While the ASTM and OPPTS test methods provide some guidance for conducting amphibian studies they are not appropriate for evaluating potential impacts of sediments on early life stage indigenous amphibians.

2.2 Amphibian Research

A number of researchers have modified established testing methods to evaluate the impacts of chemical and solar stressors on amphibian receptors in the field and in the laboratory. Topics of interest have included evaluating the impacts of pesticides and herbicides on amphibian development, the sensitivity of amphibians to metals and organic compounds, and the influence of UV radiation on the toxicity of contaminants.

2.2.1 Effects of Pesticides and Herbicides

In the majority of pesticide and herbicide studies reviewed, amphibians were generally exposed to varying concentrations of the target analyte in water and lethal and sublethal endpoints were evaluated. Allran and Karasov (2000) exposed *R. pipiens* from Gosner stage 25 (Gosner, 1960) through metamorphosis to three concentrations of the herbicide atrazine (0, 20, 200 ug/L) and three concentrations of nitrate (0, 5, 30 mg NO_3-N/L). Tadpoles were exposed in 2.5-gallon aquaria containing 7 liters (L) of treatment solution. Test solutions were renewed every 48 hours and all dead tadpoles were removed during renewal. Every 7 days, length and Gosner stage were measured for 10 randomly selected larvae from each tank. At metamorphosis individuals were collected and placed in aquaria containing 1 L of treatment solution. The aquaria were slanted to create a bank where developing juveniles could climb out of the water. Upon tail resorption, individuals were anesthetized, weighed, and euthanized after blood was drawn. The experiment terminated at 138 days, when 90% of the tadpoles completed metamorphosis. Artificial pond microcosms containing pond water, phytoplankton, periphyton, macrophytes and larval gray tree frogs (*Hyla versicolor*) have also been used to evaluate the effects of atrazine on amphibians (Diana, et al., 2000).

Berrill, et al. (1994) used similar water-only methods to evaluate the effects of three pesticides (fenitrothion, triclopyr, and hexazinone) on embryos and tadpoles of *R. pipiens*, *R. clamitans*, and *R. catesbeiana*. Pesticide composition and concentrations were selected to approximate the formulations used in forest spraying and field collected embyros and tadpoles were exposed to various concentrations of the pesticides in water for 9 days. Hatching success, time of hatching, and gross abnormalities were evaluated for embryos; mortality, length and Gosner stage were determined for the tadpoles. Fordham, et al. (2001) performed a similar evaluation using the pesticide malathion, Gosner stage 26 to 28 *R. catesbeiana* tadpoles, and a 28-day exposure period. In addition to survival, length, weight and stage measurements, tadpoles were evaluated for loss of equilibrium.

In situ testing has been conducted with caged eggs to evaluate the impact of pesticides and herbicides near the site of application. Harris, et al (1998) used *in situ* and laboratory bioassays to evaluate the potential impacts of pesticides and eutrophic conditions on amphibians in wetlands in managed orchards. The results of laboratory and *in situ* water-only exposures of *R. pipiens* and *R. clamitans* were compared to determine whether the eutrophic conditions within the wetlands offered any protection from the pesticides. Hatching success, survival, and tadpole length were measured for all tests.

Greenhouse (1976) fertilized *X. laevis* and *R. pipiens* eggs in the laboratory and exposed the resulting tadpoles to pesticides in aquaria to evaluate mortality and gross abnormalities. Birge and Just (1973) and Cabejszek and Wojcik (1968) performed similar tests with *X. laevis* tadpoles exposed to metoxychloride (an insecticide) and heavy metals and determined that frog embryos were well suited to water quality bioassays. These early tests helped to develop the FETAX assay now used to evaluate teratogenesis of various chemicals (ASTM, 2001d).

2.2.2 Sensitivity to Metals and Organic Compounds

Birge et al (2000) conducted a number of acute exposure toxicity tests with 25 species of amphibians and compared toxicity results with results for the rainbow trout (a sensitive benchmark species commonly used in toxicity criterion development). This comparison was used to evaluate the relative sensitivity of the amphibian species for a variety of metals and organic compounds. Testing protocols, like those for pesticide evaluations, consisted of water-only exposures of amphibians from fertilization through four days post-hatch. Survival was the primary endpoint measurement. Median lethal concentration

(LC-50) values were calculated for each test and compared against LC-50s calculated for the rainbow trout.

While most amphibian toxicity testing is based on water-only exposures, Savage et al. (2002) conducted tests with field-collected sediments containing polychlorinated biphenyls (PCBs). They used wood frog (*R. sylvatica)* to assess acute and chronic effects in a 42-day test. Exposures were in aquaria with 20 to 40 grams (g) of sediment and 3 L of overlying water. Test conditions were static-renewal with partial water replacement every 3 to 5 days. Tadpoles at Gosner stages 23 to 25 were used to initiate the studies. Some tadpoles were exposed directly to the sediment and others were suspended above the sediment in mesh containers to avoid direct contact. Survival, length, weight, and developmental rate (metamorphic stage) were evaluated every 7 days and swimming speed was evaluated on Day 12.

2.2.3 Effects of Ultraviolet Radiation

Ultraviolet (UV) light, particularly UV-B radiation, appears to reduce the hatching success of embryos in a manner correlated with the species-specific capability of amphibian eggs to repair UV-B induced damage. UV light may also act synergistically with other environmental pollutants. Hatch and Burton (1998) conducted a series of acute experiments with three amphibian species to evaluate the photoinduced toxicity of fluoranthene in the laboratory and outdoors. Amphibian eggs in water were exposed to fluoranthene and various intensities of UV light in the laboratory and survival, growth, and malformation measurements were made at test termination. The outdoor tests evaluated the hatching success of embryos in water exposed to fluoranthene in sunlight. Covers on some test chambers filtered out different intensities of UV light. Time to death and hatching success rates were then compared against UV light intensities.

Kagan, et al. (1984) performed a similar experiment with anthracene and alpha-terthienyl and late embryonic *R. pipiens*. Test vessels containing 20 milliliters (ml) of pond water with anthracene or alpha-terthienyl and 20 embryos were irradiated between 30 minutes and 5 hours. Controls were not irradiated. At the end of the exposure period, mortality was evaluated.

2.3 Summary

Although amphibians have been appropriately coined a keystone species as well as an indicator/sentinel member of their ecological community (Murphy et al., 2000), few standardized test methods exist for evaluating impacts to amphibians from environmental stressors. The existing test methods and the majority of research conducted with amphibians has focused on the impacts associated with water, not sediment, exposure. In addition, a variety of species, endpoints, and testing durations have been used to evaluate effects. In general, there is a lack of information regarding the potential toxicological impacts to amphibians from contaminated sediments or hydric soils in wetlands.

The purpose of this current YO817 study was to develop and validate an amphibian test method that can be applicable to the evaluation of environmental sediment and hydric soil samples and be cost-effective so that a large number of samples can be tested, if needed. The test method was also developed to be consistent with already-existing procedures for sediment tests (e.g. *H. azteca* sediment tests).

SECTION 3
TEST METHODS

The following sections describe the test methodology followed to validate the proposed SOP developed to evaluate the potential effects of sediment/hydric soil exposure to early life stage amphibians (ENSR, 2002). Laboratory toxicity testing in support of this component of the YO817 program was conducted between March and June of 2002, at the ENSR Environmental Toxicology Laboratory, Fort Collins, Colorado (CO).

3.1 Test Organisms

Rana sp. were obtained from Carolina Biological Supply in Burlington, North Carolina. During certain times of the year (approximately November through late February or March) Carolina produces eggs in their laboratory by artificial fertilization. Therefore, laboratory-produced eggs are known to be *R. pipiens*. Laboratory production drops substantially in the spring and becomes unreliable. All the organisms used in these studies were obtained from wild-collected eggs. Although *R. pipiens* was requested, the exact species of *Rana* cannot be stated with certainty, although it was believed to be *R. pipiens*. *Bufo americanus* eggs were also collected in the wild at the former South Weymouth Naval Air Station, Massachusetts.

Rana eggs from Carolina Biological Supply were received in plastic bags injected with oxygen before shipment. Eggs were left in the bags in a temperature-controlled water bath (23°C) until they began to hatch; the embryos were then transferred to an aquarium with Horsetooth Reservoir (HT) water. Hatch rate using this method was generally high (>70%). *Bufo* eggs were sent in a thermos bottle and packed with ice in a cooler. Upon receipt the eggs were immediately transferred to a shallow dish containing water from Horsetooth Reservoir. The water was aerated. Nearly 100% of the *Bufo* eggs hatched. All of the batches received are listed in Table 3-1.

After hatching, live tadpoles were transferred to five-gallon aquaria containing HT water, which was constantly renewed at a slow rate. When tadpoles reached Gosner stage 25 (disappearance of all external gill structures), the animals were fed ground Tetramin® flake food *ad libitum* on a daily basis. Organisms were generally 48 to 72-hours old when tests were initiated, although some were slightly older.

3.2 Preparation of Test Sediment

Sediment was spiked with copper (Cu), cadmium (Cd), lead (Pb), or zinc (Zn) for testing. The sediment composition was the same for all tests. The base sediment was collected from the flood plain immediately adjacent to the Cache la Poudre River (PR) north of Fort Collins, CO. PR sediment is very sandy with a relatively low total organic carbon (TOC) concentration of 1,300 mg/kg. In order to enhance the TOC levels of the PR sediment, the sediment was amended with 15% (by weight) sheep/peat garden compost. After amending with the compost, the TOC concentration was measured to be 14,000 mg/kg. Prior to use in this toxicity testing program, the PR and compost mix was thoroughly homogenized in an end-over-end tumbler for at least 30 minutes at a rate of approximately 32 rotations per minute.

Stock solutions of the divalent metals were prepared by using $CuCl_2$, $CdCl_2$, $PbCl_2$, or $ZnCl_2$. Salts were added to deionized (Milli-Q) water to prepare high-concentration solutions (e.g., 5,000 µg/L Cu as $CuCl_2$). Amended PR sediment was placed in clean, 1-gallon jars and slightly wetted with Milli-Q water. The sediment/water mixture was shaken manually to distribute the moisture and form a slightly sticky mixture. Spiking methods were conducted in general accordance with those described by Ditsworth et al. (1990). Five holes were "punched" into the sediment with pipets. The volume of stock solution needed to provide the necessary amount of metals was then placed in equal amounts in the holes. After addition of the metals, the jars were sealed and tumbled end-over-end for a minimum of 30 minutes. The jars were stored in the dark at 4°C until use. The jars were shaken manually just before use. All toxicity tests were conducted within 2 days of mixing (i.e., no sediment aging studies were conducted).

3.3 Toxicity Testing Methodology

The second phase of this YO817 project resulted in a proposed SOP (ENSR, 2002) developed for the evaluation of sediment and hydric soil using early life stage amphibians. The purpose of the SOP is to help evaluate possible effects of chemical stressors in sediments and hydric soils on amphibians in natural ecosystems. The test method uses an early life stage of a native North American species, and lethal and sub-lethal toxicity endpoints that are relevant to typical assessment endpoints considered by the Navy in their ecological risk assessments. Table 3-2 is based on the SOP presented in the *Development of a Short Term Chronic Sediment Toxicity Test Using Early Life Stage Amphibians* (ENSR, 2002) and summarizes the exposure parameters of the amphibian toxicity test used in the spiking studies.

3.4 Spiked-Sediment Toxicity Tests

A total of 19 tests were conducted during this phase of the research (Table 3-3). Of those 19 studies, 12 were studies in which single metals (Cu, Cd, Pb, or Zn) were spiked into sediment (PR amended with 15% sheep/peat) and tested as described in this section. Eight of these sediment tests were with *Rana* and four were with *Bufo americanus*. Five tests were conducted in which tadpoles were placed in test chambers containing water only, spiked with either copper (three tests) or cadmium (one test). These tests were prepared as single-replicate studies to generate data for a reference toxicant database; data from these tests are not presented here. Finally, two studies - 033 and 034 - were designed to investigate the effects of organic carbon on copper or zinc toxicity. These tests are described in greater detail in Section 3.6.

Spiked sediment and overlying water were added to 500-ml beakers on Day –1 (i.e., approximately 24 hours before organisms were added). Each beaker contained 100 mls of sediment and 175 mls of overlying water. The overlying water was HT water. The beakers with sediment and overlying water were placed in a water bath at 23±1°C and allowed to settle overnight. After the overnight settling period, tadpoles were added to each test chamber.

Each experiment included four to five metal concentrations and one control. Table 3-3 summarizes the testing program conducted during this stage of the YO817 program. The control was amended PR sediment with no metals added. Each treatment included four replicates with five organisms per test chamber, for a total of 20 organisms per treatment. All organisms used in the spiked sediment studies were ≤96 hours old. At test initiation, tadpoles were generally at Gosner stage 24 or less, with the right operculum still visible. Tadpoles usually advanced to stage 25 within one to three days, at which time feeding was initiated. Tadpoles were fed approximately 4 mg of dry TetraMin® fish food daily.

Overlying water was replaced continuously in all chambers via a flow-through system which consisted of a PVC manifold and valves that could be adjusted to introduce HT water at a very slow rate (Figure 3-1). Each test chamber received 2 to 4 volume additions every 24 hours. Tests were monitored daily for dissolved oxygen, pH, and temperature. These parameters were measured in one replicate from each treatment. Temperature in the water bath was monitored continuously. If mortality occurred in any individual test chamber that appeared to be anomalous with other chambers in that treatment, the water quality of that chamber was checked (e.g., to determine if there was low dissolved oxygen).

Although the test chambers were generally examined daily, the number of surviving organisms could often not be fully determined. Tadpoles often blended in well with the sediment and were hard to see unless they were against a strongly contrasting background (Figure 3-2). At the end of the 10-day exposure period, final water chemistry measurements were made and the number of surviving organisms was recorded. Animals were then anaesthetized with MS-222 (3-aminobenzoic acid ethyl ester, methanesulfonate salt, an anaesthetic). Body width was measured as the widest part of the tadpole body and body length was measured as the tip of the snout to the base of the tail. Width and length were recorded to the nearest 0.5 mm.

3.5 Analytical Chemistry

Sediment, water, and tissue samples were collected to quantify the amount of each metal in the various media. Sediment and overlying water samples were collected at test initiation and at test termination. In most cases, samples were composites of all test replicates, although a limited number of individual replicate samples were collected to evaluate within-treatment variability.

At test initiation, sediment was collected from each of the jars that contained the mixed, spiked sediment for each treatment. Overlying water was collected from each treatment after the overnight settling period and before organisms were added. Approximately 15 ml of water from each of the four replicates were sampled and combined. Both total recoverable (TR) and dissolved (DIS) phase water samples were collected. Dissolved phase water samples were collected by filtering overlying water with 0.45 μm syringe filters (e.g., Whatman® 25 mm GD/XP polyethersulfone filter with polypropylene housing). At the end of the test, composite samples were collected from each treatment by combining water or sediment from each replicate. After measuring width and length, selected tadpoles were also collected to measure the tissue concentration of the test metal. All tadpoles within a treatment were combined for analysis. All samples for metal analysis were placed in 50 ml "bullet" tubes (VWR brand). Water samples were preserved with nitric acid to a pH of <2. Sediment and tissue samples were frozen until analysis.

All analyses were completed according to SW-846, 3rd Edition (USEPA, 1986). Solid matrix samples (sediment and tissue) were digested according to method 3050B and trace ICP (Inductively Coupled Plasma emission) analysis was completed according to method 6010B. For water samples, digestion was according to 3005A and conventional ICP analysis was per method 6010B.

3.6 Organic Carbon Studies

Many of the wetlands at Navy palustrine wetland sites contain soils/sediments with elevated levels of organic carbon. It is known that elevated dissolved organic carbon in the water column can reduce the toxic effects of certain metals, such as copper, by binding the copper and making it biologically unavailable (i.e., see USEPA, 1999). Therefore, as part of this YO817 program, two studies were conducted to determine the relationship(s) between various levels of organic carbon and the toxicity of copper to amphibians. One study involved exposing tadpoles to sediment containing different levels of organic matter, zinc, and copper; the second study was a water-only test in which tadpoles were exposed to test water containing different levels of organic matter and copper. Each study is described below.

3.6.1 Organic Carbon – Sediment Exposures

As described in Section 3.2, the base sediment used in all of the regular spiked-sediment tests was PR sediment with 15% sheep/peat compost added, by weight. In order to assess the effects of different levels of organic carbon in the test matrix, this sediment was used as an example of a high-organic carbon sediment. Three other sediments with lower levels of organic carbon were used in this study; (1) washed Silica Sand, (2) Unaltered Poudre River Sediment, and (3) Poudre River Sediment amended with 7.5% (by weight) sheep/peat compost. The measured levels of total organic carbon in each of these sediments were: 125 mg/kg, 1,300 mg/kg, and 13,000 mg/kg, respectively.

Each of the four sediments was spiked with two different levels of copper (150 and 300 mg/kg nominal concentrations) and one concentration of zinc (1,000 mg/kg nominal). Metals were added in the same manner as the regular spiked-sediment studies described in Section 3.2. A control (no added copper or zinc) was included for each sediment. Tests were initiated with *B. americanus* and were conducted in the same manner as the spiked-sediment tests and were 10 days in duration. Organism survival was monitored daily; tadpole width and length were measured at the end of the test.

3.6.2 Organic Carbon – Water-Column Exposures

Tadpoles (*B. americanus*) were exposed to six waters containing increasing concentrations of dissolved organic carbon. The waters were reconstituted moderately hard water (U.S.EPA, 1993), HT water (unamended), and HT water amended with four different amounts of sheep/peat compost. The amended HT water was prepared by adding 100, 500, 800, or 1,500 mg of sheep/peat per liter of HT water. The sheep/peat was weighed to the nearest 0.5 g and added to 20 L of HT water in low-density polyethylene cubitainers. A stir bar was placed in each cubitainer and the mixtures were stirred vigorously for approximately 17 hours. The stir plates were then shut off and the mixtures were allowed to settle for at least four hours before use. The mixtures were carefully poured through a fine mesh net to remove any larger particles that became re-suspended during pouring.

Copper was added to each of the six waters at nominal concentrations of 40, 100, and 300 μg/L Cu as $CuCl_2$. HT water without any copper was used as the control. Each treatment consisted of four replicates with five organisms in each chamber. Tadpoles were 96 to 120-hours old at test initiation and the tests were 7 days in duration. Organism survival was monitored daily; tadpole width and length were measured at the end of the test.

3.7 Statistical Analysis

For each test, the No Observed Effect Concentration (NOEC) was determined for the acute (survival) and chronic (width and length) endpoints. Statistical analysis was completed using Toxstat version 3.5 (WEST and Gulley, 1996). Normality was first determined for data in each test using Shapiro-Wilk's Test (α=0.01). Homogeneity of variance was determined using and Bartlett's Test or the F-Test for Equality of Variance (α=0.01). The latter test was used in cases where there were only two treatments (control and lowest metal concentration). If there were more than just two test treatments and the data met the requirements for parametric analysis, then analysis of variance followed by Dunnett's Test (for an equal number of replicates) or T-Test with Bonferrroni Adjustment were used (α=0.05). If there were only two test treatments and the data were normal, then a 2-Sample T-test for Equal Variances or Unequal Variances (modified T-Test) was used.

If parametric assumptions were not met and there were more than two test treatments, then Steel's Many-One Rank Test followed by Dunn's Test (for an equal number of replicates) or the Wilcoxon Rank Sum Test (for an unequal number of replicates) were used (α=0.05). The Wilcoxon Rank Sum Test was also used if there were only two test treatments.

The 10-day median lethal concentration (LC_{50}) was calculated for each test, provided there was >50% mortality in any concentration. LC_{50}s were calculated using Probit, Spearman Karber, or Trimmed Spearman Karber methods, depending upon the condition of the data and the number of organisms surviving in each treatment. Software from the USEPA was used (USEPA, 1994). Where appropriate, LC_{50}s were calculated using all of the analytical measures available: sediment, total recoverable water (overlying), dissolved water (overlying), and tissue concentrations. In the case of the latter concentrations, if there was 100% mortality in a treatment, tissue measurements could obviously not be made and therefore LC_{50} calculations could not be completed using tissue concentrations.

The 25% Inhibition Concentrations (IC_{25}s) were also calculated using the interpolation method (Norberg-King, 1993). IC_{25}s were only calculated for the total sediment concentrations and the dissolved overlying water concentrations. However, they were calculated on all three biological endpoints: survival, width, and length.

Additional analyses were completed on the tests that evaluated the relationship between organic carbon concentration, copper and zinc concentrations, and larval amphibian toxicity. Analytical data on total and dissolved organic carbon, total and dissolved metals, hardness, and alkalinity were used as independent variables to determine which variables best predicted organism response (survival, width, and length). For the sediment tests, additional independent parameters included bulk sediment total organic carbon and bulk sediment copper or zinc concentrations. Models were developed Statistix Version 2.0 (Analytical Software, 2000). Stepwise regression was used to identify the independent variables that best predicted the biological responses.

Table 3-1
Sources of Amphibian Eggs for Validation Testing

Taxa	**Batch Number**	**Date Received**	**Date Hatched**	**Source**
Rana sp.	02-016	3/20/02	3/23/02	Carolina Biological
Rana sp.	02-017	4/4/02	4/5/02	Carolina Biological
Bufo sp.	02-018	4/19/02	4/19-20/02	Field Collected by ENSR
Rana sp.	02-019	4/19/02	4/20/02	Carolina Biological
Bufo sp.	02-029	5/17/02	5/17/02	Field Collected by ENSR

Table 3-2
Amphibian Toxicity Testing Parameters

Test Length	10 days
Test Temperature	23°C
Test Chambers	500-1000 ml beakers or aquaria with an overflow pipe or other outflow system
Age at Test Initiation	≤96 hours
Food	Approximately 4 mg dry TetraMin®in each test chamber after organisms reach stage 25
Endpoints	Survival, body width, and body length
Test Acceptability	80% survival in the controls and measurable growth in the controls

Table 3-3
Tests Conducted During Sediment-Spiking Phase

Test No.	Toxicant	Genus	Matrix	Organism Age (Hours)
8503-116-019-016	Copper	*Rana*	Sediment	48
8503-116-019-017	Cadmium	*Rana*	Sediment	48
8503-116-019-018[a]	Copper	*Rana*	Water	48
8503-116-019-019[a]	Cadmium	*Rana*	Water	48
8503-116-019-020	Lead	*Rana*	Sediment	72
8503-116-019-021	Zinc	*Rana*	Sediment	72
8503-116-019-022[a]	Copper	*Rana*	Water	72
8503-116-019-023	Copper	*Rana*	Sediment	48
8503-116-019-024	Cadmium	*Rana*	Sediment	48
8503-116-019-025	Copper	*Bufo*	Sediment	48-72
8503-116-019-026	Cadmium	*Bufo*	Sediment	48-72
8503-116-019-027[a]	Copper	*Rana*	Water	48
8503-116-019-028[a]	Copper	*Bufo*	Water	48-72
8503-116-019-029	Lead	*Rana*	Sediment	72
8503-116-019-030	Zinc	*Rana*	Sediment	72
8503-116-019-031	Lead	*Bufo*	Sediment	72-96
8503-116-019-032	Zinc	*Bufo*	Sediment	72-96
8503-116-019-033[b]	Copper/TOC	*Bufo*	Water	96-120
8503-116-019-034[b]	Copper & Zinc/TOC	*Bufo*	Sediment	48

[a]Water-only tests were conducted to gather more information for a reference toxicant database. Data from these tests are not presented in this report.

[b]Tests 033 and 034 were conducted to explore the effects of organic carbon on copper and zinc toxicity.

Figure 3-1
Flow Through System

Flow-through test system in a temperature-controlled water bath.

Figure 3-2
Close-up of Test Beaker

Test beaker in water bath. Test organism can be seen against the white beaker label.

SECTION 4
RESULTS

During the method development phase of this YO817 project (ENSR, 2002), numerous tests were conducted to determine the toxicity of various materials, including copper and cadmium, to amphibians. Using the information gathered from the water-column studies, preliminary sediment-mixing tests were conducted to determine how much of a particular metal needed to be added to sediment to achieve a certain amount of the target metal in the water column. The studies conducted during the method development phase were in the aqueous phase only, without any sediment in the test chambers or without any evaluations of the effects of organic carbon on the toxicity of the material.

The results of all validation tests are presented in the following sections. Concentrations of all analytes in sediment and water (total recoverable and dissolved) are mean values of samples collected on Day 0 (test initiation) and Day 10 (test termination). Some concentrations in the control treatments were below the detection limit for a particular analyte. In these situations, mean values were calculated by using an assumption of ½ the detection limit. Tissue concentrations are the final measurements only, presented on a wet weight basis. Table 4-1 presents a summary of statistical endpoints for the validation phase. The terms sediment and hydric soil are treated interchangeably in this section.

4.1 Test 016, Copper and *Rana*

Test 016 was conducted to evaluate potential effects associated with copper exposure to larval amphibians. Copper was added to the sediment to a target maximum copper concentration of 30 mg/kg, with lower concentrations of 20, 10, and 1 mg/kg. Actual measured sediment concentrations were approximately twice the nominal concentrations in this test (Figure 4-1). However, concentrations of copper in all matrices increased with increasing exposure concentration. Total recoverable and dissolved copper concentrations rose from 0.027 and 0.022 mg/L, respectively, in the control, to 0.39 and 0.28 mg/L in the highest treatment (64 mg/kg copper in the sediment). Since there was no significant mortality in even the highest test treatment, tissue concentrations were measured in organisms from all copper concentrations. Copper in the high treatment was present at a concentration of 16 mg/kg wet weight, compared to 2.4 mg/kg wet weight in control animals.

No lethal or sub-lethal toxicity to *Rana* was observed in Test 016. Survival, width, and length or the organisms actually increased in the higher copper treatments (Figure 4-2). None of the measured endpoints were significantly (α=0.05) less than the control in any of the test treatments (Table 4-1).

4.2 Test 017, Cadmium and *Rana*

Test 017 was conducted to evaluate potential effects associated with cadmium exposure to larval amphibians. A review of literature as well as preliminary studies in water indicated that cadmium would likely be toxic at higher concentrations than copper (ENSR, 2001). As a result, the target nominal concentrations of cadmium in sediment were much higher: 500, 1,000, 2,500, and 7,500 mg/kg. Measured sediment concentrations of cadmium were close to the nominal concentrations (Figure 4-3). Total recoverable and dissolved cadmium in the water column were near or below the detection limits in the control, but concentrations rose consistently with increasing sediment concentration to a high of 440 and 420 mg/L for total and dissolved in the highest treatment. Tissue cadmium concentrations were very low in the controls

(0.8 mg/kg) but quite high in the 2,600 mg/kg treatment (measured sediment concentration) where some organisms were still alive and contained tissue residues of 1,400 mg/kg.

There were significant (α=0.05) reductions in survival and growth in most of the cadmium treatments (Figure 4-4). Survival was reduced in the second lowest test treatment, with a resulting NOEC of 760 mg/kg cadmium (4.3 mg/L dissolved Cd) (Table 4-1). Both body width and length were significantly reduced in the lowest test treatment, resulting in a NOEC of 0.46 mg/kg cadmium sediment concentration (this was the measured cadmium concentration in the control treatment).

4.3 Test 020, Lead and *Rana*

Test 020 was conducted to evaluate potential effects associated with lead exposure to larval amphibians. Nominal sediment concentrations of lead ranged from 0 mg/kg to 20,000 mg/kg. Measured test concentrations of lead in the sediment ranged from 3.4 mg/kg in the control to 22,000 mg/kg in the high concentration. The measured concentrations of lead were very close to the nominal concentrations (Figure 4-5). Total and dissolved lead concentrations in the control treatment were near or below the detection limit. In the high concentration (22,000 mg/kg in the sediment) total and dissolved lead were measured at 36 and 14 mg/L, respectively. There was 100% mortality of *Rana* tadpoles in the two highest lead treatments (Figure 4-6), and significant (α=0.05) mortality of tadpoles was found at a sediment concentration of 6,100 mg/kg lead. Neither tadpole width nor length were reduced in the lowest sediment concentration of 2,000 mg/kg. Because all organisms were dead at test termination in the 11,000 and 22,000 mg/kg concentrations, tissue concentrations could not be determined in these two treatments.

4.4 Test 021, Zinc and *Rana*

Test 021 was conducted to establish the effects of zinc concentrations on *Rana* tadpoles in sediments with concentrations ranging nominally from 0 to 1,000 mg/kg. However, several of the replicates in this study demonstrated apparent effects due to low dissolved oxygen (DO). In the control, for example, all organism in replicates A and B were dead at the end of the test, while all organisms were alive in replicate C and 4 (out of 5) were alive in replicate D. Since DO levels were not measured in each test chamber every day, it is not possible to quantify all of the oxygen concentrations throughout the test. However, in replicate B of the 250 mg/kg (nominal) sediment treatment, DO at test termination was 1.4 mg/L; all organisms in this replicate died. Further evidence that the observed mortality was due to low DO and not to zinc toxicity came from the 10-day survival in the high zinc treatment (1,000 mg/kg) where only one organism died. Replicates where low DO had apparent detrimental effects on organism performance were not included in the statistical analysis, since inclusion of those replicates would have severely skewed the data analysis and biased the interpretation.

Since there was good survival of *Rana* in all test treatments (excluding apparent DO problems), tissue concentrations were available at all concentrations (Figure 4-7). Tissue concentrations generally increased with the sediment concentration, although there was no increase between the 100 and 130 mg/kg treatments and there was actually a slight decrease in the tissue concentration of tadpoles in the highest sediment zinc treatment (1,200 mg/kg), relative to the second highest concentration (490 mg/kg), possibly suggesting an asymptotic relationship between sediment zinc and uptake in this test. Dissolved zinc in the water column was very close to the total concentration; in some cases the reported dissolved portion exceeded the reported total portion of zinc, indicating that all of the zinc was in the dissolved form and differences were associated with analytical variability.

At the concentrations used in this test, no lethal or sub-lethal toxicity to *Rana* was noted (Figure 4-8). In general, there was an increase in survival and body size in the higher zinc treatments.

4.5 Test 023, Copper and *Rana*

As described in Section 4.1, the first spiked sediment test with copper (Test 016) had a nominal high copper concentration of 30 mg/kg and a measured sediment copper concentration of 64 mg/kg. Since there were no apparent adverse impacts to tadpoles in that test, the target concentrations in Test 023 were increased by an order of magnitude to 300 mg/kg. The measured sediment concentrations were generally similar to the nominal concentrations although the sediment copper concentration in the highest treatment was only about 67% of the nominal concentration (Figure 4-9). Water and tissue concentrations increased with each treatment although, like the sediment concentration, they were similar in the two highest treatments. This suggests that the high treatment was under-spiked with copper.

Despite dissolved water concentrations of nearly 1,000 μg/L, there were no measurable lethal or sub-lethal effects to *Rana* tadpoles. Survival in the control (70%) was less than acceptable for this test (80%). However, there was considerable variability in survival throughout the test and no evidence of a concentration-related effect on growth or survival (Figure 4-10).

4.6 Test 024, Cadmium and *Rana*

As described in Section 4.2, in the initial toxicity test with cadmium (Test 017), *Rana* tadpoles were adversely affected at all concentrations above 510 mg/kg in the sediment; sublethal effects were found even at 510 mg/kg, which was the lowest spiked concentration tested. Therefore, in the subsequent cadmium test, the target concentrations were reduced so that the highest nominal sediment concentration was 650 mg/kg and the lowest was 100 mg/kg. Actual sediment concentrations ranged from 580 mg/kg to 160 mg/kg (Figure 4-11). Total recoverable and dissolved cadmium concentrations in overlying water increased in each test concentration; however, tissue levels did not show a consistent increase in concentration with increases in sediment cadmium levels. For example, in the 300 mg/kg treatment, the tissue cadmium concentration was 40 mg/kg. However, even when there was nearly twice as much cadmium in the sediment (580 mg/kg), the tissue concentration was only 47 mg/kg.

Control survival in this test was poor; only 50% of the control animals were alive at the end of the test. In the remaining treatments, survival was at least 85% (Figure 4-12). There was also no significant reduction in tadpole length or width in any of the cadmium treatments.

4.7 Test 025, Copper and *Bufo*

The toxicity of copper in sediment to the American Toad was tested using the same spiked sediment that was used in the second *Rana* test (Test 023). As in the *Rana* test, *Bufo* tissue concentrations increased in each concentration with relatively little change between the two highest concentrations (Figure 4-13). There were no effects to survival or growth of the tadpoles.

4.8 Test 026, Cadmium and *Bufo*

Test 026 was conducted to evaluate potential effects associated with cadmium exposure to *Bufo* tadpoles. Nominal test concentrations in Test 026 ranged from 100 mg/kg to 650 mg/kg cadmium in sediment. Cadmium in water and tissue increased progressively with increasing sediment cadmium concentrations (Figure 4-14). Unlike Test 024, where *Rana* tissue levels did not appear to increase consistently with higher cadmium levels in the sediment and water, cadmium concentrations in *Bufo* tissues did rise in conjunction with higher exposure concentrations. At a measured sediment concentration of 580 mg/kg, for example, cadmium in *Bufo* tadpole tissue was measured at 200 mg/kg. However, in Test 024 with *Rana*, the tissue concentration of cadmium was only 47 mg/kg in a sediment concentration of 580 mg/kg. In the first *Rana* test with cadmium (Test 017), the tissue concentration of cadmium was 110 mg/kg at a sediment concentration of 510 mg/kg.

Cadmium did not cause any significant lethal effects to *Bufo* (Figure 4-15). Only a single mortality occurred in the test in the lowest spiked sediment concentration (110 mg/kg). Therefore, the acute NOEC was 580 mg/kg as sediment cadmium. However, both width and length were significantly reduced in all spiked sediment concentrations, relative to the control. For example, mean body widths were 4.25, 3.75, 3.80, 3.65, 3.45, and 3.22 mm in the control (0.32 mg/kg measured cadmium), 110, 180, 310, 420, and 580 mg/kg treatments, respectively. As a result, the NOEC for both sublethal measurements was <110 mg/kg.

4.9 Test 029, Lead and *Rana*

Test 029 was conducted to further evaluate potential effects associated with lead exposure to *Rana* tadpoles. As described in Section 4.3, in the first lead test with *Rana* (Test 020), the calculated NOEC was 2,000 mg/kg and significant lethal and sub-lethal effects were apparent in the higher concentrations (61,00 mg/kg or greater). Therefore, in the second round of testing, lead concentrations were reduced to determine if the effect concentration could be more accurately determined. The target high sediment concentration was 3,000 mg/kg, however, the measured concentration was 2,400 mg/kg (Figure 4-16). Survival in the control was slightly lower (70%) than acceptable (80%) which may be due to low DO in some of the test chambers (Figure 4-17). Tadpole width and length were somewhat lower in the high concentration relative to the control, but not enough to be statistically significant.

4.10 Test 030, Zinc and *Rana*

Test 030 was conducted to further evaluate potential effects associated with zinc exposure to *Rana* tadpoles. The lowest spiked zinc concentration in this test (900 mg/kg) was 75% of the highest concentration in the first zinc test (Test 021; 1,200 mg/kg). The increased nominal concentrations of zinc were needed since there were no significant effects in the first zinc study (Test 021). Measured sediment zinc concentrations in the two highest treatments (3,200 and 4,700 mg/kg) were substantially lower than the target concentrations for those treatments (4,800 and 8,000 mg/kg, respectively) (Figure 4-18). Despite these lower than expected concentrations, the zinc levels were sufficiently high to cause significant mortality in all but the lowest spiked sediment treatment (900 mg/kg) (Figure 4-19). Body length and width were reduced at this concentration, but not significantly so (all NOECs = 900 mg/kg).

4.11 Test 031, Lead and *Bufo*

Test 031 was conducted to further evaluate potential effects associated with zinc exposure to *Bufo* tadpoles. As in Test 029, the highest sediment concentration of lead (2600 mg/kg) was somewhat lower than the target concentration of 3,000 mg/kg (Figure 4-20). Water and tissue concentrations rose with sediment concentration, although the tissue concentration in the highest sediment level was lower than the second highest concentration. There were no significant reductions in survival or growth in any of the test treatments (Figure 4-21).

4.12 Test 032, Zinc and *Bufo*

Test 032 was conducted to evaluate potential effects associated with zinc exposure to *Bufo* tadpoles. Nominal zinc concentrations in the spiked sediments ranged from 1,040 mg/kg to 8,000 mg/kg. Measured sediment concentrations of zinc in the two high treatments (3,200 and 4,700 mg/kg) were lower than the nominal target sediment concentrations (4,800 and 8,000 mg/kg, respectively) (Figure 4-22). However, there were significant lethal and sub-lethal effects in the three highest zinc concentrations (Figure 4-23).

The measured zinc concentrations in *Bufo* tadpoles illustrate a pattern that is particularly evident in the zinc tests. That is, tissue concentrations of zinc decreased as the exposure concentrations increased. This trend was not observed in Test 030 with *Rana*, although the increase in tissue levels between 900 and 1,400 mg/kg (sediment concentrations) was small. In the first test with zinc (Test 021), there was a decrease in the tissue concentration of the highest treatment, relative to the second highest treatment. These data suggest that, as toxic levels of zinc are approached, uptake diminishes or the body increases zinc excretion. This consistent trend was not observed with any other metal, although tissue concentrations of lead in Test 031 did decrease in the highest concentration.

4.13 Test 033, Interaction of Copper and Organic Carbon in Water

Test 033 was conducted to evaluate the potential relationship between copper, organic carbon in water, and toxicity to *Bufo* tadpoles. In order to evaluate their relationships, copper and TOC concentrations were adjusted to provide different levels of TOC and copper.

The amount of organic carbon in solution in each treatment increased with the amount of sheep/peat compost used to prepare the solutions. Moderately hard water had only 1 mg/L TOC, all in the dissolved form (Table 4-2). The maximum TOC concentration was 18 mg/L (14 mg/L DOC) in HT water treated with 1,500 mg/L sheep/peat compost. The amount of organic carbon in the water did not have a substantial impact on the concentration of total recoverable copper in any given series of copper treatments (Figure 4-24). In the 100 μg/L copper treatment, the measured total copper concentrations varied by only 0.008 μg and the concentration in the lowest organic carbon treatment (1 mg/L TOC) was the same as in the highest organic carbon treatment (18 mg/L TOC). Dissolved copper concentrations in treatments where sheep/peat compost was added were also fairly consistent and very similar to total copper. However, dissolved copper in the two treatments that did not receive sheep/peat compost was approximately ½ of the concentration in the treatments receiving sheep/peat (Figure 4-25). This anomalous finding suggests that the presence of sheep/peat-derived organic carbon may play a role in maintaining copper in the dissolved phase and may even be a source of some of the copper.

Toxicity of copper was inversely related to TOC concentrations; higher concentrations of organic carbon resulted in decreased toxicity of the copper. In the two lowest TOC treatments (no sheep/peat added), there was 0% survival in the two highest copper treatments (Figure 4-26). In the treatments where 100 mg/L sheep/peat was added (yielding 4 mg/L DOC), there was 0% survival in the highest copper treatment (300 μg/L nominal) but 86.7% survival in the second highest treatment (100 μg/L nominal). There was 10% survival in 300 μg/L copper in the treatments containing 500 mg/L sheep/peat (yielding 6 mg/L DOC); the two highest TOC treatments yielded no significant mortality of tadpoles.

There was no significant effect on tadpole growth endpoints in the two highest TOC treatments. However, in the 100 mg/L sheep/peat treatment (4 mg/L DOC) tadpole width and length were significantly reduced in the 100 μg/L copper treatment (Figures 4-27 and 4-28). The width and length NOECs were lower than the survival NOEC, indicating sublethal toxicity. One or both chronic NOECs were also lower in the moderately hard and HT water treatments. All NOECs as total and dissolved copper concentrations are presented in Table 4-3.

4.14 Test 034, Interaction of Copper or Zinc and Organic Carbon in Sediment

Test 034 was conducted to evaluate the potential relationship between copper, zinc, sediment organic carbon, and toxicity to *Bufo* tadpoles.

4.14.1 Copper

Whereas in Test 033 the maximum concentration of TOC in water was 18 mg/L, the maximum concentration of water-column TOC in the sediment Test 034 was 223 mg/L. TOC increased in each treatment, with 7 mg/L in Silica Sand, 32 mg/L in Un-amended Poudre River (PR) Sediment, 155 mg/L in PR + 7.5% sheep/peat, and 223 mg/L in PR + 15% sheep/peat. DOC increased proportionally and both water-column TOC and DOC reflected TOC in the sediment (Table 4-4). The amount of organic carbon in the sediment had a substantial effect on measured copper in the sediment and in the water. The more organic carbon in the sediment, the higher the sediment copper concentration (Figure 4-29). This trend was especially evident in the controls (no added copper) and in the 300 mg/kg treatment. There was no detectable copper in the Silica Sand alone, but copper was detected in the Un-amended PR sediment; there was also copper present in the sheep/peat since the copper concentration increased as more sheep/peat was added to sediment.

As would be expected, as the concentration of copper in the sediment rose with the amount of organic carbon, the water-column concentration generally decreased. Total recoverable water-column copper in Silica Sand in the 300 mg/kg nominal sediment treatment was 48 mg/L at 7 mg/L TOC; total recoverable copper in the PR Sediment + 15% sheep/peat treatment (223 mg/L TOC) with the same added copper was only 2.7 mg/L (Figure 4-30). Water column copper concentrations actually increased in the PR + sheep/peat treatments when no copper was added. This increase reflects the copper already present in the sheep/peat compost. Dissolved copper concentrations also decreased with increasing organic carbon concentration (Figure 4-31).

Survival of *Bufo* was essentially an all-or-nothing response in Test 034. In the Silica Sand and Un-amended PR sediment, there was 100% mortality in both of the treatments with added copper; the NOEC in both cases was the control copper concentration (Table 4-5). In both the PR Sediment + 7.5% sheep/peat and the PR Sediment + 15% sheep/peat there was 100% survival in all treatments, including the highest copper concentration of 420 mg/kg in sediment (which resulted in 1,300 µg/L dissolved copper in the water column) (Figure 4-32).

Sub-lethal effects were observed in several treatments with 100% survival. In the 7.5% sheep/peat treatment, both tadpole width and length were affected in the highest copper treatment (300 mg/kg nominal) (Figures 4-33 and 4-34). As a result, the chronic NOEC was 130 mg/kg zinc in sediment (1,400 µg/L dissolved zinc) (Table 4-5). Width and length were also slightly lower in the second highest copper treatment (150 mg/kg nominal), but not significantly so. In PR Sediment + 15% sheep/peat, length was significantly reduced in both of the spiked copper concentrations.

4.14.2 Zinc

Figures 4-35 through 4-37 present the results of the zinc and TOC studies. The reported zinc concentrations in sediment and overlying water were also affected by the concentration of organic carbon. In the treatment with the highest concentration of organic carbon (14,000 mg/kg sediment TOC; PR + 15% sheep/peat), the measured zinc concentration (930 mg/kg) was very close to the nominal concentration of 1,000 mg/kg (Figure 4-35). In the Silica Sand treatment, however, where TOC was very low (125 mg/kg), the measured zinc concentration was only 230 mg/kg. Lower zinc concentrations in the sediment resulted in higher zinc concentrations in the water column. Total recoverable zinc in the overlying water of the 1,000 mg/kg (nominal) zinc treatment dropped from 140 mg/L in the Silica Sand treatment (7 mg/L TOC) to 5.5 mg/L in PR + 15% sheep/peat (223 mg/L TOC) (Figure 4-36). Dissolved zinc concentrations exhibited similar effects (Figure 4-37).

In the Silica Sand and Un-amended PR treatments, there was 100% mortality of *Bufo* tadpoles in the 1,000 mg/kg zinc treatment. Therefore, the lethal and sub-lethal NOECs were the control concentrations (Table 4-6). In the two TOC-amended PR treatments, there were no significant lethal or sub-lethal effects in the 1,000 mg/kg treatment (Table 4-6).

4.14.3 Modeling the Effects of Organic Carbon on Copper Toxicity

As described in the prior sub-sections, the concentration of organic carbon in the test matrix appears to have a significant impact on the toxicity of both copper and zinc. The toxicity of copper and other metals can be affect by numerous factors including not only organic carbon, but also pH, and water hardness (e.g., USEPA, 1999a). To determine what factors might make significant contributions to the observed lethal and sub-lethal toxicity of copper on larval amphibians, stepwise regression modeling was conducted. Modeling was not done on the zinc tests since only one spiked zinc concentration was tested.

Percent survival, tadpole body width, or tadpole body length were used as dependent variables. The independent variables included TOC, DOC, sediment TOC (sediment test only), hardness, alkalinity, total recoverable copper, dissolved phase copper, and sediment copper (sediment test only).

When the stepwise regression analysis is completed on variables from the water-column TOC study (Test 033), only total copper and TOC fall out as significant variables, with a coefficient of determination (r^2) of 0.54 (Table 4-7). Forcing the regression to use dissolved copper and DOC results in a significant model, but the r^2 is lower (0.42). If the regression analysis selects total copper, the resulting model has a higher r^2 (0.56) but a lower probability (0.259 vs 0.005 for the total copper and TOC only). The best regression models for both width and length in the water-column study also include total copper and TOC as the best predictors of the endpoint (Table 4-7).

Modeling of the data from the sediment TOC tests indicates that sediment TOC alone is the best predictor of any of the biological endpoints (Table 4-7). If dissolved copper in the water and DOC are forced into the model, the r^2 does not change (0.47) and the probability decreases from 0.0085 to 0.0335. This suggests that an insufficient number of copper/TOC treatments were included in the test to produce a wide range of responses.

Table 4-1
Statistical Endpoints of Spiked Sediment Studies

Test #	Metal	Taxa	Biological Endpoint	Matrix (units)	Statistical Endpoint (At 10 Days)			
					IC_{25}	NOEC	LOEC	LC_{50}
016	Cu	*Rana*	Survival	Sediment (mg/Kg)	NC	64	>64	>64
				Total Metal (mg/L)	NA	0.39	>0.39	>0.39
				Diss. Metal (mg/L)	NC	0.28	>0.28	>0.28
				Tissue (mg/Kg)	NC	16	>16	>16
			Width	Sediment (mg/Kg)	NC	64	>64	NA
				Total Metal (mg/L)	NA	0.39	>0.39	NA
				Diss. Metal (mg/L)	NC	0.28	>0.28	NA
				Tissue (mg/Kg)	NC	16	>16	NA
			Length	Sediment (mg/Kg)	NC	64	>64	NA
				Total Metal (mg/L)	NA	0.39	>0.39	NA
				Diss. Metal (mg/L)	NC	0.28	>0.28	NA
				Tissue (mg/Kg)	NC	16	>16	NA
017	Cd	*Rana*	Survival	Sediment (mg/Kg)	430	760	2600	700
				Total Metal (mg/L)	NA	2.6	7.2	5.4
				Diss. Metal (mg/L)	0.94	1.1	4.3	2.9
				Tissue (mg/Kg)	94	110	260	ND
			Width	Sediment (mg/Kg)	250	0.46[a]	510	NA
				Total Metal (mg/L)	NA	0.006[a]	2.6	NA
				Diss. Metal (mg/L)	0.54	0.011[a]	1.1	NA
				Tissue (mg/Kg)	54	0.8[a]	110	NA
			Length	Sediment (mg/Kg)	230	0.46[a]	510	NA
				Total Metal (mg/L)	NA	0.006[a]	2.6	NA
				Diss. Metal (mg/L)	0.57	0.011[a]	1.1	NA
				Tissue (mg/Kg)	51	0.8[a]	110	NA

Table 4-1 (cont'd)
Statistical Endpoints of Spiked Sediment Studies

Test #	Metal	Taxa	Biological Endpoint	Matrix (units)	Statistical Endpoint (At 10 Days)			
					IC_{25}	NOEC	LOEC	LC_{50}
020	Pb	*Rana*	Survival	Sediment (mg/Kg)	3550	2000	6100	4662
				Total Metal (mg/L)	NA	5.1	17	11
				Diss. Metal (mg/L)	0.43	0.27	0.70	0.58
				Tissue (mg/Kg)	ND	700	1600	1308[b]
			Width	Sediment (mg/Kg)	3494	2000	6100	NA
				Total Metal (mg/L)	NA	5.1	17	NA
				Diss. Metal (mg/L)	0.43	0.27	0.70	NA
				Tissue (mg/Kg)	ND	700	1600	NA
			Length	Sediment (mg/Kg)	3494	2000	6100	NA
				Total Metal (mg/L)	NA	5.1	17	NA
				Diss. Metal (mg/L)	0.43	0.27	0.70	NA
				Tissue (mg/Kg)	ND	700	1600	NA
021	Zn	*Rana*	Survival	Sediment (mg/Kg)	NC	1200	>1200	NA
				Total Metal (mg/L)	NA	3.9	>3.9	>3.9
				Diss. Metal (mg/L)	NC	3.0	>3.0	>3.0
				Tissue (mg/Kg)	NC	240[c]	>240[c]	>240[c]
			Width	Sediment (mg/Kg)	NC	1200	>1200	>1200
				Total Metal (mg/L)	NA	3.9	>3.9	NA
				Diss. Metal (mg/L)	NC	3.0	>3.0	NA
				Tissue (mg/Kg)	NC	240[c]	>240[c]	NA
			Length	Sediment (mg/Kg)	NC	1200	>1200	NA
				Total Metal (mg/L)	NA	3.9	>3.9	NA
				Diss. Metal (mg/L)	NC	3.0	>3.0	NA
				Tissue (mg/Kg)	NC	240[c]	>240[c]	NA

Table 4-1 (cont'd)
Statistical Endpoints of Spiked Sediment Studies

Test #	Metal	Taxa	Biological Endpoint	Matrix (units)	Statistical Endpoint (At 10 Days)			
					IC_{25}	NOEC	LOEC	LC_{50}
023	Cu	*Rana*	Survival	Sediment (mg/Kg)	NC	200	>200	>200
				Total Metal (mg/L)	NA	1.2	>1.2	>1.2
				Diss. Metal (mg/L)	NC	0.90	>0.90	>0.90
				Tissue (mg/Kg)	NC	79[d]	>79[d]	>79[d]
			Width	Sediment (mg/Kg)	NC	200	>200	NA
				Total Metal (mg/L)	NA	1.2	>1.2	NA
				Diss. Metal (mg/L)	NC	0.90	>0.90	NA
				Tissue (mg/Kg)	NC	79[d]	>79[d]	NA
			Length	Sediment (mg/Kg)	NC	200	>200	NA
				Total Metal (mg/L)	NA	1.2	>1.2	NA
				Diss. Metal (mg/L)	NC	0.90	>0.90	NA
				Tissue (mg/Kg)	NC	79[d]	>79[d]	NA
024	Cd	*Rana*	Survival	Sediment (mg/Kg)	NC	580	>580	>580
				Total Metal (mg/L)	NA	1.8	>1.8	>1.8
				Diss. Metal (mg/L)	NC	1.1	>1.1	>1.1
				Tissue (mg/Kg)	NC	47	>47	>47
			Width	Sediment (mg/Kg)	NC	580	>580	NA
				Total Metal (mg/L)	NA	1.8	>1.8	NA
				Diss. Metal (mg/L)	NC	1.1	>1.1	NA
				Tissue (mg/Kg)	NC	47	>47	NA
			Length	Sediment (mg/Kg)	NC	580	>580	NA
				Total Metal (mg/L)	NA	1.8	>1.8	NA
				Diss. Metal (mg/L)	NC	1.1	>1.1	NA
				Tissue (mg/Kg)	NC	47	>47	NA
025	Cu	*Bufo*	No Effects on Growth or Survival					

Table 4-1 (cont'd)
Statistical Endpoints of Spiked Sediment Studies

Test #	Metal	Taxa	Biological Endpoint	Matrix (units)	Statistical Endpoint (At 10 Days)			
					IC_{25}	NOEC	LOEC	LC_{50}
026	Cd	*Bufo*	Survival	Sediment (mg/Kg)	NC	580	>580	>580
				Total Metal (mg/L)	NA	1.8	>1.8	>1.8
				Diss. Metal (mg/L)	NC	1.1	>1.1	>1.1
				Tissue (mg/Kg)	NC	200	>200	>200
			Width	Sediment (mg/Kg)	NC	0.32[a]	110	NA
				Total Metal (mg/L)	NA	0.0025[a]	0.27	NA
				Diss. Metal (mg/L)	NC	0.0025[a]	0.16	NA
				Tissue (mg/Kg)	NC	0.25[a]	28	NA
			Length	Sediment (mg/Kg)	540	0.32[a]	110	NA
				Total Metal (mg/L)	NA	0.0025[a]	0.27	NA
				Diss. Metal (mg/L)	1.0	0.0025[a]	0.16	NA
				Tissue (mg/Kg)	170	0.25[a]	28	NA
029	Pb	*Rana*	Survival	Sediment (mg/Kg)	NC	2400	>2400	>2400
				Total Metal (mg/L)	NA	6.2	>6.2	>6.2
				Diss. Metal (mg/L)	NC	0.48	>0.48	>0.48
				Tissue (mg/Kg)	NC	870	>870	>870
			Width	Sediment (mg/Kg)	NC	2400	>2400	NA
				Total Metal (mg/L)	NA	6.2	>6.2	NA
				Diss. Metal (mg/L)	NC	0.48	>0.48	NA
				Tissue (mg/Kg)	NC	870	>870	NA
			Length	Sediment (mg/Kg)	NC	2400	>2400	NA
				Total Metal (mg/L)	NA	6.2	>6.2	NA
				Diss. Metal (mg/L)	NC	0.48	>0.48	NA
				Tissue (mg/Kg)	NC	870	>870	NA

Table 4-1 (cont'd)
Statistical Endpoints of Spiked Sediment Studies

Test #	Metal	Taxa	Biological Endpoint	Matrix (units)	Statistical Endpoint (At 10 Days)			
					IC_{25}	NOEC	LOEC	LC_{50}
030	Zn	*Rana*	Survival	Sediment (mg/Kg)	980	900	1400	1500
				Total Metal (mg/L)	NA	6.3	18	20
				Diss. Metal (mg/L)	7.2	5.2	17	19
				Tissue (mg/Kg)	ND	300	310	ND
			Width	Sediment (mg/Kg)	1200	900	1400	NA
				Total Metal (mg/L)	NA	6.3	18	NA
				Diss. Metal (mg/L)	12	5.2	17	NA
				Tissue (mg/Kg)	ND	300	310	NA
			Length	Sediment (mg/Kg)	1200	900	1400	NA
				Total Metal (mg/L)	NA	6.3	18	NA
				Diss. Metal (mg/L)	12	5.2	17	NA
				Tissue (mg/Kg)	ND	300	310	NA
031	Pb	*Bufo*	Survival	Sediment (mg/Kg)	NC	2600	>2600	>2600
				Total Metal (mg/L)	NA	6.2	>6.2	>6.2
				Diss. Metal (mg/L)	NC	0.48	>0.48	>0.48
				Tissue (mg/Kg)	NC	620	>620	>620
			Width	Sediment (mg/Kg)	NC	2600	>2600	NA
				Total Metal (mg/L)	NA	6.2	>6.2	NA
				Diss. Metal (mg/L)	NC	0.48	>0.48	NA
				Tissue (mg/Kg)	NC	620	>620	NA
			Length	Sediment (mg/Kg)	NC	2600	>2600	NA
				Total Metal (mg/L)	NA	6.2	>6.2	NA
				Diss. Metal (mg/L)	NC	0.48	>0.48	NA
				Tissue (mg/Kg)	NC	620	>620	NA

Table 4-1 (cont'd)
Statistical Endpoints of Spiked Sediment Studies

Test #	Metal	Taxa	Biological Endpoint	Matrix (units)	Statistical Endpoint (At 10 Days)			
					IC_{25}	NOEC	LOEC	LC_{50}
032	Zn	*Bufo*	Survival	Sediment (mg/Kg)	1700	1200	2700	2100
				Total Metal (mg/L)	NA	18	64	49
				Diss. Metal (mg/L)	34	17	64	35
				Tissue (mg/Kg)	NC[e]	250[f]	170[f]	ND
			Width	Sediment (mg/Kg)	1600	1200	2700	NA
				Total Metal (mg/L)	NA	18	64	NA
				Diss. Metal (mg/L)	29	17	64	NA
				Tissue (mg/Kg)	NC[e]	250[f]	170[f]	NA
			Length	Sediment (mg/Kg)	1600	1200	2700	NA
				Total Metal (mg/L)	NA	18	64	NA
				Diss. Metal (mg/L)	28	17	64	NA
				Tissue (mg/Kg)	NC[e]	250[f]	170[f]	NA

[a] NOEC concentrations for this test and endpoint are from the control treatment; LOEC concentrations are the lowest treatment containing added test material; some NOEC concentrations may be calculated using ½ the detection limit.

[b]This value should be considered to be an estimate, as calculations were based on a limited amount of tissue data.

[c] Measured tissue concentration in the high treatment was 240 mg/Kg Zn. However, the highest body burden was in the second highest test concentration at 270 mg/Kg Zn.

[d] Measured tissue concentration in the high treatment was 79 mg/Kg Cu. However, the highest body burden was in the second highest test concentration at 80 mg/Kg Cu.

[e] Although there was sufficient organism response to calculate an IC_{25}, tissue zinc concentrations were inversed and no reliable estimate could be calculated (see footnote f).

[f] Measured tissue concentrations of zinc actually decreased with increasing exposure concentrations, therefore, the tissue LOEC is actually less than the NOEC.

NC = Not calculated due to lack of negative organism response.

ND = Not calculated due to lack of tissue concentration data.

Tissue concentrations presented on a wet weight basis.

Table 4-2
Total and Dissolved Organic Carbon in Test 033 Treatments

Treatment	Organic Carbon (mg/L)	
	Total	**Dissolved**
Moderately Hard Water	1	1
Horsetooth Reservoir (HT) Water (Unamended)	3	3
HT Water + 100 mg/L Sheep/Peat	5	4
HT Water + 500 mg/L Sheep/Peat	7	6
HT Water + 800 mg/L Sheep/Peat	12	9
HT Water + 1500 mg/L Sheep/Peat	18	14

Table 4-3

Lethal and Sub-Lethal Copper No Observed Effect Concentrations (NOECs) for Test 033 Treatments

Treatment	**Copper Form**	**NOECs (μg/L)**		
		Survival	**Width**	**Length**
Moderately Hard Water	Total	31	31	<10[a]
	Dissolved	14	14	<10[a]
Horsetooth Reservoir (HT) Water (Un-amended)	Total	32	<10[a]	<10[a]
	Dissolved	14	<10[a]	<10[a]
HT Water + 100 mg/L Sheep/Peat	Total	84	35	35
	Dissolved	84	30	30
HT Water + 500 mg/L Sheep/Peat	Total	86	86	86
	Dissolved	88	88	88
HT Water + 800 mg/L Sheep/Peat	Total	260	260	260
	Dissolved	260	260	260
HT Water + 1500 mg/L Sheep/Peat	Total	270	270	270
	Dissolved	240	240	240

[a] Significant effects at the lowest added copper concentration. NOEC is the control, where Cu was less than the detection limit of 10 μg/L.

Table 4-4
Total and Dissolved Organic Carbon in Test 034 Treatments

Treatment	Organic Carbon		
	Total in Sediment (mg/kg)	**Total in Water (mg/L)**	**Dissolved in Water (mg/L)**
Silica Sand	125	7	6
Poudre River Sediment (PR) Un-amended	1300	32	13
PR + 7.5% Sheep/Peat	13000	155	128
PR + 15% Sheep/Peat	14000	223	187

Table 4-5
Lethal and Sub-Lethal Copper No Observed Effect Concentrations (NOECs) for Test 034 Treatments

Treatment	Copper Form	NOECs		
		Survival	**Width**	**Length**
Silica Sand	Sediment Total (mg/kg)	<1[a]	<1[a]	<1[a]
	Water Total (μg/L)	<10[a]	<10[a]	<10[a]
	Water Dissolved (μg/L)	<10[a]	<10[a]	<10[a]
Poudre River Sediment (PR) (Un-amended)	Sediment Total (mg/kg)	5.2	5.2	5.2
	Water Total (μg/L)	<10[a]	<10[a]	<10[a]
	Water Dissolved (μg/L)	<10[a]	<10[a]	<10[a]
PR + 7.5% Sheep/Peat	Sediment Total (mg/kg)	250	130	130
	Water Total (μg/L)	4500	2400	2400
	Water Dissolved (μg/L)	2400	1400	1400
PR + 15% Sheep/Peat	Sediment Total (mg/kg)	420	420	8
	Water Total (μg/L)	2700	2700	39
	Water Dissolved (μg/L)	1300	1300	36

[a] Significant effects at the lowest added copper concentration. NOEC is the control, where Cu was less than the detection limit of 1 mg/kg (sediment) or 10 μg/L (sediment).

Table 4-6

Lethal and Sub-Lethal Zinc No Observed Effect Concentrations (NOECs) for Test 034 Treatments

Treatment	Zinc Form	NOECs		
		Survival	Width	Length
Silica Sand	Sediment Total (mg/kg)	2.3	2.3	2.3
	Water Total (µg/L)	0.02[a]	0.02[a]	0.02[a]
	Water Dissolved (µg/L)	0.063	0.063	0.063
Poudre River Sediment (PR) (Un-amended)	Sediment Total (mg/kg)	26	26	26
	Water Total (µg/L)	0.02[a]	0.02[a]	0.02[a]
	Water Dissolved (µg/L)	0.11	0.11	0.11
PR + 7.5% Sheep/Peat	Sediment Total (mg/kg)	700	700	700
	Water Total (µg/L)	7.7	7.7	7.7
	Water Dissolved (µg/L)	5.5	5.5	5.5
PR + 15% Sheep/Peat	Sediment Total (mg/kg)	930	930	930
	Water Total (µg/L)	5.5	5.5	5.5
	Water Dissolved (µg/L)	3.4	3.4	3.4

[a] Significant effects at the lowest added zinc concentration. NOEC is the control, where Zn was less than the detection limit of 0.02 mg/L.

Table 4-7

Regression Models Predicting Survival, Body Width, or Body Length based on Various Independent Variables

Test	Biological Endpoint	Model	Coeff. of Determination (r^2)	Prob.
Water-Column	Survival	% Surv. = -2.61473(Tot Cu) + 0.03984(TOC) + 0.68577	0.54	0.0050
Water Column	Survival	% Surv. = -2.54067(Diss Cu) + 0.06046(DOC) + 0.5522	0.42	0.0057
Water-Column	Survival	% Surv. = 3.50895(Diss Cu) + 0.04157(DOC) – 5.49542(Tot Cu) + 0.73412	0.56	0.037
Water-Column	Width	Width (mm) = -9.14782(Tot Cu) + 0.1852(TOC) + 2.19255	0.63	0.0005
Water-Column	Length	Length (mm) = -13.6188 (Tot Cu)+ 0.26323(TOC) + 3.28356	0.62	0.0006
Sediment	Survival	% Surv. = 0.00525(Sed TOC) + 28.9678	0.47	0.0085
Sediment	Width	Width (mm) = 0.0001898(Sed TOC) + 1.35116	0.34	0.028
Sediment	Length	Length (mm) = 0.00031(Sed TOC) – 0.01075(Sed Cu) + 3.32029	0.56	0.040

Figure 4-1
Measured Copper Concentrations in all Matrices for Test 016

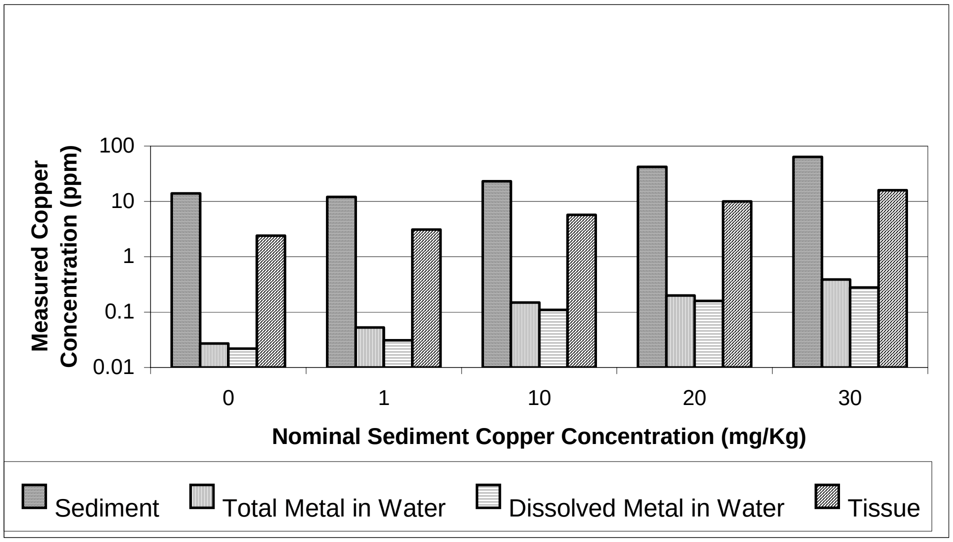

Figure 4-2
Summary of Biological Responses from Test 016

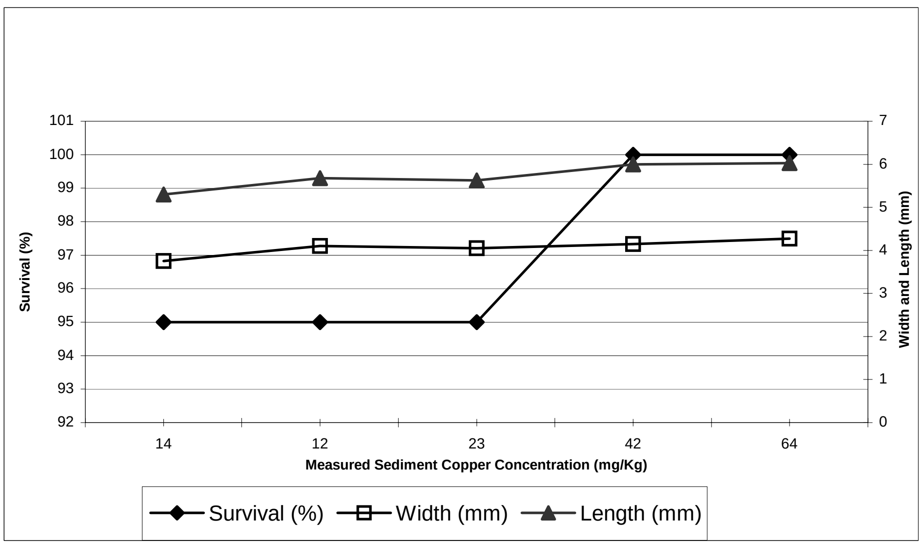

Figure 4-3
Measured Cadmium Concentrations in all Matrices for Test 017

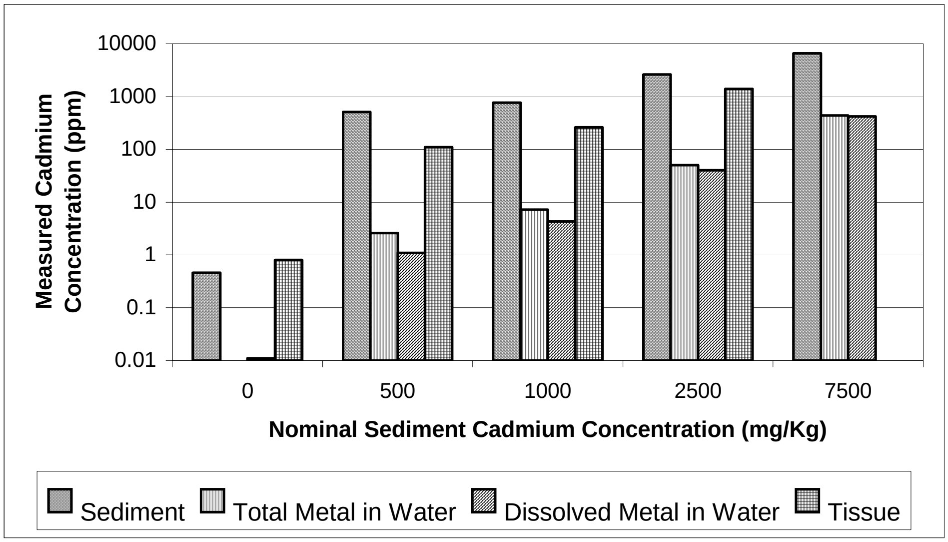

Figure 4-4
Summary of Biological Responses from Test 017

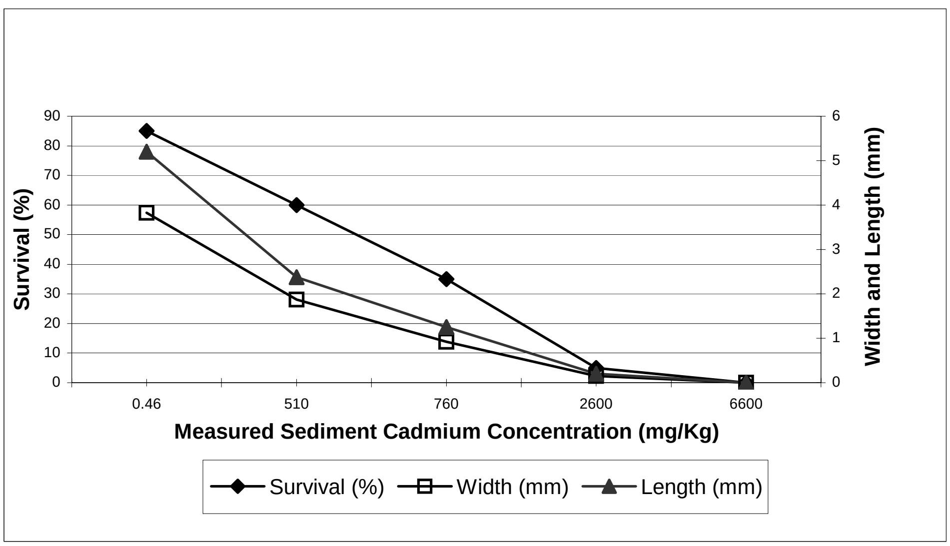

Figure 4-5
Measured Lead Concentrations in all Matrices for Test 020

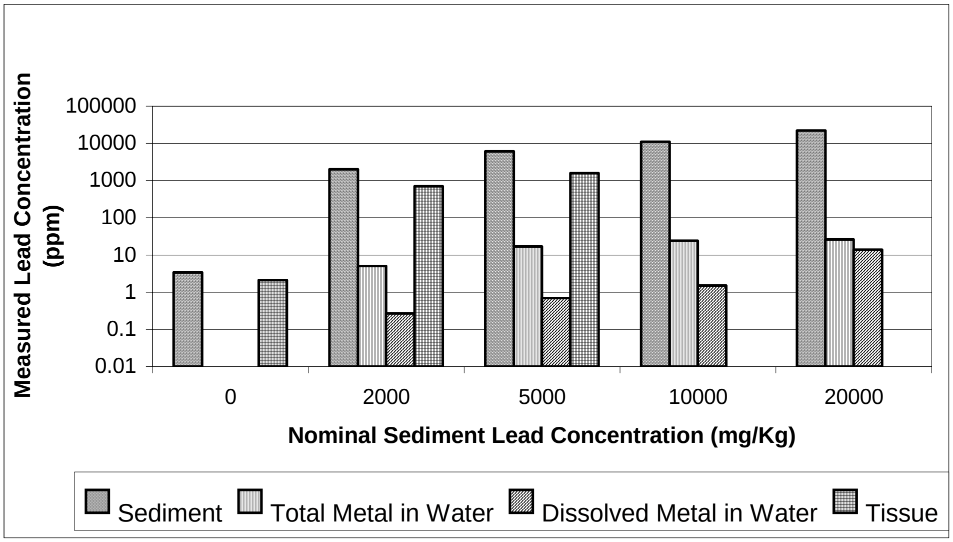

Figure 4-6
Summary of Biological Responses from Test 020

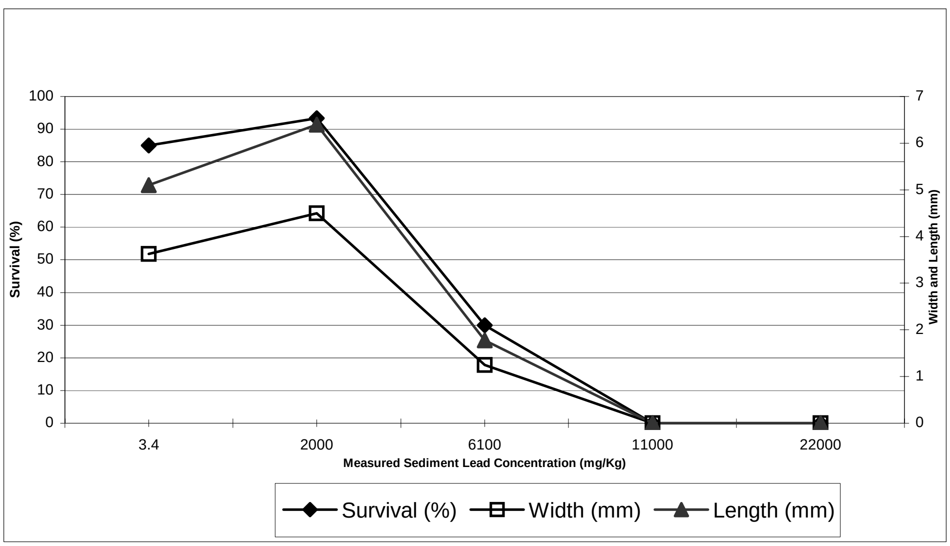

Figure 4-7
Measured Zinc Concentrations in all Matrices for Test 021

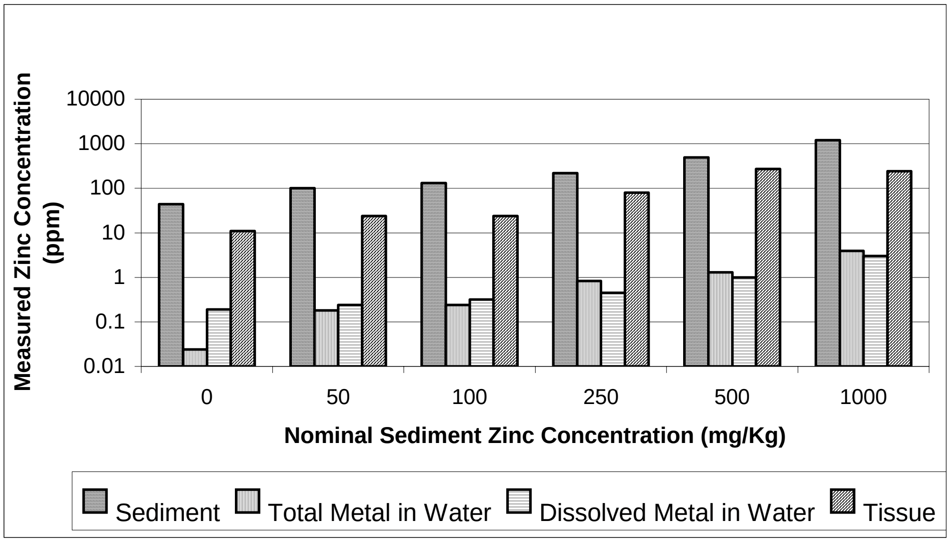

Figure 4-8
Summary of Biological Responses from Test 021

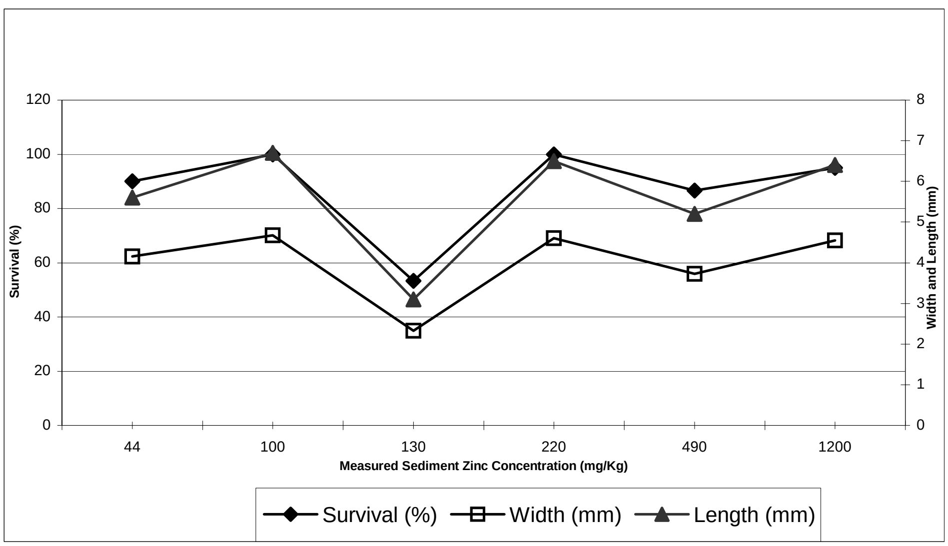

Figure 4-9
Measured Copper Concentrations in all Matrices for Test 023

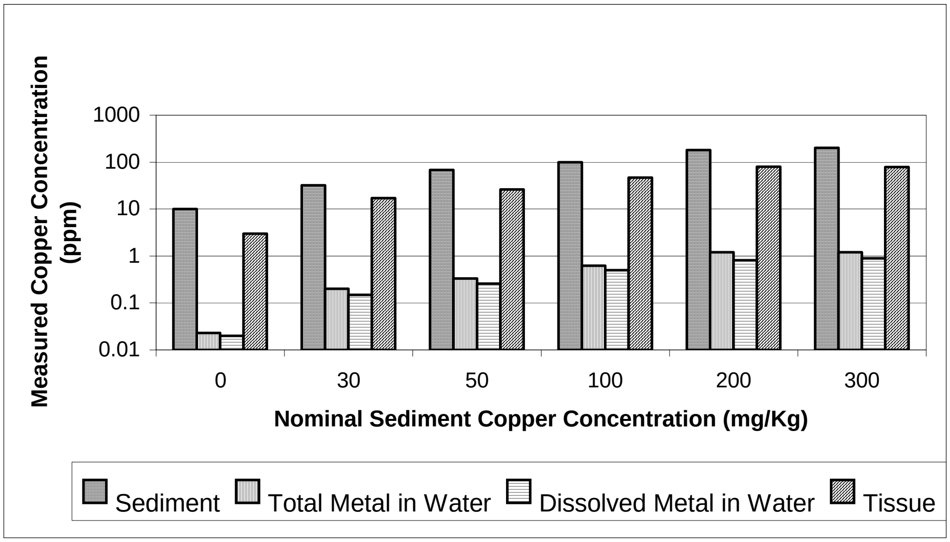

Figure 4-10
Summary of Biological Responses from Test 023

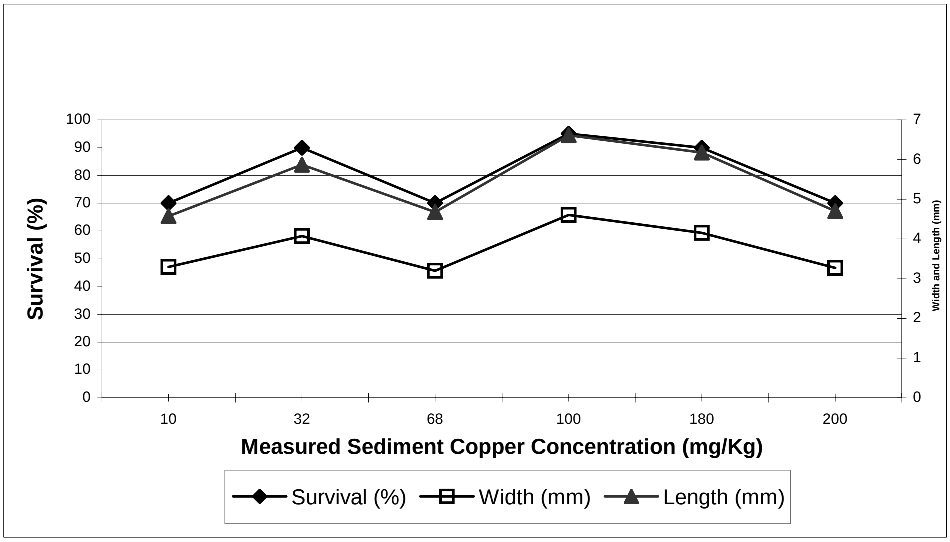

Figure 4-11
Measured Cadmium Concentrations in all Matrices for Test 024

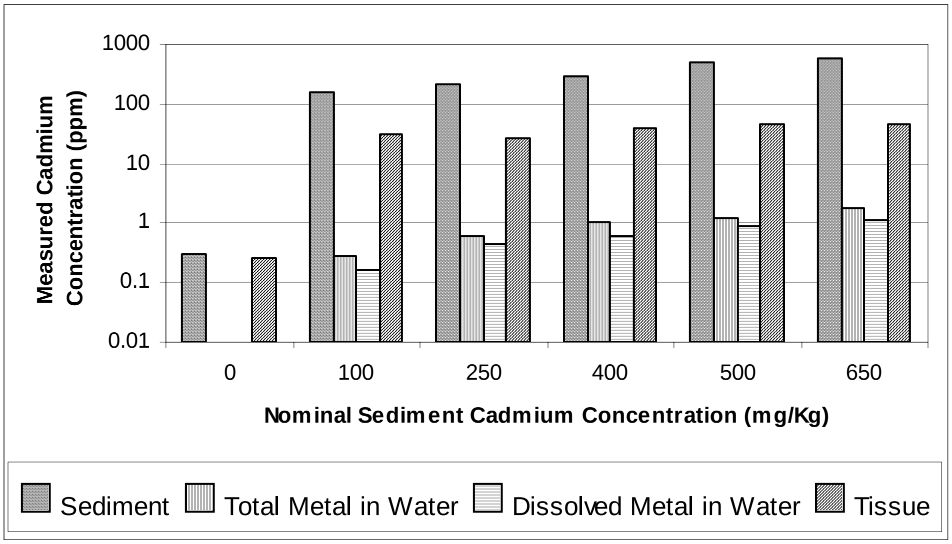

Figure 4-12
Summary of Biological Responses from Test 024

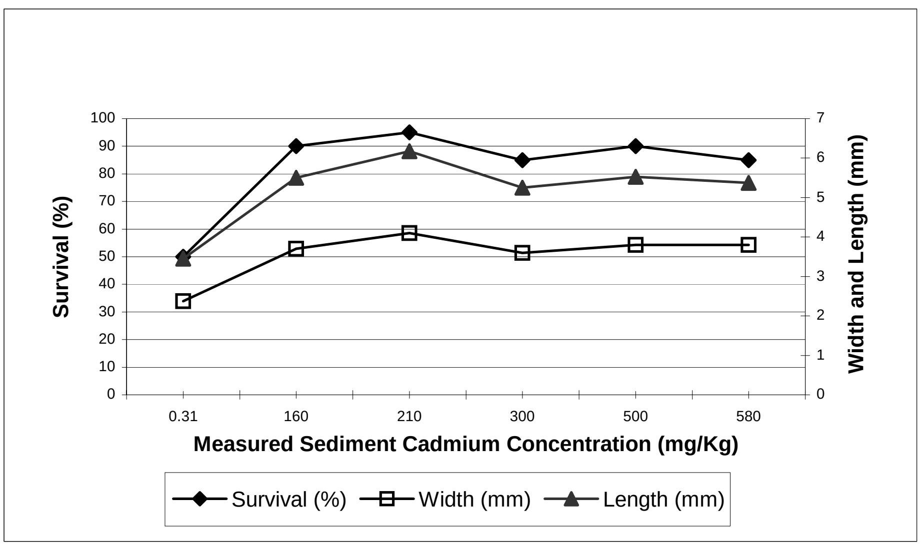

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Figure 4-13
Measured Copper Concentrations in all Matrices for Test 025

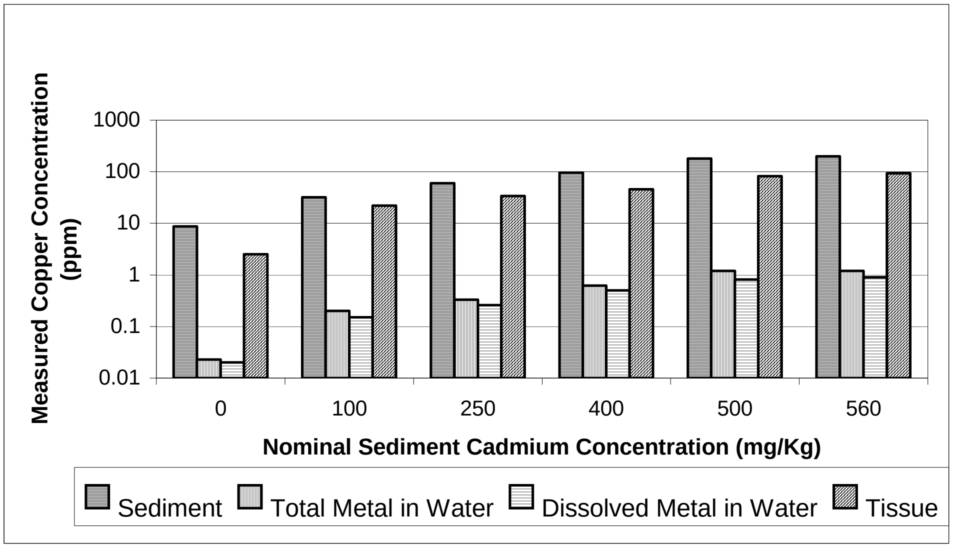


Figure 4-14
Measured Cadmium Concentrations in all Matrices for Test 026

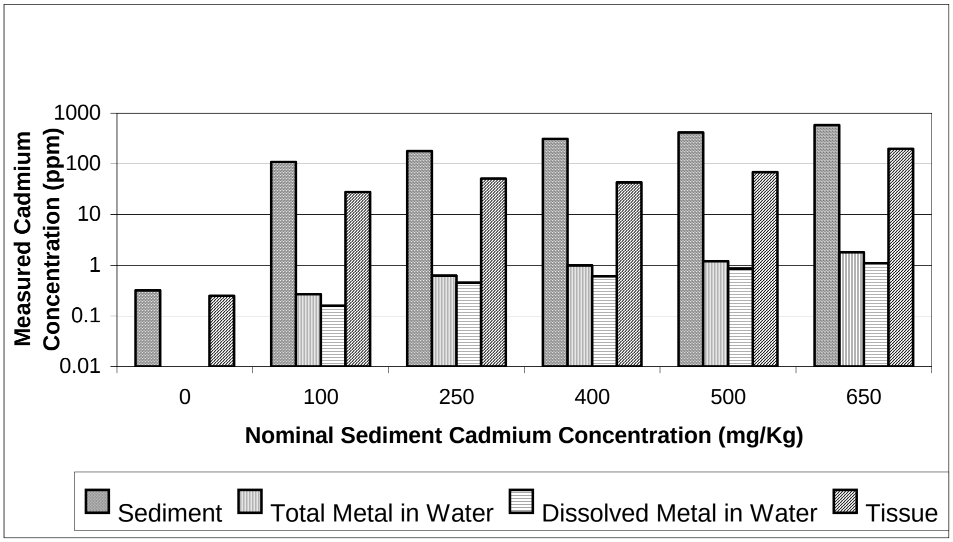


Figure 4-15
Summary of Biological Responses from Test 026

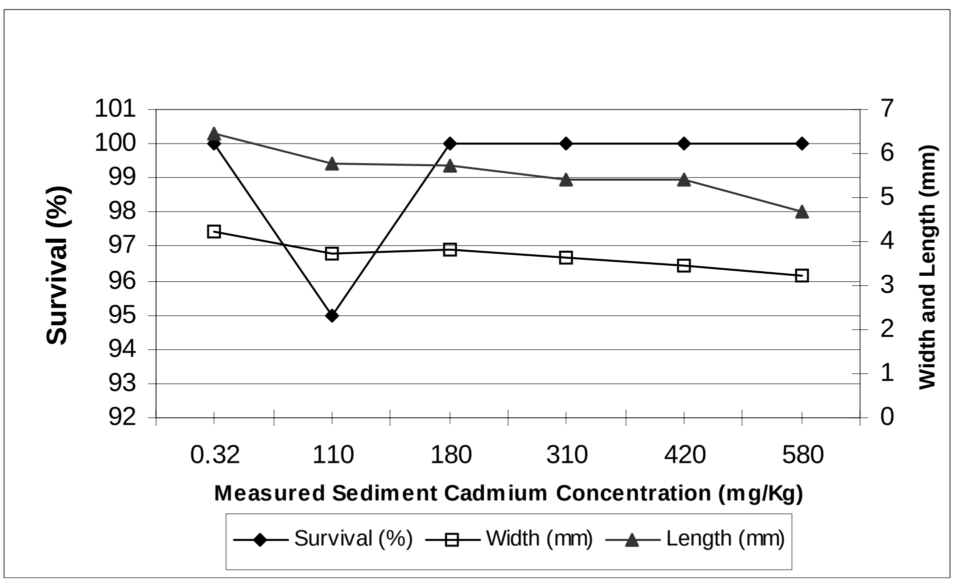


Figure 4-16
Measured Lead Concentrations in all Matrices for Test 029

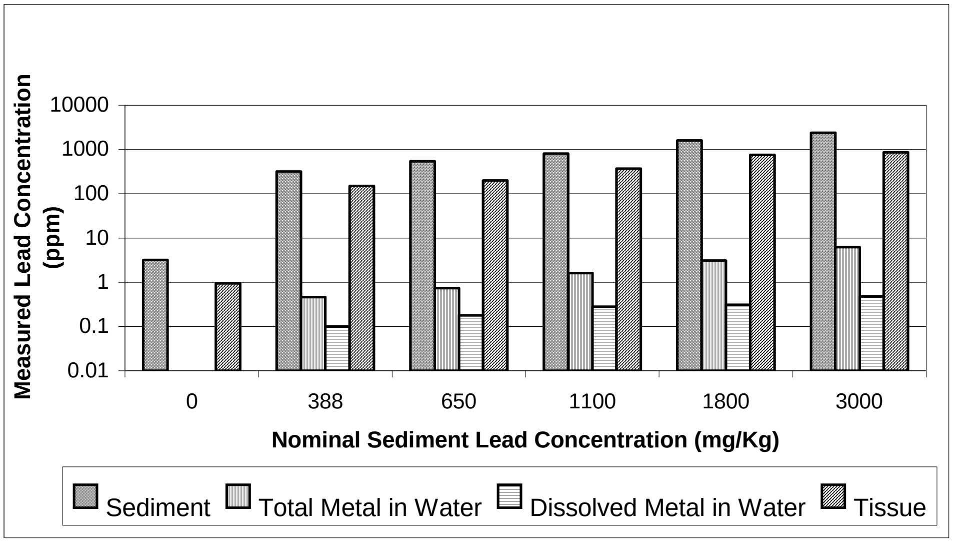


Figure 4-17
Summary of Biological Responses from Test 029

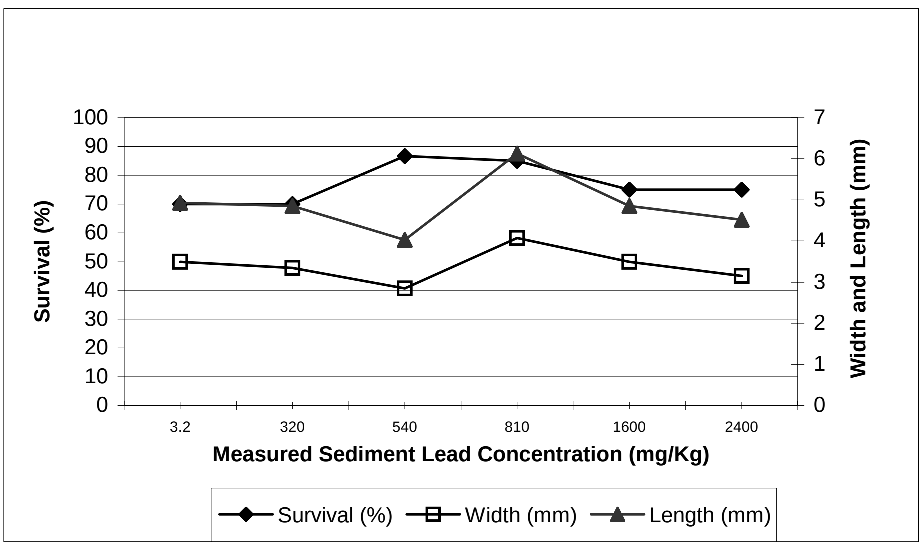


Figure 4-18
Measured Zinc Concentrations in all Matrices for Test 030

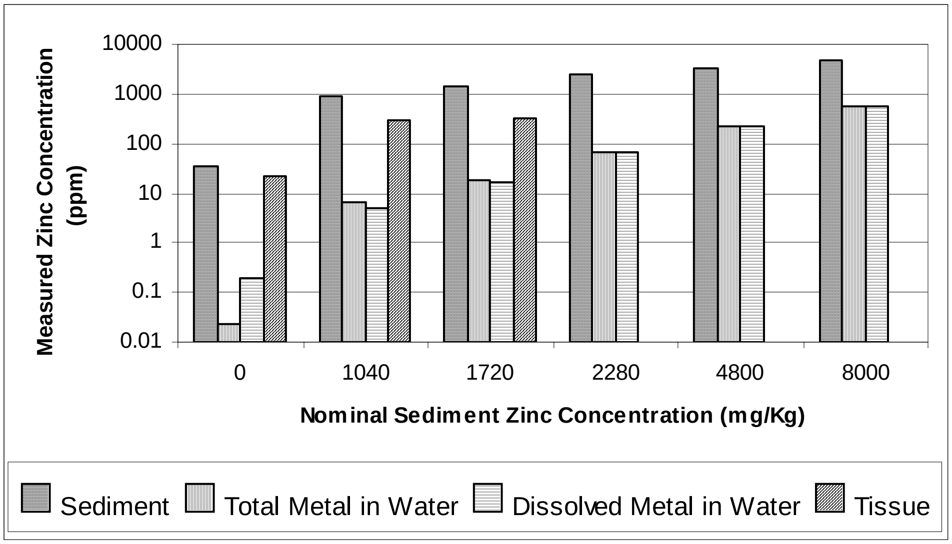


Figure 4-19
Summary of Biological Responses from Test 030

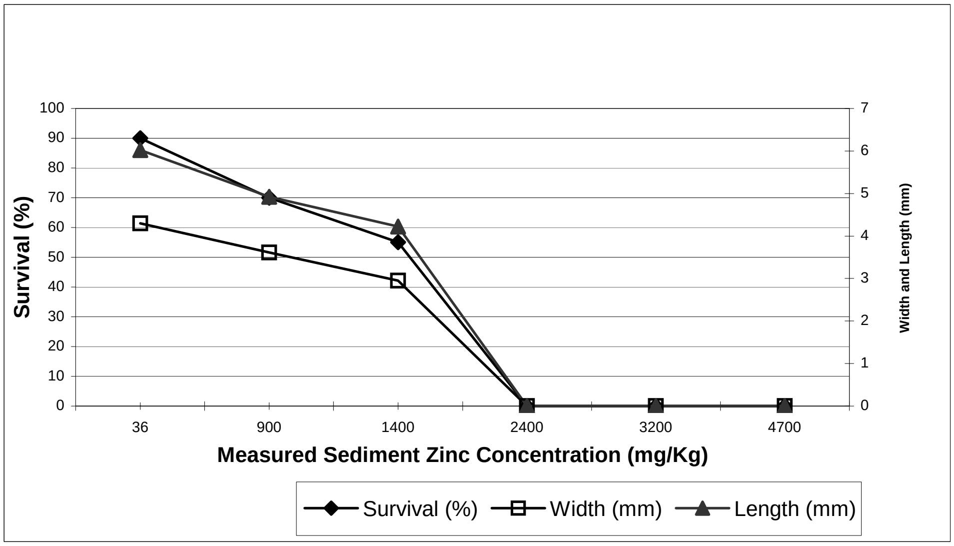


Figure 4-20
Measured Lead Concentrations in all Matrices for Test 031

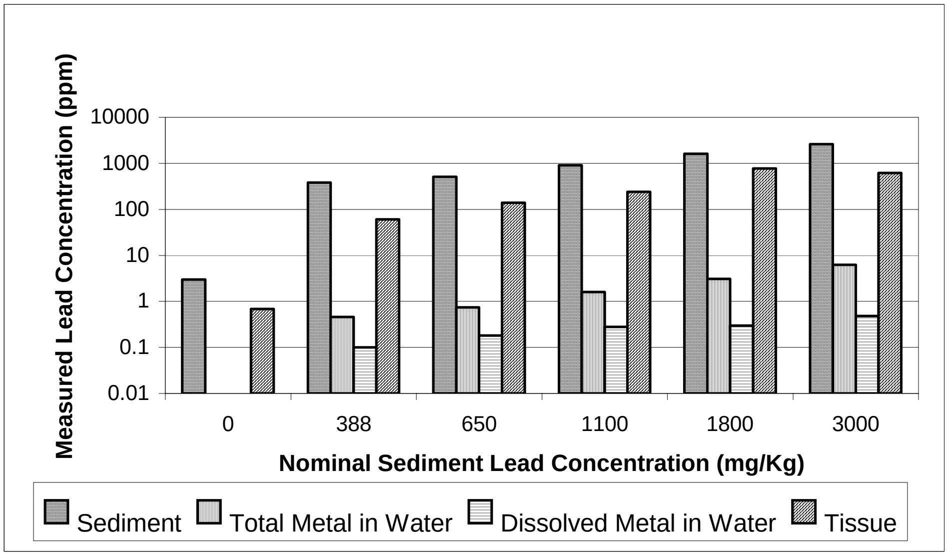


Figure 4-21
Summary of Biological Responses from Test 031

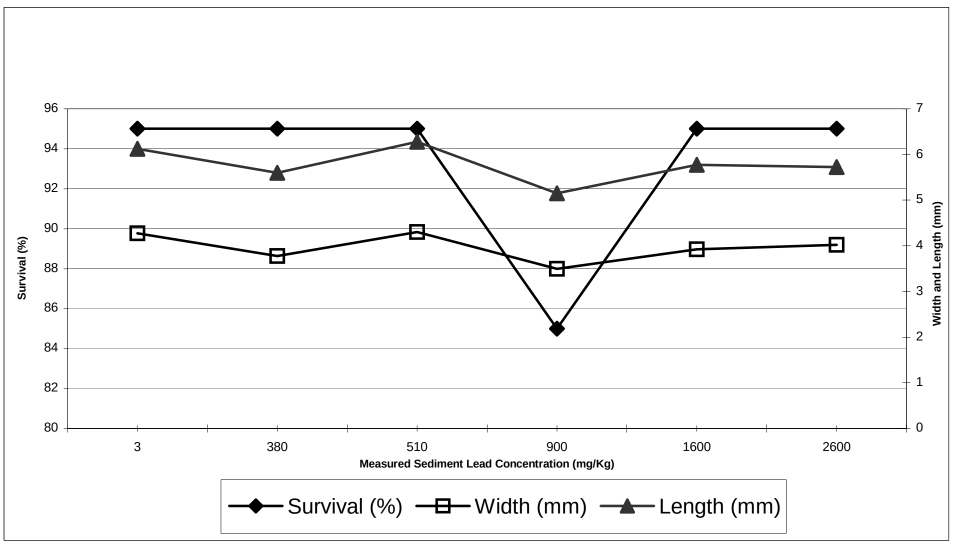


Figure 4-22
Measured Zinc Concentrations in all Matrices for Test 032

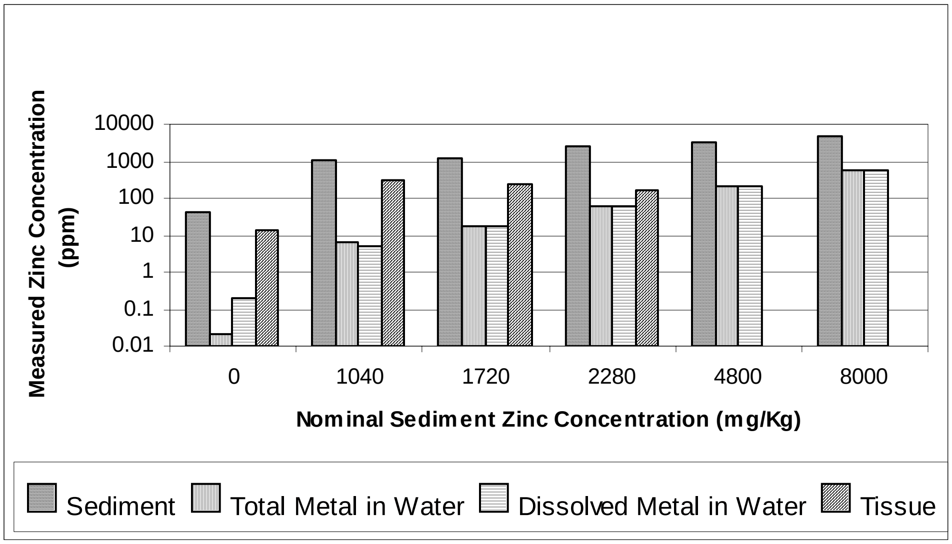


Figure 4-23
Summary of Biological Responses from Test 032

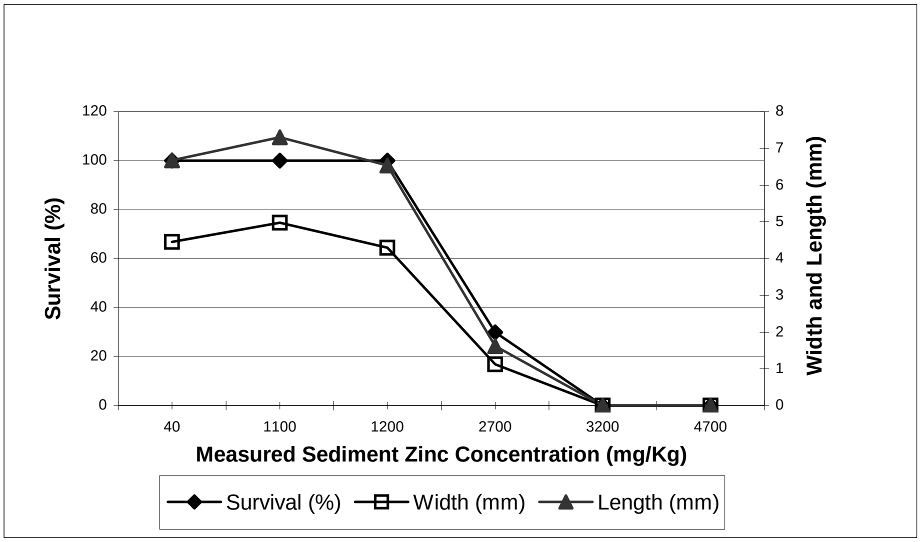


Figure 4-24
Total Recoverable Copper in Water in Test 033

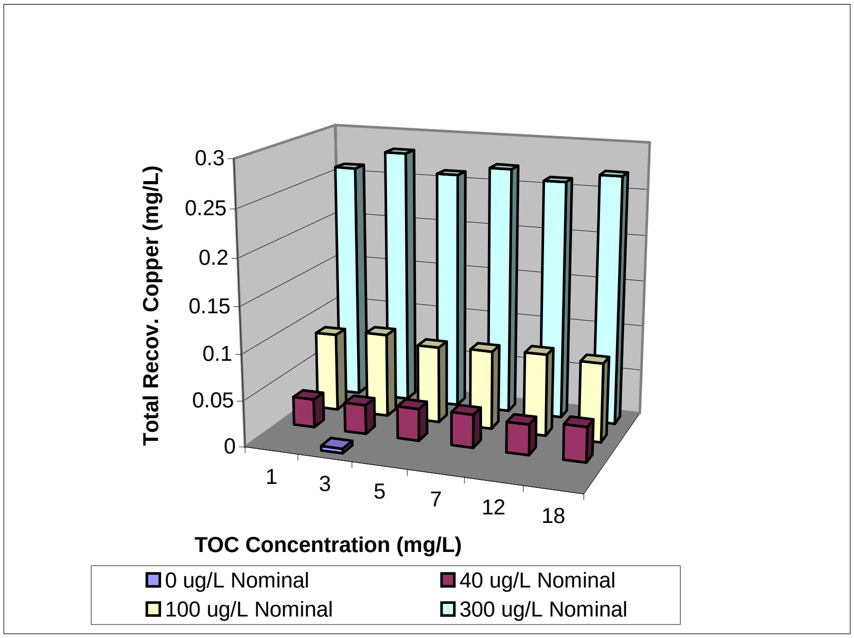
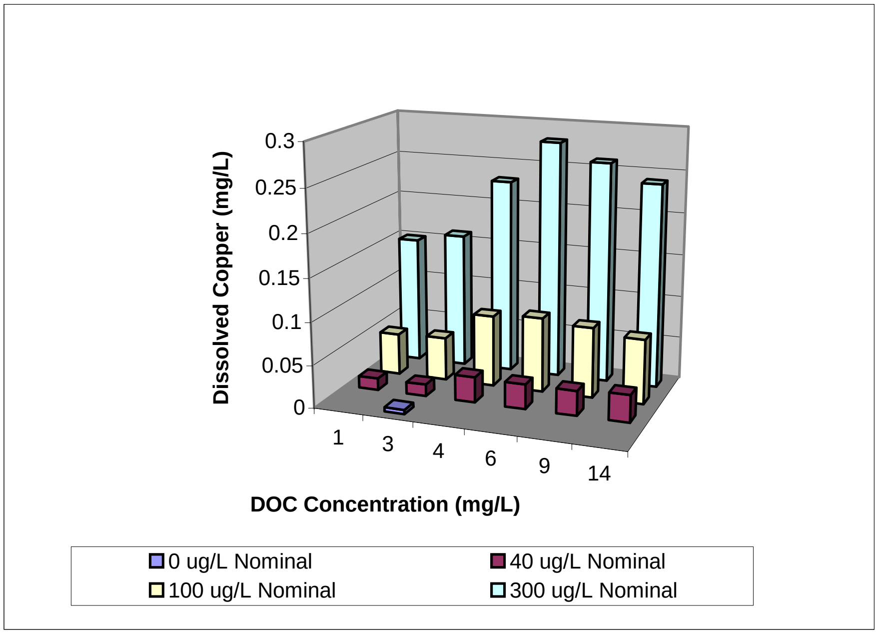


Figure 4-25
Dissolved Copper in Water in Test 033



Note: Test 033 was a water-only exposure.
Nominal concentrations are for copper in overlying water.

Figure 4-26
Survival of Bufo in Test 033

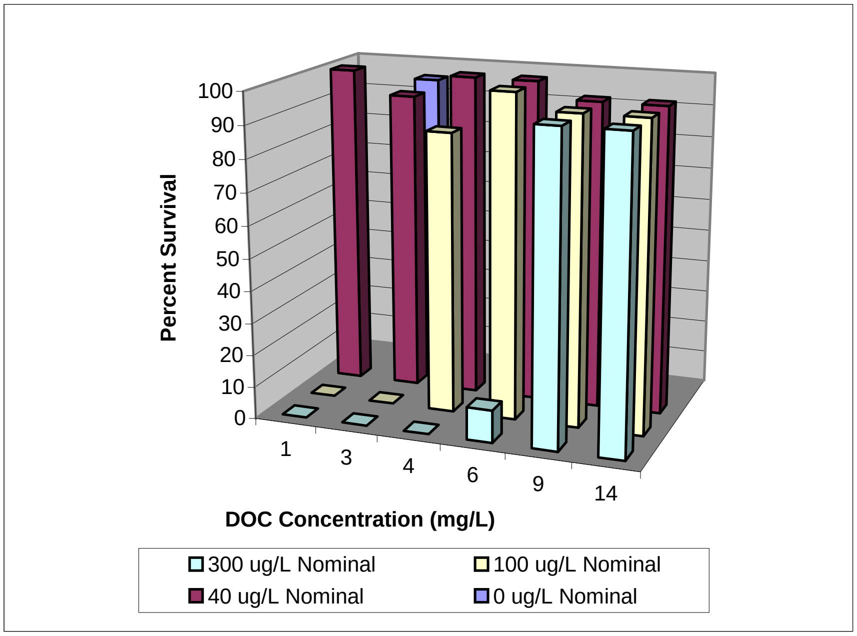
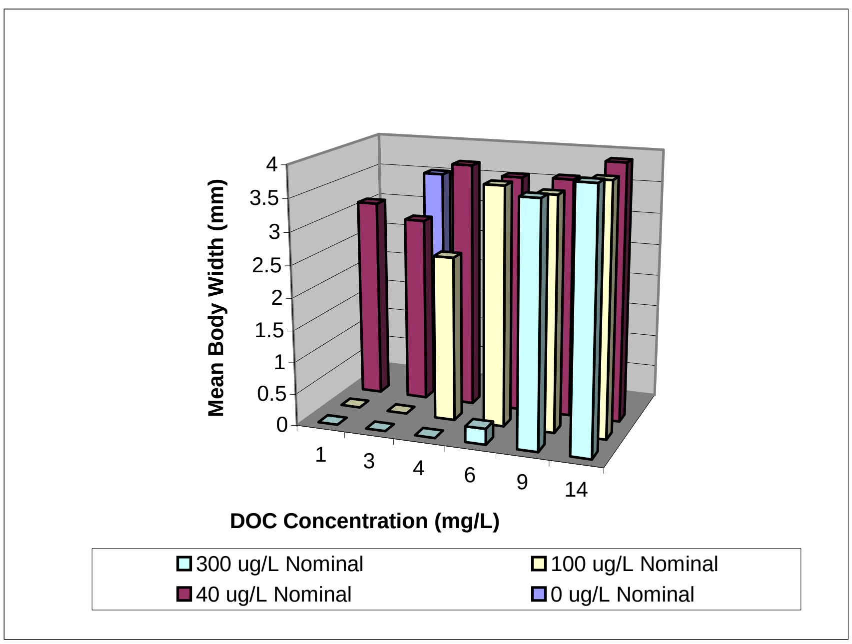


Figure 4-27
Mean Body Width of Bufo Tadpoles in Test 033



Note: Test 033 was a water-only exposure.
Nominal concentrations are for copper in overlying water.

Figure 4-28
Mean Body Length of Bufo Tadpoles in Test 033

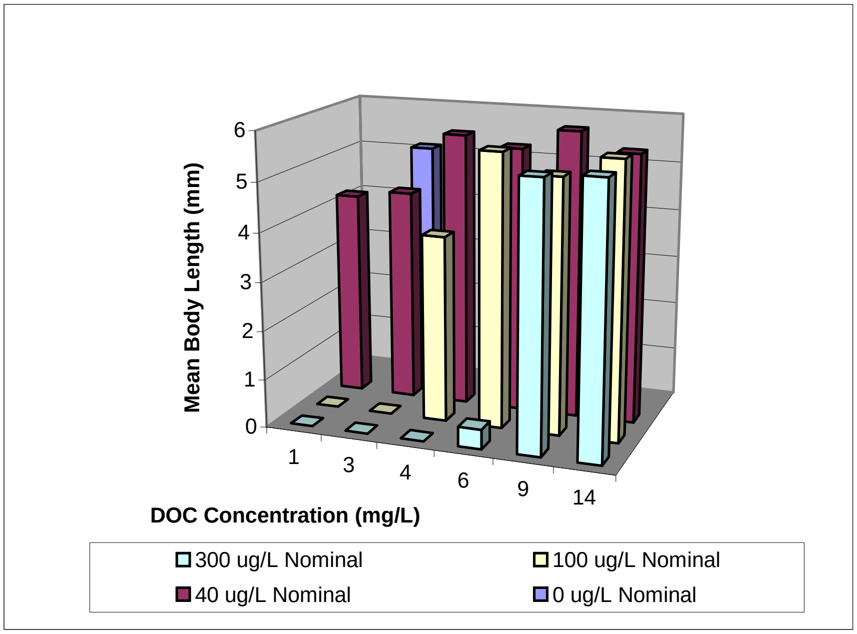
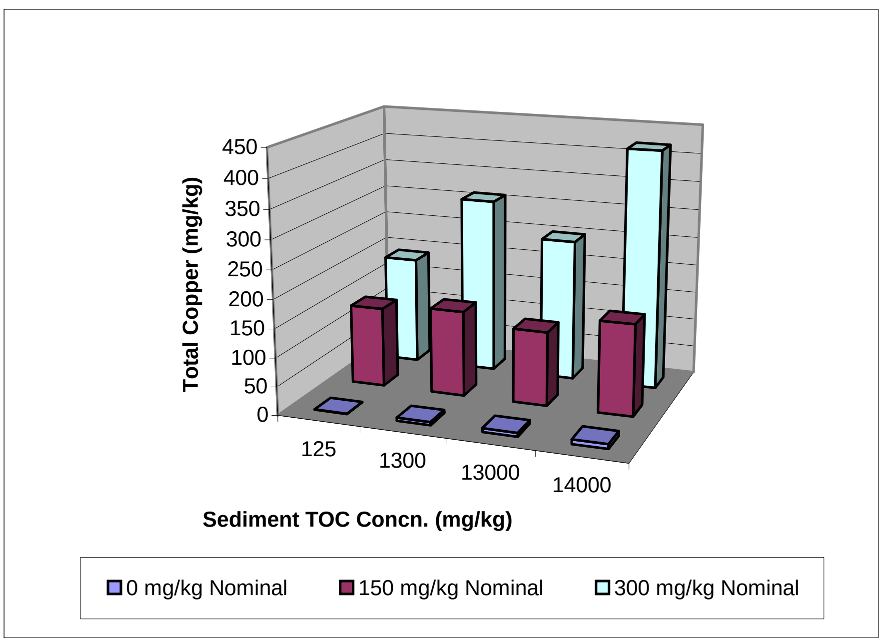


Figure 4-29
Total Copper in Sediment in Test 034



Note: Test 033 was a water-only exposure.
Nominal concentrations are for copper in overlying water (Test 033) and copper in sediment (Test 034).

Figure 4-30
Total Recoverable Copper in Water in Test 034

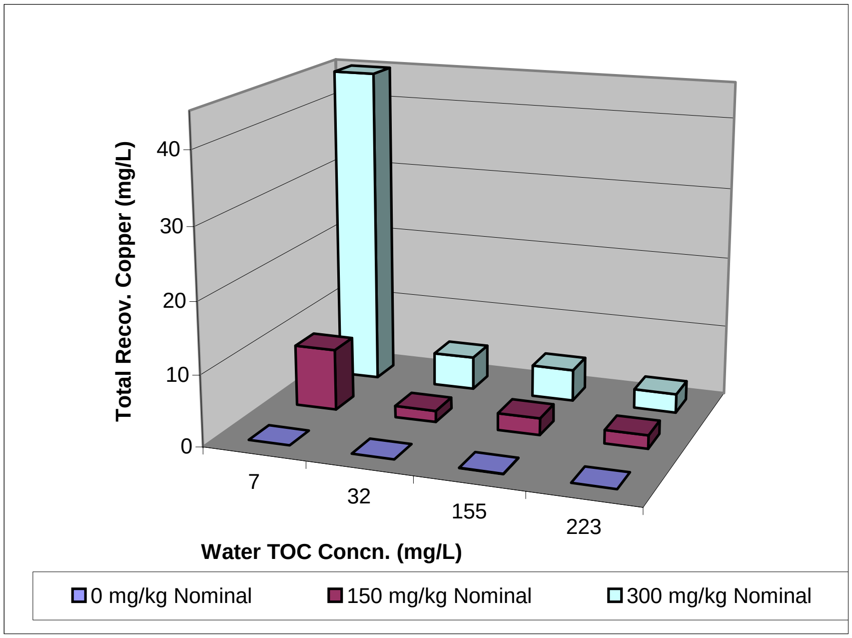
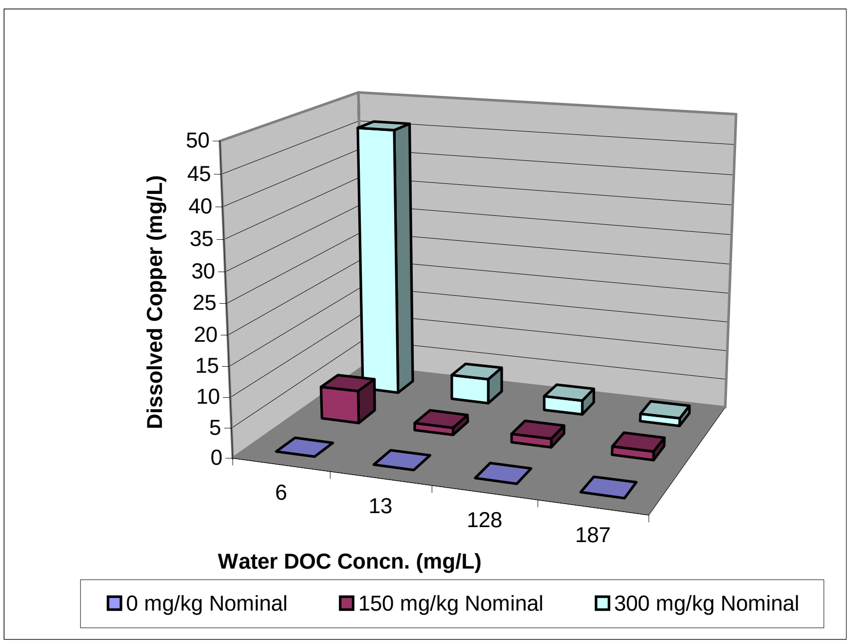


Figure 4-31
Dissolved Copper in Water in Test 034



Note: Nominal concentrations are for copper in sediment.

Figure 4-32
Survival of Bufo in Test 034

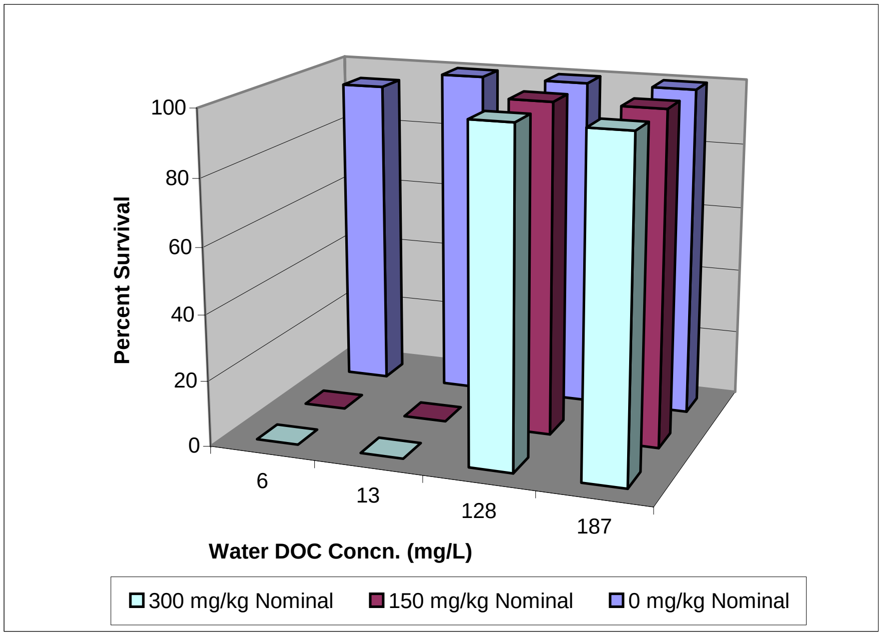
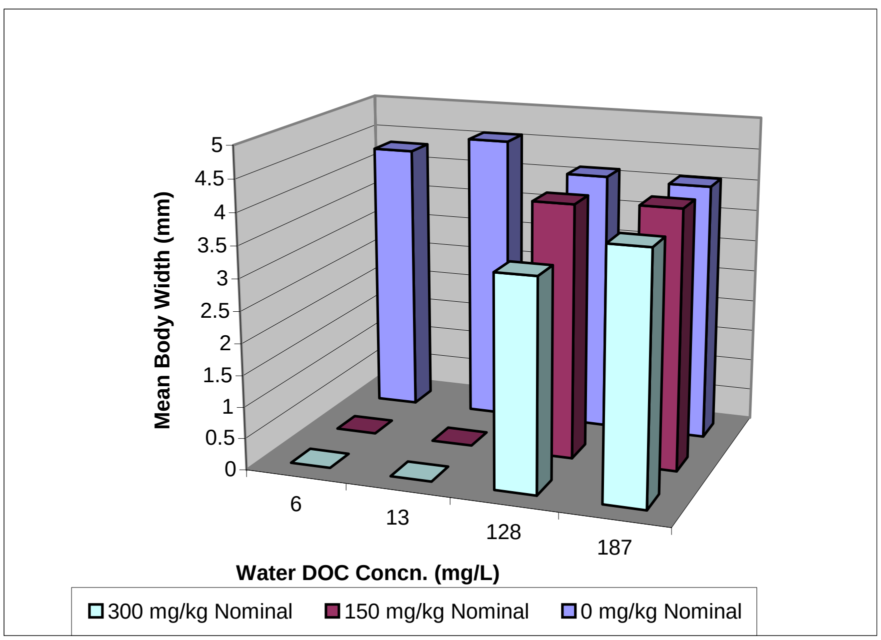


Figure 4-33
Mean Body Width of Bufo Tadpoles in Test 034



Note: Nominal concentrations are for copper in sediment.

Figure 4-34
Mean Body Length of Bufo Tadpoles in Test 034

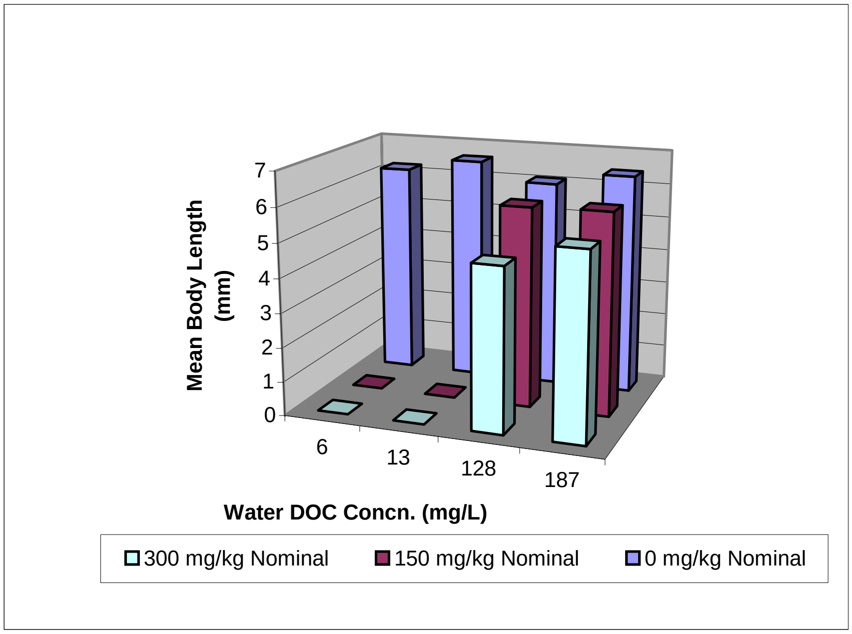
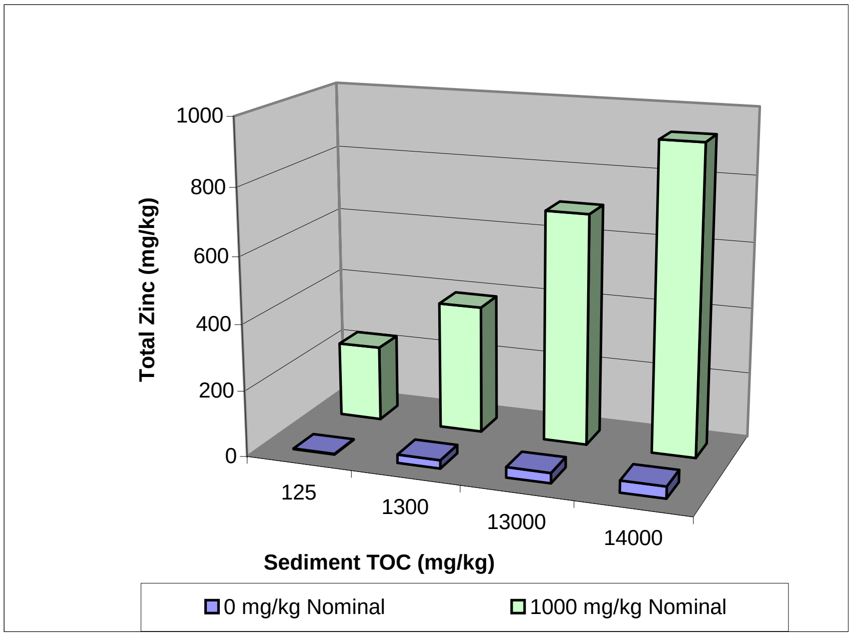


Figure 4-35
Total Zinc in Sediment in Test 034



Note: Nominal concentrations in Figure 4-34 are for copper in sediment.
Nominal concentrations in Figure 4-35 are for zinc in sediment.

Figure 4-36
Total Recoverable Zinc in Overlying Water in Test 034

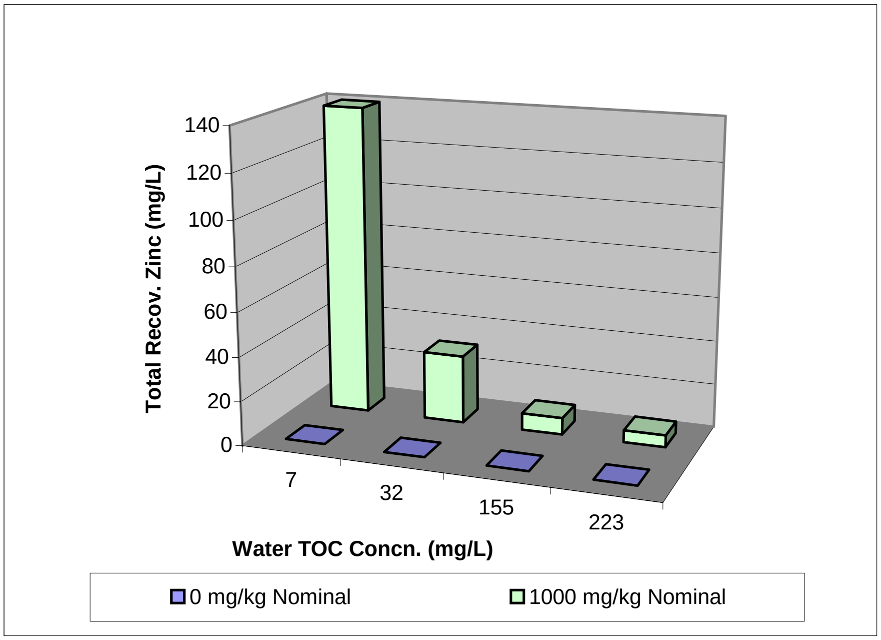
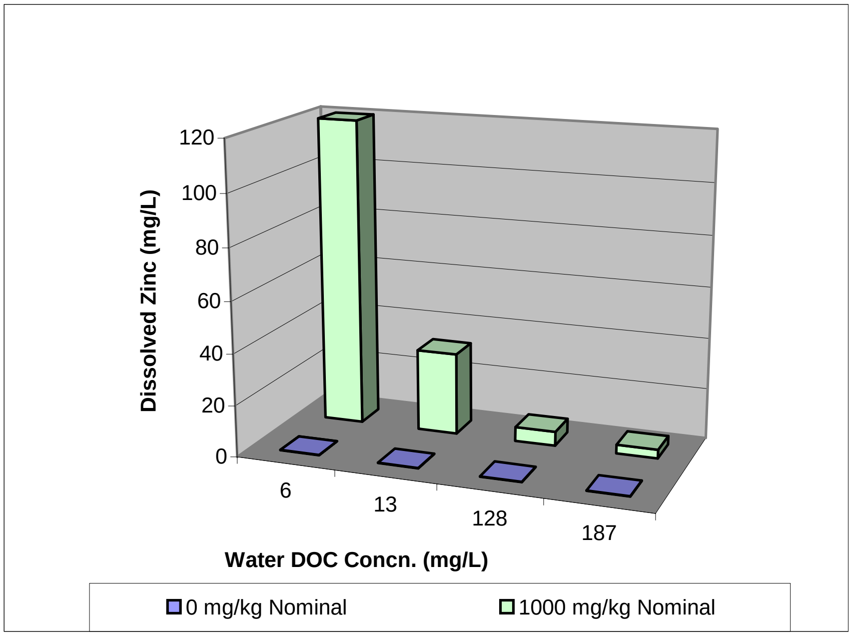


Figure 4-37
Dissolved Zinc in Overlying Water in Test 034



Note: Nominal concentrations are for zinc in sediment.

SECTION 5
DISCUSSION

The purpose of this phase of the YO817 study was to evaluate the toxicity of four metals to larval amphibians exposed to sediment/hydric soil. One of difficulties in determining the toxicity of individual chemicals in field-collected sediments is that many sediments often contain a mixture of organic and inorganic materials that may be toxic to test organisms or, if not directly toxic, interact to modify the toxicity of other chemicals. To avoid this potential concern during this phase of the YO817 study, target analytes were spiked to a natural sediment containing ambient levels of background contaminants that are not toxic to test organisms. This spiked sediment was used to approximate the hydric soil typically found in larval amphibian breeding pools.

The test procedures used for this phase of the YO817 study were developed during earlier phases of the study to establish a standard, short-term testing method for amphibians (ENSR, 2002). The test method uses recently hatched, early life-stage tadpoles since studies during the method-development phase strongly indicated that younger animals were significantly more sensitive to inorganic toxicants than older organisms. Certain test parameters generally followed those established by USEPA and ASTM for benthic macroinvertebrate organisms (i.e., *Hyalella azteca* and *Chironomus tentans*), including test temperature and test length. Biological endpoints evaluated in this amphibian study included survival, body width, and body length.

Four divalent metals were tested this phase of the YO817 program: copper, cadmium, lead, and zinc. Two rounds of tests were conducted with *Rana* sp. and one round of tests was conducted with *Bufo americanus*. Although several water-column tests were previously conducted with copper and cadmium (ENSR, 2002), it was difficult to predict what the toxicity of these metals, as well as lead and zinc, would be in a sediment matrix with a much higher organic carbon content. The first round of tests with *Rana*, therefore, was intentionally set with a broad range of copper concentrations in an attempt to bracket the effects concentrations. This technique was unsuccessful with copper and zinc where there were no significant effects during the first round of testing. In the second round of testing, significant effects were observed for zinc, but no effects were observed for either *Rana* or *Bufo* exposed to copper, even though the target sediment concentration was increased by an order of magnitude.

In addition to copper, lethal and sub-lethal effects concentrations were determined for cadmium, lead and zinc. A summary of the statistical endpoints is provided in Table 5-1. From these endpoints, it appears that for the endpoints evaluated during this study, both *Rana* and *Bufo* tadpoles are generally more tolerant of copper, cadmium, lead, or zinc than test organisms typically used in establishing ambient water quality criteria or sediment quality threshold values (Tables 5-2 and 5-3). The lowest cadmium IC_{25} calculated during this study was 0.54 mg/L (540 μg/L). The EPA chronic criterion for cadmium is 0.25 μg/L at a hardness of 100 mg/L as $CaCO_3$. In this study the hardness of the overlying water was generally high (400-600 mg/L) after the overnight settling period but then tended to drop once flow-through had begun. Even adjusted to a hardness of 500 (the maximum allowed in algorithms for hardness-dependent water quality criteria), the chronic criterion is still much lower than the lowest effects level from this study. Similarly, the lowest calculated zinc IC_{25} was 7.2 mg/L (7,200 μg/L), which is 60 times the chronic criterion of 118 μg/L at a hardness of 100 mg/L as

$CaCO_3$. A similar phenomenon was observed with the evaluation of amphibian endpoint data relative to sediment quality benchmarks.

The effects of organic carbon on the toxicity of copper and zinc were substantial. In the PR Sediment + 15% sheep/peat, which was the standard sediment used for all spiked studies, the TOC was approximately 14,000 mg/kg, or roughly 1.5%, resulting in a water-column DOC concentration of approximately 187 mg/L. At this TOC/DOC level, even a dissolved copper concentration of 1,300 μg/L (several orders of magnitude above the copper AWQC) was insufficient to induce significant measurable negative effects. However, when Un-amended PR Sediment was used, resulting in a DOC concentration of 13 mg/L, 100% mortality was observed at 1,200 μg/L dissolved phase copper. Although hardness in the PR Sediment + 15% sheep/peat treatment was also higher than in the PR Sediment, a shift in hardness alone would be insufficient to provide the level of protection observed.

Even at a TOC level of 1.5%, the amended PR Sediment used in this study may not be representative of the hydric soils often encountered in wetlands on Naval facilities. Such wetland sediments may contain organic carbon levels of 5% or higher. Given the information gathered in the series of studies described here, such wetlands could potentially harbor relatively high concentrations of some metals, such as copper, without causing short-term chronic toxicity to amphibians. However, it is not known whether exposure to very high levels of metals, even if they are sequestered by sediment organic matter, could cause subtle, long-term toxicity to amphibians which might, in-turn, affect amphibian populations.

Tissue residue IC_{25}s presented in Table 5-1 are low effect thresholds representative of metals concentrations in larval amphibians associated with the tested endpoint. For instance, the lowest IC_{25} for cadmium in tissue (51 mg/kg wet weight) is the IC_{25} associated with the *Rana* length measurement endpoint. Concentrations in tissue above this IC_{25} benchmark are presumed to be associated with adverse growth (i.e., length) effects for the tested organism.

Table 5-1
Summary of Statistical Endpoints

Metal	Taxa	Lowest IC_{25}			Lowest LC_{50}	
		Sediment (mg/kg)	**Dissolved Water (mg/L)**	**Tissue (mg/kg)**	**Sediment (mg/kg)**	**Dissolved Water (mg/L)**
Cd	*Rana*	230[a]	0.54[b]	51[a]	700	2.9
	Bufo	540[a]	1.0[a]	170[a]	>580	>1.1
Cu*	*Rana*	64[a,b,c]	0.28[a,b,c]	16[a,b,c]	>64	>0.28
	Bufo	200[a,b,c]	0.9[a,b,c]	93[a,b,c]	>200	>93
Pb	*Rana*	3490[a,b]	0.43[a,b,c]	NA	4662	0.58
	Bufo	NA	NA	NA	NA	NA
Zn	*Rana*	980[c]	7.2[c]	NA	1500	19
	Bufo	1600[b]	28[a]	170[a]	2100	35

NA = Effect insufficient for point estimates
a – length statistical endpoint
b – width statistical endpoint
c – survival statistical endpoint
*Lowest NOEC or LOEC values are presented. No effects were observed for copper exposures.

Table 5-2

Comparison of Surface Water Screening Benchmarks to Lowest Statistical Endpoints

Analyte (ppb)	**Chronic Values**				**Acute Values**			
	Chronic AWQC		**Lowest IC_{25}**		**Acute AWQC**		**Lowest LC_{50}**	
Inorganics	**Hardness 100 mg/L**	**Hardness 500 mg/L**	***Bufo***	***Rana***	**Hardness 100 mg/L**	**Hardness 500 mg/L**	***Bufo***	***Rana***
Cadmium	0.25	0.84	1,000	540	2	11	> 1100	2,900
Copper	9	35.4	NA	280*	13	61.2	NA	> 280*
Lead	2.5	13.7	NA	430	65	352	NA	580
Zinc	120	462	28,000	7,200	120	458	35,000	19,000

NA - not analyzed
ppb - parts per billion
AWQC - Ambient Water Quality Criteria (dissolved phase)
IC - Inhibition Concentration
LC - Lethal Concentration
Hardness measured in mg $CaCO_3$/L
AWQCs from USEPA, 2002.
*NOEC/LOEC for dissolved phase

Table 5-3
Comparison of Sediment Screening Benchmarks to Lowest Statistical Endpoints

Analyte	Lowest IC_{25}		Low Effect Levels			Lowest LC_{50}		Severe Effect Levels		
	Rana	***Bufo***	**MIN**	**MAX**	**Source**	***Rana***	***Bufo***	**MIN**	**MAX**	**Source**
Inorganics					**Minimum/Maximum**					**Minimum/Maximum**
Cadmium	230	540	0.6	1.2	LEL (OMOE)/ERL (NOAA)	700	> 580	4.98	9.6	Consensus PEC/ERM (NOAA)
Copper	64*	NA	16	34	LEL (OMOE)/ERL (NOAA)	> 64	NA	110	270	SEL (OMOE) at 1% TOC/ERM (NOAA)
Lead	3,490	NA	31	46.7	LEL (OMOE)/ERL (NOAA)	4,662	NA	128	218	Consensus PEC/ERM (NOAA)
Zinc	980	1,600	120	150	LEL (OMOE)/ERL (NOAA)	1,500	2,100	410	459	ERM (NOAA)/Consensus PEC

NA = Not Analyzed
*highest inhibition concentration (IC) used in study without detectable effect
ERL – Effects Range Low
ERM – Effects Range Median
LEL – Low Effects Level
PEC – Probable Effects Concentration
SEL – Severe Effects Level
Sources:
NOAA – National Oceanic and Atmospheric Administration.1999. Screening Quick Reference Tables.
OMOE –Ontario Ministry of the Environment, 1996. Guidelines for the Protection and Management of Aquatic Sediment Quality in Ontario.

SECTION 6
SUMMARY AND CONCLUSIONS

This report presents a focused evaluation of the SOP developed to evaluate the potential effects of sediment/hydric soil exposure to early life stage amphibians. The purpose of the SOP development and validation studies was to develop and refine a test methodology that can be incorporated into the development of a standardized risk assessment protocol for evaluating potential risks to amphibians at sites owned and/or operated by the United States Navy.

The methods and results of the validation testing are summarized below:

- Tadpoles of two North American anurans, *Rana* (likely *pipiens*) and *Bufo americanus* were used to assess the toxicity of copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn) in hydric soils.
- Natural sediment was amended with approximately 15% (by weight) sheep manure/peat compost and then spiked with solutions containing salts of the four divalent metals of interest.
- Flow-through tests were conducted for 10 days; the biological endpoints measured were survival, body width, and body length.
- Control organisms generally showed good survival although low levels of dissolved oxygen in some test chambers may have caused mortality unrelated to the levels of metals added to the sediment.
- In sediment containing 15% sheep/peat, no effects from Cu were found even though sediment Cu concentrations were as high as 200 mg/kg, and dissolved Cu levels in the water were close to 1,000 μg/L.
- Chronic effects sediment concentrations, as measured by IC_{25}, ranged from 230 mg/kg for Cd to 3,490 mg/kg for lead; IC_{25}s for dissolved metals ranged from 430 μg/L for lead to 28,000 μg/L for zinc.
- Copper and zinc toxicity is strongly associated with the amount of organic carbon in the test. High levels of sediment organic carbon bind these metals, retaining them in the sediment and decreasing concentrations in the water column. Some uncertainty is associated with the contribution of copper and zinc from the total organic carbon source (sheep/peat). A data gap representing the dissolved and total metals concentrations in sheep/peat exists, as well as the bulk metals concentrations in sheep peat is currently being filled.
- In general, the results of this phase of the YO817 study confirmed the results of the Phase I Literature Review (ENSR, 2001), which suggested that relative to the toxicity testing endpoints evaluated herein, amphibian test thresholds were generally substantially higher than AWQC and other literature-derived benchmarks.

Given the information derived from these studies, it appears that this testing methodology could effectively be used to evaluate potential hydric soil/amphibian breeding pool toxicity at Navy sites. It is recommended that this SOP for conducting sediment toxicity tests with amphibians be incorporated into the ecological risk assessment process used by the Navy. The purpose of the SOP is to help evaluate possible effects of chemical stressors in sediments and hydric soils on amphibians in natural ecosystems. This test method uses an early life stage of a native North American species, and lethal and sub-lethal toxicity endpoints that are relevant to typical assessment endpoints considered by the Navy in their ecological risk assessments.

SECTION 7
REFERENCES

Allran, J.W. and W.H. Karasov, 2000. Effects of Atrazine and Nitrate on Northern Leopard Frog (*Rana pipiens*) Larvae Exposed in the Laboratory from Post-hatch through Metamorphosis. *Environ. Toxicol. Chem*. Vol. 19, No. 11, pp. 2850-2855.

American Society for Testing and Materials (ASTM), 2001a. Test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates. E 1707-00. pp. 1109-1225 *in* Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides.

American Society for Testing and Materials (ASTM), 2001b. Standard guide for conducting acute toxicity tests on aqueous ambient samples and effluents with fishes, macroinvertebrates, and amphibians. E 1192-97. pp. 405-417 *in* Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides.

American Society for Testing and Materials (ASTM), 2001c. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. E 729-96. pp. 175-196 *in* Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides.

American Society for Testing and Materials (ASTM), 2001d. Standard guide for conducting the frog embryo teratogenesis assay-Xenopus (FETAX). E 1439-98. pp. 776-791 *in* Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides.

Analytical Software. 2000. Statistix7. Analytical Software, Tallahassee, FL.

Berrill, M., S. Bertram, L. McGillivray, M.Kolohon, and B. Pauli. 1994. Effects of low concentrations of forest-use pesticides on frog embryos and tadpoles. *Environ. Toxicol. Chem*. Vol. 13, No. 4, pp. 657-664.

Birge, W. and J. Just, 1973. Sensitivity of vertebrate embryos to heavy metals as a criterion of water quality. *U.S. Nat. Tech. Inform. Serv*. PB Rep., Issue No. 226850/6GI.

Birge, W.J., A.G. Westerman, and J.A. Spromberg, 2000. Comparative Toxicology and Risk Assessment of Amphibians. *In*: Ecotoxicology of Amphibians and Reptiles. Eds. D.W. Sparling, G. Linder and C.A. Bishop. SETAC Technical Publication.

Cabejszek, I. and J. Wojcik, 1968. Trial application of tadpoles *Xenopus laevis* for estimation of harmfulness of pesticides in water. *Rocz. Panstw. Hig*. ,4, pp. 499-506.

Diana, S.G., W.J. Resetarits, D.J. Schaeffer, K.B. Beckmen, and V.R. Beasley, 2000. Effects of atrazine on amphibian growth and survival in artificial aquatic communities. *Environ. Toxicol. Chem.* Vol. 19, No. 12. pp. 2961-2967.

Ditsworth, G.R. and D.W. Schults, 1990. Preparation of benthic substrates for sediment toxicity testing. *Environ. Toxicol. Chem*. 9:1523-1529.

ENSR, 2001. Draft Deliverable No. 1: Literature Review and Development of Amphibian Screening Values. Document Number 09070-045-106. October 2001.

ENSR, 2002. Draft Deliverable No. 2: Development of Short-Term Chronic Sediment Toxicity Test Using Early Life Stage Amphibians. Document Number 09070-045-211B. May 2002.

Fordham, C.L., J.D. Tessari, H.S. Ramsdell and T.J.Keefe. 2001. Effects of malathion on survival, growth, development, and equilibrium posture of Bullfrog tadpoles (Rana catesbeiana). Environ.Toxicol.Chem. 20.1.179-184.

Gosner, K.L., 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica. 16:183-190.

Greenhouse, G. 1976. The Evaluation of Toxic Effects of Chemicals in Fresh Water by Using Frog Embryos and Larvae. *Env. Pollut*. Vol. 11, pp.303-315.

Harris, M.L., C.A. Bishop, J. Struger, B. Ripley and J.P. Bogart. 1998. The functional integrity of northern leopard frog (Rana pipiens) and green frog (Rana clamitans) populations in orchard wetlands. II. Effects of pesticides and eutrophic conditions on early life stage development. Environ.Toxicol.Chem.17.7.1351-1363.

Hatch, A. and G.A. Burton, 1998 Effects of photoinduced toxicity of fluoranthene on amphibian embryos and larvae. *Environ. Toxicol. Chem*. Vol. 17, No. 9. pp. 1777-1785.

Kagan, J, P.A. Kagan, and H.E. Buhse, 1984. Light-dependant toxicity of alpha-terthienyl and anthracene toward late embryonic stages of *Rana pipiens. Journal of Chem. Ecol*., Vol. 10, No. 7, pp. 1115-1122.

Murphy, J.E., C.A. Phillips, V.R. Beasley, 2000. Aspects of Amphibian Ecology. *In*: Ecotoxicology of Amphibians and Reptiles. Eds. D.W. Sparling, G. Linder and C.A. Bishop. SETAC Technical Publication.

National Oceanic and Atmospheric Administration (NOAA).1999. Screening Quick Reference Tables. http://response.restoration.noaa.gov/cpr/sediment/squirt/squirt.html

Norberg-King, T.J., 1993. A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0). NETAC Technical Report 03-93, United States Environmental Protection Agency, Duluth, MN.

Ontario Ministry of the Environment (OMOE), 1996. Guidelines for the Protection and Management of Aquatic Sediment Quality in Ontario, Ontario Ministry of the Environment, Queen's Printer for Ontario; 23 pp.

USEPA. 1986. Test methods for evaluating solid waste. SW-846, Third Edition. United States Environmental Protection Agency, Office of Solid Waste and Emergency Response.

USEPA, 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth Edition. EPA/600/4-90/027F.

USEPA, 1994. USEPA Toxicity Data Analysis Software. Version 1.5. United States Environmental Protection Agency, Cincinnati, OH.

USEPA, 1996. Ecological effects test guidelines, OPPTS 850.1800 Tadpole/ sediment subchronic toxicity test. EPA 712-C-96-132. Office of Prevention, Pesticides, and Toxic Substances.

USEPA, 1999. Integrated Approach to Assessing the Bioavailability and Toxicity of Metals in Surface Waters and Sediments. EPA–822-E-99-001. Office of Water.

USEPA, 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. Second Edition. EPA/600/R-99/064. Office of Water.

USEPA, 2002. National Recommended Water Quality Criteria: 2002. EPA-822-R-02-047. November 2002.

WEST, Inc. and D.D. Gulley, 1996. Toxstat Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, WY.